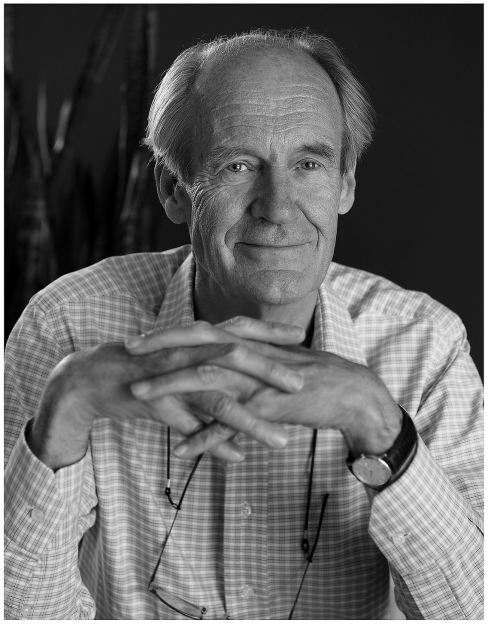
Tomas Höckfelt



Tomas Hökfelt

Born:

Stockholm, Sweden
June 29, 1940

Education:

Karolinska Institutet Stockholm, Medicine Candidate (1962)
Karolinska Institutet Stockholm, PhD (1971)
Fogarty Fellow, National Institutes of Health, Bethesda, MD (1981/1982)

Appointments:

Docent, Histology, Karolinska Institutet (1969–1979)
Professor of Histology and Cell Biology, Karolinsk Institutet (1979–2016)
Associated member/member of the Nobel Committee for Physiology or Medicine (1981–1991, 1994–1996)

Honors and awards:

Associate of Neuroscience Research Program (1977–1989)
Foreign Associate, National Academy of Sciences USA (1984)
Member of Swedish Royal Academy of Sciences (1985)
Member of Academia Europaea (1989)
Member, Accademia Nazionale Dei Lincei (1993)
Member, American Academy of Arts and Sciences (1996)
Member, Royal Danish Academy of Sciences and Letters (1996)
Foreign Member, Chinese Academy of Sciences (2000)
Honorary Member, Hungarian Academy of Sciences (2004)
Foreign Associate, Institute of Medicine/National Academy of Medicine USA (2006)
Foreign Member, The Norwegian Academy of Science and Letters (2021)
Doctor *honoris causa* at several universities
Hilda and Alfred Eriksson's Prize, Royal Academy of Sciences, together with K. Fuxe (1979)
The Artois-Baillet Latour Health Prize, together with V. Mutt (1987)
The Bristol-Myers Award for Distinguished Achievement in Neuroscience Research, together with W. Nauta and T. P. S. Powell) (1988)
Anders Jahre's Prize (1990)
The Eli Lilly Preclinical Research Award (1991)
Grand Medaille, Academie des Sciences (Paris) (2007)
Golden Kraepelin Medal (Munich) (2012)
The Arvid Carlsson Prize (2022)

Tomas Hökfelt is a histochemist with a focus on chemical signaling in the nervous and endocrine systems using a variety of approaches: the formaldehyde fluorescence (Falck-Hillarp) method for monoamines, autoradiography for amino acids, immunohistochemistry, and in situ *hybridization. These techniques have been complemented with electrophysiology and standard molecular biology. He has explored the localization and role of neuropeptides and their coexistence with classic transmitters with special interest in pain and mood behavior, as well as neurodegeneration and systems important for control of food intake. Over many years, he studied rodents but has more recently analyzed human postmortem brains, also from depressed patients. A goal has been to search for novel strategies to treat human diseases.*

Tomas Hökfelt

Prologue

The reason for me being invited to write this article can be spelled Nils-Åke Hillarp. He was recruited from Lund to our Histology Department at Karolinska Institutet (KI) in 1962, passed away in 1965, just 49 years old, but still changed my life and that of several others, as will be told. Of course, many other people have been important. This article is dedicated to all of the wonderful and talented PhD students (more than 20), postdoctoral fellows and guest scientists (altogether about 75), mentors, worldwide colleagues, and, last but not least, technicians and secretaries, with whom I have collaborated for nearly six decades. During some periods, my research group included more than a dozen members, often thanks to U.S. National Institutes of Health (NIH) and Canadian Medical Research Council (MRC) grants to support U.S. and Canadian postdocs. In later years, Chinese PhD students and postdoc fellows played a particularly important role. Through the years, many of the ideas and projects in the laboratory sprung out of my collaborators' minds (motivation is strong when working on own ideas!). Also, antibodies have been the scientific oxygen in my professional life, and many of them have been generously supplied by colleagues around the globe. I will first go through my life and research chronologically, the first part describing the period before my Fogarty scholarship at NIH, Bethesda, USA, the second part covering research after returning to Sweden. I will present both interesting (to me) results as well as some interesting errors. The last part deals with special topics.

My Family and Early Life

My mother Sonja was born in Stockholm; her father died early. Her mother Olga was a wonderful (grand-)mother who supported herself and her two children by running a small cab company; she also had a garden with delicious raspberries. There was neither time nor financial resources for higher education. My father Paul's family had a small farm in Småland, a poor region from where many Swedes emigrated to the United States. My father was born, however, in southern Lapland (Lapponia), high up in the North in the small village of Vinliden. The reason was that my grandfather, Gustav Karlsson, had become a missionary in Lapland, a region even poorer than Småland. During my military service in the North of Sweden in 1960, my father visited me, and we made a tour, including Vinliden. His birth house was still there but uninhabitable. We met an old lady who

remembered the Karlsson family, and we were invited for coffee and buns. A very moving event.

The family moved back to their farm, called *Hök*argården (the origin of my family name), in Landsbro in Småland. My grandfather regularly alone went back to Lapland for further missions, whereby his return to the farm resulted in my father getting five siblings. As the eldest child, my father became responsible for the farm and only completed four years in school. As a young man, he moved to Stockholm and worked as an errand boy in his uncle's trading company. In 1937 he married my mother. I was born in 1940, my brother Stefan in 1944, and my second brother Paul (Jr.) in 1953.

Choosing a career in business with several job changes and mostly living abroad, Stefan and Paul always asked at our fairly rare meetings what I was doing. Their somewhat compassionate reaction to my attempts to explain was always, "so just the same." I attended school in Stockholm for six years. My real interest was sports—ice hockey, swimming, basketball, and more—but these activities stopped after our family moved to Germany. Here I started to play golf in 1955. Two years later, I was down to a 7 handicap. But then I hit a caddie: from a high tee, my 150 m shot made a perfect curve and the boll dropped right on the poor boy's head (concussion but fast recovery), as close as I ever got to a hole-in-one. (An interesting example of the impact of "free fall"; a second one will follow.) After that, I never played a decent round of golf. A tough but I guess fair punishment. This said, I played many rounds of golf with my father up till his too early passing away.

Still, I had some use of my swimming: during my military service, I won the regiment championship in three disciplines. The lieutenant under whom I served was initially very much against my participation in this event. After my "victories," he suddenly became amicable. My grandson Gustav, who was initially coached by my son Patrik, has taken over the competitive swimming baton. Gustav now holds some Swedish records in backstroke, thanks to a generous four-year stipend from George Washington University, Washington, D.C., where he greatly improved his skills and was appointed team captain of the GWU swimming team. In all fairness, my granddaughter Hedda is also great. She has studied digital cultures at Lund University and now works as a social media coordinator in a company manufacturing electric boats.

While raising a family in Stockholm, my father was employed by the Swedish National Association of Farmers (SNAF), trading agricultural products. In the early 1950s, he was offered a job by a large international company, Bunge and Born, based in Buenos Aires, as head of their Scandinavian and German operations. This precipitated our family's move to Germany. After 10 years the family moved back to Sweden, with my father now serving as the CEO of SNAF (well done considering only four years of school).

Frankfurt am Main and Hamburg

In the autumn of 1953, our family landed in Frankfurt. This meant a dramatic change in lifestyle and economic resources; and a transfer from a clean and orderly Sweden, not harmed by war, to a Frankfurt badly damaged by bombs. Because I did not speak German, I was placed into a private school in a small village 10 miles outside of Frankfurt, with hens and ducks in the street, a bakery (wonderful bread!) in one corner of the inner yard, and a dung hill at the opposite corner.

My mother had always been ambitious on my behalf. Before moving to Germany, she insisted that I learn the German irregular verbs; so I studied and she examined. The first exam in the class was actually irregular German verbs. It turned out that German kids also had difficulties with them. Thanks to my mother, I wrote the best test, even though I could not speak a single sentence in German. Half a year later, I moved to a school in the city, Goethe Real Gymnasium. There was a shortage of teachers and lecture halls, and half the pupils occupied the rooms during the morning and the other half in the afternoon. A sick teacher meant cancelled lectures and free time. By that point, I was pretty fluent in German. To improve my language skills, my parents allowed my brother Stefan and me to go to the movies two or three times a week (foreign movies were dubbed into German). So I have heard them all—John Wayne, Richard Widmark, Cary Grant, and many more—"speaking German."

In 1955, my father's company moved to Hamburg, and five very pleasant years followed, after which I finished up with abitur in 1960. I very much enjoyed and appreciated attending the Ernst-Schlee-Gymnasium, a fine school with great teachers and class mates. Several teachers had served in the war with different types of experiences. Our Latin teacher "survived" because he was sitting in the trenches, by heart reciting the works of the great Latin poets. Another teacher, in physics, had a badly hurt leg, but he stays in my mind for another reason: his demonstration of gravity from the roof of the school, with a stopwatch in his right hand and a stone in the other became a memorable event: while pressing the stone, he dropped the watch (my second example of "free fall").

Every second year, the class went for a week to a school home on the island of Föhr in the North Sea. We wandered over the wet land exposed to ebb and flow of the tides, a wonderful and unique experience. A special event was the personal washing procedure, which took place twice during the visit. We pupils stood in a long queue in front of a single outdoor shower with the gymnastics/sports teacher holding a stopwatch in his hand: shower for 60 seconds, then soaping, followed by another 60 seconds of rinsing. Very efficient, and cost effective. What we suspected was confirmed just a couple of years ago when visiting my old classmates in Hamburg: our teacher had been an SS officer during the war.

Return to Sweden

After abitur in Hamburg, I went back to Sweden for university studies. The move from Sweden in 1953 had been somewhat of a nightmare, losing my comerades, no more ice hockey, new language and much more, but I now realized that the seven years in Germany had indeed widened my horizons. I had experienced a people that had been hit hard by war and now was rebuilding their country. Travelling with my family I also early on got to know many other European countries and their customs and practices. Indeed, these years shaped my life.

The grading system for university access was different in Germany and Sweden, and my total points were not enough to get into medical school. So my mother (again) and I summarized my German curriculum and sent that to the University Chancellor in Sweden for evaluation. My first choice was to study medicine. One influence behind my choice was my uncle *Bernt* Hökfelt, who has been a bit of a hero for me. Bernt had a small *sports* car, which I always included, along with my uncle, in my evening prayer. In contrast to my father, Bernt had been able to go through all school classes, and after matriculation he was admitted to KI. Bernt made his thesis on noradrenaline (NA), adrenal gland, and more with Ulf von Euler in the Department of Physiology as supervisor; he then transferred to Karolinska Hospital. Here, Rolf Luft had created an excellent Department of Endocrinology and been appointed the first professor in endocrinology in Scandinavia. Together with Nils-Åke Hillarp my uncle published, in 1953, results that would end up in textbooks: there are separate adrenaline and NA cells in the adrenal medulla. My uncle later moved to Malmö to become the first professor of endocrinology at Lund University.

With the uncertainty of the value of my German degree in mind, I had also applied for other academic fields upon my return to Sweden, including at the Royal Technical High School (KTH). On a Monday in August 1960, I had an appointment with the professor in Technical Physics. When I opened the daily newspaper, I found my name on the list of students accepted to KI. I immediately cancelled my appointment at KTH, which was a big relief. In my official examination book, it is written that I had been admitted by a personal decision of the university chancellor. Thus, the German school curriculum was of a high quality, and the summary had been useful.

Karolinska Institutet: Medical Studies

In 1960, after three summer months of military service, I entered KI to start the class in anatomy/histology. The first examination was in histology. I was so nervous that the examiner, Professor Lars Gyllensten, asked me to take a walk and come back in 10 minutes. Already also an established novel writer, Lars Gyllensten had started has literary career with a prank: Gyllensten

wrote together with Torgny Greitz, later chair of radiology at the Karolinska Hospital, under pseudonyms, a small, positively reviewed pamphlet. When exposed as a joke, the blame was initially on the critics, but the future would prove them right: Gyllensten became one of Sweden's most famous authors and permanent secretary of the Swedish Academy, the body that awards the Nobel Prize in Literature, and later was named chair of the Nobel Foundation. In 1971, he stepped down as professor of histology at KI, a position that I eight years later successfully applied for.

In 1966, I had completed all medical courses, except one: oto-rhino-laryngology. I also had worked as a deputy physician for a couple of months in a mental hospital. In parallel, I had started modest experiments in the Histology Department and was involved in teaching. And, importantly, I had married Lil, and soon Patrik (1964) and Paula (1967) were born. This was economically a difficult time, and the very generous support provided by my father was essential for my possibility to early form a family and to pursue a research career.

Nils-Åke Hillarp

Hillarp was appointed professor in the Department of Histology at KI in 1962. He came from Lund University, where he had made several seminal contributions to neuroscience and endocrinology (see Hökfelt, 2010). A more detailed article on Hillarp has been written by Annica Dahlström (1996). Hillarp's last project before moving to Stockholm was the development of a novel method: the formaldehyde fluorescence method (FAM), also known as the Falck-Hillarp technique, allowing demonstration of the monoamine transmitters dopamine (DA), NA, and, less efficiently, serotonin (5-hydoxytryptamine, 5-HT) in brain and peripheral tissues in a microscope—a revolution in neuroscience. Hillarp worked out the method in Lund together with his younger colleague and former student Bengt Falck, and with a team around Arvid Carlsson in Gothenburg (Falck et al., 1962); and they published the first study with the title "Cellular localization of brain monoamines" (Carlsson et al., 1992). A new field, "chemical neuroanatomy" was born.

The Amine Group and PhD Theses: Exciting Times

It is a special privilege for a young scientist to be lucky and work in a lab, where everybody feels that new knowledge is continuously generated, and to see things no one else has seen before. That is what happened in 1962 to the 10 students Hillarp recruited after his arrival at KI, the first ones being Kjell Fuxe and Annica Dahlström, followed by Torbjörn Malmfors (passed away 2022), Karl-Axel Norberg (passed away 2021), Gösta Jonsson (passed away 2019), Bertil Hamberger, Tomas Hökfelt, Lars Olson, Charlotte

Sachs and, the last, Urban Ungerstedt. We all defended our thesis, but sadly we did so *after* Hillarp has passed away because of a malignant melanoma. Six of Hillarp's initial 10 students advanced to become full professors. This was achieved despite limited resources, because Hillarp gave us great projects and excellent guidance, because of our hard work, and because the 10 of us stuck together and helped each other by forming the Amine Group (AG). The premature departure of our leader and supervisor, however, allowed us to flourish independently from early on.

When setting up FAM at KI, it was recognized that diffusion of the amines in the tissue was a critical issue. To that end, the samples were freeze-dried and reacted with para-formaldehyde (PFA) vapors. Moreover, the humidity of the PFA powder was monitored, and all steps up to embedding in paraffin were carried out in a climate-controlled room, a protocol developed by Bertil Hamberger, Torbjörn Malmfors, and Charlotte Sachs and reported in a one-page, key paper in 1964.

My own project under Hillarp's supervision was to localize the monoamine transmitters at the ultrastructural level—that is, in the electron microscope (EM)—with special focus on the brain. I was given this task, because I had trained with Professor Ove Nilsson, an excellent electron microscopist. I defended my thesis in 1968, but was saddened that Hillarp never knew of my success at confirming the results he had predicted. (Hillarp always wrote protocols for his students, including number of animals, drugs, doses, times, and expected results, and the results he had proposed were always correct.) Being the last student joining Hillarp, his illness left him with very little time to spend on me. Also sadly, several years after Hillarp's passing away I was told by another AG member that he during the daily coffee pause had looked around and asked for me. When noting my absence, he said "well, Tomas is apparently not that interested in research." I was too busy perfusing rats in the basement and had no time to attend the coffee pause. So my advice to young scientists is this: don't miss the coffee hour with your supervisor.

The background for my thesis project was that Keith Richardson, as well as Eduardo de Robertis and Amanda Pelligrino De Iraldi and several more had demonstrated that *peripheral* sympathetic, noradrenergic nerve terminals contain a special type of synaptic vesicles (SVs) (diameter about 500 Å), which have a reserpine-sensitive, electron-dense core, thus in all probability representing NA. In addition, there were large dense core vesicles (LDCVs) (diameter about 1,000 Å) that, especially after glutaraldehyde exposure, had a dense core of variable opaqueness. It had been suggested that these LDCVs may represent the amine storage sites in the anterior hypothalamus—that is, the brain (Pellegrino de Iraldi et al., 1963). Hillarp, however, was convinced that the bulk of monoamines in the brain was in the small SVs. Both Dr. De Robertis and Amanda visited the AG very early. I remember Dr. De Robertis sitting in the middle as we formed a circle around

him, competing to impress him with our data, as he took notes. Amanda was the first ever foreign visitor who stayed for a longer time with the AG. We were so young and greatly impressed by this elegant and somewhat exotic lady from a far-away country.

SVs with a dense core, however, had *not* been demonstrated in the *brain* and, surprisingly, *not* in sympathetic nerves in *rat iris*. I worked hard with different fixatives (including 0.5% potassium permanganate, $KMnO_4$). I even asked Professor von Euler for advice, and he suggested that alkaline pH may retain the amine in the vesicles. So I used alkaline phosphate buffer and, indeed, it worked in the rat. My short paper on rat iris was just published in 1966, when everything changed for me. Keith Richardson in a short *Nature* paper showed that high (3%) concentrations of $KMnO_4$ allowed demonstration of SVs with a dense core in the *rat iris* (Richardson, 1966). This confirmed the idea that the amine rapidly diffuses out of the vesicle and that a super-fast/-strong fixation is needed. I immediately repeated Richardson's results on the rat iris and expanded the analysis to the entire sympathetic neuron, and the brain.

The main results from analyses of $KMnO_4$ fixed tissues were a series of mostly single-authored papers published from 1966 to 1969 reporting on the following: (1) the dense core both in SVs and LDCVs reflects stored NA, based on *in vivo* and *in vitro* experiments conducted together with Gösta Jonsson, our methods expert (1966); (2) the dense core in the SVs in nerve endings in the rat iris disappears after preganglionic electrical stimulation combined with inhibition of tyrosine hydroxylase (TH) synthesis (and of course after reserpine) (1967), and here help from Torbjörn Malmfors and his technician was critical; (3) the first ultrastructural histochemical demonstration of a transmitter (monoamine) in classic SVs (and LDCVs) in the brain, in nerve terminals, and cell bodies in the locus coeruleus (LC) (Hökfelt, 1967) (previous evidence was based on subcellular fractionation studies by de Robertis's and Victor Whittaker's groups); (4) the presence of noradrenergic SVs in the cell body, dendrites, and axons of sympathetic neurons (i.e., in all parts of the neuron), providing morphological support, for example, for dendritic NA release (1969) (unfortunately, however, I did not suggest that mechanism); (5) the ultrastructural identification of DA nerve terminals in the striatum and that they represent 12–15% of all nerve endings (Hökfelt, 1968)—I incubated thin slices with alpha-methyl-NA, a compound that is resistant to breakdown, to increase intravesicular amine levels and thus facilitating detection, which was the slice technique I learned from Bertil Hamberger; (6) a lesion of the ascending nigro-striatal DA bundle reduced the number of nerve endings with SVs with a dense core in the striatum to a few percent, confirming their dopaminergic nature; together with Urban Ungerstedt (1969); (7) the presence of monoaminergic type 1 synapses in the hypothalamus, but not in association with DA nerve endings in the striatum, which served as evidence for nonsynaptic striatal

DA release (Hökfelt, 1968; Torbjörn Malmfors scrutinized this big manuscript), and further, in the hypothalamus (periventricular zone) about 4% were monoaminergic and about 5% in the suprachiasmatic nucleus (mainly serotonin) (Hökfelt, 1968); and (8) the nerve endings of the tubero-infundibular DA (TIDA) neurons in the external layer of the median eminence (ME) abut the portal vessels, suggesting release into the blood (1967, *cf.* below). I also may add a paper not included in my thesis showing that a single nigral DA neuron can give rise to some 500,000 nerve endings in the striatum, an enormous divergence (Andén et al., 1966). This was later beautifully shown by Wakoto Matsuda, Takeshi Kaneko, and colleagues who in 2009, using a virus tracer, could visualize the entire axonal arborization of a single nigrostriatal DA neuron.

Taken together, my work showed that the monoamine synapses in most regions represent only a small population of all nerve endings in the brain, as also reported in parallel autoradiographic studies by George Aghajanian, Floyd Bloom, and others. I was able to fulfill the main task that Hillarp had given to me, that is, to demonstrate the ultrastructural localization of monoamines in the brain. Nils-Åke Hillarp not being around anymore, I had the privilege to visit Arvid Carlsson in Gothenburg to discuss my results and get advice. Moreover, I would not have been able to carry out my thesis work without Waldtraut Hiort, one of the many technicians at the Histology Department at the time. She had extensive experience in all details and steps involved in EM.

Studies Parallel to My Thesis Work

Selected Studies with FAM

Along with my first PhD student, Åke Ljungdahl I made some modifications of FAM, using the first ever Vibratome and formalin perfusion. This approach resulted in beautiful micrographs without the disturbing cracks seen in freeze-dried tissue after processing for FAM (Hökfelt and Ljungdahl, 1972). Thus, it is possible to retain amines at their storage sites after perfusion even with *liquid* formalin. This finding was appreciated by Olavi Eränkö in Helsinki, a pioneer in histochemistry, who actually before Falck and Hillarp had shown that liquid formalin fixation results in fluorescent NA cells in the adrenal medulla. How the Vibratome method was later combined with the glyoxylic acid (GA) reaction, a new method developed in Lund, has been described by Anders Björklund (see Volume 10). With this modified method, we for the first time visualized DA fibers in limbic cortical areas, including dense fiber patches in the entorhinal cortex (Hökfelt et al., 1974), confirming the pioneering biochemical work of Ann Marie Thierry, Jacques Glowinski, and colleagues, who showed, surprisingly, a cortical DA innervation (1973).

Early Neuroendocrine and Pharmacological Studies

Parallel to these studies, I started to work with Kjell Fuxe focusing on neuroendocrine and pharmacological experiments with FAM. To work with Kjell was for me a learning experience, like a pre-postdoctoral period with Kjell as supervisor, before having finished my thesis work.

The neuroendocrine studies focused on the role of monoamines in the control of hormone secretion from the anterior pituitary, research pioneered by Charles Sawyer and colleagues in the United States. Kjell had in 1963 reported an intense fluorescence in the external layer of the ME, the first evidence for involvement of DA in the brain control of pituitary hormones. Kjell suggested that I should help him in this project, and the first joint, short paper on these TIDA neurons was published in 1966.

Using semiquantitative fluorescence combined with TH synthesis inhibition, we monitored the rate of DA depletion in the ME as an indirect measure of activity and turnover of DA, using different models and drugs (estrous cycle, ovariectomy, hormones, DA agonists, and more) (Fuxe et al., 1967). Our results strongly suggested that DA inhibits the release of luteinizing hormone (LH), yet others had an opposite view. Thus, both at meetings and in reviews of our papers, we felt resistance (e.g., one reviewer said: "what's the evidence that this green stuff in the median eminence is dopamine?"). But Kjell was fearless and argued against our famous opponents. Eventually Kjell's PhD student Anders Löfström, in 1977, reported objective quantification of the intensity using microfluorimetry and could show that, in fact, DA turnover in the lateral palisade zone of the ME is low only at proestrus (i.e., preceding and allowing ovulation), confirming our subjective evaluations. Further support was given by Nira Ben-Jonathan, John Porter, and colleagues, who in 1977 reported that portal plasma DA levels were at the lowest levels during proestrus (i.e., less inhibition).

We speculated that DA released from nerve terminals in the ME, through axo-axonic contacts, inhibits luteinizing hormone-releasing hormone (LHRH). Interestingly, our immunohistochemical studies, several years later, in 1975, showed a distinct overlap between TH-positive(+) and $LHRH^+$ nerve endings in the external layer of the ME.

I also proposed an alternative hypothesis involving structural changes: DA may expand the surface area occupied by tanycytes along the portal vessels, in this way preventing LHRH nerve endings from releasing their hormone (Hökfelt, 1973). This was indirectly supported by Björn Meister's demonstration of the DA- and cyclic adenosine-3':5'-monophospate (cAMP)-regulated phosphoprotein (DARPP-32) in tanycytes in close relation to both TH^+ and $LHRH^+$ nerve endings (Meister et al., 1988). DARPP-32 was discovered in the Paul Greengard laboratory and is known to be expressed in DAceptive neurons that possess D1 receptors. Taken together, we may have been correct in the view that DA inhibits LH secretion.

In 1968, J. H. van Maanen and Peter Smelik provided evidence that DA may also inhibit prolactin release, and in 1974, Robert MacLeod and Joyce Lehmeyer were able to provide final evidence for the view that DA, in fact and surprisingly, is the prolactin inhibitory factor (PIF) and thus directly acts on the lactotrophs. This was a major discovery that "cleared the air." So PIF is the only principal anterior pituitary "controller" that is not a peptide. In addition, as mentioned earlier, my own demonstration of DA terminals directly abutting the portal vessels in the external layer supported that view. Kjell and I also pursued this issue with our CA-synthesis-inhibition approach and reported in 1969 that prolactin administration increases TIDA neuron activity during, for example, pregnancy and lactation, but, without going into detail, this time we barked up the wrong tree in terms of site of action. Thus, as we suggested, DA is a coordinator inhibiting both prolactin and LH release, but by different mechanisms and at different levels (pituitary vs. ME). My previous PhD student Christian Broberger has together with his postdoctoral fellows recently shown that the frequency of TIDA neuron oscillations is important for prolactin secretion.

Our neuroendocrine research took a new turn when Barry Everitt from Cambridge joined, introducing us to novel models, like sexual behavior, and adding important tools and perspectives to our work. Barry would come back as guest professor a second time and remain a collaborator (see the section "The Hypothalamic/Neuroendocrine Theme"). Barry became master of Downing College, Cambridge; a fellow of the Royal Society and Honorary Doctor at KI; and in 2020 and 2021, the first ever non–U.S. citizen president of SfN.

The collaboration with Kjell resulted in a long series of papers on the neuroendocrine role of hypothalamic monoamines and several reviews. At international meetings, often in Italy and sometimes organized by Luciano Martini and colleagues, we had the privilege to meet some of the pioneers in neuroendocrinology, from the United States, Charles H. "Tom" Sawyer, William "Fran" Ganong, Samuel "Don" McCann (of course, I never dared to address them by their nickname) and, from Europe, Geoffrey Harris, Claude Kordon, János Szentágothai, Béla Flerkó, Béla Halász, and many more. Later, after the discovery of the releasing factors by Roger Guillemin's and Andrew Schally's teams, my exploration of the neuroendocrine hypothalamus would continue (see the section "Neuropeptides and Coexisting Messenger Molecules").

An exciting event for Kjell and me was Dr. Guillemin's visit at KI shortly before the final discovery that thyrotropin-releasing hormone (TRH) is "just" a tripeptide. Dr. Guillemin, knowing that TRH is a small molecule, contacted Kjell and said he had seen his data on the DA system in the ME. He suspected that TRH could in fact be DA. We told him we could easily test that. So he came all the way to KI with a small tube containing TRH, and we put a drop on an object slide and reacted it with PFA vapors. Upon

inspection in the fluorescence microscope, the droplet remained nonfluorescent. Thus, TRH was not DA.

During my career, I have made several blunders. One happened in relation to the Fifth International Congress of Endocrinology in Hamburg in 1976. I was invited to be a member of the Program Committee, representing the Nordic countries. It was a big honor, and I was certainly the youngest committee member by far. I contacted dozens of colleagues in the five Nordic countries, asking for names of potential speakers and collected an impressive list of names. I arrived at the hotel and presented myself to the concierge and told him about the committee meeting. But he couldn't find my name. Finally, he returned, excusing himself and saying "die Herren sind schon gestern angekommen" (i.e., "the gentlemen [of the committee] already arrived yesterday"). I was one day late! I checked my plane ticket—yes, I should have traveled the day before, but the check-in also had overlooked the wrong date. When I entered the meeting room, I was met with disparaging looks, and I heard a senior German professor saying, "he could at least have excused himself." Principally, the whole program had been decided the day before, without considering my proposals. When I returned to my room on the 10th floor of the hotel, I seriously considered jumping out of the window.

One more, less sad story, is also related to neuroendocrinology. Umberto Scapagnini studied medicine at the University of Catania, Sicily, and did postdocs in the United States, among other places in San Francisco, working with Dr. Ganong on the monoaminergic control of the stress axis. I met him at several conferences. After returning to Italy, he became full professor at the University of Catania and later moved into politics eventually becoming the mayor of Catania. In 2003, I was invited to give a lecture at that university. A young female scientist was appointed to take me on a tour through the city. We arrived at City Hall with a statue of an elephant outside, the symbol of the city. I mentioned to my young colleague that I actually know your mayor. The girl looked skeptical and said, "so let's go and visit him." I thought it was not a good idea, and we walked toward a big avenue. We heard music from a distance that grew stronger. A parade with many people was approaching headed by a big, live elephant and Umberto Scapagnini. Still surprised that I did what I did: I took some steps into the street and said, "Hi, Umberto." He looked at me and gave me a big smile and warmly said, as only Italians can, "Tomas, Tomas, come here, join me." So I ended up heading the parade through Catania, arm-in-arm with Umberto and after the elephant. To be honest, I felt a bit satisfied having provided convincing evidence that I did indeed know the mayor.

In a different set of pharmacological experiments, along with the Gothenburg group (Nils-Erik Andén, Hans Corrodi and colleagues), Kjell and I and other AG members analyzed the effect of several psychoactive drugs on the turnover of the monoamines using the TH synthesis inhibition

approach. This strategy was based on Arvid Carlsson and Margit Lindquist's 1963 pioneering paper strongly suggesting that chlorpromazine is a DA receptor blocker. The Gothenburg group used biochemistry, and we used the histochemistry protocol to look at the monoamine systems in the fluorescence microscope. We concluded, for example, that apomorphine activates receptors for DA (1967) and clonidine receptors for NA (1970), that LSD stimulates serotonin receptors (1970) and that ergot alkaloids stimulate DA receptors (1973). This latter finding would turn out to have implications for the treatment of Parkinson's disease.

I defended my PhD thesis in December 1968. So what to do after that? I had planned to complete my medical studies, but the prospect to remain at the department did not seem good, even if Kjell Fuxe had been appointed professor in 1968. Thus, at least one AG member had a permanent position. The selection process for Kjell's professorship was a drama. In the first round, Nils Ringerts, an eminent cell biologist and later secretary of the Nobel Committee, was listed first and Kjell second by the faculty committee. However, after Kjell's formal complaint, the faculty changed the order. My aim was to become, like my Uncle Bernt, a clinical endocrinologist. However, I had also applied to attend a neuroscience course in Boulder, Colorado.

The Neuroscience Research Program: The Second Study Program

In 1962, leading U.S. scientists joined forces to promote interactions among researchers working on the nervous system, and they established the Neuroscience Research Program (NRP). It was sponsored by MIT and initially based in Boston and led by Dr. Francis O. Schmitt of Harvard University. One may say that this initiative represented the foundation of a new discipline: modern neuroscience. In 1969, the NRP announced the second Intensive Study Program in Boulder, Colorado. I applied and, to my surprise, was accepted. I arrived in Boulder on July 20, 1969, and together with some of the faculty and other students, watched the first landing of man on the Moon. An exciting start for my first visit to the United States. It was, of course, an unbelievable experience listening to wonderful lectures from scientists that I knew only by name (and some not at all). This firework of (neuro)science at its best made me finally choose basic research. That same year, I applied for a research position at the Swedish Medical Research Council and was successful, thus making it possible for me to pursue a career in research.

I would later, once more, be surprised by the NRP. In the mid-1970s, I was invited to become an associate of the NRP, which meant annual visits to Boston (and after 1981 to New York and Rockefeller University when Gerald Edelman took over as scientific chair) with the benefit of listening to brilliant scientists as well as to attend so-called work sessions organized

on specific hot topics that were then summarized and published in the *NRP Bulletin*. In fact, I got the privilege of co-organizing such a session, together with Geoffrey Burnstock, in November 1978. Also participating were Michael Gershon, Leslie Iversen, Hans Kosterlitz, Joseph Szurszewski, Frederic Worden, and NRP Staff coordinator Key Dismukes. The topic was "nonadrenergic, noncholinergic autonomic transmission mechanisms," which also included my presentation of neuropeptides and their coexistence with classic transmitter. The proceedings were swiftly published in the *NRP Bulletin* in 1979.

Amino Acid Neurotransmitters

In 1966, I attended the first major symposium of my life, "Structure and Function of Inhibitory Neuronal Mechanisms," a Wenner-Gren Center International Symposium in Stockholm. Virtually all famous scientists in the field attended, and at that meeting glycine as transmitter was born, "delivered", as it were, by Robert Werman and Morris Aprison. I was baffled by the tough critique expressed by some participants. This lecture and much discussion on GABA primed me for amino acid transmitters. At that time, however, there was no reliable histochemical technique available to demonstrate, in the microscope, which neurons utilize such transmitters.

The finding, in my thesis, that only about 15% of all nerve endings in the striatum were dopaminergic was surprising to me. To be honest, looking in the fluorescence microscope with FAM, it seemed that there were only DA fibers. This raised my interest in the identity of the remaining 85%. Leslie Iversen had published, in 1968, two papers with Michael Neal and Sol Snyder on uptake of tritiated GABA in synaptosomes. So why not try autoradiography, as already carried out with tritiated monoamines by several groups?

Åke Ljungdahl and I started autoradiographic studies after incubation of brain slices with ^{3}H-GABA and direct isotope injections into the brain and spinal cord. At least half a dozen experiments failed over the first half year, but fortunately we followed Thomas Edison's adage that "Our greatest weakness lies in giving up. The most certain way to succeed is always to try just one more time" (Thomas A. Edison). So, we decided on one more, final experiment (without having read Edison). Thanks God, this one was successful, and we could report the labeling of (the expected) GABA neurons in cerebellum, both Golgi type neurons in the granular layer, and basket cells in the molecular layer (Hökfelt and Ljungdahl, 1970). Parallel studies using a similar approach yielded corresponding results in both retina (Bernt Ehinger) and cerebral cortex (Floyd Bloom and Leslie Iversen). We also obtained positive results in the spinal cord by injecting either tritiated GABA, glycine or glutamate, although the latter mainly accumulated in glial cells.

In 1970, I was surprisingly invited to present these data at a high-profile electrophysiological meeting in Basel organized by Leo Hösli. All of the big names were there. I had prepared a dozen slides and was very nervous, and got even more so when the chair, David Curtis, announced my lecture saying, "you have two minutes." I presented 10 slides but am not sure that the electrophysiologists understood anything, except perhaps John Eccles who worked with János Szentágothai and was interested and knowledgeable in anatomy. Floyd Bloom and Leslie Iversen understood, of course. Floyd presented, at the same meeting, the first data on the effect of cAMP on Purkinje cells. John Eccles, acting as chair (and a hero of mine), gave some positive comments on Floyd's talk but still recommended not take these results "too seriously."

Floyd Bloom and Leslie Iversen are both pioneers in neuroscience. They were close to us thematically, but we really never collaborated on a specific topic. Leslie, like us, moved early into substance P research. His focus was on the receptors, which he continued after assuming a position as director of a newly established Merck, Sharp, and Dohme (MSD) Research Centre in Terlings Park, United Kingdom. My wife Lil and I had the privilege to be invited to the festive inauguration of the center. The MSD big shots were flying in from the United States (flying the last leg in two parallel helicopters, in case one were to crash, I think someone told me). Our own flight was complicated by a last-minute strike in Sweden, making it necessary for us to fly from Copenhagen at 10 am. Our plan was to drive for five-plus hours from Stockholm to Halmstad, from where my father would take us to the Copenhagen Airport. Unfortunately, we overslept the alarm clock and woke up at 2 am instead of midnight. This time, we made the 500 km journey in 3.5 hours and arrived, thanks to my father, just in time to catch the plane to London.

Eventually, Åke and I decided to give up mapping GABA neurons with autoradiography. One reason was that Eugene Roberts had started to purify the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD), for immunohistochemistry (IHC); and in 1973–1975, Roberts, Chung Wu, and their collaborators published pioneering papers on the distribution of GABA neurons with this method. This was in 1983 followed up by Jon Storm-Mathisen, Ole Petter Ottersen, and colleagues using antibodies to the transmitters GABA, glycine, and glutamate themselves. More recently, antibodies to the vesicular transporters for GABA and glutamate (VGLUT1-3), generated by several groups, have emerged as valuable tools for studying glutamatergic neurons in the microscope.

The Immunohistochemical Era (1969–present day)

In the present day (2020–2021), there are hardly any physical gatherings, only virtual video meetings, because of the Covid-19 pandemic. I keep wondering

what my life would have been in a video-only world with no physical meetings? In fact, my scientific life changed because of the (live) Fourth International Congress on Pharmacology, which was held July 14–18, 1969, in Basel. This was the first time that I, together with Kjell Fuxe, met Menek Goldstein, professor at New York University School of Medicine. He told us that he had purified enzymes in the catecholamine (CA) synthesis pathway and made antibodies. He asked if we would be interested in a collaboration using the immunofluorescence method. Menek mentioned a then freshly published paper by Laurie Geffen, Bruce Livett, and Robert Rush (Geffen et al., 1969), reporting staining of chromaffin cells in the adrenal medulla and sympathetic neurons with antibodies to dopamine ß-hydroxylase (DBH). We gratefully accepted Menek's offer, and I started immediately to set up the method with help from excellent technicians (including first Ann-Cathrin Swenson/Radesäter, and later Annie Nygårds, Katarina Åman, and others). Also, I soon realized that fluorescein-isothiocyanate (FITC)-labeled antibodies were commercially available in Stockholm, thanks to the work carried out at the Department of Microbiology headed by Professor Astrid Fagraeus. I got help and advice from her younger colleagues Peter Biberfeld and his wife Gunnel.

The Immunofluorescence Method/Immunohistochemistry

The immunofluorescence method already had been introduced by Albert Coons and collaborators in 1942 and was widely used in the field of microbiology and later also by the pioneer in protein tracing, Richard Nairn. The insight of a possible broader applicability started decades later with the purification of DBH by Stanley Friedman and Seymour Kaufman in 1965 and the generation of anti-DBH antibodies by James Gibb, Sydney Spector, and Sidney Udenfriend in 1967, followed by the previously mentioned paper by Geffen and collaborators. Why this delay of some three decades? One reason was perhaps the lack of suitable (pure) immunogens, so the purification of DBH was indeed a pioneering effort.

I have tried to find out how the Australian group started the immunofluorescence project and have been told the following story by Bruce Levitt and Robert Rush (and they have graciously allowed me to include their story in this article): Bruce had begun dating a girl named Barbara. He was aware that her father was head of pathology at Monash, but was not familiar with his research. At the time, pathology announced an introductory course on IHC. Bruce suggested to Laurie Geffen that they should both enroll, but Laurie replied, "No Bruce, you enroll!"

The next weekend, Barbara's father appeared in the living room and asked Bruce, "What are you working on?"

Bruce answered, "purifying DBH together with Robert Rush in the Geffen/Austin laboratories in Physiology/Biochemistry to raise anti-DBH antibodies."

After a thorough interrogation, Bruce getting more and more impressed and curious, the father suggested that Bruce should use their anti-DBH antibodies to localize the enzyme with the immunofluorescence method. Bruce agreed and informed him that he had already enrolled in the course to be run by pathology. Barbara's father happened to be Richard Nairn, the noted pioneer in immunofluorescence protein tracing. So, choosing the right girlfriend seems important.

IHC meant an unprecedented advance in our ability to study the cellular and subcellular localization of proteins; and even smaller molecules, like peptides and even very small molecules like 5-HT, the latter pioneered by Harry Steinbusch, the late Albert Verhofstad, and Henk Joosten in the Netherlands (Steinbusch et al., 1978). There were at least two questions: Can one really make specific antibodies to such small molecules, and are 5-HT/amino acids retained in the tissue after perfusion with liquids? Albert had previously spent some time in our lab looking at the adrenal gland, his favorite tissue, with our antibodies to the CA-synthesizing enzymes. Not being familiar with the brain, he and Harry sent 5-HT antibodies to me in 1978 and asked for my opinion. Having seen the weak, and rapidly fading 5-HT-induced fluorescence in neurons visualized with FAM, I could not believe what I saw in the microscope: It was absolutely wonderful, a highlight in my "microscopy life." Clearly, this was the way to visualize 5-HT neurons.

With "immunocytochemistry" as key word 733,134 hits appeared in PubMed on March 4, 2021, and 737,903 on May 27, 2021 (i.e., more than 50 publications per day). A useful method, to say the least.

Methodological Improvements

The initial publication by Geffen et al. (1969) was followed in 1970 by papers from Hartman and Udenfriend and our group. These studies suffered from suboptimal quality of illustrations, the main reason being the mild fixation used (acetone, chloroform, methanol, alcohol) to retain immunogenicity (Fuxe et al., 1971). Having worked with fixatives and transcardial perfusion for my electron microscopic studies, I started to test all possible fixatives and perfusion. The conclusion was that "ordinary formalin" gave excellent results without compromising immunogenicity (Goldstein et al., 1972; Hökfelt et al., 1973b). The 1972 immunohistochemical mapping of NA neurons by Boyd Hartman, Doris Zide, and Sidney Udenfriend using DBH antibodies also introduced an important piece to the puzzle: the addition of Triton X-100 to the diluted antibody not only "reduced nonspecific binding" but also enhanced antibody penetration, so that not only cell bodies but also terminals and dendritic processes could be visualized (Hökfelt et al., 1973a). The introduction of monoclonal antibodies to the field in 1979, pioneered by Claudio Cuello, represented a novel tool, also useful for double-labeling

experiments. Subsequent method modifications—for example, using peroxidase labeling—were reported by Paul Nakane and Barry Pierce in 1966 and by Ludwig Sternberger and colleagues in 1970, as well as the tyramide signaling amplification method by Joe Adams in 1992. The latter offered higher sensitivity and allowed use of up to tenfold higher dilutions of the antisera. Some old "not-so-good" antisera became useful. Today, genetically modified mice expressing a certain tagged protein are often used for identifying neuronal (and glial) subpopulations.

Specificity Controls

Specificity of antibody labeling became more critical when expanding the method to fields outside microbiology. While studies on the CA-synthesizing enzymes did not present problems, this became an issue with the neuropeptides, that is, small molecules that had to be ligated to larger molecules like bovine serum albumin before immunization. Thus, the rabbit may generate antibodies also to the carrier protein. And it would turn out that there are families of structurally similar peptides, opening up for cross-reactivity. We acknowledged this in an early paper on substance P (Hökfelt et al., 1975b) by using the term substance P-*like* immunoreactivity, as suggested by my coauthor Göran Nilsson. This was good advice because, for example, several more tachykinins were subsequently identified. However, the real specificity problems arose when turning to the G protein-coupled receptors (GPCRs), where antibodies very often show false positives.

In those early days, specificity controls were based on testing cross-reactivity with different known peptides and, especially, preadsorption of the antibody with the immunogen. We noted that this was not enough, and more recently the introduction of peptide/receptor knock-out (KO) mice offers superior specificity controls. As does the possibility to compare with results from *in situ* hybridization (ISH) (localization of mRNA transcripts), especially if double-labeling could be achieved on the same section (IHC+ISH). Several colleagues have published on this problem.

In-House Problems (Our Errors)

In spite of our insight specificity being a problem, we (and others) have made some serious mistakes. For example, antibodies to the C-terminal part of cholecystokinin (CCK) cross-react with the calcitonin gene-related peptide (CGRP): these peptides share a C-terminally amidated phenylalanine, and a glycine in the 5th last amino acid position. Not much similarity, but still enough for cross-reactivity. Fortunately, we detected the error, and in a 1986 paper with Gong Ju as first author, we corrected our mistake.

Unfortunately, a second case was more serious: we reported in *PNAS* in 1983 that corticotropin-releasing factor (CRF) and peptide histidine-

isoleucine (PHI) coexist in rat hypothalamus, allowing for some interesting speculations on functionality. In 1986, I received for review a manuscript from *Neuroendocrinology* with the title: "Colocalization of PHI- and CRF-immunoreactivity in neurons of the rat hypothalamus: a surprising artefact" by Fred Berkenbosch and colleagues. This was a shock, but eventually we managed to present an explanation (Hökfelt et al., 1987a). Coauthor Jan Fahrenkrug showed that the antiserum we used and believed to be specific for the N-terminal portion of PHI, in fact contained a subpopulation of C-terminally directed antibodies that also reacted with rat CRF: rat CRF and PHI share a common C-terminally amidated isoleucine. So only one amino acid is identical and was sufficient to cause cross-reactivity. Why did we not spot the problem in our control adsorption experiments? The answer: because we used for adsorption the *ovine* peptide that does not have a C-terminal leucine, and because at that time, only the ovine CRF peptide was available—the sequence of rat CRF was reported by Wylie Vale and co-authors six months after our 1983 publication. So, staining was not blocked with ovine CRF suggesting specificity. Had we waited until the rat CRF sequence was known, we would have avoided this serious mistake. As always, timing is important.

Remapping the Monoamine Systems: The Adrenaline Neurons

Using Menek Goldstein's antibodies to the 'monoamine enzymes', we published a series of papers essentially confirming the Dahlström-Fuxe map of monoamine localization in the rat brain. (Dahlström and Fuxe, 1964). A member of the AG, passing by me sitting at the microscope looking at immunostained CA neurons, remarked "why are you doing this, everything has already been discovered?" But it turned out there was more to see. Of particular importance (for us) was the discovery of a third, albeit small, CA system based on the antibodies to phenyletholamine N-methyltransferase (PNMT), which allowed identification of two adrenaline (epinephrine) neuron groups, C1 and C2, in the medulla oblongata (Hökfelt et al., 1974).

In 1980, the Australian scientists Peter Howe, John Chalmers, and colleagues described a third adrenaline group, C3, and showed, with double FAM-immuno labeling, that the adrenaline cells do not show formaldehyde-induced fluorescence. In 1985, George Foster, a postdoctoral fellow from the United Kingdom, reported interesting differences during brain development: PNMT appeared *before* TH. We summarized the main findings of our work on the CA systems in a review article in 1984 in a special issue of *Science* on neuroscience edited by Solomon H. Snyder. Sol is a brilliant scientist who has made many fundamental discoveries (one of them would have been enough to make a great career), and who over decades has been very supportive, always generous, and interested.

The function of adrenaline system was difficult to define, but involvement in blood pressure control was explored by Kjell Fuxe and colleagues, and several other groups. In 1974, we suggested that C1 neurons represent a vaso*depressor* system, whereas other groups (Donald Reis, John Chalmers, Alan Sved, and Patrice Guyenet) provided evidence for a vaso*pressor* system, involved in the baroreflex. It is now clear that our interpretation was wrong as discussed in Hökfelt (2010). In general terms, roles of the C1 adrenaline neurons include involvement in pain, hypoxia, blood loss, and more, and these neurons have fittingly been called the body's "emergency medical technicians" (Gyenet et al., 2013).

Two major papers on the CA enzymes, the "crowning" of our work with Menek's antibodies, were published in volume 2 of the *Handbook of Chemical Anatomy* series: *Classical Transmitters in the CNS, Part I*. This series was initiated by Anders Björklund after discussions with Elsevier. Anders then invited me to join as coeditor. I was delighted to accept, and we started with the first volume in 1983, the last one, number 21 on DA, was published in 2005. The series ran for two decades. Anders and I are deeply thankful to all colleagues who contributed with excellent chapters to the 21 volumes and to those who served as coeditors (Michael Kuhar, Larry Swanson, Christer Owman, Floris Wouterlood, Anthony van den Pol, Masaya Tohyama, Floyd Bloom, Rémi Quirion, Harry Steinbusch, Jan de Vente, Steven Vincent, Ole Petter Ottersen, Jon Storm-Mathisen, Leszek Kaczmarek, Harold Robertson, Stephen Dunnett, and Marina Bentivoglio). Volume 1 was on methods, and we were both grateful and proud when Professor Walle Nauta accepted to write the foreword.

The second volume, in 1984, then focused on the CA systems in the rat brain and included, for example, a detailed map of the DA system by Anders and Olle Lindvall, a comprehensive comparison of all four enzymes with focus on the adrenaline neurons (Hökfelt et al., 1984a), a complete map of the TH-positive (+) systems showing coronal sections from about 180 levels (Hökfelt et al., 1984b). For the latter study, I traveled to Lund with stained sections to work with Anders, a memorable and exciting experience. All photos (half or full coronal rat brain sections) were taken by Ragnar Mårtensson, a medical hospital photographer in Lund. Inspired by Anders, Ragnar had built a new microscope variant with a scanning condenser that allowed for seamless photos of half a rat brain section (instead of merging some 50 single photos to a montage). This method is also described in the same volume. This was a remarkable progress for that time. We were amazed and delighted with the outcome.

To work with Anders was a treat. As a PhD student, Anders had revived brain research in the Histology Department in Lund. After having made a thesis with a methodological angle, Anders then carried out, mainly with Olle Lindvall, outstanding histochemical studies on, especially, the DA systems

in the rat brain, and thereafter became a pioneer in brain transplantation research, again together with Olle—the rest is history.

I also immensely enjoyed working with Menek Goldstein. He had, after being sheltered against the Holocaust in Poland, escaped to Switzerland, where he received his PhD in Bern in 1955. He then moved to the United States and worked at New York University School of Medicine from 1957 onward. Menek was a sweet and considerate human being. When Kjell and I visited the United States, Menek took care of us. For example, we attended the CA meeting in wonderful Asilomar in 1996. Menek arrived a day earlier and reserved the best room of the hotel facility for Kjell and me, a big room with an open fireplace. (We understood that Arvid Carlsson did not live in a similarly elegant room.) A lunch at the crowded deli on Second Avenue was imperative when visiting New York. With Menek as principal investigator (PI), I twice received support from NIH, which made much of our collaboration possible. Menek was awarded a doctor honoris causa at KI. He mostly lived alone and, in 1997, was found dead, sitting in the chair in front of his computer with an NIH application on the screen.

Neuropeptides and Coexisting Messenger Molecules (1974–present day)

Neuropeptides are the most diverse signaling system in the nervous system with more than 100 peptides and at least twice as many receptors. The immunohistochemical studies on the CA neurons might perhaps not have revealed so many new things, but this technique was our inroad to the peptidergic systems. At this point, Kjell Fuxe and I took different paths. That occurred quite naturally, also because Kjell had been joined by a brilliant Italian guest scientist, Luigi Agnati, who would become Kjell's close collaborator for decades to come.

All of my PhD students and most postdocs worked on neuropeptides, with major foci being peripheral neurons, especially dorsal root ganglia, brain, and coexistence of neuropeptides and classic neurotransmitters. These systems offered a wealth of new information, simply because hardly anything was known about their anatomical or/cellular localization. For example, immunohistochemical analysis of the parvocellular hypothalamic hormones expressing the releasing and inhibitory factors discovered by Roger Guillemin's and Andrew Schally's teams, provided surprising information. They were, as expected, found in nerve endings in the external layer of the ME but, surprisingly, also in many other brain regions. For example, in 1975, we detected TRH in nerve terminals throughout the brainstem, and as caudally as around spinal motoneurons. Could they originate from the hypothalamic TRH neuronal cell bodies? They did not. We showed with Olle Johannsson and other colleagues in 1981 that TRH in the spinal ventral horn is produced by medullary neurons projecting to the spinal cord having

5-HT as the main principal transmitter. This wide brain distribution of the peptides was demonstrated, in parallel, using highly sensitive radioimmunoassays (RIA) (Michael Brownstein, Miklós Palkovits, Seymour Reichlin, Keith Porter, and associates). TRH was also found in peripheral tissues, such as pancreas and gastrointestinal tract, as shown by John Morley in 1979; in ß-cells as shown by Ivor Jackson and Ronald Lechan as well as Hitoshi Kawano and colleagues, both in 1984; and in enteroendocrine cells and nerves in the intestine, as shown by our Japanese postdoc Yoshihiro Tsuruo in a 1988 article.

Altogether these findings heralded what now appears to be a rule: neuropeptides are cogwheels in many large machineries. The same neuropeptide is used in many parts of the nervous and other systems. The functional effects of peptides then depend on the type of GPCRs and G proteins involved. The name given to a peptide following its discovery has in some cases turned out to be a "misnomer," because it may reflect the tissue or organ from which the neuropeptide was first isolated, or the very first function associated with the peptide. Viktor Mutt, a pioneer in peptide research at KI and another hero of mine as well as of many more colleagues, discovered dozens of peptides. He recognized this problem and started to give "his" peptides neutral names—for example, galanin was named after its first (*g*lycine) and last (*alanin*e) amino acid.

Dorsal Root Ganglion Neurons and Pain

This project eventually would encompass a large number of collaborators. In the early phase, Zsuzsanna Wiesenfeld-Hallin and her team, including Xiao-Jun Xu, carried out the physiological experiments. Zsuzsanna was already an established and skillful pain physiologist using the flexor reflex as a pain model. I was fortunate and grateful to work with her over several decades, given that our collaboration gave meaning to our histochemical results.

Robert Elde was my second guest scientist, from the University of Minneapolis. We joined forces to study somatostatin (SST) in dorsal root ganglion (DRG) neurons, and he also raised antibodies to enkephalin. He later returned for a second period and made me aware of confocal microscopy, among many other things, and helped us to acquire perhaps the first microscope of this type in Sweden. Bob served as dean at the University of Minneapolis and was elected doctor honoris causa at KI.

Jan Lundberg had already started PhD studies with Annica Dahlström in Gothenburg. After a lecture in Gothenburg, Jan approached me and asked if he could join us. His arrival transformed our lab. He approached the work on peripheral systems with an enormous amount of energy and competence. It is an interesting question, where our group would have been, if I had not given that *live* lecture in Gothenburg. Jan had the gift of doing the right thing at the right time. His wife Ingeborg was expecting their first

child, when the Swedish annual moose hunt started. Jan is from Värmland, a county where hunting is top priority, so this was really a difficult situation: attend birth or hunt? After much agony, Jan went back to Värmland, shot a moose, and returned home just in time to witness the birth of his daughter.

Strategic as always, half-way through his thesis work, Jan transferred to the Pharmacology Department to work with Anders Änggård and his cat salivary gland model, an ideal preparation to monitor the stimulus-induced release of coexisting neuropeptides. There, he early on became professor and successfully supervised some 20 PhD students over 15 years (approximately the same number as I have had over 40 years). He then joined the Astra drug company, eventually becoming Astra/AstraZeneca's head of world-wide research, a position that he later assumed at Eli Lilly in Indianapolis. Jan and I share coauthorship of more than 100 papers (but only he owns a Ferrari).

Antibodies to peptides had been generated for RIAs, and many colleagues were prepared to share these reagents. The first antibodies that we tested were raised against SST and substance P. SST was discovered in 1973 in Roger Guillemin's laboratory as the growth hormone release-inhibiting factor, but we received the antibody in 1974 from Dr. Akira Arimura, a close coworker of Dr. Andrew Schally, thanks to support by Rolf Luft.

The substance P antibody was a different story. It was raised at KI by Göran Nilsson working in Börje Uvnäs's laboratory. The project was initiated after Susan Leeman, Michael Chang and Hugh Niall identified, in 1970/1971, substance P as an undecapeptide (11 amino acids), 40 years after its discovery by Ulf von Euler and John Gaddum in 1931, a true breakthrough. Bengt Pernow, who defended his thesis on substance P in 1953 with Ulf von Euler as supervisor, realized the opportunity. He and Börje Uvnäs, decided to send Göran to Rosalyn Yalow's laboratory to learn RIA and how to generate antibodies. Yalow had together with Solomon Berson developed RIA in the 1950s, and she received the Nobel Prize for this achievement in 1977 (Berson passed away in 1972), together with Guillemin and Schally.

Completely ignorant of this background, I had a meeting with Göran in 1974, when he told me about the antibodies that he had generated for RIA. Would I be interested in testing them for IHC? Guess if I was! This was a very fortunate offer, but peptide antibodies turned out to be more complicated than those directed against the CA enzymes. Göran had immunized 10 rabbits that were boosted four times. I analyzed serum from all rabbits and from all blood drawn. The immune response of the rabbits was indeed variable: some rabbits did not respond at all, there was one winner and some were useful; and the strongest staining was mostly seen with the blood drawn after the third and fourth boost. I right away understood that differences in antibody quality are a fundamental factor and realized that there always may be a better antibody around the corner.

Substance P had been associated with sensory neurons and pain already in the 1950s, first by Fred Lembeck of the University of Graz in Austria. Thus, I first applied the antibody to sections of the spinal cord. I got help from Jan-Olof Kellerth, a student of Ragnar Granit, with surgery and perfusion of the cat spinal cord. The result was spectacular: the superficial layers were shining like a semi-solar eclipse outlining the superficial layers, suggesting that peptide was present in primary sensory afferents. A second highlight in my "fluorescence microscopy life." In agreement, fibers were also found in the skin and in cell bodies in the sensory ganglia, that is, as expected from sensory neurons. Importantly, the substance P^+ cell bodies constituted only some 15–20% of all neurons and were always of small size, both in cat and rat, suggesting a relation to pain sensation (Hökfelt et al., 1975b). A parallel paper was submitted to *Brain Research*, but having never worked on the spinal cord, I placed the ventral horn upward in the figures. The founder and editor in chief of this journal, Professor Konrad Akert of the Brain Institute in Zürich, politely asked if I could consider turning the photos 180 degrees with the dorsal horn appropriately oriented? And so I did, and suddenly I became a "spinal cord scientist."

Together with Bob Elde, we demonstrated in 1976 that SST is also present in small DRG neurons, but in a population different from substance P. We were surprised to find that SST, an inhibitory transmitter, is expressed in DRG neurons at all. Were these neurons inhibiting pain? To help address this question, Bob generated antibodies to enkephalin using a peptide supplied by Lars Terenius. This was the start of my long and fruitful collaboration with Lars, a pioneer in the opiate field. The enkephalins are endogenous ligands for morphine receptors and were discovered by John Hughes, Hans Kosterlitz, and coworkers as first reported in a *Nature* paper in 1975 (for history, see the chapter by Huda Akil and Stanley J. Watson, Jr. in Volume 8). That *Nature* paper evoked enormous interest (and hope)—hundreds of labs started projects based on this discovery, including our group. With these antibodies, we could demonstrate the cellular localization of enkephalins. These peptides were not present in DRG neurons, but in spinal dorsal horn interneurons, and in many brain regions (Elde et al., 1976). In 1977, we reported that spinal enkephalin$^+$ interneurons intermingled with substance P afferents, providing a morphological basis for endogenous pain defense. This result fitted nicely with the parallel demonstration by Thomas (Tom) Jessell and his coworkers that morphine/enkephalin inhibits substance P release. In fact, Leslie Iversen's group in Cambridge along with Tom, Claudio Cuello, Piers Emson, and others were important early players in the substance P field, showing, among other things, for the first time that substance P is also present in human DRG neurons.

We explored many aspects of substance P expression. Stephen Brimijoin, a senior and eminent scientist based at the Mayo Clinic, wished to come to study axonal transport of substance P in human nerves, which he would

arrange to provide. Long before Steve's planned arrival in Sweden, I traveled to a meeting in the United States. So we decided that Steve should send the nerve(s) to the conference center. And, indeed, I received a thermos with ice and the tissue sample in a bottle. To ensure the tissue quality, I took the thermos to the hotel kitchen each day and asked the staff to fill it with ice. Once I returned to Sweden, I opened the thermos, still with ice, but no bottle. In the kitchen they had thrown out the *bottle with all ice* and added new ice. What an embarrassment! Things got much worse when Stephen arrived and I confessed to the mishap. He then pulled up his trousers and showed the scar caused by the removal of a piece from his *own* sural nerve.

Our work on peptides in DRG neurons was the beginning of our efforts to phenotype these neurons. We showed that they are not only of different sizes but also heterogeneous with regards to chemistry. Several years later, in 1982 and 1983, Susan Amara, Geoffrey Rosenfeld, and colleagues made a remarkable discovery: the calcitonin gene generates, by splicing, a second tissue-specific peptide only expressed in the nervous system: CGRP. This turned out to be the most abundant and arguably the most important peptide in DRG neurons. We demonstrated that CGRP is coexpressed with substance P and sensitizes a substance P-induced pain reaction (Wiesenfeld-Hallin et al., 1984), possibly via inhibition of a peptidase breakdown of substance P, as suggested in a 1985 paper together with Pierre Le Greves and LarsTerenius. The 1984 paper, published in a low-impact factor journal, is one of my most interesting publications: intrathecal injection of substance P alone at the lumbar level caused scratching for a few minutes (assumed to be a pain response), but CGRP alone did not induce any effect at all. Combining the two peptides, however, resulted in scratching for up to 30 minutes, a dramatic potentiation—and the first direct evidence that CGRP is associated with pain. More recent research found unexpected and important roles of the CGRP system in several peripheral systems, and the U.S. Food and Drug Administration (FDA) approved CGRP antibodies for the treatment of migraine, a major breakthrough both for patients and peptide research alike. This major advancement was reviewed by the two main players, Lars Edvinsson and Peter Goadsby, in 2019. Of note, Lars is a Swedish colleague working at Lund University.

Our work on neuropeptides in sensory neurons and dorsal horn had obvious clinical implications. Looming—then and now—over research on this topic was the issue of the generally inefficacious treatment of, especially, neuropathic pain. There had "always" been morphine, the gold standard, but with serious side effects and lower efficacy for neuropathic pain. Might the discovery of substance P and/or enkephalin(s) lead to new pain treatments? In 1975, James Henry and colleagues had shown that substance P excites spinal dorsal horn neurons supporting a role as a pain transmitter. Thus, a substance P antagonist could very well be a "pain killer." Hopes were high, until Pfizer finally succeeded in developing a small-molecule

substance P antagonist that passed the blood-brain barrier: it worked in rats but not in humans, a big disappointment. My personal explanation is that the DRG neurons are not only releasing substance P, but also CGRP and the excitatory amino acid glutamate (and even further transmitters). So, maybe it is not enough to just block substance P (neurokinin) receptors?

The Endocrine, Autonomic, and Gut Nervous Systems, and Coexistence

The autonomic nervous system is classically divided into two parts: sympathetic and para-sympathetic neurons using, respectively, NA and acetylcholine as neurotransmitter. In the mid-1970s, I started a collaboration with (the late) Lars-Gösta Elfvin, an anatomist who had trained with Fritiof Sjöstrand. This collaboration took me back from the sensory to the autonomic nervous system, which I had studied in my thesis. Elfvin's work focused on autonomic ganglia and included cat and guinea pig, The original question was whether these ganglia have adrenaline ($PNMT^+$) neurons (they did not). While working on this project, I did some unplanned experiments by applying peptide antisera on some sections of *guinea pig* sympathetic ganglia. It turned out that this species is wonderful for IHC—the antibodies cross-reacted and all stainings were much stronger than in rat. This work involved several PhD students, including Marianne Schultzberg, Jan Lundberg, Carl-Johan Dalsgaard, and the late Björn Lindh (the latter two were Elfvin's PhD students). Marianne Schultzberg was a PhD student with Tamas Bartfai at Stockholm University, and Tamas contacted me about her when he was leaving for a postdoc with Paul Greengard at Yale in 1976. As described next, Marianne got a new project and completed her thesis in 1980.

In the endocrine system, we, in 1974/1975, found SST in cells of the islands of Langerhans, thus a new pancreatic hormone, and we also found SST is parafollicular cells in the thyroid gland and in endocrine cells in the stomach mucosa, as well as in nerves in lamina propria and the myenteric plexus (Hökfelt et al., 1975a). Similar results on the Langerhans islets were reported by Stephen Bloom/Julia Polak, Anthony Pearse, Lelio Orci, Paul Goldsmith/William Ganong, and collaborators. In 1979–1980 articles, in the adrenal medulla, we reported expression of the pentapeptide enkephalin in the chromaffin cells, also found in the human adrenal medulla—that is, a new putative hormone or regulator had been identified in addition to NA and adrenaline.

We then started to phenotype the autonomic nervous system and found, like in the DRGs of the sensory system, a distinct heterogeneity regarding chemical phenotype: (1) presence of SST in NAergic ganglion cells, the first example of coexistence of a peptide with a classical transmitter (Hökfelt et al., 1977); (2) in the same year, sensory substance P fibers in sympathetic ganglia, establishing a connection between sensory and autonomic functions;

(3) in 1978, a preganglionic enkephalin innervation in sympathetic ganglia); and (4) subpopulations of nerve terminals/neurons in prevertebral ganglia defined by neuropeptides neuropeptide tyrosine (NPY, SST, and vasoactive intestinal polypeptide [VIP] as reviewed by Elfvin et al. [1993]).

A start of a major project was the observation by Jan Lundberg and colleagues that VIP is expressed in cholinergic neurons in cat sympathetic ganglia (1979), followed by functional data showing how acetylcholine and VIP cooperate in producing vasodilation (Lundberg et al., 1980). In a series of papers, we also defined the subcellular localization of neuropeptides vs. classic transmitter in these systems and provided strong evidence that peptide storage is exclusive to LDCVs (see Fried et al., 1985). Interestingly, as shown in my thesis, the LDCVs can store monoamines. But could they also store amino acid transmitters? Immunoelectron microscopy on the dorsal horn by Adalberto Merighi, a champion in ultrastructural IHC, working with Julia Polak and Dionysia Theodosis reported that GABA (1989) and glutamate (1991) can be detected only in the SVs (vs. CGRP only in LDCVs). If true, a distinct difference exists between classic transmitters like amino acids and monoamines, the latter being stored both in SVs and LDCVs (my thesis work).

Regarding the functional roles of coexistence, Jan and colleagues in 1982 presented evidence that NPY in sympathetic neurons primarily inhibits the release of NA—that is, it exerts a presynaptic effect (Lundberg et al., 1982). Jan summarized much of this work in a comprehensive, single-author article published in *Pharmacological Reviews* in 1996. In 1976, Geoffrey Burnstock wrote a visionary paper raising the transmitter coexistence and cotransmission issue, although his own work mainly focused on purines and classical neurotransmitters. Lily Yeh Jan and Yuh Nung Jan explored in 1979 cotransmission in elegant studies on bull frog ganglia, revealing involvement of an LHRH-like peptide. To be honest, transmitter coexistence had already been shown in studies on nonvertebrates (but not involving neuropeptides), for example, in 1974 by Michael Brownstein working in the Axelrod laboratory on Aplysia. Mike was one of Julius Axelrod's many brilliant postdocs (also see the section "National Institute of Mental Health")

Finally, Marianne Schultzberg went on to describe in detail the peptidergic innervation of the gut (Schultzberg et al., 1980), in parallel with Frank Sundler and associates in Lund and with Marcello Costa and John Furness and collaborators at Flinders in Australia, the latter complementing their beautiful histochemistry with elegant electrophysiological analyses.

The Peptidergic Brain and More Transmitter Coexistence

While these studies of the autonomic nervous system showed important principles, a main interest for me was the brain. After remapping the CA neurons, we generated complete rat brain mappings of three neuropeptides:

substance P in 1978, first author Åke Ljungdahl (parallel to Claudio Cuello and Ichiro Kanazawa); SST in 1984, Olle Johansson first author (but James Finley, Peter Petruzs, and colleagues already had published an SST map in 1981); and galanin in 1986, Tor Melander first author (parallel to Gerhard Skofitsch and David Jacobowitz). We also published two neuropeptide receptor maps: the NPY Y1 receptor in 2002, with Jutta Kopp as first author (parallel to Janice Urban and colleagues) and NPY Y2 in 2006 with Davor Stanic as first author. We also paid special attention to the hypothalamus, a brain region that, as noted earlier, has been central to our efforts in the neuroendocrine field.

The substance P map is the first detailed map of a brain peptide, which I made together with Åke Ljungdahl, already mentioned and a clever young person with whom it was a treat to work. Åke went on to clinical work, becoming an expert in Parkinson's disease. He sadly succumbed to lung cancer in 2003. We learned the brain together, sitting side by side at two microscopes with the König and Klippel Rat Brain Atlas in between. A short report of the substance P map was published in *Science* in 1975 (Hökfelt et al., 1975b), but it took us three more years to publish the full 82-page paper in *Neuroscience* (Ljungdahl et al., 1978). Something important happened in between: in the 1975, we reported only a few substance P^+ cell bodies and wondered from where all the dense fiber plexuses originated? Could they come from sensory ganglia, similar to the belief held at one point that brain NA may originate from peripheral sympathetic ganglia present in nerves following the blood vessels?

The substance P cell body issue was resolved by intraventricular (i.vt.) application of colchicine, and in the 1978 paper, we observed more than 30 substance P^+ cell groups in different brain regions, explaining the extensive fiber networks in most parts of the brain. In the same year, the first substance P pathway in the brain was reported by Claudio Cuello and colleagues. How did colchicine reveal substance P cell bodies? Annica Dahlström and George Kreutzberg of the Max Planck Institute in Munich had in 1968/1969 shown that this "microtuble-destroyer" blocks axonal transport of NA (Dahlström) and acetylcholine esterase (AChE) (Kreutzberg). Thus, the centrifugal transport of peptides produced in the cell bodies was inhibited, leading to accumulation in the soma. In fact, colchicine i.vt. became a standard for peptide mapping studies. But already in 1973, Julien Barry and colleagues in France used colchicine to detect hypothalamic LHRH neurons.

The use of the colchicine approach deserves discussion, since the method has been so widely used. Do the cells really produce or utilize the same peptide under normal conditions, or does colchicine induce synthesis of novel peptides? Is the detection dependent on only the inhibition of axonal transport? ISH could later contribute to this discussion: we can almost always see the peptide transcript in cells (without colchicine), where the peptide itself only appears after colchicine treatment. There are exceptions: DRG

neurons are fairly unique in that colchicine is not needed to show peptides in cell bodies with IHC. There are also cases in which colchicine changes both peptide/enzyme and transcript levels, as shown by Roser Cortes and colleagues in 1990.

After the discovery of NA-SST coexistence in sympathetic neurons, we wondered if this was a unique situation and therefore looked for more examples. The presence of multiple substance P neurons in the medullary raphe nuclei and adjacent regions was strongly reminiscent of the 5-HT neurons described by Dahlström and Fuxe in 1964. Having tested the splendid Harry Steinbusch-Albert Verhofstad 5-HT antibody I asked if we could use their antibody. They agreed but I would first have to ask their chair, Professor Rudolf Nieuwenhuys, a pioneer in the field of comparative neuroanatomy. So, I traveled to Nijmegen and, after a lengthy discussion, I got permission to use the antibody. We reported in *Neuroscience* in 1978 that many medullary descending 5-HT neurons coexpress substance P. In the same year, this coexistence was also published by Victoria Chan-Palay, Gösta Jonsson, and Sanford Palay. In 1980, together with Lana Skirboll and Jens Rehfeld, we reported that many mesencephalic DA neurons coexpress cholecystokinin (CCK), findings that were subsequently followed up by Kim Seroogy, a postdoctoral fellow from the United States, who also discovered additional coexisting peptides in DA neurons. Thus, we became convinced that peptide-classical transmitter coexistence is real and, in fact, may be a rule. I was invited to write a review for *Nature* (Hökfelt et al., 1980). Not yet having realized the importance of this journal, I had to be reminded twice by the *Nature* editor before we prepared and submitted the manuscript.

We are often asked to present a list of our most important publications. This 1980 *Nature* paper would be very high on such a list, because it summarizes the early phase of neuropeptide research and peptide-classical transmitter coexistence, including Jan Lundberg's functional and, in any case for us, fundamental studies. Notably, it made our group known also locally at KI. I had just been appointed professor, and now the senior and famous colleagues took notice of us and got an idea of what we were doing. I had never believed that a *Nature* paper could be so important. I therefore contacted *Nature*, 10 years later, and asked if I could write a 10-year perspective. In fact, I right away submitted a fairly complete draft, finishing up by saying (something like) "if monoamines were the transmitters of 1960s and GABA of the 1970s, we hoped that the 1980s would be the decade of the peptides, but then glutamate appeared and grasped the attention." After several weeks the editor responded (again something like): "If now glutamate is so important, why don't you write a review on that transmitter?" Fortunately, Eric Kandel, who insisted I should be sole author, showed mercy and supported an improved version (Bob Elde helped, too) for publication in *Neuron* (Hökfelt, 1991).

National Institute of Mental Health (1981–1982)

The 1970s and 1980s had been very productive, but the group had been small. My first student, Åke Ljungdahl, had defended his thesis in 1980, and I had two more ongoing PhD students. Also, Lana Skirboll, a postdoc from Yale, had made important contributions to establish the DA-CCK coexistence, findings that she followed up with electrophysiological studies published in 1981. She also established methods to combine IHC and retrograde tracing using fluorescent dyes (1983) introduced by Hans Kuypers and Marina Bentivoglio. Lana returned to the United States and joined NIH to run a lab but soon moved into science policy, eventually becoming director of, first, the Office of Science Policy of the National Institute of Mental Health (NIMH) and, then, of the NIH under the NIH director Harold Varmus, managing a range of important policy issues. Under NIH Director Elias Zerhouni, she helped to create the NIH Roadmap for Medical Research. She then moved to the private sector, to Sanofi. Lana and her husband Leonard were great hosts during our time at NIH.

At KI, Steven Vincent from the University of British Columbia (UBC) had arrived in 1981 for postdoctoral studies. During his stay Steve reported a wealth of interesting data, on peripheral and central dynorphin pathways (1982); the coexistence of two peptides in human cortex (1982); glutamate decarboxylase (GAD) in pancreatic ß-cells (1983); and, together with Lana Skirboll, in *Science*, a GABAergic hypothalamic projection to the neocortex (1983). Steve brought with him an NADPH-diaphorase staining protocol, which he called the "magic stain," showing beautiful neurons in cortex. Later, after returning to UBC, Steve reported, in the *Proceedings of the National Academy of Sciences* in 1991 with Hope and colleagues, that this staining in fact showed nitric oxide synthase (NOS), the enzyme that had just been discovered by Ted Dawson, Sol Snyder, and colleagues.

In 1980, I received an invitation to visit the NIH in Bethesda, Maryland, as a Fogarty Scholar, and I felt it would be good for me to be exposed to a different research environment. The Fogarty International Center was established by the U.S. Congress in 1968 and named after Congressman John E. Fogarty, who during his almost three decades in Congress "was a champion for NIH and for the value of research."

I had never done postdoc studies, and there was no tradition in the AG to undertake a postdoc challenge abroad. I was at this point 41 years old, a bit late for "postdoctoral training." My host at NIMH was Michael Brownstein, who is incredibly bright and was chief of the Molecular Brain Laboratory, previously headed by Julius Axelrod, the 1977 Nobel laureate. The invitation came as a big surprise, but I did accept. We leased our house in Stockholm and arrived in Bethesda in September 1981. My technician, Gun Norell, had traveled to Bethesda before us, so that when I arrived the laboratory on the 10th floor of Building 10 was ready and outfitted with a

cryostat and a Swedish flag in the window of the door. Thus, everyone knew where the Swedes were.

The first thing we had to do was buy a car. After signing the contract for an old, but good-looking Mercury and walking out of the door, I saw over my shoulder how several colleagues patted my salesman on the shoulder and congratulated him. It wasn't long before I had to bring the car to the repair shop, and I understood why.

My goal at the NIH was to work with Mike Brownstein to set up what was, at that time, a novel histochemical technique, ISH, to map the transcripts for various neurotransmitter-related molecules. In 1981, only a single study related to messenger molecules had been published (by Roland Pochet, Jean Pasteels, and colleagues). As noted, my lab and Mike and his collaborators at NIH had in parallel been mapping the distribution of many transmitters including neuropeptides, we with IHC, and they using highly sensitive biochemical techniques and the elegant punch method developed by Miklós Palkovits. So now we were going to go for the transcripts. At that time, however, it was deemed necessary to have a plasmid for the molecule you wanted to investigate. There were only a few such plasmids available, one being for the enkephalin precursor, at Mount Sinai in New York. As time passed, however, it became obvious that we would not have access to any plasmid, and our original plans evaporated.

In spite of this setback, I was very busy setting up IHC in Bethesda. I started a collaboration with Lou Sokoloff who, together with Seymour Kety, had made a major contribution with the introduction of the 2-deoxyglucose (2-DG) technique to monitor activity in brain sections, and whose work also created a basis for positron emission tomography (PET). One unresolved question raised by his work was as follows: where is the 2-DG localized at the cellular level? Cutting frozen tissue in a cryostat may cause diffusion/redistribution. With Carolyn Smith, Gun Norell, Lou Sokoloff, and colleagues, we worked out an autoradiographic method showing the cellular localization of 2-DG (1983). This method also could be combined with IHC (1984). Even if these papers have left few traces, it was an honor to collaborate with Lou Sokoloff, a wonderful and generous person, and a pioneer in neuroscience.

We Fogarty Scholars had simple offices on the top floor of the Stone House, which was certainly fine with me. In fact, it was a tremendous courtesy in view of the general shortage of space at the NIH. Mike Brownstein's own office in Building 10 was a platform a meter above the stereotaxic instrument, and Mike had to climb up using a small ladder. This elevated floor was so close to the roof that Mike could not use a normal chair but had installed an old car chair without legs that allowed him to sit and work in his "office." Dr. Axelrod still came in and had a desk in one corner. I felt it would have been good for some of the PhD students at KI to see this, particularly those who were not happy until they had their own office.

During my time at NIMH, there was a second Swedish Fogarty Fellow, Professor Lars Svennerholm from Gothenburg University, a neurologist and pioneer in the field of gangliosides. Lars was not happy with his office. He felt that a Fogarty Scholar should be housed in one of the nice rooms on the second floor. He approached several of the NIH directors and, in fact, he succeeded: after several months, the Fogarty Scholars moved down from the attic. But this was too late for Lars, he already had moved back to Gothenburg. To my biggest surprise, I got a wonderful office with two open fireplaces designated, I guess, for Lars.

Overall, I am forever grateful for the invitation to visit NIH as a Fogarty Scholar. The weekly presentations in the Stone House by heads of the various NIH branches were a treat, and the exposure to this unique, biggest-in-the-world research organization strongly influenced my professional life and way of thinking. I like to see my research life as before (small team) and after (eventually large group) NIH.

The visit to the United States and Bethesda was also a big adventure for the family. Our children, Patrik and Paula, were exposed to a very different school system. They liked it and performed well, even though the U.S. history curriculum was very complex, and Paula struggled with studying French from English. Patrik learned to enjoy football. When back in Sweden he, together with some friends, started a team (Danderyd's Mean Machines) and eventually the first ever American football league in Sweden (still ongoing). Lil drove the kids to school. One day they were stopped by a big, impressive motorbike police officer outside the school: the car lacked the tax badge (I had it in my pocket and was, as pretty often, in Sweden). Paula was scared to death, also because Lil had just swept a coat over her nightgown, and Paula wondered if she really could enter the school building under these circumstances. But the officer was polite and understanding. Patrik got his driver's license one year earlier than would have been possible in Sweden. Around Christmas we drove, with the Mercury, to New York (yes, the Mercury still made it, even if one window could not be fully closed) and embarked on a flight for a one-week vacation in the Bahamas—a highlight for the family.

Return to Sweden

We returned to Sweden in late spring 1982. It did not start well. I was told that I would not get any salary for the coming three summer months. This was because I had not worked during the preceding nine months. Since the summer months in those days were, in principle, holidays for academics, the summer salaries had to be "earned" during the previous nine months. Bengt Pernow, at that time president at KI, stepped in and solved the problem. But worse was to come. Paula, our daughter, had had several infections in Washington. Back home in Sweden, we spent some summer days

on the southern West coast visiting my parents. Despite wonderful weather, Paula did not want to join us on the beach. I had to go back to NIH for one more month, and I needed to find out what was wrong before departing. In the late afternoon the day before departure to Washington, D.C., we went to the local hospital. After a blood test, the doctor told us that it did not look good, but he was not sure. Only a specialist at the university hospital in Lund/Malmö could give a correct diagnosis. I drove the 100 miles and dropped the sample in a box around midnight. Early the next morning, we learned that Paula had acute leukemia, and our lives changed. The trip to Washington was canceled and a horrible period started with initial visits to Karolinska Hospital. Was a transplantation possible? The first test said no, but after retesting it changed to a yes. The chemotherapy was efficient, and we were told that the relapse risk was 85%. We were recommended, and chose, transplantation. This initially was successful, but then a graft vs. host reaction set in with an enormous force, bleeding, and unbearable pain that ensued for weeks. In spite of the extensive and committed work from doctors and nurses, Paula passed away on March 31, the day before her 16th birthday. We wondered, and still do, whether we did the right thing to go for transplantation. We were deeply impressed and grateful for the heroic efforts of the doctors and nurses at KI Huddinge Hospital, and we were touched and truly comforted by the many letters of condolences from colleagues around the world. However, the loss of our daughter (sister) will always be on our mind, and there is not a single day when Lil and I do not think about her. We were comforted by having our son Patrik who later married Ulrika, and they gave us the two already mentioned grandchildren Gustav and Hedda.

Return to Research at KI

Even though my return to Sweden had started with a tragedy for our family, the coming two post-NIH decades saw an upswing of research. Tremendously talented and hard-working Swedish PhD students and post-docs from abroad joined my lab in increasing numbers, creating an exciting and (almost always) collegial and interactive atmosphere, just like in the Hillarp days. Together we generated a wealth of interesting results and published many and, hopefully, some rather important papers.

The first major project after my return centered on galanin in the brain, a peptide discovered in 1983 by Kazuhiko Tatemoto, Viktor Mutt, and colleagues at KI. One of the co-authors on the original paper was Åke Rökaeus in the Department of Pharmacology. He had continued the tradition of Göran Nilsson and raised antibodies against galanin, and now offered them to me for immunohistochemical analysis. I made a preliminary mapping, and we published a short paper with Åke Rökaeus as first author in 1984.

At this point Tor Melander came to my lab from the United States with experience in experimental brain research obtained in Joseph Well's lab at the University of Vermont. Immediately after my return from my NIH period, the lab was fairly deserted. My daughter had just passed away, and so the mood was low. Tor's arrival was a boost to my mood, and he wanted to continue the 2-DG studies I had begun at NIMH with Louis Sokoloff. However, eventually and fortunately, Tor agreed to do the full galanin mapping, and he did it virtually by himself (1986), in parallel with Skoftisch and Jacobowitz at NIMH (1985). Our main interest from Tor's work was the coexistence of galanin with classical transmitters in many systems. What caught our special attention was the galanin expression in NAergic neurons in the LC and serotonergic neurons in the dorsal raphe nucleus (DRN) (Melander et al., 1986) as well as in cholinergic forebrain neurons (1985).

Many excellent researchers contributed to our studies on galanin in the brain, including Tamas Bartfai, a truly brilliant colleague with extensive experience from both academia and big pharma. After working at Roche in Basel, he was appointed professor in biochemistry at KI, but he decided to move to the Scripps Research Institute in La Jolla as chair of neuropharmacology. He and Ulo Langel became important players in the galanin field and created the first galanin receptor ligands that were used all over the world. Tamas and colleagues also made important contributions to the work on sensory neurons and pain, as will be described later. Tamas has throughout been a most valued adviser and inspiring colleague. My collaboration with Tamas has resulted in more than 40 publications.

Anders Björklund happened to visit our lab in 1984, and Tor Melander and I showed him some of Tor's galanin stainings. "These are cholinergic forebrain neurons," Anders said. I had never worked in this region of the brain, so this was new and exciting information for both Tor and me, especially since these neurons had been associated with Alzheimer's disease (AD). Together with Tamas and Gilberto Fisone, a PhD student in Tamas's lab and today chair of our department at KI, we showed that galanin inhibits acetylcholine (ACh) release in the rat (1987) and monkey (1991) (ventral) hippocampus. Marcelo Villar, a postdoc from Buenos Aires, took on an incredible amount of experimental work ranging from studies of peptide localization in DRG neurons and hypothalamus, and using diverse methods including hypophysectomy and brain lesions. After Marcelo returned to Buenos Aires, he soon became rector of the Austral University. He and Roser Cortes, from Barcelona, found that the cholinergic forebrain neurons, like the DRG neurons upregulate galanin synthesis after axonal lesions (Cortes et al., 1990). We hypothesized that in AD, degeneration starts in some nerve terminals (axonal/synaptic damage) initiating galanin upregulation and release from intact terminals, inhibiting acetylcholine (ACh) release and aggravating the disease. We proposed that a galanin antagonist

may initially in the disease process have therapeutic effects by counteracting reduction of ACh levels.

This work on AD was continued by other groups, with studies of human brain tissue, in particular by Victoria Chan-Palay and by Elliott Mufson and their colleagues. They could not detect galanin in cholinergic forebrain neurons, which, without colchicine, was also the case in rats. (No one has used colchicine with the human brain.) However, and interestingly, Victoria discovered in 1988 a galaninergic hyperinnervation of cholinergic forebrain neurons. This was confirmed by Mufson's group, who suggested that galanin exerts trophic effects. We felt that we were at a dead end, and Tamas Bartfai suggested that we should leave AD and focus on depression, in view of galanin's coexistence in NA and 5-HT neurons. Kjell Fuxe, Luigi Agnati, and Sven Ove Ögren were already working on this hypothesis. Later, Sven Ove, an expert in animal behavior and pharmacology with a past as a successful drug developer at Astra/AstraZeneca, and I extensively collaborated on the galanin project.

Our focus for functional studies thus was directed toward galanin in LC and DRN. It was obvious that an electrophysiological approach might reveal interesting results. Eventually and thanks to Vincent Pieribone, a U.S. postdoc, electrophysiology was introduced in our laboratory and, in 1995, we were able to publish our first paper on LC (Pieribone et al., 1995). Sten Grillner, an outstanding electrophysiologist who headed the Nobel Institute of Neurophysiology at KI was the expert coauthor. What a big moment for our lab this was. When writing the manuscript, however, we discovered that the groups of Albert Dresse and Peter Illes had already shown that galanin inhibits the spontaneous firing of LC neurons. Our electrophysiological studies of galanin were then led by Zhi-Qing David Xu, my second Chinese PhD student, who defended his thesis on this project in 1997. In brief, using receptor selective ligands obtained from colleagues at AstraZeneca, we proposed involvement of inhibitory *GalR1* autoreceptors activated by galanin released from dendrites in the LC. The work to support this hypothesis was carried out by Xiasong Ma and David (2001) and by Evelyne Vila-Porcile, Marc Landry, and colleagues (2009). Somato-dendritic release of peptides is an accepted concept thanks to the extensive work by Mike Ludwig, Gareth Length, and colleagues on oxytocin and vasopressin in the magnocellular nuclei in the hypothalamus. In 1998, David showed a similar effect of galanin on DRN 5-HT neurons. David stayed with me for almost two decades before returning to Beijing. We still collaborate.

Even if our focus was on GalR1, there was also evidence for involvement of GalR2 in depression-like behavior. Thus, Xiaoying Lu, Tamas Bartfai, and colleagues provided evidence that GalR2 mediates antidepressant effects (Lu et al., 2005). Some years later, we could together with Sven Ove Ögren confirm this view in a rat model using swim stress and ISH (Kuteeva et al., 2008). Taken together and overall, the two galanin receptors appear

to have opposite effects, GalR1 being pro- and GalR2 being antidepressive. These results from animal experiments were the basis for our later studies of the human brain.

Clearly, it would be critical to understand the localization of the galanin receptors. Though many antibodies have been generated against these receptors, it remains unclear to this day whether any one of them is specific, even if some generate "convincing" stainings. The general specificity of GPCR antibodies has been tested by Martin Michel (Amsterdam) and of galanin receptor antibodies by Tamas Bartfai (Scripps) and Barbara Kofler (Salzburg) and their collaborators. False positive labeling occurred with many antibodies examined at that time.

We also have been working on NPY which, like galanin, is coexpressed in the LC as described in some detail by Vicky Holets, a U.S. postdoc (Holets et al., 1988). NPY was also discovered in the Mutt laboratory, in 1982. The NPY1 and NPY2 receptors are particularly abundant. In this case, we have been more successful with the receptor antibodies. The NPYR1 mapping by PhD student Jutta Kopp was a heroic enterprise. She first mapped the Y1 receptor using our in-house antibody and basically had finished the work. At this point, I received antibodies from John Walsh and Helen Wong at UCLA, where John had established an NIH-supported antibody core facility. They generously sent me many antibodies, among others a dozen raised against NPYR1. Two of these (here called #1 and #2) were particularly interesting. Scanning revealed that #1 and #2 showed extensive, but completely different staining patterns; #1 showed the few systems that Jutta had already discovered, but in addition the whole brain was "covered" by fluorescence, an enormous difference. So we decided to work with #1 and Jutta now had to redo everything. After exceptionally hard work, she produced a likely complete and absolutely wonderful map (Kopp et al., 2002).

Before manuscript submission there had been some highly relevant questions that made me very nervous: First, was the staining specific? To address this question, Jutta studied an NPYR1-KO mouse generously supplied by Thierry Pedrazzini in Lausanne. Second, is the NPYR1 distribution in mouse similar to what Jutta had seen in rat? Thus, Jutta had to make a fairly detailed pilot study of the mouse brain, and yes, the staining patterns for NPYR1 were similar in mouse and rat; and none of the #1-induced staining could be seen in the KO mouse. However, the wide staining with antibody #2 remained in the Y1 KO mouse—strongly suggesting a lack of specificity, false positive staining. Had we studied only antibody #2, without checking a KO mouse, we might have reported the wrong map. Jutta was then able to publish an 89-page paper in *Neuroscience* and defend her thesis.

Neuroscience is a journal that was started in the mid 1980s, with Professor David Smith of University of Oxford as founding editor and chief editor for many years to come. We published many of our most important

papers in his journal. David, a brilliant scientist, was always helpful and constructive, and he generously allowed publication of Jutta's very long Y1R paper, as well as our already mentioned and similarly long substance P mapping paper, and even foldouts for some micrograph montages.

The *In Situ* Hybridization Era (ongoing)

If IHC was our main tool from 1970 and onward, ISH became a second basic, complementary method some 15 years later. After my failure at NIMH it was Martin Schalling who took on the challenge to establish ISH in our group. He joined my group, even if he had to support himself by working as a psychiatrist. He managed to introduce a TH insert into a vector in the lab of Rolf Ohlsson in Umeå and proceeded to collect the data that resulted in our first ISH paper (Schalling et al., 1986). However, Martin realized that the method needed significant improvements and decided to make a tour in the United States, visiting a series of labs, including Michael Brownstein/Scott Young (who at that time had started up the oligo approach = no plasmid needed) at NIMH in Bethesda. Martin returned and developed a new protocol based on the discussions he had with Scott Young and others. The first papers using this protocol were published 1987 and 1988 (e.g., Schalling et al., 1988), whereby the help by the late Håkan Persson and his student Anders Ericsson was most valuable. Subsequently, Åke Dagerlind and Stefan Brené came on board to help develop and publish protocols in 1996.

Martin defended his thesis in 2002 and succeeded in obtaining a competitive European Molecular Biology Organization (EMBO) postdoctoral fellowship to work in David Housman's laboratory at MIT, where he completely shifted gears and became a geneticist. After returning to Sweden, he established a large group working on the genetics of psychiatric disorders. Taken together, Martin and his coworkers' contributions were essential for the survival and productivity of our lab—in the years to come, the ISH method was to be used in many studies. For the past year or so we have been using the RNAscope method, and we have complemented ISH with quantitative polymerase chain reaction (qPCR).

Continuation of the Studies of DRG Neurons and Pain

The failure of a substance P antagonist in treating pain in humans made us look for alternatives, and the two novel peptides, galanin and NPY, seemed interesting to us. Galanin is normally visible in only a few neurons in DRGs under normal conditions, as first shown by the Polak/Bloom and Skofitsch/Jacobowitz labs in 1986. Subsequently, we observed a dramatic upregulation (100-fold) of galanin, mainly in small/midsize DRG neurons after peripheral nerve injury (Hökfelt et al., 1987b). Of note, Shehab and Atkinson already in 1986 demonstrated increased expression of a peptide,

VIP, in DRG neurons after nerve injury. After the dramatic upregulation of galanin, again published in a low-impact factor journal, I became convinced that galanin research should be a priority. Regarding NPY, this peptide shows even lower expression in normal DRGs but is also strongly upregulated after axotomy, mainly in large DRG neurons, as first demonstrated by Satoshi Wakisaka, Keith Kajander, and Gary Bennet in 1981.

In our continuing fruitful collaboration with Zsuzsanna Wiesenfeld-Hallin, the physiological experiments now focused mainly on galanin and NPY. Tamas Bartfai got rapidly involved in this work. Most collaborators were, however, guest scientists. Marcelo Villar extended the original finding by elegant studies reporting quantitative results on galanin and other peptide levels. The RIA analyses were carried out by Elvar Theodorsson and showed that, contrasting galanin, CGRP and substance P levels are decreased after nerve injury (Villar et al., 1989). Marc Landry, an excellent histochemist from Bordeaux, provided great methodological upgrades to our lab. When I asked Marc why he chose our lab for postdoctoral studies, he said "I listened to a lecture of yours in Paris and really liked your tie." Val Verge, from Canada, an elegant lady, who introduced elegant and for us novel quantitative methods, also complemented our pain research with aspects on growth factors. Andrew Bean, from the United States, contributed to the development of methods as well as to cloning and work on growth factors. Jacquie Corness, a PhD student from Canada, took on the task to explore molecular mechanisms underlying the nerve injury-induced upregulation of galanin. With help from Peter Burbach, Utrecht University, she demonstrated an involvement of leukemia inhibitory factor (LIF) and NGF (1996). Thus, Jacquie followed up the pioneering studies of LIF and nerve injury by Yi Sun, Story Landis, and Richard Zigmond, which they had published in 1993. Nora Kerekes focused her PhD work on growth factors and DRG neurons using DRG cell cultures and electrophysiology. Importantly, together with Francoise Mennicken and Dajan O'Donnell from AstraZeneca Montreal and Russell Hill, Nora showed that a large proportion (about 75%) of DRG neurons express GalR2 and that galanin, through this receptor, increases excitability (Kerekes et al., 2003).

During this period, many researchers in our lab were Chinese. Ju Gong Ju was my first Chinese guest scientist. Expelled from the university due to the cultural revolution, he was now back as professor in Xi'an eager to catch up in research. LKB, a Swedish company, asked if I was willing to host Ju Gong. When I heard of his comparatively old age, I expressed doubt (today I feel ashamed, thinking of my own age when I write this). Fortunately, LKB convinced me, and Gong Ju, a lovely man, arrived in 1986. He became a real asset, carrying out all experimental work himself, and writing the manuscript, the first one published in 1987. Gong Ju also liked singing, and he even learned some Swedish songs.

Xu Zhang (Chang) and his wife Lan Bao followed. Chang was a student of Gong Ju and had previously learned EM from Björn Afzelius, another Swedish pioneer, at Stockholm University. (I was responsible for a PhD course in EM at KI for several years. The highlight of the course was a lecture by Björn and Arvid Maunsbach, professor of anatomy and another student of Fritof Sjöstrand, dealing with published errors in ultrastructural research. The students and I loved their presentation.) So Chang came to me to study peptides in DRG neurons at the ultrastructural level. He produced a massive amount of data, with the help of his wife, Lan Bao—her contribution was initially unbeknownst to me. His results provided evidence that galanin was upregulated in CGRP neurons, alike replacing CGRP in the LDCVs, and he outlined the peptidergic secretory pathway in DRG neurons in a beautiful 1995 article, and much more. Chang completed his thesis in 1994. We have more than 60 publications together. Chang and Bao returned to China, first to Ju Gong and the Fourth Military Medical University in Xi'an. Chang then received one of seven positions at the Chinese Academy of Sciences (CAS) in Shanghai aimed at recruiting young successful Chinese scientists working abroad, a project masterminded by Jian-Ping Wu and Mu-Ming Poo. (I was proud that someone from our laboratory was selected.) In Shanghai, both Chang and Lan Boa embarked on successful careers, Chang being elected academician of the CAS at a young age.

Other Chinese collaborators were, as mentioned, Zhi-Qing David Xu, who also worked on galanin in DRGs. Using ISH, he studied galanin during development and found prenatal expression not only in DRGs but also in the ear, eye, nose, and, surprisingly, during bone formation, suggesting a developmental role for this peptide (Xu et al., 1996). Ru-Rong Ji studied growth factors and neuropeptides after inflammation and the effect of antisense nucleotides on peptides and pain behavior. He then went on to work with Clifford Woolf at Harvard and is now professor at Duke and a leading pain researcher. Tiejun Sten Shi completed his thesis on regulation of galanin and NPY in different mononeuropathic pain models, including a quantitative analysis based on stereology of the effect of nerve injury on the actual number of DRG neurons in mice. Mice, in contrast to rats, exhibit a fairly pronounced and rapid loss of neurons (Shi et al., 2001). Sten served as the lead person for our pain research program for a long time. Mingdong Zhang joined later to work on calcium-binding proteins, and more. Hong-Xiang Ruby Liu was, like Ru-Rong Ji, a postdoctoral fellow from the laboratory of the legendary Professor Ji-Sheng Han in Beijing, well known for his fundamental studies on acupuncture.

The work by Marc Landry and Hong-Xiang then gave us the insight that two galanin receptors are involved: GalR1 on glutamatergic spinal interneurons is *anti*nociceptive (Landry et al., 2006). By contrast, GalR2 on DRG neurons is *pro*nociceptive (Liu et al., 2001). These findings suggest that pain alleviation could be achieved by galanin, or better a selective GalR1 *agonist*,

given intrathecally (or by a compound passing the blood-brain-barrier), or alternatively by a GalR2 *antagonist* that is administered peripherally.

The main conclusion from our extensive work on galanin and pain is that we have defined a new endogenous antinociceptive system that protects against neuropathic pain, which is *intrinsic to* a population of *DRG neurons*. This system is activated by nerve injury and involves GalR1 and upregulation of, among other molecules, inhibitory galanin (and downregulation of the excitatory peptides substance P and CGRP in the same neuron subpopulation). Interestingly, after nerve injury, galanin has an initial pronociceptive phase that involves GalR2 and is part of the warning system. So the effect of galanin is biphasic, via two galanin receptors. We speculated that when the protective mechanism(s) is fatigued, neuropathic/chronic pain will arise. A second endogenous antinociceptive system had previously been recognized: dorsal horn interneurons expressing, for example, the enkephalins (opioid peptides) (their receptors also being targets for morphine). This system is activated by inflammatory pain as shown, for example, by Ronald Dubner and Mary Ann Ruda (1992) and Eberhard Weihe and colleagues (1994). Inflammatory pain upregulates expression of substance P and CGRP in DRG neurons.

Having often stressed the importance of studying human tissues, long after submission of this article, I got a major shock: Tavares-Ferreira, Price, and colleagues reported in a single-cell transcriptomic study that the only galanin receptor they could detect in human DRGs was GalR*3* (Tavares-Ferreira et al., 2022). So I will have to rethink my view on how to treat pain in humans based on galanin receptors. My hope is that also in the human spinal cord GalR1 is present in dorsal horn interneurons, as in rat.

In animal experiments, intrathecal administration of galanin/GalR1 *agonists* does reduce pain, in accord with our hypothesis. However, peripheral injection of GalR2 *antagonists* did not show pain relief (unpublished work by Tiejun Sten Shi and Mingdong Zhang). We have tried to explain this as follows: GalR2 stimulation results in activation of phospholipase Cß (PLCß), which increases intracellular Ca^{2+}, resulting in increased glutamate release and pain. After blockade of GalR2 alone, pain persists, possibly because DRG neurons also express several other receptor types, that are parallelly upregulated after nerve injury and equally activate PLCß. One example is cholecystokinin B (CCKB) receptors, as shown by Xu Zhang and colleagues in 1993. To reduce pain, it therefore would be necessary to block GalR2 and several other receptors at the same time. An alternative could be to directly inhibit PLCß, that is, the common intracellular target for these receptors, including GalR2. Preliminary results published by Shi and colleagues in 2008 (*PNAS*) suggest that this may be a plausible idea.

Parallel analysis of the involvement of the NPY system in pain has revealed many similarities with galanin, as reviewed by Pablo Brumovsky (Brumovsky et al., 2007) and more recently by Tyler Nelson and Brad Taylor,

in 2021. Detailed information on localization of NPY-Y1 and -Y2 receptors in DRGs and spinal cord was first reported by Xu Zhang and later by Pablo Brumovsky and colleagues, in 1999 and 2005, respectively.

At this point, an important question was whether these galaninergic mechanisms operate in humans. Zhang Xu showed upregulation of galanin in monkey DRGs after nerve injury as well as regulation of other peptides (Zhang et al., 1993). Thanks to Zsuzsanna Wiesenfelt-Hallin's contact with two Canadian colleagues (Jonathan Dostrovsky and Andres Lozano, Toronto) and their generosity, we got access to human DRGs (unlesioned [donated] or brachial plexus injury). Thankfully, Marc Landry and colleagues showed robust galanin expression in normal human DRGs, and possibly an increase of expression after brachial plexus injury, reported in 2003. Taken together, these results suggested that similar galaninergic mechanisms may operate in rodents and primates/humans, which supported our conviction that the galanin system is an important target for developing drugs for the treatment of pain. A big relief.

The Hypothalamic/Neuroendocrine Theme

Neuroendocrinology has its roots in neurosecretion—that is, the release of messenger molecules from neurons into blood, and therefore hormones. Pioneered by great scientists like Ernst and Berta Scharrer, Wolfgang Bargmann, and many more, early studies were focused on the magnocellular hypothalamic neurons in the paraventricular (PVN) and supraoptic (SON) nuclei. The classic book by Geoffrey Harris entitled *Neural Control of the Pituitary Gland*, published in 1955, may represent the start of modern neuroendocrinology. This was followed by the work of Roger Guillemin and Andrew Schally and their colleagues reporting the structure of several such "controlling" molecules—that is, the releasing and inhibitory hormones.

The fact that the hormones discovered by Guillemin and Schally's teams were peptides made it possible to study them by IHC. Thus, we initiated a second wave of neuroendocrinology at KI, in which my PhD students together with guest scientists made important contributions. Björn Meister focused on the many peptides in neurons in the arcuate nucleus, partly working with Barry Everitt, and together they in 1986 published a comprehensive review on this tiny nucleus. Sandra Ceccatelli studied nitric oxide synthase, growth factors, and peptides in the PVN, partly together with Björn and Marcelo Villar. Sandra, after a short postdoc period with Donald Pfaff at Rockefeller, came back to KI to join Sten Orrenius in the Department of Environmental Medicine. She later returned to our Neuroscience Department to become our first female chair.

In 1995, Martin Schalling imported a new mouse line from the Jackson Laboratories to KI: the anx/anx mouse that lost weight and survived for only three weeks or so. He generously shared this "anorectic" project with us,

initiating research related to food intake. This resulted not only in Christian Broberger's thesis focusing on the arcuate nucleus (Broberger et al., 1997) and the lateral hypothalamus, but also two further theses with Martin as main supervisor for Ida Nilsson and Jeanette Johansen who explored possible mechanisms underlying the anx/anx phenotype.

Christian took a postdoctoral position with David McCormick at Yale and learned electrophysiology, a method that he then successfully combined with histochemistry, resulting in a novel understanding of synaptic signaling in the arcuate nucleus and publications in high-profile journals (and a professorship at Stockholm University).

For over three decades, our lab has had a fruitful collaboration with Laura Calza's team in Bologna, working on a palette of projects mainly associated with hormones, in particular the thyroid axis, and neuropeptides, especially galanin, both at the hypothalamic and spinal level, as well as using tissue cultures. Laura's lab has many skills that we lack, and it has been a privilege to interact with her and her team.

We have had a long-standing collaboration with the Endocrinology Department headed by Rolf Luft, who initiated our work on SST. With Anna-Lena Hulting, we have analyzed pituitary tumors, and the effect of leukotrienes on the release of pituitary hormones (in collaboration with Bengt Samuelsson).

In 2016, Tibor Harkany moved to our department to work on hypothalamus and neuropeptides. Tibor's efforts represent a wonderful continuation of the neuroendocrine tradition at KI, using, for example, single-cell analysis of hypothalamic neurons (Romanov et al., 2017), perhaps one could say a third wave, now based on modern molecular–biological methods. Tibor is a stellar scientist with an extraordinarily wide competence and great ability to win big grants. His move was an enormous boost for the Neuroscience Department, and for me personally, in terms of intellectual (and economic) support, including recruiting (and paying for) PhD and postdoc students (Joanne Bakker, Martino Caramia) for our group. In 2015, Tibor accepted a generous offer to move to the Medical University of Vienna, retaining 20% involvement at KI.

Human Postmortem Tissues (2010–present day)

Having carried out animal experiments for many decades and trying to relate our results to illnesses like pain and mood disorders, I every now and then contemplated the relevance of animal experiments. So some 10 years ago I started a project with the question: how similar is the chemical makeup of rodent vs. human nervous tissues? Are results from animal studies relevant when embarking on the (very costly) development of drugs for human clinical treatment? I approached Miklós (Miki) Palkovits on this topic. Miki had established the Human Brain Bank in Budapest, and I have long enjoyed a

professional relationship with him, since my time at NIMH. I know Miki to be a master of brain anatomy and brain dissection, and a true scholar.

Together with postdoc Erwan LeMaitre and colleague Rochellys Diaz-Heijtz and, later, PhD student Swapnali Barde, we embarked on the analysis of galanin and its three receptors primarily in two human brain regions: LC and the DRN, using ISH and qPCR (Le Maitre et al., 2013). Importantly, these are big tissue samples where the NA and 5-HT neurons represent only a small population of all neurons. We found strong galanin expression in NAergic LC neurons (like in rat and mouse), and in the DRN region, but not in 5-HT neurons (as in rat, but not mouse). Regarding receptor distributions, we need more exactly defined, microdissected regions or even single-cells studies to gain a clear picture of their localization (under way).

The next project concerned five human male and female brain areas from controls and subjects who had committed suicide (Barde et al., 2016). The samples were obtained from Naguib Mechawar, who heads the brain bank at the Douglas Hospital (McGill) in Montreal: LC, DRN, the medullary raphe region, and two prefrontal cortex (PFC) regions were studied using RIA, qPCR, and bisulfite pyrosequencing (for DNA methylation). The results provided unique information on the distribution of the transcript and peptide, and on differences in their expression patterns in depressed vs. control brains. For example, transcript levels for galanin and GalR3 were higher in depressed DRN and LC than in controls, and in all cases, these increases were paralleled by a decrease in methylation (i.e., evidence for epigenetic mechanisms).

We had, together with a dozen research groups, obtained a European grant (acronym NEWMOOD) to explore various aspects of depression, headed by the leading psychiatrist William (Bill) Deakin of University of Manchester, and with Hungarian colleagues, including György Bagdy and Gabriella Juhász. Here a candidate gene study revealed that variants in genes for galanin and its three receptors confer increased risk of depression and anxiety in people who experienced childhood adversity or recent negative life events (Juhasz et al., 2014). Together, the two studies suggest that the galanin system may be involved in major depression disorder.

With Mathias Uhlén we published an RNAseq study in *Science* (2020) comparing the mouse, pig, and human brains with Evelina Sjöstedt as first author. Most recently, we have analyzed, with RNAseq and RNAscope, 17 regions of the human prefrontal cortex with a focus on neuropeptide systems, including galanin, where Wen Zhong, a biostatistician, has made a terrific contribution (submitted). By integrating RNA-seq, ISH, and single-cell data we could propose, for example, microcircuits involving galanin and galanin receptors in glutamtergic pyramidal neurons. Mathias is a great scientist and entrepreneur alike, and the Swedish scientist who by far has received the biggest grants in Sweden, by the Knut and Alice Wallenberg Foundation. With part of this money, Mathias funded the Science for Life

Laboratory, a unique resource and core facility for Swedish research now housing some 800 scientists offering support, for example, for RNAseq studies and genetic analyses.

Taken together, results from our lab and others, and from both animal and our human studies, have provided a coherent, *hypothetical* view of the involvement of galanin in mood regulation: Galanin synthesized in LC neurons is present in noradrenergic nerve terminals in cortex but also locally in their dendrites. Stress leads to increased firing of LC neurons and upregulation of galanin synthesis. If firing becomes excessive, galanin will be released from dendrites and activate inhibitory GalR3/GalR1 autoreceptors to prevent overexcitation. We and others have proposed that this is part of the resilience machinery (Weinschenker and Holmes, 2016; Hökfelt et al., 2018). Exhaustion of this protective mechanism may result in depression. Interestingly, like in the pain models, the two galanin receptors may have opposite effects.

The just-mentioned analysis of human PFC suggests that galanin also plays a role in the anterior cingulate cortex, in this case coexisting as an antagonist in glutamatergic pyramidal neurons. Here, galanin may surveil and suppress the excitability in this circuit. This is interesting in relation to the recent successful treatment of depression with ketamine, a noncompetitive NMDA antagonist. Thus, John Krystal and colleagues (Krystal et al., 2019) have proposed "a paradigm shift for depression research and treatment"—that is, from the old monoamine hypothesis to involvement of glutamatergic mechanisms. Maybe we can maintain an interest in both?

Some Reflections on Progress in the Neuropeptide Field

The discovery of an increasing number of neuropeptides accelerating from the 1960s raised interest and hope in this new type of signaling molecules, amplified by the discovery of corresponding receptors. Eventually, it was realized that the peptides and their receptors represent the most diverse of all transmitter systems (>100). Important insights include the fact that peptides coexist with classic transmitters and thus are auxiliary messengers, which are primarily released when neurons fire at high rates or burst fire. This explains the early observation that peptide KO mice do not necessarily present a phenotype different from wild types. Findings suggest that neuropeptides are essential only under certain circumstances, such as stress, injury, seizure, and addiction (Hökfelt, 1991). From this growing body of evidence, thoughts about developing new therapeutic strategies emerged. These included, for example, the use of substance P antagonists for treatment of pain, and use of antagonists to CRH for relief of stress. Although CRH receptor antagonists that pass the blood-brain barrier have been developed, they, and many antagonists at other receptors, often elicit serious side effects, like liver toxicity. Merck's failure with a neurokinin 1 antagonist for treatment of depression was a major setback for the whole

field. But there are some successes with, for example, CGRP antibodies/antagonists to treat migraine, orexin/hypopcretin antagonists for insomnia, and substance P antagonists for chemotherapy-induced nausea. Our own hope for the treatment of pain and depression based on galaninergic mechanisms has not materialized. But we have not given up. Nonetheless, the development of pharmacological tools for influencing peptide signaling still has limitations compared with the monoamine systems, in which each metabolic and signaling step is known, offering a multitude of targets.

Other Projects

We have over three or so decades also pursued several other lines of research, as listed next.

Neuropeptides in Glia

It had long been known that neuropeptides are expressed in glia. Our own work with Laura Calza, Ruud Ubink, and Norbert Halasz focused on galanin and NPY, and we have reviewed this topic (Ubink et al., 2003).

Basal Ganglia

Together with Mario Herrera-Marschitz (Mario is a wonderful scientist without initial resistance; "let's do it" and we did) and Urban Ungerstedt, using Urban's pioneering microdialysis method, we focused on peptides in basal ganglia, involving many postdocs: Marie-Noëlle Castel, Patrizia Morino, Rafael Rodriguez-Puertas, Zhi-Bing You, and Sonia Gomez-Urquijo.

Adrenergic Receptors

Anthony Nicholas, a postdoc from the United States, initiated a major study on adrenergic receptors based on ISH. Together with Vincent Pieribone, he explored the distribution of alpha-1, alpha-2, beta-1, and beta-2 adrenergic receptor mRNAs in the rat brain. We have summarized the results in a review (Nicholas et al., 1996).

Neurodegeneration

Thanks to the generosity of U.S. and Swiss colleagues, including Dr. Stanley Prusiner of the University of California, San Fransisco, we had the possibility to analyze several models of neurodegeneration, including Alzheimer and prion mouse models, with this work showing dramatic changes in peptide expression and carried out by my PhD student Margarita Diez (e.g., Diez et al., 2000).

SNAP-25 and the Metabolic Syndrome

Christina Bark working as a postdoc with Michael Wilson discovered two isoforms of SNAP-25: SNAP 25a and -b. After returning to Sweden, she created a unique mouse model in which SNAP-25b had been replaced by SNAP-25a. This SNAP-25b-deficient mouse present a distinct metabolic disease phenotype, especially after Western diet, including serious liver pathology (Valladolid-Acebes et al., 2015). Following up using three-dimensional imaging, Csaba Adori has been able to describe dramatic changes in the sympathetic liver innervation both in this mouse model and human fat liver (Adori et al., 2021).

Autoantibodies

Autoantibodies increasingly have been implicated in various diseases. Searching for such antibodies, we applied sera from human subjects with a particular disease onto sections of a formalin-fixed rat brain, hoping to find binding to discrete structures (neurons/glia) in specific brain regions, results that may give disease-related clues. Sergui Fetissov used sera from patients with anorexia and bulimia nervosa and found labeling of arcuate neurons expressed in food-intake-controlling alpha-melanocyte-stimulating hormone$^+$ neurons (Fetissov et al., 2002). Serguei in 2009 reported on serum from patients with the autoimmune polyglandular syndrome type 1, where one serum (with anti-AADC autoantibodies) stained nigral DA neurons even at a dilution of 1:1,000,000 (in collaboration with Olle Kämpe). Together with Henning Vaeröy, a Norwegian forensic psychiatrist, Serguei found that sera from violently aggressive subjects harbor antibodies reactive to adrenocorticotropic hormone (2018).

In collaboration with the Finnish sleep researcher Markku Partinen, we studied sera from narcoleptic patients. Peter Bergman and Csaba Adori showed that several sera from such patients reacted with hypothalamic melanin-concentrating hormone$^+$ and POMC$^+$ neurons, and together with Hungarian scientists a possible role of the immunogen in the fine regulation of sleep was reported (Bergman et al., 2014). Finally, together with Krister Kristensson, Marina Bentivoglio, and others, my PhD student Mingdong Zhang analyzed viral invasion of the brain and could show that the H1N1 virus is transported to nuclei involved in sleep regulation and causes narcoleptic-like sleep (Tesoriero et al., 2016).

Kidney Research

Ulla Kopp is Swedish and married to Gerald Dibona, both eminent kidney physiologists working at the University of Iowa. Combining her experimental kidney model with IHC, Ulla explored among other things, the role of

peptides and prostaglandins in renal nerve activity. I was surprised about the many similarities with our work on DRGs and pain. As a bonus, Ulla was a super-skilled organizer who, like a general, oversaw our move to new labs.

Committees

The Nobel Committee

In 1981, I was elected into the Nobel Committee (NC; today 18 members). The NC handles the many (today up to 800) proposals and meets with the Nobel Assembly (final vote, 50 members) to discuss the results of the crucial, extensive evaluations of the top candidates, followed by the Nobel Prize announcement the first week in October and a ceremony on December 10. The deliberations carried out by these two Nobel bodies are supported by the secretary, who also is a member of the NC, and during most of my period it was Jan Lindsten (1979–1990).

Already my first year on the NC was exciting, the prize for neuroscience was awarded to David Hubel, Torsten Wiesel, and Roger Sperry. The prize awarded to Köhler and Milstein for monoclonal antibodies in 1983 was important for its potential clinical relevance, and it also provided new reagents for our immunohistochemical work, as said. However, who could anticipate that such antibodies would revolutionize biomedicine, including the treatment of breast cancer and rheumatoid arthritis, and more? In fact, according to a 2021 report by Asher Mullard in *Nature Reviews: Drug Discovery*, the 100th monoclonal antibody product was approved by the U.S. Food and Drug Administration (FDA) in 2021, and in 2019, 9 of the top 20 therapeutics by sales were antibodies, with cumulative earnings of US$75 billion. This prize certainly was awarded to scientists who have "conferred the greatest benefit on mankind" (Alfred Nobel's testimony).

In 1986, the prize was awarded to Rita Levi-Montalcini for the discovery of nerve growth factor (NGF) and to Stanley Cohen for the discovery of epidermal growth factor (EGF). Even if well-received, some colleagues felt that one scientist had been left out: Viktor Hamburger. Why was Hamburger not included? There are many factors that contribute to deciding on an award. Foremost among them being reviews by specialists in the field, but also the nominations, including how many and by whom. As stated by Professor Bengt Pernow, previous chair of the NC and KI president: "We had been discussing the field of growth factors for around 5 years before Levi-Montalcini's prize and asked five or six scientists to review the field for us as a normal part of the evaluation" (Williams, 1995). Apparently these reviews had not favored Hamburger. Why not? Perhaps Hamburger received fewer, if any, nominations than Levi-Montalcini? If so, this will remain a secret until 2037, when the archives including the 1986 Nobel Prizes can be accessed (after application).

Other exciting events for us neuroscientists were during *my time in the Nobel assembly* (ending 2006) the Nobel Prizes in Physiology and Medicine given to Erwin Neher and Bert Sakmann in 1991 (the introduction of the patch clamp technique), to Stanley Prusiner in 1997 (the discovery of prions), to Arvid Carlsson, Paul Greengard, and Eric Kandel in 2000 (discoveries concerning chemical signaling in the nervous system), and to Richard Axel and Linda Buck in 2004 (the discoveries of odorant receptors and the organization of the olfactory system).

The IBRO Dargut and Milena-Kemali Foundation for Research in Neuroscience

The IBRO Dargut and Milena-Kemali Foundation was established in 1996 by Dargut Kemali, a professor of psychiatry in Naples, to honor the memory of his wife Milena Kemali. Milena was an anatomist/histochemist, and I gave her advice. On one visit to Naples, she drove me from the airport respecting traffic rules (which is not always the case in Naples), but after leaving the city she drove against three red lights. I couldn't help but ask why? The answer was (something like) this: "These red lights are not needed but were put up by a relative to the mayor, a clear case of nepotism." I had the privilege, together with Marina Bentivoglio, to assist with setting up the Dargut and Milena-Kemali Foundation. The Foundation, now taken over by IBRO, supports a course and awards a prize every other year with a lecture presented at the Federation of European Neuroscience Societies (FENS) Forum.

The Gruber Prize Selection Committee

In 2004, the Peter and Patricia Gruber Foundation established a substantial prize in neuroscience. Sol Snyder invited me to become a member of the first selection advisory board, and the first prize was presented to Seymour Benzer at the SfN meeting in San Diego the same year. It was a special honor to be part of this group, who met in the sitting room of Peter and Patricia Gruber's private flat on the west side of Central Park in New York City. To get to know the Grubers, a wonderful and modest couple with a strong philanthropic engagement, was inspiring. The Prize Lecture continues to be an important highlight at the annual SfN meeting.

The Brain Prize Selection Committee (2010–2012)

The Brain Prize is the brainchild of Nils Axelsen, former department chair and scientific director of Statens Serum Institute, Denmark, and at that time vice chair of the board of directors of the Lundbeck Foundation. Having been asked to explore the possibility of creating a major international

prize in neuroscience, Nils evaluated many award-giving foundations and suggested a prize in the field of basic and clinical brain research, with the name The Brain Prize. Nils proposed that this prize should (i) be awarded each year to one or more scientists after evaluation of nominated candidates by a selection committee; (ii) stimulate European and particularly Danish neuroscience; and (iii) be supported by an independent foundation, with Nils as chair of the board of directors. Anders Björklund and I assisted with recruiting the first selection committee.

The original emphasis was on European scientists and research carried out in Europe, but collaborating researchers from, for example, the United States also could be included. In this spirit, two U.S. colleagues were on the first selection committee (Huda Akil and Fred Gage), and Colin Blakemore acted as chair, and I as vice chair. This was a wonderful committee. I was happy to see three Hungarian scientists receive the first prize (György Buszáki, Tamás Freund, and Péter Somogyi). The third prize went to six scientists behind optogenetics. Because there was no strict limitation on the number of awardees, we could honor the several scientists behind this important methodological advancement. Now the prize is available to scientists all around the world, and the award is 10 million DKK (around US$1.6 million). It is recognized by the scientific community as one of the world's major scientific awards.

International Alliance for Translational Neuroscience

In 2012, the International Alliance for Translational Neuroscience (IATN) was founded in Beijing, a vision of Xiaomin Wang, professor at the Capital Medical University (CMU), assisted by Zhi-Qing David Xu, my previous PhD student. This organization eventually encompassed eight universities around the world. I had the honor of helping prepare the founding documents. Over five years, scientists from these universities met and discussed important issues in the field of basic and clinical neuroscience. A highlight was the Xianshan Science Conference 506th session, International Forum on Brain Disorders, in Beijing, in 2014. I was invited to many unique events in China, with Zhi-Qing David Xu often being a wonderful cohost. These included a visit to an illuminated Great Wall on a dark late evening and the opera "Impression West Lake" performed on the lake and directed by Yimou Zhang (who also choreographed the opening ceremony at the 2008 Beijing Olympic games). Both were unforgettable experiences. It was a privilege to be involved in this exciting enterprise and interact with Chinese colleagues.

The Dagens Nyheter Affair (1995)

Toward the end of this article, the worst experience in my professional career, an event that made me seriously think of quitting, shall be told. Early

summer 1995, I got a call from a journalist, BGA, from *Dagens Nyheter*, the largest morning newspaper in Sweden. BGA asked me, surprisingly, about the situation with my U.S. grants. I guessed he was alluding to an NIH grant with Menek Goldstein as PI, and I responded "fine." Thinking more about it, I remembered that I had received mail from NIH referencing an "audit." Not knowing what to do, I had left the letter on my secretary's desk for a couple of months. At that time, my secretary helped several other groups in the department, and it occurred to me that someone might have seen the mail and noted the word "audit" and contacted the journalist.

At the end of the summer, BGA called me again and said he wanted to go through my correspondence. KI is a governmental institution and all correspondence is open to the public. In the follow-up interview, I slowly realized that it was not simply a matter of my U.S. grant: BGA suspected that I had been bribed by the Italian pharmaceutical company Fidia to promote the selection of Rita Levi-Montalcini for the Nobel Prize, which she, as said, received in 1986. He actually said that he was aiming for the prestigious annual Prize for Investigative Journalists and that he wanted to put an end to all hush-hush associated with the Nobel Prize.

BGA mainly based his accusation on a Fidia check deposited to my account in a U.S. bank in the amount of US$5,000. I still had that account from may stay at NIH 1981/1982 and had received this money from my coorganizer Erminio Costa for the organization of a symposium on "Neuropeptide-Catecholamine Interactions" at the Fifth International Catecholamine Symposium, held in Gothenburg, Sweden, in June 1983. Costa had raised the money from Fidia, and it was to be used to pay travel costs for eight speakers. Instead of changing the check into SEK (Swedish krona) and paying the speakers in cash (SEK), I deposited it in my U.S. account and wrote a check to the speakers (most from the United States). I could present, to the *Dagens Nyheter's* editor (and BGA), written confirmation by all of the participants that they had received travel reimbursement (altogether US$4,600). The rest I used for my travel to Gothenburg.

Fidia was a small Italian pharmaceutical company, producing and selling GM1, a ganglioside. Fidia gave grants to work on the mechanism of action of GM1 (I did *not* receive a grant) and supported high-profile meetings in the neuroscience area. Fidia also awarded a modest prize (US$2,000), that several distinguished colleagues had received, including me. Fidia both paid travel and lodging at the meetings but almost always required a manuscript for the proceedings book. I accepted several invitations and sometimes even my wife was invited.

Some time after the interview, I understood that the story was soon going to be published. At this point, I just asked BGA for one favor: please tell me a day in advance of publication so that I could warn my elderly mother. I had been invited to a meeting in Amsterdam to give a talk on

Sunday in the Dutch Academy of Sciences, and I told BGA that I would be in my office on Saturday until around 1 p.m. before leaving for the airport. I did not hear anything and flew to Amsterdam and gave my talk Sunday morning. After that, I called Stockholm and then got to know that *Dagens Nyheter* had published what was going to be the first of four one- to two-page articles with me as target. An hour later, I came down with a terrible pain attack—a kidney stone. After treatment at a hospital, I could return to the meeting. But even if that acute pain was gone, the coming months, even years, would turn out to cause chronic pain and frustration.

Completely inexperienced, I did not know how to handle the situation but was advised to keep quiet. Sten Grillner and Nils Ringertz, chair and secretary of the NC, respectively, wrote a response, in which they explained the principles of the work in the NC and Nobel Assembly. What helped me overcome the trauma were the wonderful and thoughtful letters from many colleagues, from all around the world, who expressed support and even indignation on my behalf. Things soon calmed down, but I of course was heavily afflicted and incapacitated, pondering whether I could proceed with my research career. What did the colleagues *really* think, the relatives of my Swedish collaborators, and the public in any case? (I did receive some anonymous mails suggesting that I should be ashamed.)

Decisive support came from a small note in *Science*, one of the most prestigious journals, by Nigel Williams (1995), for me one of the most important articles ever published. Nigel interviewed eminent and knowledgeable scientists, by phone, and asked what they thought about the serious accusations in the *Dagens Nyheter* articles. In fact, my case had been published in newspapers around the world. One important voice was Professor Bengt Pernow, as said, a member of the NC, who was quoted as saying, "We had been discussing the field of growth factors for around 5 years before Levi-Montalcini's prize and asked five or six scientists to review the field for us as a normal part of the evaluation, *but Hökfelt was not one of them"* (Williams, 1995). This, along with the fact that the Nobel Prize was given for "growth factors" (and not *nerve* growth factors) and that the laudation in the Concert Hall was presented by Kerstin Hall, professor of endocrinology at KI, certainly confirmed that I did not play a major role in the decision of the Nobel Prize (I, of course, *voted for* the prize). Previously, the secretary of the NC had not allowed me to reveal that I was *not* one of the reviewers, but now Bengt Pernow had made this public. As said, these reviews are extensive and in depth, up to 30 pages or so, and are the basis for the work by the NC and the decision by the Nobel Assembly, so I felt "cleared" from the accusations. The editor in chief of *Dagens Nyheter*, later published some retractions: "There were no bribes" and "The laureate is not questioned" but still "We stand by the basic message . . . and I haven't regretted we published the articles" (Williams, 1995). One may wonder, what the "basic message" really was, or even what it was intended to be. I felt great gratitude to Nigel

Williams. I have today made considerable efforts to get in contact with him, to express my appreciation, but so far I have failed.

Shortly after the note in *Science*, I was contacted by a journalist working for an influential investigative Swedish television program that was produced by a media star, Robert Aschberg. They had read the note in *Science* and realized that I had been wrongly accused of taking a bribe, and they wanted me to confront BGA in their program. It felt good, of course, to get a chance to publicly clear my name, and I was tempted to accept. But knowing myself, I thought that I would probably mess things up. So I said no thanks. I also considered contacting the Swedish "press ombudsman." In fact, I spent a lot of time writing a long and detailed response to all accusations, but never submitted.

Traveling and Meetings

Meetings have always been an important and fun part of my life. Therefore, visits to the United States and other countries have been important. I have always been grateful for invitations and for the warm hospitality of my many hosts, too many to mention here. During one period, I often visited Japan and would like to thank some wonderful Japanese colleagues for many memorable meetings: Professors Yutaka Sano, Yasuhiko Ibata, and Nobutaka Hirokawa. I have already mentioned some special Chinese colleagues. Of the many meetings I attended over five decades, some, for various reasons, remain special to me and will briefly be mentioned here.

Nerve Cells, Transmitters, and Compartments Seminar

This seminar took place at the Pontifical Academy on October 9–14, 1978. The symposium was held in the Vatican Gardens, and I was humbled by the list of the brilliant participating scientists, whom I had never met but only read about. Several topics were discussed, ranging from growth factors through amino acid transmitters, opioid peptides, cotransmission, and sympathetic ganglia, to the brain-mind problem.

This seminar was somewhat overshadowed by excitement over the possibility that the participants would have the opportunity to meet the Pope, Paulus VI, who had been in office since 1963, for 15 years. Unfortunately, the pope passed away on August 6, 1978, so that chance faded away. Surprisingly, a new pope, Johannes Paulus I, was elected on August 28, again raising the hope of a personal encounter. Unbelievably, Johannes Paulus I passed away on September 28, just a few days before the symposium was to begin. Our seminar took place, but with the Vatican in mourning and with certain restrictions. When departing from the Vatican we, the participants, saw the white smoke raising over the conclave of the Cardinals. The Cardinals had elected Johannes Paulus II who remained in office until 2005, that is, for

more than a quarter of a century. The timing of my once-in-a-lifetime opportunity to meet a pope had been exceptionally unfortunate, indeed.

Central Regulation of the Endocrine System

Already in 1979, Kjell Fuxe and I had the great honor to organize a Nobel Symposium (#42) together with and thanks to Professor Rolf Luft. The meeting was attended by leaders in the field, three Nobel laureates (Axelrod, Schally, and von Euler) and three to be named: Martin Rodbell talked about early evidence for G-proteins, findings heralding the Nobel Prize that he would share with Alfred Gilman in 1994. Paul Greengard talked about protein phosphorylation; and Arvid Carlsson, surprisingly, talked about neuropeptides and their effect on gross behavior. But he would soon return to DA and other classical transmitters; and, as said, share the Prize with Greengard and Eric Kandel in 2000. This was a fantastic meeting and an honor to meet all these brilliant scientists at such an early stage in my career.

Coexistence of Neuronal Messengers: A New Principle in Chemical Transmission

I had the privilege to organize, together with Kjell Fuxe and Bengt Pernow, this symposium in Stockholm in 1985. It was supported by the Wallenberg Foundation for International Cooperation in Science, Astra Pharmaceuticals, and the Nobel Foundation. The meeting was attended by many outstanding colleagues. It was very exciting and a great honor, as well as a true highlight of my professional life.

Symposium Commemorating the 90th Anniversary of the Nobel Prize

The Nobel Foundation decided to celebrate the 90th anniversary of the Nobel Prize with pomp and circumstances. All previous laureates were invited (and more than 140 accepted) and four parallel, one-day symposia were held on December 7, 1991. One was on neuroscience, and Kjell Fuxe, Gunnar Grant, Sten Grillner, Lars Olson, and myself had the honorable assignment to organize the program. The 1991 Awardees, Erwin Neher and Bert Sakmann, served as chairs. Two participating laureates shared the 90th birthday with the Nobel Prize: Linus Pauling and Charles Huggins—impressive, to say the least. Two speakers were brilliant recruitments to the neuroscience field, Leon Cooper (from physics) and Gerald Edelman (from chemistry), together with many old heroes. It was a great day.

Finally, What About Grants?

Today, it is obvious that grants are important, and they must be *big* grants. This was not always so. Governmental and institutional support in the form

of position, PhD students, technicians, and secretary represented a reliable basis for research. Very importantly, during the 1980s and 1990s, many foreign guest scientists came to our lab with their own support and salary, for example, from NIH, the Canadian MRC, and Chinese bodies. I also want to gratefully acknowledge several further sources, some unexpected. In Sweden, the (Medical) Research council has supported me uninterruptedly from 1969 to this day (more than 50 years). Another source was the Wallenberg Foundations, after the Research Council, the largest donors to Swedish research. Their support to the research community here in Sweden cannot be overestimated. I also was lucky to obtain NIH grants, as co-PI, one in the 1970s focused on substance P with my Swedish mentor Bengt Pernow as PI, and later grants for the immunohistochemical analysis of the monoamine neurons, with Menek Goldstein of NYU as PI. I was grateful, impressed, and surprised by the U.S. generosity to support researchers in Sweden.

Even more surprising was another U.S. grant: It started, without any application, with a message from Dr. Perry Molinoff formerly at the University of Pennsylvania; at that time, he was the head of neuroscience discovery at Bristol-Myers Squibb (BMS). He informed me that I was being considered for an unrestricted neuroscience grant. However, before making the award, he and a group from BMS wanted to make a site visit to KI. They suggested a date and planned to visit me in the morning. I realized that the group was too large for me to pick them up by my car at the hotel. So I gave them, I thought, exact instructions for how to get to me. I was standing, dressed in white shirt and tie, in the window of our building starting 8.00 a.m., looking over the campus and waiting for their arrival. It was raining, and with every minute I got more and more nervous, and saw the money slowly fading away in the grey sky. Finally, around 10 a.m. a group crossed the campus; they had been left at the main entrance that is East on the campus (our building is West), with umbrellas and carrying/dragging their luggage. Now I was definitely sure about the failure. But it ended well. After a short presentation, they left for Arlanda airport, and the company jet that took them to Copenhagen. Some time later, I learned that I would have US$100,000 for each of the next five years. It was a big sum, in the range of my MRC grant. This meant a lot. Thanks Perry, and thanks BMS.

I had 10 years earlier very good reasons to be extremely grateful to BMS or, more specifically, to their Prize Committee: I was awarded the first BMS Award for Distinguished Achievement in Neuroscience Research together with Drs. Walle Nauta and Thomas Powell. It was an incredible honor for me to share the award with these giants in neuroanatomy. Incredible also was what happened when my wife and I were traveling to attend the prize ceremony at noon in The Pierre Hotel in New York. I had since long agreed to give three lectures at College de France in Paris, and the last one was at 5 p.m. the day before the prize ceremony in New York.

This seemed like an impossible situation, until BMS came back and told me that my wife and I could fly with the Concorde from Paris to New York. The supersonic plane would only take three-and-a-half hours and would land at JFK at 11 a.m. on the day of the Ceremony. We arrived by public transport in good time at Charles de Gaulle (together with the passengers traveling in Bentleys and similar luxury cars) and boarded. After having been seated for 20 minutes or so we were told to disembark—there was a problem with a vault on the aircraft. I am always the nervous type, but this was too much. Heart racing: what if we arrived too late and BMS had paid these terribly expensive tickets? Eventually the problem was fixed and Air France guaranteed arrival at JFK at 11:45 am, so we decided to fly. I was completely exhausted and slept the whole trip, and did not note the breaking of the sound barrier nor enjoyed the caviar and champagne (but Lil did). A waiting driver took us to New York City and, at exactly 12:30 p.m., we ran into The Pierre, convinced that half the ceremony lunch had passed. However, we had forgotten that the lunch was preceded by a 30-minute cocktail session. We joined the last guests on their way to the lunch, and slipped unnoticed into our chairs. No one seemed to have missed us. An unbelievable relief, after unbelievable stress and an unbelievable journey in an unbelievable plane.

Concluding Remarks

I consider myself an explorer, some would say stamp collector, providing information on the localization of chemicals in the nervous system, information to be used for functional studies. But to see, with one's own eyes in the microscope, what no one else has seen before has given me an enormous joy, even if just a twinkle in the universe. Much work has focused on neuropeptides. Four of them, galanin, NPY, CCK, and VIP, were discovered at KI, in the Viktor Mutt Laboratory, and it has been an honor to follow up Viktor's groundbreaking work.

I have been awarded several prestigious honors and have always felt a bit embarrassed. But these experiences have stimulated me to work harder to show that I perhaps was worthy such distinctions. My school period in Germany made me familiar with its history, including Otto von Bismarck, the mighty chancellor who united Germany. He once said (among many other quotes): "A statesman . . . must await until he hears the steps of God sounding through events, then leap up and grasp the hem of His garment." By replacing some revered words, this has characterized much of my career: I have listened to and learned from many inspiring and inventive colleagues and collaborators, including more than 20 PhD students and some 75 postdocs, and then I have put all my energy into every project. Things have sometimes gone wrong, but I believe that our results have mostly been correct. Nine of my PhD students became full professors (Marianne Schultzberg,

Martin Schalling, Björn Meister, Jan Lundberg, Sandra Ceccatelli, Christian Broberger, and Nora Kerekes; and, in China, Zhang Xu, a member of the Chinese Academy of Sciences, and Zhi-qing David Xu.

And it all began with one person, Nils-Åke Hillarp. It is hard to overestimate the importance of a single person for advances in biomedicine (in Hillarp's case, achieved in three years). Times have changed, as has the process of conducting science and publishing results. In the old days, with our simple techniques, it could be that a postdoc left after two years with 20 papers. Today, especially in the good labs, postdocs may leave with one publication after four years or more. Hopefully, it is clear that I, during my six decades in research, have been supported by many to whom I owe sincere thanks, but here I would like to express my gratitude to Solomon Snyder, Eric Kandel, Jean-Pierre Changeux, and Huda Zoghbi for support, advice and taking interest in our work over decades; to Paul Greengard for support also in personal matters; to Tamas Bartfai for collaboration and friendship; to Anders Björklund, Robert Elde, and Tibor Harkany for reading and providing valuable input to the draft; and, finally, to Tom Albright for extensive editing. I appreciate and am honored by the invitation from Drs. Larry R. Squire and Tom Albright to contribute to their unique series *The History of Neuroscience in Autobiography*. It is and will remain *the* account of neuroscience research carried out over the past seven decades or so. Finally, none of the things described in this article could have been achieved without my wife Lil.

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