A Paradigm Shift in Brain Research

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As late as the 1950s, it was assumed that communication between nerve cells in the brain occurred predominantly, if not entirely, by electrical impulses. A decade later, the theory of chemical transmission, which until then had been thought to occur only in the peripheral nervous system, had gained strong entrance for the central nervous system. This paradigm shift opened up an enormous new perspective in brain research, not least by facilitating the study of brain function by means of chemical tools, which in different ways could modify the chemical signaling between nerve cells. Moreover, such tools sometimes turned out to be useful as therapeutic agents. Thus for the first time, a variety of disorders in the central nervous system could be treated effectively.

In 1955 and '56, I took a sabbatical in Bernard B. Brodie's famous Laboratory of Chemical Pharmacology at the National Heart Institute in Bethesda, Maryland, during a very dramatic period in which drug research was undergoing a revolution and neuropsychopharmacology was *in statu nascendi*. This was only 3 years after the discovery of the antipsychotic action of chlorpromazine and 1 or 2 years after the rediscovery of the antipsychotic action of reserpine (reported by Indian psychiatrists three decades earlier). At this stage, I was introduced by Brodie and his collaborator, Parkhurst Shore, to the most modern methods of biochemical pharmacology available at that time, as well as into the hottest area of neuropsychopharmacology.

Brodie's background was in organic chemistry, but he had specialized in drug metabolism, which he had pioneered by developing a multitude of methods for measuring the levels of drugs and their metabolites in tissues and body fluids. At the time of my visit the prototype of a new instrument, the spectrophotofluorimeter had been constructed in Brodie's laboratory by Robert Bowman in collaboration with Sidney Udenfriend. This instrument was to revolutionize the measurement not only of drugs but also of several endogenous compounds of great physiological interest. It combined a high sensitivity with specificity. For several decades this instrument dominated biochemical pharmacology, but has now been surpassed by even more sophisticated equipment.

The antipsychotic actions of chlorpromazine and reserpine, and the finding that D-lysergic acid diethylamide (LSD) seemed to possess affinity for serotonin receptors, inspired Brodie and his colleagues to experiment with these drugs to find out more about their relation to serotonin. These experiments culminated in the seminal discovery that reserpine has a dramatic effect on the tissue storage of serotonin. This happened only a few months before I joined Brodie's group.

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L-DOPA and Dopamine: Rosetta Stone?

In Brodie's lab we demonstrated a direct effect of reserpine on serotonin release from blood platelets (*1*). After returning to my home university in Lund, Sweden, as associate professor of pharmacology, I discovered, together with Nils-Åke Hillarp and other collaborators, that reserpine caused depletion of the adrenal medullary hormones as well as of noradrenaline in other tissues, including the brain (*2–4*) (Fig. 1). These findings offered a plausible explanation for the hypotensive action of reserpine, and this was confirmed by experiments in which, after reserpine treatment, stimulation of sympathetic nerves no longer caused release of the neurotransmitter noradrenaline. Shortly before, Hillarp had described the catecholamine-storing organelles in the adrenal medulla. I had the hunch that Brodie and Hillarp were working on the same thing, without knowing of each other.

To investigate the mode of action of reserpine on the central nervous system, my colleagues and I administered DOPA to reserpine-treated rabbits and mice. The idea was that L-DOPA, a precursor of noradrenaline, should be able to replenish the reserpine-depleted stores of this amine. We then discovered the central stimulant action of DOPA as well as its ability to reverse the akinetic and sedative action of reserpine (*5*) (Fig. 2). However, when we analyzed the brains of the animals treated with reserpine and DOPA, we found them still fully depleted of noradrenaline. Further analysis revealed that the behavioral action of L-DOPA closely correlated with the accumulation of dopamine in the brain (Fig. 3). Moreover, our studies disclosed that dopamine is a normal

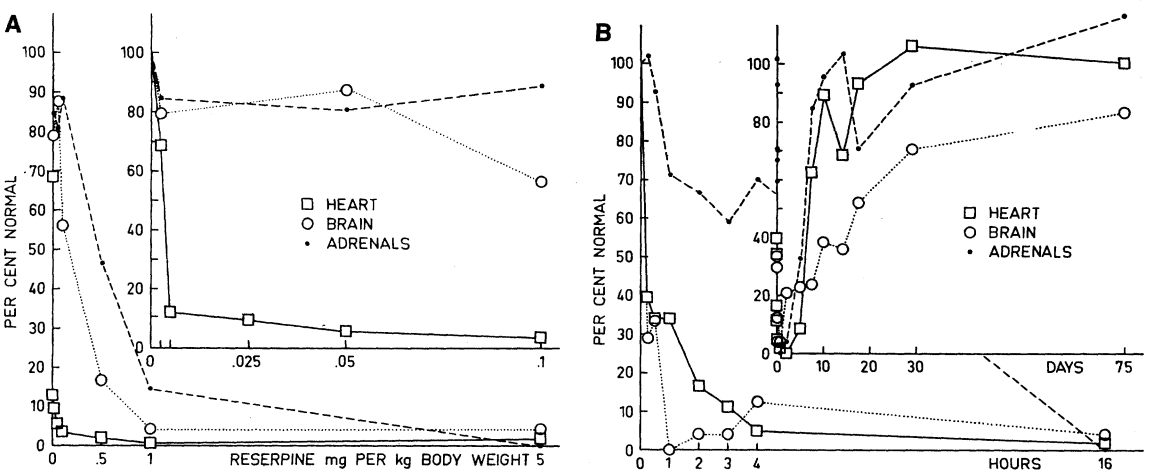

Fig. 1. Effects of reserpine on catecholamine levels. (**A**) Noradrenaline levels in heart and brain and adrenaline in the adrenal medulla of rabbits 16 hours after various intravenous doses of reserpine. (Inset) Effects in a lower dose range. [From (*4*)] (**B**) Noradrenaline levels in heart and brain, and adrenaline in the adrenal medulla, of rabbits during the first 5 hours after treatment with reserpine (5 mg/kg intravenously). (Inset) Time course over 75 hours. [From (*4*)]

brain constituent and is released by reserpine, like noradrenaline and serotonin (Fig. 4). The data suggested to us that dopamine is not just a precursor to noradrenaline, as was generally believed at that time, but that it is an endogenous agonist in its own right. This notion received further support when we discovered the marked difference in regional distribution between dopamine and noradrenaline, the former being largely in the basal ganglia. We therefore suggested that the parkinsonism induced by reserpine is due to dopamine depletion, which can be restored by L-DOPA treatment, and that dopamine is involved in the control of extrapyramidal motor function (*6–8*).

We had discovered in an experimental animal model a phenomenon later described in Parkinson's patients and vividly depicted in the book *Awakenings*, written by Oliver Sacks and later made into a film. In fact, our discovery that animals, made almost immobile and deeply sedated by drug-induced depletion of dopamine, could then instantaneously be restored to full mobility and wakefulness by repletion of dopamine through treatment with its precursor L-DOPA, was one of the most astounding experiences in my scientific career. My colleagues and I felt as though we had come across something like a "Rosetta Stone," which would provide the key to the chemical language of the brain. This was in 1957. It took another 10 years for L-DOPA to become the standard for the treatment of Parkinson's disease, mainly through contributions by the Austrians W. Birkmayer and O. Hornykiewicz, the Canadians A. Barbeau and T. Sourkes, the Japanese I. Sano, and the Greek-born American G. Cotzias.

Our experiments in the late 1950s provided the first direct evidence for a role of an endogenous agonist, present in brain tissue, in animal behavior, thus foreshadowing the paradigm shift from electrical to chemical signaling between nerve cells in the brain. As might be expected, these observations and interpretations at first met with considerable skepticism by some of the most prominent representatives of this field (*9*).

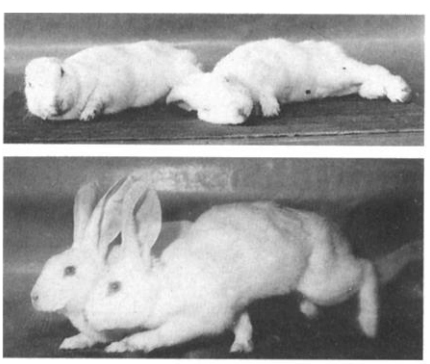

Fig. 2. Reversal of reserpine's effects by DOPA. (**Top**) Rabbits treated with reserpine (5 mg/kg intravenously). (**Bottom**) The same rabbits 15 min after D-L-DOPA (200 mg/kg intravenously). [From (*41*)]

Chemical Transmission in the Brain

Hillarp and I decided to further increase our efforts to demonstrate chemical transmission in the brain. I had just been appointed professor and chairman at the Department of Pharmacology, University of Gothenburg. We agreed that Hillarp should join me to work on catecholamines. We focused on two problems: (i) the investigation of a possible active amine-uptake mechanism by the adrenal medullary granules and its inhibition by reserpine, and (ii) the development of a histochemical fluorescence method to visualize catecholamines in tissues and demonstrate their occurrence in specific neural pathways. Both of these projects were successful (*10, 11*). In my opinion these discoveries had a considerable impact on the scientific community's acceptance of the concept of chemical transmission in the central nervous system and on the development of monoaminergic synaptology. Since detailed accounts of this work have been given elsewhere (*12, 13*), they will not be repeated here.

The concept of chemical transmission made it possible to clarify the mode of action of numerous drugs on the central nervous system (*9*) (see Fig. 5). For example, our subsequent work on chlorpromazine and other antipsychotic agents led us to propose that these agents act by blocking receptors for dopamine and, partly, receptors for noradrenaline and serotonin (*14*). These results became the starting point for the much-debated dopamine hypothesis of schizophrenia (*15*) (which has received direct support in recent studies with modern imaging techniques).

Zimelidine, the First SSRI

The major, so-called tricyclic antidepressants, discovered in the late 1960s, were found to act by blocking the reuptake of noradrenaline and serotonin by nerve terminals, thus enhancing the transmission mechanism. At an early stage this effect was generally believed to be mediated entirely by blocking noradrenaline reuptake. However, in 1968 we discovered that many antidepressants also could block the reuptake of serotonin (*16, 17*), and this led us to develop a compound that selectively blocked the reuptake of serotonin without acting on noradenaline. Such agents are now known as SSRIs (selective serotonin reuptake inhibitors). This first agent was called zimelidine, whose preclinical properties we described in a patent published in 1972 (*18*). Zimelidine was an active antidepressant agent with a very favorable side-effect profile, apart from a very rare but serious side effect that rather soon led to its withdrawal from the market. Several other SSRIs were soon developed, among which Prozac is especially well known, in part because of the 1993 book *Listening to Prozac* by P. D. Kramer. In this book Prozac is described not only as a treatment for depression and a variety of anxiety disorders (as had been amply demonstrated for many SSRIs), but also as means for changing the personality of people with psychological problems and for improving their function. Kramer was especially astonished by the fact that disturbances that would have taken several months of psychotherapy to control could be alleviated within a few days of treatment with Prozac. This favorable action of Prozac on people who are not mentally ill is a fascinating but controversial issue. In any event, the SSRIs represent a major therapeutic advance as well as a milestone in rational drug development.

The development of zimelidine was based

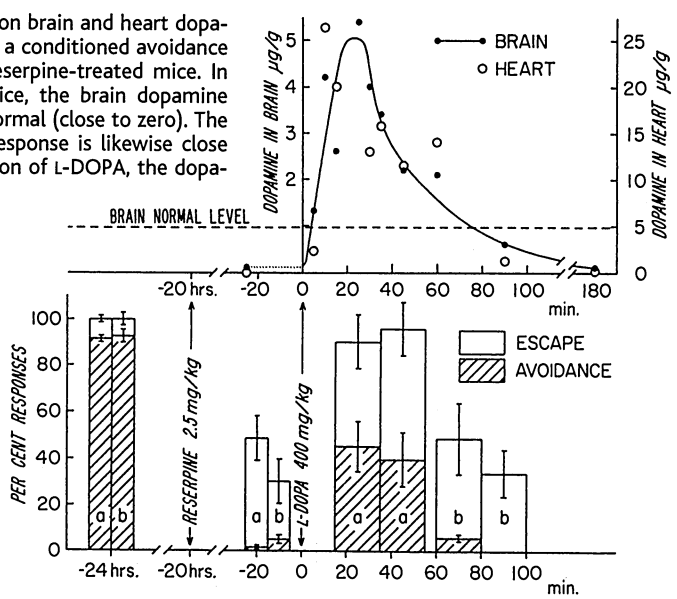

Fig. 3. Effect of L-DOPA on brain and heart dopamine levels (**top**) and on a conditioned avoidance response (**bottom**) in reserpine-treated mice. In the reserpine-treated mice, the brain dopamine levels are much below normal (close to zero). The conditioned avoidance response is likewise close to zero. Following injection of L-DOPA, the dopamine levels increase dramatically and the conditioned avoidance response is markedly restored. This behavioral response coincides with the rise in dopamine levels. Under these conditions the treatment with reserpine does not cause any appreciable increase in noradrenaline levels (not shown). [From (*42*)]

on our discovery that certain antihistamines possess serotonin-reuptake blocking properties, although without being selective. The most powerful agents among these were the pheniramines and diphenhydramine (*19*). We started from the pheniramines to develop zimelidine. The Lilly scientists started out from diphenhydramine and developed Prozac, which acts very much like zimelidine, although devoid of its serious side effect.

More recently our research group, which consists of both medicinal chemists and pharmacologists, has been engaged in a number of projects. A few of these are mentioned below. For a more detailed account, see (*9*).

Dopaminergic autoreceptors as drug targets. One area that has attracted our interest for many years deals with the dopaminergic autoreceptors. In the early 1970s we developed a method to measure the first, rate-limiting step in the synthesis of catecholamines in vivo (*20*) and discovered a very simple negative feedback mechanism in the control of dopamine function. This mechanism seemed to be mediated by dopamine receptors located on the dopamine neuron itself (*21*), which we called "autoreceptors" (*22*). We also proposed that agents with selective action on autoreceptors might prove useful as research tools and as therapeutic agents. We speculated that an influence on these receptors could be milder than that caused by the currently used drugs, which predominantly act on postsynaptic receptors, located on nerve cells downstream from dopamine neurons. Several years later our collaboration with skillful organic chemists led to the discovery of 3-PPP, a drug whose (-)-enantiomer is a partial dopamine receptor agonist with some selectivity for the dopaminergic autoreceptors (*23, 24*). In collaboration with Carol Tamminga of the Maryland Psychiatric Research Center, this drug has been tested in schizophrenic patients and found to have antipsychotic properties (*25, 26*), although it is not yet clear whether this agent can be used as an antipsychotic agent in clinical practice. Recently a partial dopamine receptor agonist, called aripiprazole, has been shown to possess antipsychotic properties in phase III clinical trials on schizophrenic patients (*27*).

Our research group has also developed preferential dopamine autoreceptor antagonists. A number of compounds with similar profiles but somewhat variable properties have been prepared (*28–30*). Preliminary clinical observations with one compound, called OSU6162, confirm our animal data showing that this agent has some unique stabilizing properties. It can favorably influence signs of hyperdopaminergia, such as the dyskinesias and choreatic movements that occur in Huntington's patients and in Parkinson's patients treated with L-DOPA, and can also improve signs of hypodopaminergia, such as dystonias. Similarly, preliminary observations in schizophrenic patients indicate that this agent can favorably influence both positive and negative symptoms. These effects occur without any concomitant signs of hypodopaminergia, such as extrapyramidal side effects, or interference with the anti-Parkinson action of L-DOPA. Thus, after more than 30 years of autoreceptor research, there is reason to hope that it will ultimately result in drugs with improved therapeutic properties, useful in the treatment of both neurologic and psychiatric disorders.

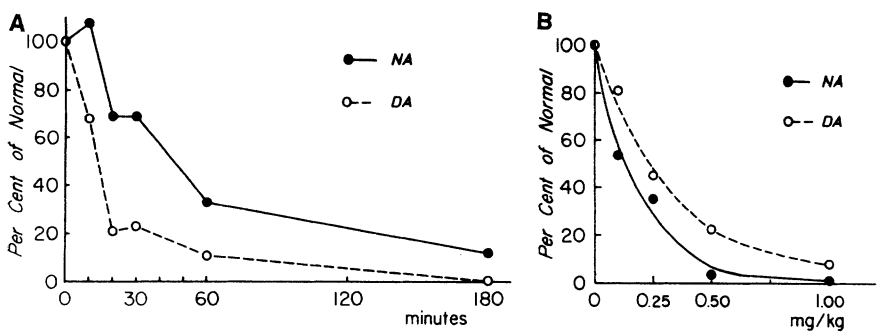

Fig. 4. Effect of reserpine on dopamine (DA) and noradrenaline (NA) levels in rabbit brain. (**A**) Time course after 1 mg /kg intravenously. (**B**) Dose-response curves 16 hours after the injection. [From (*43*)]

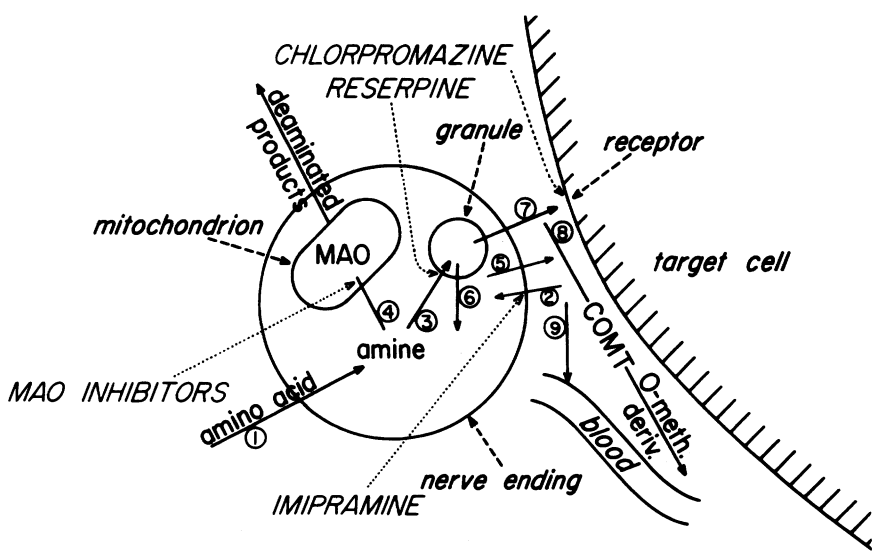

Fig. 5. Model of a monoaminergic transmission unit, depicting the chief sites of action of four major classes of psychotropic drugs. Reserpine blocks the transport of monoamines into the granules (vesicles). Chlorpromazine blocks the dopaminergic (and noradrenergic) receptors. Imipramine blocks the transporters for noradrenaline and serotonin at the level of the nerve-cell membrane. Monoamine (MAO) inhibitors block MAO in the mitochondria. [From (*44*)]

Interplay Among Cortex, Striatum, and Thalamus

For more than 10 years, our group has been interested in the interplay between the cerebral cortex, the striatum, and the thalamus. We started by trying to design a model that explained a paradox: Antipsychotic agents exert powerful actions on the cerebral cortex, yet they act mainly on targets which are very sparse in the human cerebral cortex, that is, dopamine D2 receptors. On the basis of available pharmacological and neuroanatomical evidence, we postulated that dopamine acts to inhibit the striatal complexes (the dorsal and ventral striatum and the corresponding dorsal and ventral pallidum), which in turn exert a predominantly inhibitory function on the thalamus, leading to a reduced sensory input to the cerebral cortex and a concomitant reduction of arousal. As is generally recognized, arousal is controlled by the ascending reticular formation, which operates in close linkage to the sensory input. We assumed that dopamine is predominantly inhibitory on striatal neurons, and thus an increased dopaminergic tone should counteract the inhibitory impact of the striatal complexes on the thalamus and, consequently, enhance the relay of sensory information to the cortex and raise the level of arousal. If the transmission through the thalamus becomes excessive, the integrative capacity of the cortex may break down, and confusion or psychosis will ensue. In the striatum, dopamine is counterbalanced by a powerful corticostriatal glutamatergic system derived from all parts of the cerebral cortex (*31*). We have tested this model in various ways and incorporated other neurotransmitters into it (*32–36*) (Fig. 6).

Using this approach, we have been able to elucidate the mode of action of a drug developed at Marion Merrell Dow (now part of Aventis).

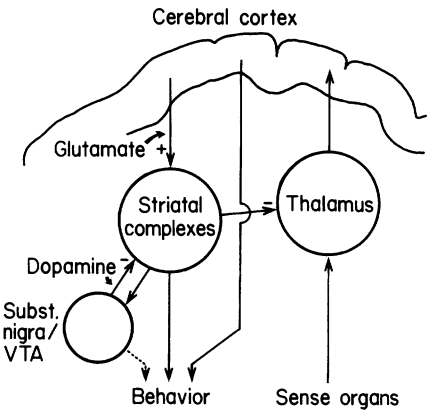

Fig. 6. Schematic representation of the hypothesis that the cerebral cortex can protect itself from an overload of information and from hyperarousal by means of feedback loops engaging the striatal complexes and the thalamus (as well as the mesencephalic reticular formation, not shown). The feedback loops are postulated to be modulated by the mesencephalostriatal dopaminergic pathways. [From (*31*)]

This agent, M100,907 has been characterized as a 5HT2A receptor antagonist. We have found it to work in low dosage in an animal model of psychosis that can be induced by lowering glutamatergic function (*37–39*). Recently this compound has also been found to possess antipsychotic properties in schizophrenic patients (*40*). These and other observations support the view that in addition to dopamine, glutamate and serotonin are also critically involved in schizophrenia. It is likely that other neurotransmitters, such as noradrenaline, acetylcholine, and γ-aminobutyric acid (GABA), will soon be added to this list.

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REVIEW: NEUROSCIENCE

The Neurobiology of Slow Synaptic Transmission

Paul Greengard

Nerve cells communicate with each other through two mechanisms, referred to as fast and slow synaptic transmission. Fast-acting neurotransmitters, e.g., glutamate (excitatory) and γ-aminobutyric acid (GABA) (inhibitory), achieve effects on their target cells within one millisecond by virtue of opening ligand-operated ion channels. In contrast, all of the effects of the biogenic amine and peptide neurotransmitters, as well as many of the effects of glutamate and GABA, are achieved over hundreds of milliseconds to minutes by slow synaptic transmission. This latter process is mediated through an enormously more complicated sequence of biochemical steps, involving second messengers, protein kinases, and protein phosphatases. Slow-acting neurotransmitters control the efficacy of fast synaptic transmission by regulating the efficiency of neurotransmitter release from presynaptic terminals and by regulating the efficiency with which fast-acting neurotransmitters produce their effects on postsynaptic receptors.

There are about 100 billion nerve cells in the brain, and on average each of these cells communicates directly with 1000 others. A vigorous debate went on from the 1930s through the 1960s as to whether communication across the synapses between nerve cells was electrical or chemical in nature. The electrical school of thought held that the nerve impulse or action potential was propagated along the axon to the nerve ending, changed the electrical field across the plasma membrane of the postsynaptic cell, and thereby produced a physiological response. The chemical school believed that when the action potential came down the axon to the nerve terminal, it caused the fusion of neurotransmitter-containing vesicles with the presynaptic plasma membrane, releasing the neurotransmitter, which then diffused across the synaptic cleft and, through activation of a (hypothetical) receptor, produced a physiological response. The chemical school won this debate: over 99% of all synapses in the brain use chemical transmission. On the basis of those earlier studies, I became interested in the biochemical mechanisms by which neurotransmitters, through activation of their receptors, produce their physiological effects within their postsynaptic, target nerve cells.

We know today that there are two categories of chemical transmission between nerve cells, which we refer to as fast and slow synaptic transmission. About half of the fast synapses in the brain are excitatory, and most of these fast excitatory synapses use gluta-

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