A HALF-CENTURY OF NEUROTRANSMITTER RESEARCH: IMPACT ON NEUROLOGY AND PSYCHIATRY

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by

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BEGINNINGS

My encounter with dopamine followed upon an incredible sequence of fortunate events. I had been working on calcium metabolism, using radioactive isotopes, which had then just become commercially available. This work had resulted in my doctoral thesis in 1951 and a series of subsequent papers, including two doctoral theses by students of mine. It had become somewhat visible internationally, resulting for example, in an invitation to a Gordon Conference in New England in 1955. The reason why I left this research field was that in connection with a competition for an associate professorship in pharmacology the expert committee let me know that in their opinion calcium metabolism did not occupy a central position in pharmacology. I therefore turned to Professor Sune Bergström who was at that time head of the Department of Physiological Chemistry of the University of Lund, Sweden. This department was located in the same building as our Pharmacology Department. Professor Bergström had already been very helpful in several instances when I had a professional problem of some kind. Incidentally, Dr. Bengt Samuelsson was at that time working with Professor Bergström in the same department. Thus the three Swedes who were to become Nobel Laureates in the period 1980–2000, happened to be working under the same roof for a few years.

I asked Sune Bergström if he could help me to get in touch with an outstanding American laboratory working in the area of biochemical pharmacology, which I felt had a great future. He wrote to his friend Dr. Bernard Witkop, a highly talented chemist working in the National Institutes of Health in Bethesda, Md. This letter was forwarded via the late Dr. Sidney Udenfriend, to his boss the late Dr. Bernard B. Brodie (Fig. 1), head of the famous Laboratory of Chemical Pharmacology of the National Heart Institute. That is how I came to work under Dr. Brodie for about five months, starting in August 1955. The timing of my arrival there was extremely fortunate. Brodie and his colleagues had just a few months before made a breakthrough discovery, namely that the administration of reserpine, a recently introduced

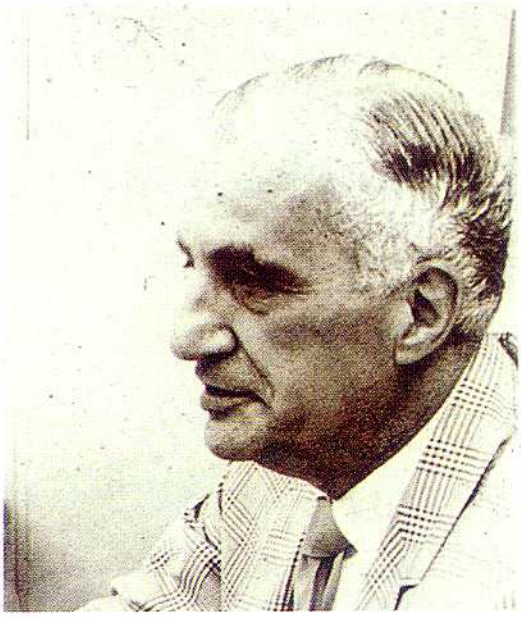

Figure 1. Bernard B. Brodie (1907–1989)

antipsychotic and antihypertensive drug, caused the virtually complete disappearance of serotonin from the brain and other tissues (Pletscher *et al.* 1955, 1956, Fig. 2).

"APPRENTICE TO GENIUS"

Brodie was a remarkably charismatic and intensive person. He was generally called Steve Brodie. This referred to a saloon keeper named Steve Brodie, who at the beginning of the previous century had jumped off the Brooklyn Bridge in order to win a bet. Bernard Brodie, too, was a sensation seeker who

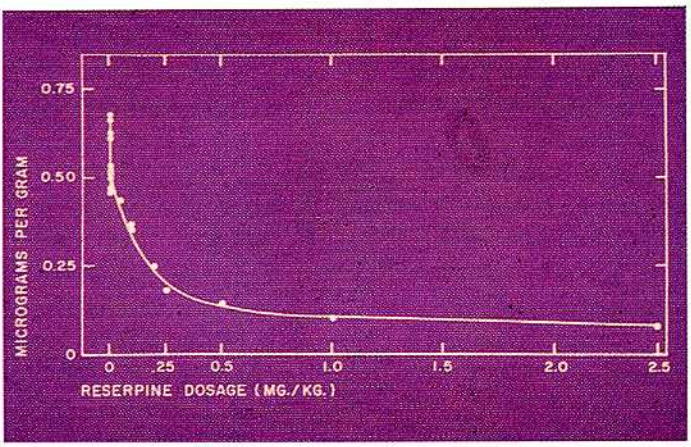

Figure 2. Brain level of serotonin four hours after various intravenous doses of reserpine. From Pletscher *et al.*(1956).

in his youth had started on a career as a boxer but later switched to become an organic chemist. He then confined his sensation seeking to non-physical adventures. He liked to call himself a gambler. He had gained a tremendous reputation as a pioneer in the area of drug metabolism and should perhaps rightly be called the father of modern biochemical pharmacology. A large number of his apprentices, coming from various parts of the world, later became prominent figures in pharmacology (see Kanigel, "Apprentice to Genius"). In the 1950s, after hearing about the sensational clinical actions of the new antipsychotic drugs and the ability of the hallucinogenic LSD to block serotonin effects on various peripheral organs he decided to enter the field of psychopharmacology. While knowing very little about the brain he had a tremendous trump card in being able to determine for the first time serotonin and similar molecules in the brain, using the prototype of a new instrument developed in his own lab together with Sidney Udenfriend and Dr. Robert Bowman. This instrument, the spectrophotofluorimeter, was to replace previous bioassays and to revolutionize drug research and neurotransmitter pharmacology for several decades.

This research soon led to the breakthrough discovery just mentioned, that is, the depletion of serotonin stores by reserpine treatment. For the first time a bridge seemed to have been built between the biochemistry of the brain and some important brain functions, with some obvious neuropsychiatric implications.

Brodie and his colleagues, especially Dr. Parkhurst Shore, generously introduced me into the new analytical methods and the use of the new instrument. I proposed to Brodie that we investigate the effect of reserpine on the catecholamines in view of their chemical similarity to serotonin. But Brodie thought this would be waste of time. He was so sure that serotonin was the target to focus upon.

A "ROSETTA STONE"?

But I felt that a look at the catecholamines might be worth while. To get started quickly I would then need a partner specialized in the catecholamine field. Again I was incredibly lucky. Of all the people working in that field at the time the most clever partner in such a project was located in my home University, the University of Lund: Professor Nils-Åke Hillarp (Fig. 3). I wrote to him from Bethesda and proposed a collaboration, and he agreed. Thus a most fruitful collaboration started, lasting until his untimely death in 1965. Hillarp's personality was different from that of Brodie in many respects, but they were similar in terms of brilliance, charisma and intensity. His background was histology and histochemistry, but his knowledge extended far into physiology and biochemistry.

In the spring of the following year Hillarp and I got the first results. We demonstrated the depletion of catecholamines from the adrenal medulla of rabbits following treatment with reserpine (Carlsson and Hillarp 1956). This was before I had acquired my own miracle instrument, the so-called Aminco-

Figure 3. Hillarp. Photo: Georg Thieme.

Bowman Spetrophotofluorimeter. The only instrument we had for the determination of catecholamines was a colorimeter, using the method of von Euler and Hamberg (1949). But we did not need any instrument because be absence of a colour development in the samples from reserpine-treated rabbits could be seen with the naked eye.

The same results were obtained when we analyzed heart and brain, in the latter case using our new instrument. We also found that sympathetic nerves no longer responded to nerve stimulation following reserpine treatment, apparently due to depletion of transmitter (Carlsson *et al.* 1957a). Thus depletion of catecholamines could be the cause of the behavioral inhibition induced by reserpine. To investigate this we gave DOPA (3,4-dihydroxyphenylalanine) to reserpine-treated animals and thus discovered the dramatic reversal of the reserpine-induced syndrome by this catecholamine precursor (Carlsson *et al.* 1957b, Fig. 4). The reason we used the precursor was that the catecholamines are unable to penetrate from the blood into the brain, because of the blood-brain barrier.

We then analyzed the brains of DOPA-treated animals and much to our disappointment we were unable to detect any restoration of noradrenaline levels. Experiments with monoamine oxidase inhibitors clearly showed that a monoamine rather than DOPA itself was responsible for the behavioral response, and thus we were forced to look for the intermediate in the conversion of DOPA to noradrenaline: dopamine.

At that time dopamine was considered to be without any interest because of its low physiological activity, when tested on various smooth-muscle preparations. We had to develop a method for determining dopamine because no such method was available at the time (Carlsson and Waldeck 1958). We could then show that dopamine occurs normally in the brain in an amount

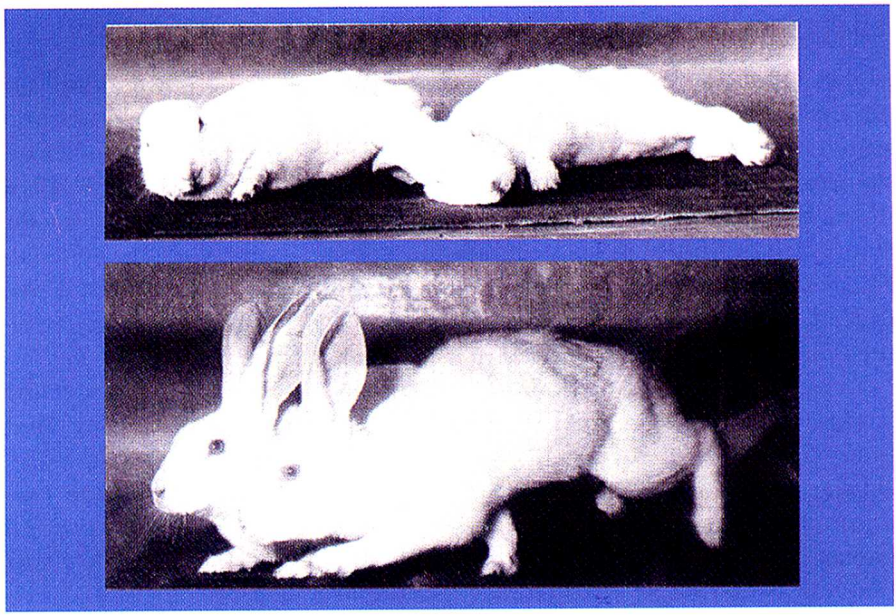

Figure 4. Rabbits treated with reserpine (5 mg/kg intravenously).before (top) and after DL-DOPA (200 mg/kg intravenously, bottom). From Carlsson (1960). Photo: Tor Magnusson.

somewhat higher than that of noradrenaline, that it is brought to disappear by reserpine treatment, and that the antireserpine action of DOPA is closely correlated to the restoration of dopamine levels in the brain. We also showed that the restoration of serotonin levels by treatment with its precursor 5-hydroxytryptophan did not lead to any reversal of the reserpine syndrome (Carlsson *et al.* 1958).

The classical method in physiology to prove a function of a natural constituent, is to remove the constituent in question and demonstrate a loss of function, and then to reintroduce the constituent, and demonstrate a restoration of the same function. We thought we had done this in the case of dopamine. We could easily exclude possible alternative explanations, such as a role of noradrenaline and serotonin and a direct action of L-DOPA.

In fact, our enthusiasm made us think that now we had found the Rosetta stone that would give us access to the chemical language of the brain.

Later we found the unique distribution of dopamine in the brain, with an accumulation in the basal ganglia, that is structures known to be involved in motor functions. This, taken together with the fact that a characteristic side effect of reserpine is to mimic very faithfully the syndrome of Parkinsonism and to induce a similar symptomatology in animals, led us to conclude that depletion of dopamine will induce the Parkinson syndrome and that treatment with L-DOPA will alleviate that syndrome by restoring the dopamine level. All this I presented at the First International Catecholamine Symposium in October, 1958 (Carlsson 1959, Bertler and Rosengren 1959).

A BATTLE IN LONDON

A year and a half later, in March 1960, a Ciba Foundation Symposium on Adrenergic Mechanisms was held in London (Vane *et al.* 1960). I then presented the same data and some additional support obtained from studies on the action of monoamine oxidase inhibitors. At this meeting practically all of the most eminent experts in this area participated. The central figure was Sir Henry Dale, a Nobel Laureate aged 85 but still remarkably vital. He dominated the scene, and the participants, many of whom were his former students, treated him with enormous respect, like school children their headmaster, although many of them had indeed reached a mature age.

To better understand how our dopamine story was received at this meeting it may be useful to recapitulate briefly the development following Otto Loewi's discovery of chemical transmission in the frog heart (Loewi 1921). During the following decades evidence accumulated, supporting the existence of chemical transmission in various parts of the peripheral nervous system. Dale and his collaborators played an important role here. They had, however, been fiercely attacked by a number of neurophysiologists, who argued in favour of an electrical transmission across the synapses. The most eminent proponent of this view was Sir John Eccles. The debates between Dale and Eccles had been quite vivid, as witnessed by several attendants of these debates between what was called "the sparks" and "the soup". Despite the sometimes harsh wordings the debates between Dale and Eccles over the years ended in mutual respect and admiration, as clearly indicated in the correspondence of more than twenty years between the two (see Katz 1996, Girolami *et al.* 1994). Doubts about a chemical transmission were particularly strongly expressed concerning the central nervous system. In the mid 1950s, however, Eccles had placed one foot in the "soup" camp, based on his own observation that a recurrent collateral of the motor neurone, impinging on the so-called Renshaw cells, seemed to operate by cholinergic transmission. This was, however, a very special case, given the fact that motor neurons are cholinergic. Apart from this finding, as pointed out by McLennan (1963) in his monograph "Synaptic Transmission", there was no evidence in favour of chemical transmission in the central nervous system.

At this meeting in London the debate that followed upon our paper entitled "On the biochemistry and possible functions of dopamine and noradrenaline in the brain" and a subsequent special discussion session, revealed a profound and nearly unanimous skepticism regarding our points of view. Our data as such were not questioned. Actually some confirmatory animal experiments were reported at the meeting, and I referred to a paper by Degkwitz *et al.* (1960), in which an anti-reserpine action of DOPA in humans was reported. Dale expressed the view that L-DOPA is a poison, which he found remarkable for an amino acid. Marthe Vogt concluded that the views expressed by Brodie and us regarding a function of serotonin and catecholamines, respectively, in the brain would not have a long life. W.D.M. Paton referred to some unpublished experiments indicating that the catecholamines are locat-

ed in glia. In his concluding remarks John Gaddum stated that at this meeting nobody had ventured to speculate on the relation between catechol amines and the function of the brain. But this was what I had insisted upon throughout the meeting, so the clear message to me was that I was nobody!

In retrospect I believe almost everybody would agree that our story and its implications were straight forward and obvious. How come that these eminent experts rejected the whole thing? I have no definite answer. Clearly the pharmacologists had great difficulty in accepting that dopamine could be an agonist in its own right, given its poor physiological effect on smooth-muscle preparations. The idea of DOPA being a mysterious poison probably came out of some experiments reported at the meeting where large doses of this amino acid, given to experimental animals together with a monoamine oxidase inhibitor, could cause paralysis, convulsions and death. In addition, I believe that the previous "sparks-and-soup" debates still had some impact. In these debates some elaborate criteria for a neurotransmitter had been formulated. Our data were of a different kind and these criteria were not applicable.

In this regard I and my collaborators, like my mentor Steve Brodie, simply had the advantage of being ignorant and not so much burdened by dogma.

A PARADIGM SHIFT

But it wouldn't be long until the scene would change dramatically. Hillarp also attended the London meeting. On our trip back to Sweden we agreed we should increase our efforts to convince the world that chemical transmission does indeed exist in the brain. Our idea was that Hillarp join me to work full time on research in our new and well-equipped department of pharmacology of Göteborg University, where I had been appointed professor and chairman the year before. We managed to obtain a grant from the Swedish Medical Research Council to set Hillarp free from his teaching duties in Lund. He could start full-time research in Göteborg already in the autumn of 1960.

We felt that the ability of catecholamines to yield fluorescent conversion products might be useful for their visualization in the microscope. We first tried a modifcation of the trihydroxyindole method (Carlsson *et al.* 1961). It worked beautifully for the adrenal medulla but not in other tissues. Hillarp then turned to another reaction that had been used for the quantitative assay of indoleamines, using formaldehyde as a reagent. Together with his skillful research assistant, the late George Thieme he worked out a model system, in which they managed to optimize the reaction conditions. (These experiments were reported by Falck *et al.* 1962). Subsequently, together with his former student Bengt Falck, Hillarp used air-dried preparations of iris and mesenterium, and discovered that the reaction worked beautifully, thus permitting the visualization of noradrenaline in adrenergic nerves and serotonin in mast cells in the fluorescence microscope. This led to an intense collaboration between our Department of Pharmacology in Göteborg and Hillarp's original Department of Histlogy in Lund, and finally, after Hillarp's move to

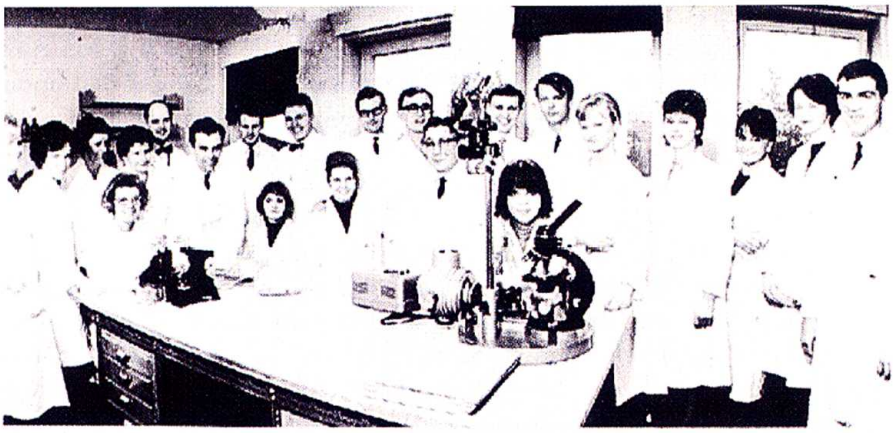

Figure 5. Group picture, taken January 1965, showing the group of young researchers recruited by Hillarp after his move to the Karolinska Institute in 1962. From Dahlström and Carlsson (1986). Photo: Lennart Nilsson.

take over the chair of histology at the Karolinska Institute in 1963, with an enthusiastic group of young students in his new department (see Fig.5). Thus within a few years the neuronal localization of dopamine, noradrenaline and serotonin in the central and peripheral nervous system was clearly established (Fig. 6). Moreover, the major monoaminergic pathways could be mapped (Fig. 7), and the site of action of the major psychotropic drugs clarified (see Dahlström and Carlsson 1986, Carlsson 1966, Fig. 8).

As mentioned, a large number of people were engaged in this effort. Sadly, many of these people have passed away, in many cases prematurely. Among these Georg Thieme (Fig. 9) has already been mentioned. Margit Lindqvist

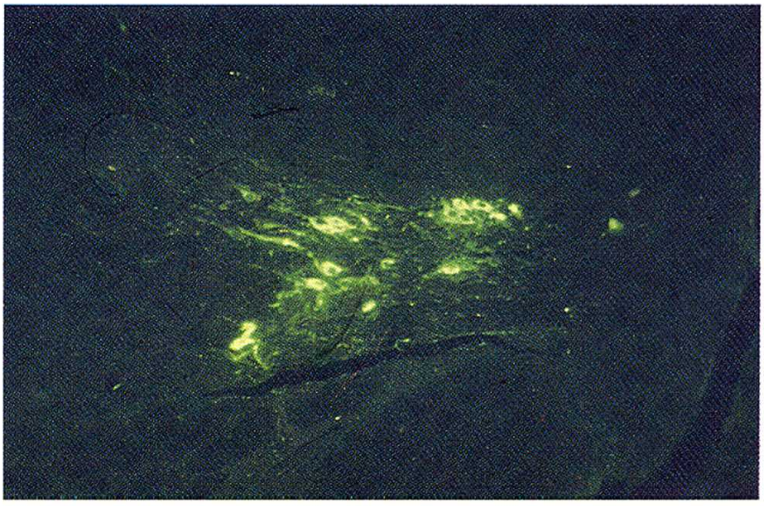

Figure 6. Dopaminergic cell bodies in rat substantia nigra. Green fluorescence developed following treatment with formaldehyde vapour. Courtesy of Annica Dahlström.

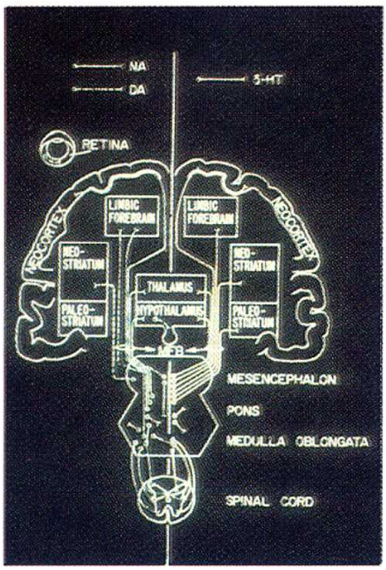

Figure 7. Monoaminergic pathways in brain. From Fuxe and Andén (1966).

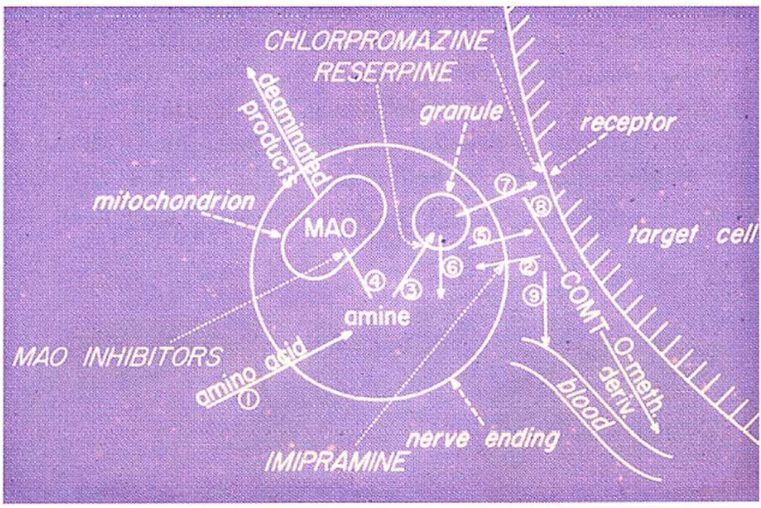

Figure 8. Scheme of monoaminergic synapse, with the sites of action of major classes of psychotropic drugs indicated. From Carlsson (1966).

(Fig. 10), a very skilful laboratrory assistant, who matured to become a qualified research worker, played an enormous role already from the outset of my scientific career. Nils-Erik Andén (Fig. 11) and Jan Häggendal (Fig. 12) were originally students of mine who became outstanding pharmacologists and largely contributed to characterize both central and peripheral monoaminergic transmission (for some of their early work, see Andén, Carlsson and Häggendal 1969). Hans Corrodi (Fig. 13), a very skilful organic chemist, who moved to Sweden because of his love for the mountains in northern Sweden,

Figure 9. Georg Thieme (1926–1996)

Figure 10. Margit Lindqvist (1924–1978)

contributed much to clarify the chemistry of the formaldehyde histofluorescence method and to many other projects, especially the development of the first selective serotonin reuptake inhibitor SSRI (see below).

In February 1965 an international symposium entitled "Mechanisms of Release of Biogenic Amines" was held in Stockholm (v. Euler *et al.* 1966), with most of the major figures of that research field participating. In his Intro-

Figure 11. Nils-Erik Andén (1937–1990)

Figure 12. Jan Häggendal (1932–1992)

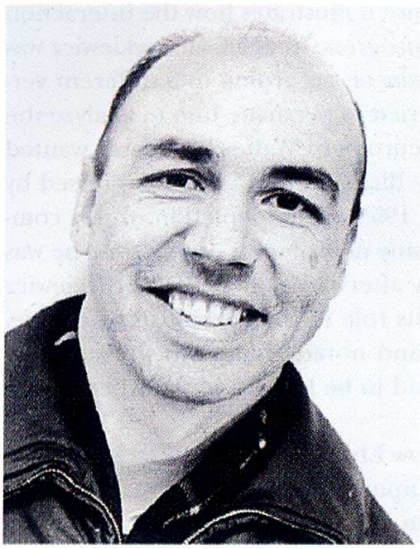

Figure 13. Hans Corrodi (1929–1974)

ductory Remarks Professor Uvnäs mentioned that "...these amines play an important role as chemical mediators in the peripheral and central nervous system...". None of the participants of this symposium expressed any doubt on this point. It looks as though a paradigm shift had taken place between 1960 and 1965.

It goes without saying that the concept of chemical transmission has had a profound impact on practically every aspect of brain research. In so far as neurology and psychiatry are concerned, a couple of examples are summarized below.

"AWAKENINGS"

Following our above-mentioned proposal of a role of dopamine in Parkinsonism some important parallel and apparently independent developments took place in Austria, Canada and Japan. These will now be briefly commented upon, starting out with Austria.

Later in the same year as the Symposium on Adreenergic Mechanisms, there appeared in Klinische Wochenschrift a paper in German, describing a marked reduction of dopamine in the brains of deceased patients who had suffered from Parkinson's disease and postencephalitic Parkinsonism (Ehringer and Hornykiewicz 1960). This was soon followed by a paper by Birkmayer and Hornykiewicz (1961), in which a temporary improvement of akinesia was reported following a single intravenous dose of L-DOPA to Parkinson patients.

As far as I can gather from an autobiography of Hornykiewicz (1992) as well as a personal communication from him, the following had happened. I

wish to mention this in some detail, because it illustrates how the interaction of different minds can lead to important progress. In 1958, Hornykiewicz was approached by a mentor Professor Lindner or, according to a different version, by his chief Professor Brücke, who tried to persuade him to analyze the brain of a Parkinson patient, which the neurologist Walter Birkmayer wanted to be analyzed for serotonin. Presumably Birkmayer had been impressed by Brodie's already mentioned discovery in 1955 of the depletion of this compound by reserpine, and in contrast to many neurologists at that time he was aware of its possible implications. Shortly afterwards, in 1959, Hornykiewicz read about our work on dopamine and its role in the Parkinson syndrome. He then decided to include dopamine and noradrenaline in the study. In fact, in the subsequent work serotonin had to be left out initially because of some technical problems.

Hornykiewicz and his postdoctoral fellow Ehringer were now facing a challenge, because they had no adequate equipment to measure dopamine. But they managed to overcome this problem by using the purification of the brain extracts by ion exchange chromatography that our research group had worked out. The subsequent measurement was performed using the colorimetric method of Euler and Hamberg. Although this method by itself is highly unspecific, specificity could be obtained by using our purification step together with our finding that dopamine is by far the dominating catecholamine in the basal ganglia, where it occurred in high concentrations. They had to work up several grams of tissue and to concentrate the extracts by evacuation to dryness. Following this heroic procedure they were richly rewarded, because the samples from the Parkinsonian brains, in contrast to the controls, turned out to be colourless, as revealed by the naked eye!

The corresponding development of Parkinson research in Canada is summarized in a paper by Barbeau *et al.* (1962), presented at a meeting in Geneva in September the previous year. The main findings of the Canadian workers was a reduction of the urinary excretion of dopamine in Parkinson patients and an alleviation of the rigidity of such patients following oral treatment with L-DOPA.

In Japan some remarkable progress was made, which has not been adequately paid attention to in the Western countries (see reviews by Nakajima 1991, and Foley 2000). In a lecture on the 5th of August, 1959, less than a year after my lecture at the International Catecholamine Symposium mentioned above, the basic concept regarding the role of dopamine in the basal ganglia in Parkinson's disease was presented by I. Sano (1959). In this lecture data on the distribution of dopamine in the human brain were presented for the first time. In a lecture in Tokyo on the 6th of February, 1960, Sano reported on reduced amounts of dopamine in the basal ganglia of a Parkinson patient, analyzed post mortem, and in the same year he published a paper describing alleviation of rigidity in a Parkinson patient following intravenous administration of DL-DOPA (Sano 1960).

Thus treatment of Parkinson patients with DOPA was initiated simultaneously in three different countries only a few years after the discovery of the

anti-reserpine action of this agent and the subsequent formulation of the concept of a role of dopamine in extrapyramidal functions. While this treatment led to results of great scientific interest, it took several years until it could be implemented as routine treatment of Parkinson patients. The reason was that the treatment regimens used initially were inadequate and led to but marginal improvement of questionable therapeutic value (Hornykiewicz 1966). It remained for George Cotzias (1967) to develop an adequate dose regimen. After that L-DOPA treatment rapidly became the golden standard for the treatment of Parkinson's disease.

When I had seen Cotzias' impressive film demonstrating the effect of escalating oral doses of L-DOPA at a meeting in Canada I hastened back to Göteborg and initiated studies together with Drs. Svanborg, Steg and others, which quickly confirmed Cotzias' observations (Andén *et al.* 1970), like in many other places at the same time. This success story was soon afterwards told to the general public by Oliver Sacks in "Awakenings" (Sacks 1973), which became a bestseller and was also made into a movie.

ROLE OF SEROTONIN IN DEPRESSION: ZIMELIDINE, THE FIRST SSRI

The so-called tricyclic antidepressants, with imipramine as the prototype, were serendipitously discovered in the late 1950s, thanks to Kuhn, a psychiatrist and a keen clinical observer. In the early 1960s these agents were found to block the reuptake of noradrenaline by nerve terminals, thus enhancing the adrenergic transmission mechanism. In 1968, we discovered that many antidepressants also could block the reuptake of serotonin (Carlsson *et al.* 1968), and this prompted us to develop a compound that selectively blocked the reuptake of serotonin without acting on noradrenaline. Such agents are now known as SSRIs. This first agent was called zimelidine, whose preclinical properties we first described by Berntsson *et al.* (1972). Zimelidine turned out to be an active antidepressant agent with a very favourable side effect profile (Carlsson *et al.* 1981), apart from a very rare, but serious side effect, presumably based on an immunological mechanism, that led to its withdrawal from the market. But zimelidine was followed by several other SSRIs, among which Prozac is especially well known, not least because of a bestseller titled "Listening to Prozac", authored by P.D. Kramer (1993). In this book Prozac is stated to be able to treat not only patients with depression and a variety of anxiety disorders, as had previously been amply demonstrated for many SSRIs, but also to be able to change the personality of people with psychological problems. Kramer was especially astonished by the fact that disturbances, which would have taken several months of psychotherapy to control, could be alleviated within a few days of treatment with Prozac. This favourable action, making people feel and function better, even if they were not mentally ill in the conventional sense, is a fascinating but, needless to say, controversial issue. Less controversial is probably the 25 % reduction in suicide rates in Sweden in the 1990s, apparently related to the introduction of the SSRIs (Isacsson 2000). In any event the SSRIs represent a major thera-

peutic advance as well as a milestone in rational drug development (Carlsson 1999).

The development of zimelidine was based on our discovery that certain antihistamines are serotonin-reuptake blocking agents, albeit non-selective. The most powerful agents among these were the pheniramines and diphenhydramine (Carlsson *et al.* 1969). We started out from the pheniramines and developed zimelidine. The Lilly scientists started out from our diphenhydramine data and developed Prozac, which was found to act very much like zimelidine, though devoid of its serious side effect.

DOPAMINERGIC STABILIZERS – A NOVEL PHARMACOLOGIC PRINCIPLE

In 1963, Margit Lindqvist and I presented the first evidence supporting the view that the most important group of antipsychotic agents, represented by agents such as chlorpromazine and haloperidol, act by blocking receptors for dopamine, and to some extent also receptors for noradrenaline (Carlsson and Lindqvist 1963, Fig.14). This conclusion has later been confirmed and extended in numerous laboratories, and techniques have been developed to screen for such agents in test tube experiments. One might have expected then that this should have led to the development of drugs with stronger efficacy and less side effects. Unfortunately, this has not happened.

We have hypothesized that the cause of this failure is that treatment with dopamine receptor antagonists can hardly avoid the serious and unpleasant side effects induced by dopamine hypofunction. Even though there is evidence of elevated dopaminergic activity in schizophrenia, this may be limited to psychotic episodes. In fact, we may be dealing with an instability of the

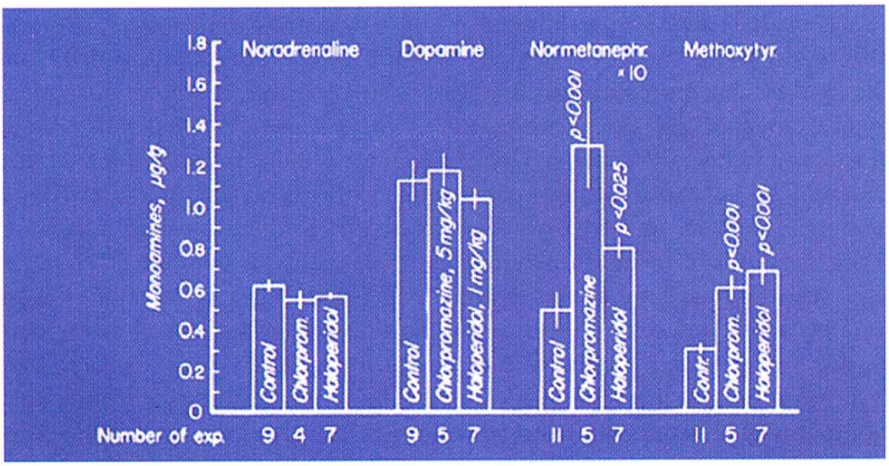

Figure 14. Accumulation of of the basic catecholamine metabolites normetanephrine and 3-methoxytyramine, enhanced by treatment with major neuroleptic agents following monoamine oxidase inhibition. From Carlsson and Lindqvist 1963.

dopamine release rather than a continuously elevated baseline. Thus, between psychotic episodes, the patient would then suffer from a dopaminergic hypofunction, especially during treatment with the currently used antipsychotic agents, showing up as a severe disturbance of the reward system and of cognition, and also as motor disturbances. This may make it impossible to attain an adequate dose level (for discussion and references, see Carlsson *et al.* 2001).

We believe that we can now get around this problem by using a new principle of intervention that we call dopaminergic stabilization. The underlying mechanism is complicated but in principle it rests on the existence of mutually antagonistic subpopulations of dopamine D2 receptors, as regards the final functional outcome. For example, the presynaptically located dopaminergic autoreceptors are inhibitory on the overall dopaminergic activity. Dopaminergic stabilizers are dopamine D2 antagonists or partial D2 agonists capable of occupying mutually opposing receptor subpopulations in such proportions as to leave the normal baseline dopaminergic activity level essentially unchanged. This leads to stabilization by dampening fluctuations of dopamine release, simply because fewer dopamine receptors are unoccupied and thus available for the endogenous neurotransmitter.

Using the dopaminergic stabilizer (-)-OSU6162 (Fig. 15), developed by our research group, partly in collaboration with Upjohn (now merged into Pharmacia Corporation), we have demonstrated the stabilization phenomenon in experimental animals (Fig. 16) and, in preliminary clinical studies, its pharmacotheraputic potential in L-DOPA-induced dyskinesias in Parkinson patients, in Huntington's disease (Fig. 17), and in schizophrenia (Tedroff *et al.* 1999, Ekesbo 1999, Gefvert *et al.* 2000).

The partial dopamine receptor agonist preclamol ((-)-3-PPP) has likewise a dopaminergic stabilizer profile. This agent was discovered by our research group and is in development in collaboration with Dr. Carol Tamminga and her colleagues at the Maryland Psychiatric Research Center (Lahti *et al.* 1988).

Our experience with dopaminergic stabilizers suggests that research into

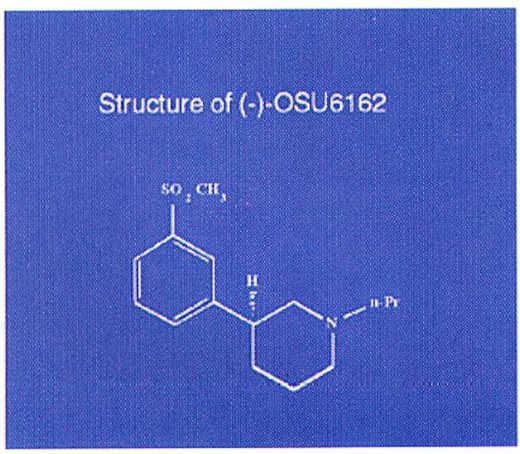

Figure 15. Chemical structure of (-)-OSU6162.

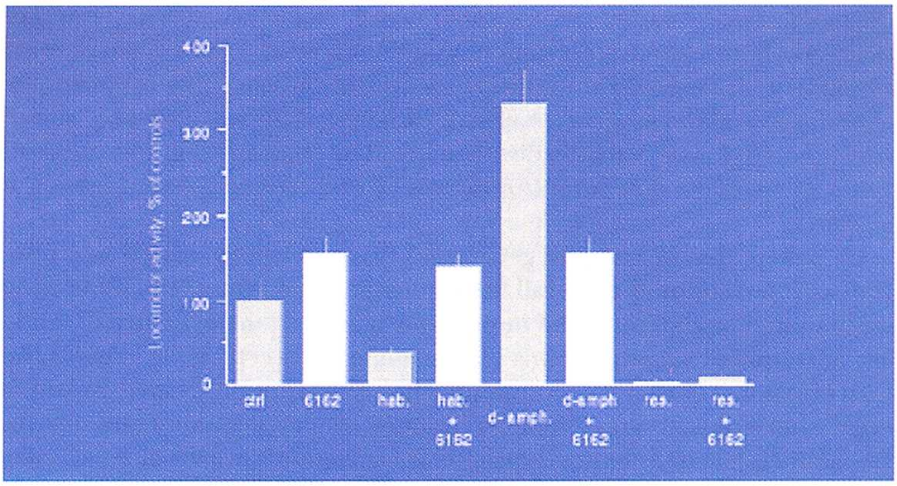

Figure 16. Stabilizing action of (-)-OSU6162 in rats. Filled bars: no treatment with (-)-OSU6162. Open bars: (-)-OSU6162. "Ctr". Actively exploring control rats. "hab": Rats habituated to their environment. "d-amph": rats treated with d-amphetamine. "res": reserpine.

Note. Treatment with one and the same dose of (-)-OSU6162 can induce stimulation of behavior when baseline activity is low (habituated rats) and inhibition when the activity is high (d-amphetamine pretreatment).

neurotransmitter pathophysiology has until now focussed too much on the hyper- versus hypofunction dichotomy. Although the instability concept is by no means new, there has not been much of a goal- directed strategy aiming to stabilize neurocircuits involved in neuropsychiatric disorders. Our preliminary data suggest that such an approach can lead to enormous gains in the treatment of a great variety neurological and psychiatric disorders.

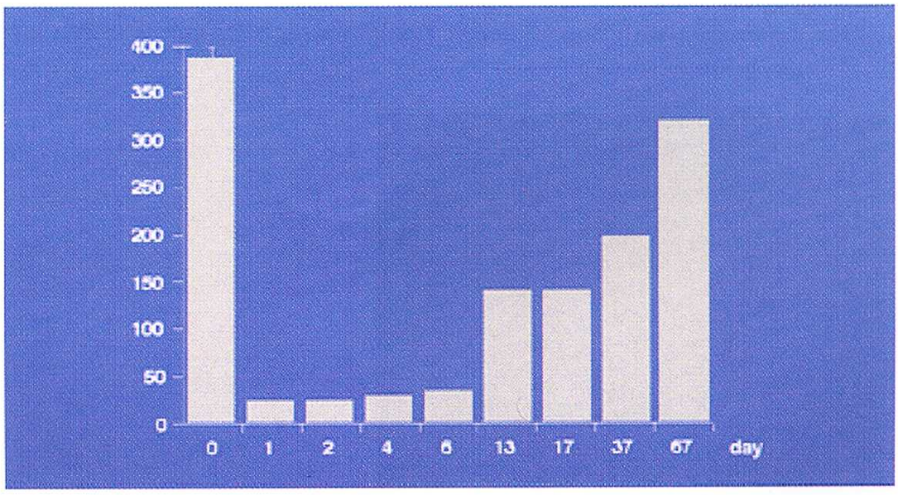

Figure 17. Choreatic events at baseline and following 0.5 mg/kg (-)-OSU6162 as an intravenous infusion during 30 minutes to a patient with Huntington's disease. From Tedroff *et al.* (1999).

OUTLOOK

During the past half-century brain research has been dominated by biochemical approaches, in contrast to the previous half-century, which had a strong electrophysiological emphasis. This switch is understandable in view of the entrance of the neurohumoral transmission concept into brain research in conjunction with the spectacular progress of molecular biology. However, it must be recognized that the brain is not a chemical factory but an extremely complicated survival machine. In order to bring all the forthcoming biochemical observations into a meaningful framework it will prove necessary to emphasize more strongly aspects of neurocircuits and connectivity and to do so both at the microscopic and macroscopic level. For example, the old questions dealing with neurocircuits within a cerebral region such as the cortex and those addressing the interaction between the different regions will in all probability come into focus more strongly in order to make full use of the new knowledge gained from neurotransmitter physiology and molecular biology. Here the new imaging techniques in conjunction with advanced computer-dependent statistics involving pattern recognition derived from a wealth of data with great complexity will probably prove extremely useful and very much help to bridge the gap between animal and human observations. If nothing else, such approaches will help to reveal the enormous width of our present ignorance of the human brain.

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