An Ulf von Euler "school" at the Department of Physiology I, Karolinska Institutet

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I. Ulf von Euler – Life and Scientific career

Ulf Svante von Euler-Chelpin was born in Stockholm in 1905. His background was in part highly academic, and cosmopolitan: His mother, Astrid Cleve, is said to have been the first female in Sweden to receive a doctoral degree, in 1898. Later, she became a professor in botany and geology. Cleve's father, Per Teodor, a professor of chemistry, discovered the elements erbium, holmium and thulium.

Ulf's father, Hans von Euler-Chelpin, was of German origin and distantly related to the famous Swiss mathematician Leonhard Euler, active in Petersburg during the eighteenth century. Hans started out as a painter in an artist colony in Bavaria. Changed his mind and went on to Berlin to work with Otto Warburg for a doctorate in chemistry. And after that to Stockholm to work with the Swedish Nobel Laureate in Chemistry Svante Arrhenius. In 1927 Hans himself received a Nobel Prize in chemistry. So it is no wonder that Ulf von Euler excelled early. At the age of 17 he published his first scientific paper, as co-author with his father. An important inspiration in his scientific development was probably to participate in the Twelfth International Physiological Congress in Stockholm (1926), where he had the opportunity to meet all the great physiologists of the time (I. Pavlov, E.H. Starling, J. Barcroft, Otto Loewi and many others).

Ulf von Euler's early scientific training took place in the laboratory of Göran Liljestrand, professor and chairman of the department of pharmacology at Karolinska institutet. Ulf reported that Liljestrand had a strong influence on his scientific career: "it is hard to imagine a more helpful and stimulating teacher and co-worker". Their seminal discovery of pulmonary hypoxic vasoconstriction is widely referred to as the von Euler-Liljestrand mechanism.

In 1930 von Euler defended his Thesis and then, supported by a Rockefeller Foundation Fellowship, went to London to work in the laboratory of Henry Dale, at the time the leading expert on the chemical basis for autonomic neurotransmission (and in 1936 recipient of the Nobel Prize for Physiology or Medicine).

After returning to Karolinska institutet, von Euler was appointed assistant professor in pharmacology, and in 1939 professor and chairman of the department of physiology, a position he held until his retirement in 1971.

From 1957 to the end of his life in 1983, he was chief editor of Acta Physiologica Scndinavica, a position in which he succeeded Göran Liljestand.

Scientific production

Ulf von Euler's scientific productivity is amazing; altogether he published 465 papers over a period of six decades. His 'Leitmotiv' was clear: to identify the chemical signals that mediate control of physiological events. Three of his discoveries that have turned out to be of the most fundamental biological importance:

1. Substance P

In 1930 von Euler made his first major discovery while he worked in Dale's laboratory. In collaboration with a senior colleague, J.H. Gaddum, he noted that extracts of brain and intestine behaved in part similarly, in part differently from the reference substance, acetylcholine. Both contracted the gut in an organ bath, and both lowered the blood pressure of the anesthetized rabbit, but while the effects of acetylcholine were abolished by atropine, those of the extracts were not. The impure preparations of the unknown substance were labeled 'Powder P'. And thus, essentially by accident, the active 'principle' later became known as 'Substance P'. By intuitive appreciation of its potential biological importance, von Euler patiently followed up his discovery by describing the polypeptide nature of Substance P, methods of its purification and assay, its general distribution in the body and the biological effects of the, still relatively crude, preparations available at the time. (Avh. BengtPernow (1953).

The breakthrough came much later, when the chemical nature of Substance P became fully elucidated by Chang & Leeman, some forty years after von Euler's original discovery, and pure Substance P became available. By immunohistochemistry and electron microscopy, Substance P could now be shown to occur selectively within many neurons in brain and periphery, stored in large dense core vesicles (Hökfelt et al. 1980). But Substance P also occurred in glial cells as well as in some cells in the immune system. The exact physiological roles of Substance P still remain to be finally established. One of them is probably to act as a neurotransmitter and/or modulator, notably of signals from primary afferent pain fibres. The report that a Substance P receptor antagonist has clinical efficacy in treatment of major depression could unfortunately not be confirmed in the large Phase 3 study but, instead, the same compound is now used for treatment of chemotherapy-induced nausea; and Substance P can produce release of several cytokines, suggesting that it may have a range of other

functions both in the nervous and in the immune systems (Hökfelt et al. 2003). Thus, for example, release of Substance P from perivascular C-fibre terminals has been shown to be involved in pressure-induced changes in vascular smooth muscle tone (Scotland et al., 2004, Baylie and Brayden 2011). The Substance P accidentally discovered by von Euler has thus turned out to be an important first member of an intriguing, now rapidly expanding class of 'neuropeptide signal substances'.

2. Prostaglandins

Ulf von Euler's second major discovery was made in 1934, after he had returned to Karolinska institutet. In extracts of seminal fluid or prostate or vesicular glands he found an unknown 'principle' which, similarly to Substance P, mimicked the effects of acetylcholine on test preparations, but whose effects were atropine-resistant. Somewhat unfortunately (because it was first found in extracts of the prostate), he named it 'prostaglandin'. During the next few years he was able to define prostaglandin as an unsaturated, lipid-soluble, nitrogen-free organic acid. He subsequently described tissue sources, methods of extraction and purification and basic pharmacological properties of the available, still crude, preparations. (Avh. Rune Eliasson 1959) The breakthrough came when the biochemist Sune Bergström proceeded further with the chemical analysis. Using a newly developed technology, Bergström succeeded in the late 1950s in the first purification of a prostaglandin. von Euler wrote in the journal Progress in Lipid Research that" a discovery is in principle like an invention, or even a piece of art…. It is sometimes said that the prostaglandins lay dormant for some 20 years after their discovery. This is not exactly true, since Sune Bergström took over in 1945 where I left it, and with consummate skill and perseverance conducted the chemical work to isolation and identification, thus starting the second stage of prostaglandin history." Together with Bengt Samuelsson, Bergström showed that 'prostaglandin' is not a single substance, but a family of biologically active compounds of the highest importance for many physiological functions, for example in the reproductive and circulatory systems, and also plays key roles under pathophysiological conditions (Gaetano et al. 2010; Schulzke et al. 2010; Tusgaard et al. 2011). For these discoveries, Bergström and Samuelsson shared the Nobel Prize in Physiology or Medicine 1982 (Avh. Per Hedqvist 1970).

3. Noradrenaline as sympathetic transmitter.

Ulf von Euler's third major discovery concerned the identity and mode of intraneuronal storage of the sympathetic catecholamine transmitter. In 1946 he settled a 40-year old

controversy concerning its identity, by showing that in most sympathetic nerves the monoamine transmitter is not adrenaline, as earlier assumed, but its non-methylated homologue and precursor, N-Ohne-Radikal-adrenaline (noradrenaline). That (synthetic) noradrenaline was more closely 'sympathomimetic' than adrenaline had been shown already in 1910. But there was no evidence at that time that noradrenaline occurs naturally in the body; it was dismissed as a mere 'synthetic curiosity'. von Euler tackled the problem by using classical pharmacological differential bioassay to determine the adrenaline and noradrenaline content of extracts of sympathetic nerve trunks or their terminals in target tissues. It became soon clear to him that the 'sympathomimetic' principle in the extracts was not adrenaline; "after many trials, doubts and guesses, it became plausible that it was noradrenaline". This conclusion was reached by colorimetric and fluorimetric methods, after separation of the compounds by paper or ion exchange chromatography. Noradrenaline did not occur in the nerve-free placenta, but was present in sympathetic nerve trunks and all sympathetically innervated target tissues, in proportion to their sympathetic nerve terminal density, and largely disappeared following denervation. Noradrenaline was, thus, clearly located strictly within sympathetic nerves, probably mainly in the terminals.

The finding that the sympathetic catecholamine transmitter is not adrenaline but its immediate precursor, noradrenaline, raised interest in the possibility that the immediate precursor of noradrenaline, dopamine, might also have signaling competence. This was shown by Arvid Carlsson (who shared the Nobel Prize in Physiology or Medicine 2000) to be the case (Iversen, SD & Iversen, LL, 2007).

As a consequence, we now know that catecholaminergic transmission utilizes three classes of monoamine signals: adrenaline, its precursor, noradrenaline, and its precursor, dopamine, each with a different distribution and physiological role. Interestingly, the original full report of von Euler's seemingly simple, but truly important discovery, was first published in Acta Physiologica Scandinavica (von Euler 1946).

Until 1946 one did not even know what sympathetic nerve terminals look like. That year Hillarp in Lund showed by classical histological staining (methylene blue, silver impregnation) that they look like 'beads on a string', forming a long series of swellings ('varicosities') 1μm in diameter separated by 4 μm small caliber intervaricose sections. Fifteen years later Hillarp and Falck developed a fluorescence histochemical method which

actually visualized noradrenaline in the nerve terminals and showed it to be concentrated in the varicosities.

What particularly intrigued von Euler was that sympathetic nerve terminals appeared to contain noradrenaline at extremely high (up to 30 mM) concentrations, similar to the levels characteristic of catecholamines in chromaffin cells. How could sympathetic nerve terminals store this extremely powerful (potentially 'poisonous') biological agent at such high concentrations safely, yet be ready to release it from the store in small fractions, on demand? Surely it could not exist in free solution. Might sympathetic nerves store its catecholamines as 'granules' in vesicles, as chromaffin cells had been shown to do, by Nils-Åke Hillarp's group. Together with Hillarp, von Euler showed by differential centrifugation of homogenates of sympathetic nerves that a large part of the noradrenaline was sedimentable, i.e., occurs in particulate form. By studying the properties of successively purified preparations of 'nerve granules', von Euler made pioneering contributions to a new field, the cell and molecular biology and pharmacology of isolated 'nerve granules' for the biosynthesis, uptake, storage and release of noradrenaline (Avh. Hugo Lagercrantz 1971). These studies have turned out to have wider implications, because they were the first to address several universal mechanisms for intraneuronal synthesis and storage vesicular storage of (almost) all neurotransmitters (Stjärne1999). But von Euler never committed himself regarding the mechanism and exact role of 'nerve granules' for nerve impulse-induced (exocytotic or non-exocytotic?) noradrenaline release.

The Nobel Prize

To the very end, even after his retirement as chairman of the department of physiology in 1971, von Euler continued to actively pursue his research line within the catecholamine field. As recognition of his discoveries in this area, and of their impact on the development of other fields, such as brain function in health and disease, or clinical hypertension, von Euler shared the Nobel Prize in Physiology or Medicine 1970 with Bernhard Katz and Julius Axelrod (Langmoen and Apuzzo 2007;Raju 1999).

Figure 1. 1970: Celebration at the department after the announcement of the nobelprize in Physiology or Medicine to Ulf von Euler. From left: Ulf von Euler, Britte Backman (secretary), Marianne Wennmalm, Arne Åström (in back), Fjodor Lishajko (in back) Åke Wennmalm, Lennart Stjärne.

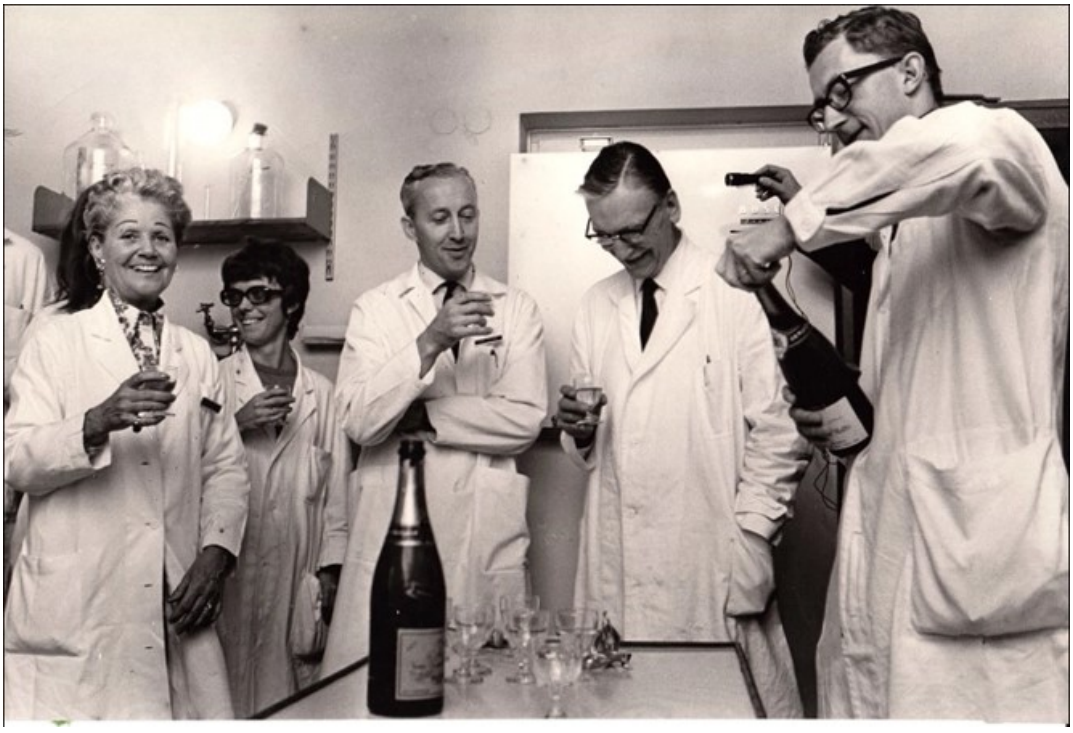

Figure 2: Sekretary Britte Backman, Laboratorieassistent Ingrid Dahlin, Docent Arne Åström, Ulf von Euler, Docent Per Hedqvist.

Figure 3 From left: Laboratorieassistent Ulla Spendrup?, Lennart Stjärne, Åke Wennmalm, Arne Åström (in back), unknown, Marianne Wennmalm, Ingrid Östman (in back), sekretary Britte Backman, unknown, Per Hedqvist (not visible), Ulf von Euler.

Ulf as a person

Towards the end of his life, von Euler could look back on a uniquely successful research career. Already as a young scientist he had had the genius - and the good fortune - to make three major discoveries. Each had grown, over time, to become one of the 'hottest' fields of the life sciences. How he experienced this he has commented on in a witty and instructive Editorial in Circulation (von Euler 1962), that reveals some aspects of his philosophical outlook on science, such as on the art of recognizing a 'discovery' from an 'observation', concluding: *"We must always guard the liberties of the mind and remember that some degree of heresy is often a sign of health in spiritual life"*. And doesn't this sound familiar? *"There is a certain tendency in our time toward collection of data, especially with the aid of new and ingenious machines with a large output capacity. Some of these machines can easily produce miles of records. They are also expensive and the press photographers have a definite liking for them. In order to pay off the large grants received for the purchase of such machines the poor scientist has to produce a number of papers which will make it seem worthwhile for the granting body to have allowed such a large sum."*

To those who have had the privilege of receiving their scientific training by Ulf von Euler, there can be no doubt about his greatness as scientist and teacher. He was not an empire-builder or a founder of schools for dogmatic adherents to his own views, but a believer in freedom in research. “Always keep an open mind – but, equally, always believe in the least sensational explanation”. New ideas in young colleagues were encouraged with a degree of enthusiasm adapted to his own intuitive faith in their soundness. Whenever disbelieving, his comment might be:” It would be most interesting if you turn out to be right…”. His openness of mind and genuine scientific curiosity, and his knack for finding the right experimental approach to test the validity of a new hypothesis made it a very educating experience to work close to him.

As a person, von Euler was aristocratic, a citizen of the world, carrying on with dignity and style the intellectual and cultural tradition that he had inherited. His dry humour and mild sarcasm made his company stimulating, sometimes even delightful. He had the great privilege to keep his full creative potential to the very end, living to see his scientific offspring mature, and rightly enjoying international recognition as a Grand Old Man of neurochemical information transfer.

Tributes to Ulf von Euler

1. **A Nobel Conference**:

1n 1980 four earlier Ulf von Euler disciples (Lennart Stjärne, Per Hedqvist, Hugo Lagercrantz and Åke Wennmalm) were granted the privilege of celebrating a double 75-year old anniversary: that of the man and of the field, in the form of The Second Nobel Conference of the Karolinska Institute, on ‘Chemical Neurotransmission 75 years’, in honour of Ulf von Euler. The meeting was held in Stockholm in December of 1980.

The aim was not a merely formal celebration. It was widely felt that the field of chemical neurotransmission had grown so vast that a personal confrontation of the various specialists: electron microscopists, biochemists, electrophysiologists, neuropharmacologists, clinicians and more might be necessary to bridge the growing gap between those working in ‘neurotransmitterology’. Leading experts on key aspects of the subject area (including The Nobel Laureates of 1970: Ulf von Euler, Bernard Katz and Julius Axelrod) accepted the invitation. The meeting was extremely successful as reflected in these closing remarks by Bernard Katz: “It is easy to speculate without knowledge. What we *do* know is that exciting areas have been opened up in the field of neurotransmission during the last decade, and that

some of the problems discussed at this conference will provide work of great interest and practical use for generations ahead."

The **Book** on the Meeting: Chemical Neurotransmission 75 years. (Eds.: Lennart Stjärne, Per Hedqvist, Hugo Lagercrantz, Åke Wennmalm). 1981. Academic Press. London. New York, Toronto, Sydney, San Fransisco.

2. Annual Ulf von Euler Lectures

The same Ulf von Euler disciples (Lennart Stjärne, Per Hedqvist, Hugo Lagercrantz and Åke Wennmalm) formed an Ulf von Euler Lecture Foundation (financed in part by surplus from the Nobel Conference), with the aim to invite a leading expert within a scientific field related to Ulf von Euler, to deliver an Annual Ulf von Euler Lecture (preferably on February 7, Ulf's birthday). The first Lecture was given in 1985. The series has been extremely successful; invited speakers have felt honored and delivered beautifully. And quite a few of them have, later, obtained a Nobel Prize in Physiology or Medicine themselves.

Beyond Ulf von Euler: A Symposium on Molecular & Cellular Mechanisms of Neurotransmitter Release

(Excerpt from the Preface to the Symposium): "The current breathtaking pace of the development of research adds to the excitement but makes it increasingly difficult for the individual neuroscientist to keep up with the news and appreciate its implication for neuroeffector transmission. We (Lennart Stjärne, Paul Greengard, Sten Grillner, Tomas Hökfelt, David Ottoson) were therefore extremely happy that leading authorities within the field accepted our invitation to come to Stockholm to compare notes at a Wenner-Gren International Symposium on "Molecular and Cellular Mechanisms of Neurotransmitter Release" held September 1-4 in 1993. This book reflects the tremendous progress in areas discussed at the Symposium. Research on quantal release has accelerated greatly as the result of new technical approaches including patch clamp analysis of single channel conductance's as well as of exocytosis, calcium imaging with high temporal and spatial resolution, and direct recording of release of single quanta from visualized boutons. It is now evident that calcium signaling serves multiple functions and that transmitter exocytosis follows more than one pathway. It has been shown beyond reasonable doubt, at least for "fast" transmitters, that active zones in different neurons function as binary units (i.e. permit each nerve impulse to release the contents of only one of the docked vesicles) but also differ by orders of magnitude in probability of monoquantal release.

It has recently been shown that transmitter exocytosis in nerves shares a number of features with the constitutive exocytosis that has been demonstrated in many kinds of cells. Comparison between model systems such as yeast cells and isolated components of the secretory machinery in animal neuronal and non-neuronal cells has turned out to be extremely fruitful. This approach has helped reveal how soluble and membrane-bound factors enable the nerve impulse to cause the two "skins" (i. e., the membranes which limit the vesicles that store transmitters, and that which forms the wall of nerve terminals) to transiently fuse and create a channel through which the *guttula* (i.e., transmitter package) is disgorged. In more modern terminology, this research is now beginning to clarify both the molecular and cellular mechanisms that make a single vesicle at the active zone "exocytosis-competent", such that the nerve impulse can release its content as a "transmitter quantum", and cause the vast majority of the vesicles, even those apparently docked at the active zone, to ignore the nerve impulse.

The multiple analytical breakthrough lines described in this volume will obviously have far-reaching implications for our views on many forms of plasticity of synaptic transmission (e.g, long-term potentiation in the hippocampus) and ultimately for our understanding of higher functions such as learning and memory.

This book will be of broad interest to graduate students and seasoned neuroscientist, and we (the Editors) hope that the excitement felt by the participants in the Symposium will be shared by the readers.

Concluding remarks (Charles Stevens, Salk Institute): "Physics reached its Golden age in the nineteen twenties. This Symposium may mark the beginning of a Golden Age for Neuroscience."

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