ON THE EVE OF THE NEUROTRANSMITTER ERA IN BIOLOGICAL PSYCHIATRY

Alfred Pletscher

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In the mid-1950s, at the beginning of the developments described in this paper, only three biogenic amines – acetylcholine, noradrenaline, and 5-hydroxytryptamine (5-HT, serotonin) – were known to occur in the brain, and monoamine receptors existed only as hypothetical constructs. Although the role of biogenic amines in brain function, including their involvement in the action of neuropsychotropic drugs, was virtually unknown, there was already a considerable amount of information about the biosynthesis and metabolism of these substances. At the National Heart Institute of the National Institutes of Health (NIH) in Bethesda, Maryland, alone there were two research groups, one led by J. Axelrod and the other by S. Udenfriend, carrying out pioneering biochemical work on biogenic amines, i.e., catecholamines and 5-HT. In addition, Bernard B. Brodie (then head of the laboratory of biochemical pharmacology at

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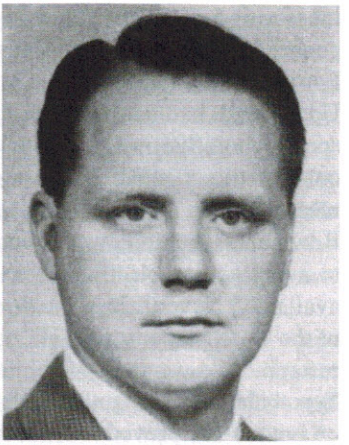
Alfred Pletscher was born in Switzerland in 1917 and holds both an MD and a PhD in chemistry. After eight years of clinical training, he was a member of the research department of F. Hoffmann La Roche Inc. between 1955 and 1978. He spent the greatest part of 1955 as a visiting scientist at the National Heart Institute in Bethesda, Maryland (USA), and he acted as research director of Hoffmann-La Roche in Nutley, New Jersey (USA), in 1957, and in Basle (Switzerland), from 1967 to 1978. As well, Pletscher has been professor of psychophysiology at the University of Basle, chairman of the department of research of the university clinics, and president of the Council of the Swiss National Science Foundation and the Swiss Academy of Medical Sciences.

Pletscher was elected a fellow of CINP in 1958. He presented papers on "Biogenic Amines," on "Pharmacologic and biochemical basis of some somatic side effects of psychotropic drugs," on "Differences between neuroleptics and tranquilizers regarding metabolism and biochmical effects" (Pletscher, de Prada and Foglar), on "Mechanism of action of neuroleptics" (Pletscher and de Prada), and on "Alterations of cerebral monoamines by aromatic amino acids and effect of decarboxylase inhibitors" (Pletscher and Bartholini) at the 4th, 5th and 7th Congresses respectively. He coauthored a paper on "Metabolism of C14-L-3,4-dihydroxyphenylalanine after inhibition of extracerebral decaboxylase in rats and man" (Bartholini, Pletscher, and Tissot), at the 6th Congress. He gave Moderator's report on Working Group 1 (Experimental and clinical biochemistry) at the 4th Congress, and was a formal discussant (Pletscher, Steiner and Voelkel) in the Third Symposium (Comparison of abnormal behavioural states of animals and man) at the 1st Congress.

the NIH) and his associates had just presented their interesting pharmacological findings with 5-HT. These led our group later on to formulate the hypothesis that reserpine might exert its action by the liberation of endogenous 5-HT.

I joined Brodie's laboratory in early March 1955 as a visiting scientist from F. Hoffmann-La Roche of Switzerland, a pharmaceutical firm that had just hired me. I felt fortunate that I could work in a laboratory with the leading experts in transmitter research of the time and learn directly from Drs. Brodie and Parkhurst A. Shore about their findings:

- that reserpine and 5-HT potentiated the action of hypnotics,
- that there was an antagonism not only between 5-HT-, but also between reserpine-induced potentiation of hypnotics and lysergic acid diethylamide (LSD, a 5-HT-antagonist), and
- that reserpine administration produced an enhancement of the urinary excretion of 5-hydroxyindolacetic acid (a major metabolite of 5-HT) in dogs.



Parkhurst A. Shore

Reserpine, an alkaloid of the *Rauwolfia serpentina* plant which was used in Indian folk medicine as a tranquilizing agent, had just been isolated, synthesized, and pharmacologically characterized by researchers at CIBA S.A. in those days. Clinically, the drug had beneficial effects in arterial hypertension, and initially it showed promise for psychiatry as one of the first neuroleptics in the treatment of schizophrenia.

RESERPINE AFFECTS BRAIN SEROTONIN

When I started my work in Brodie's group, I was assigned the task of proving that reserpine affected endogenous 5-HT stores in animal tissues. Brodie and Shore's findings with reserpine in dogs indicated that reserpine had an effect on endogenous 5-HT stores, but with the methodology used – which measured the concentration of a 5-HT-metabolite in the urine – they could provide only indirect evidence for reserpine's 5-HT-liberating effect. Therefore, experiments with animal tissues had to be performed. We decided to look first at the gastrointestinal tract, which contains relatively large amounts of 5-HT, and to carry out the experiments on rabbits, a species of animal with particularly high gastrointestinal 5-HT concentrations. A colorimetric method for the detection of 5-HT, which proved to be better than the previously used biological assays, had just become available from Udenfriend's laboratory. However, I had to modify it for use in intestinal tissue because there were interfering substances in the tissue extracts. By employing the modified method, we found to our great delight that reserpine in doses between 0.25 and 5 mg/kg caused a profound, dose-dependent decrease of serotonin (1). Thus, the hypothesis of a 5-HT-releasing action of reserpine was confirmed.

However, it was still uncertain whether reserpine also acted in the same way on brain 5-HT stores as on the 5-HT stores of the gastrointestinal tract, because intestinal 5-HT is in the enterochromaffine cells, i.e., in non-neuronal cells. The colorimetric method I used for

measuring 5-HT in the gut was not sensitive enough for this research because in the rabbit, the concentration of 5-HT in the brain is more than an order lower than in the gastrointestinal tract. Fortunately by that time, in a laboratory close to ours, R.L. Bowman, in collaboration with Udenfriend, had constructed a new type of instrument which was to become known as the spectrophotofluorimeter. With this new device, which had a higher sensitivity than the colorimetric method, it became possible to identify specific biological compounds and measure them quantitatively. The spectrophotofluorimeter that was available for us in Bowman's laboratory was an early model, an open prototype that needed careful manipulation to avoid getting electric shocks. (Later on, safe spectrofluorimeters became commercially available). Nevertheless, the device enabled us to design a method for the specific estimation of the small amounts of 5-HT present in the brain (2). Already the first injection of 5 mg/kg reserpine I gave to the rabbits caused a practically complete depletion of 5-HT in the hypothalamus (3), together with marked behavioural sedation. Even more, the reserpine effect on brain 5-HT proved to be dose-dependent. Thus, in 1956 it was demonstrated for the first time directly that a psychotropic drug, reserpine, had an effect on the concentration of a neurotransmitter in the brain. It became a cornerstone of the hypothesis that 5-HT has a role in brain function (4) and that the liberation of 5-HT in the brain is responsible for the psychotropic action of reserpine. The findings that only those Rauwolfia alkaloids that had a sedative action decreased cerebral 5-HT (5) provided further substantiation for the role of serotonin in psychotropic effects.

NEUROBIOLOGICAL EFFECTS OF A MODERN ANTIDEPRESSANT

Nathan S. Kline

During my stay at the NIH, while working with reserpine, another drug, developed by F. Hoffmann-La Roche in the course of the company's anti-tuberculosis program, came to our attention. By the time it came to us, the compound, iproniazid (Marsilid), was reported to have psychotropic effects, and there was even some evidence from Nathan S. Kline and his colleagues that it had a beneficial action on depressed mood. But even before iproniazid's psychotropic effects were noted, E.A. Zeller's group had shown that the drug is a specific inhibitor of monoamine oxidase (MAO), one of the main metabolizing enzymes of monoamines such as 5-HT in the brain. Prompted by these findings we decided to perform experiments with iproniazid as well. In the course of this research we found that, after the injection of iproniazid into rabbits (and later in Basel, into mice and rats also), cerebral 5-HT content increased, and that pretreatment with the drug not only attenuated the reserpine-induced decrease of 5-HT in the brain, but was associated with behavioural stimulation by reserpine. The attenuation of reserpine-induced changes in brain 5-HT was for me an expected finding, but the associated behavioural changes came as a surprise because iproniazid alone did not produce overt behavioural changes. Reserpine alone caused deep sedation, and the

administration of iproniazid after reserpine treatment (when the 5-HT stores were already depleted) did not influence reserpine-induced sedation at all (6, 7, 8). Later on, we encountered similar findings with the combined administration of the monoamine oxidase inhibitor, iproniazid and tetrabenazine. It was on the basis of these observations that we assumed that the reserpine-induced sedation was not due to a decrease of the stored, functionally inactive 5-HT, as previously believed, but rather to the decrease in the availability of free (active) 5-HT, in the synaptic cleft, as a result of the rapid breakdown of released 5-HT; and that the reserpine-induced behavioural stimulation after pre-treatment with iproniazid was due to the excess of free 5-HT at the synaptic cleft resulting from the interference with the breakdown of the reserpine-released neurotransmitter by the monoamine oxidase inhibitor (6, 7, 8).

The experiments with iproniazid and with the iproniazid-reserpine combination also supported the hypothesis that the action of psychotropic drugs is mediated by biogenic amines and that biogenic amines play a role in the functioning of the brain. I remember how interested Kline was when I demonstrated to him, on the occasion of his visit to our laboratory, that the sedative effect of reserpine was "reversed" by pretreatment with iproniazid. As he told me later, this experiment reassured him that his clinical findings regarding the antidepressant effect of iproniazid were on solid ground. The MAO-inhibitors were initially quite successful as antidepressants, but their triumph was of relatively short duration, mainly because of adverse effects (e.g., hypertensive crises resulting from the inhibition of the breakdown of tyramine contained in food). The original MAO-inhibitors have to a great extent been replaced by the tricyclic and other antidepressant drugs, but some of the early inhibitors are still used as antidepressants. Also, in recent years the MAO-inhibitors have seen a certain revival in the treatment of depression, at least outside of the United States, with the discovery of isoforms of MAO (MAO-A and B) and the availability of reversible MAO inhibitors with a relatively specific action on MAO.

A NEW CLASS OF RESERPINE-LIKE COMPOUNDS

Back in Switzerland towards the end of 1955, we started a screening program at F. Hoffmann-La Roche for 5-HT-releasing synthetic compounds, in order to substantiate the "monoamine hypothesis" of psychotropic effects and detect new neuroleptics of the reserpine type. We soon found a class of substances, the benzo(a)quinolizines, which attracted our interest. Some of these drugs, for example tetrabenazine, caused a marked decrease of cerebral 5-HT (also noradrenaline and dopamine) in animals, which could be attenuated by pretreatment with iproniazid. Tetrabenazine also displayed reserpine-like sedation, which was "reversed" by pretreatment with iproniazid (9, 10). Although the benzoquinolizines seemed to be more selective for the brain than reserpine, they had a shorter duration of action with regards to both 5-HT-release and behavioural effects. Nevertheless, our findings with these first groups of synthetic 5-HT-releasing agents reassured us that we were on the right track with our monoamine hypothesis. Today, tetrabenazine is still used occasionally in the treatment of Huntington's chorea, and the radiolabelled compound and some of its derivatives (e.g. dihydrotetrabenazine) serve as specific ligands for the vesicular monoamine transporter, especially VMAT-2.

THE USE OF BLOOD PLATELETS AS PERIPHERAL MARKERS IN PSYCHIATRY

The spectrofluorimetric method of 5-HT determination allowed us to perform experiments with blood platelets, which had been shown to contain 5-HT. In the platelet, as in the brain, reserpine causes a profound, long-lasting depletion of 5-HT (11). Also, repeated administration of iproniazid to humans was found to lead to a marked increase of platelet 5-HT content. The detection of the 5-HT-storage organelles (dense bodies) in platelets of animals (12) and humans (13), and the description of some of their pharmacological properties (14), revealed other similarities between platelets and monoaminergic neurons as well. These and other findings (such as those regarding the 5-HT transporter at the plasma membrane) led psychiatrists to use platelets as peripheral markers of mental disorders. Such investigations seem to be of interest for determining the in vivo activity (e.g., on 5-HT-uptake and MAO-B activity) of neuropsychotropic drugs in humans. However, regarding the use of platelets as markers of psychiatric disorders, the results have been controversial, and further investigations using accurate, standardized methodology are needed in order to reach definitive conclusions.

FUTURE DEVELOPMENTS

Our results in Brodie's laboratory had an impact on research at F. Hoffmann-La Roche. They contributed to our decision to concentrate our efforts on the new field of neuropsychopharmacology, in which Roche had not been active before. This, in turn, led not only to the discovery of the 5-HT-releasing benzoquinolizines but also to the discovery of the benzodiazepines (15, 16), such as chlordiazepoxide and diazepam, which became important tools for elucidating the role of the GABA-ergic system in the brain. In collaboration with Birkmayer, Vienna, another advance was made in the improvement of levodopa treatment by combining the substance with an extracerebral decarboxylase inhibitor (benserazide). The rationale for this combination was based on the discovery that the decarboxylase inhibitor enhanced the levodopa-induced rise of cerebral dopamine, whereas it decreased the level of dopamine in the extracerebral tissues (17, 18). Levodopa, combined with a decarboxylase inhibitor, such as Roche's Madopar and Sinemet, are still being used as the standards in the drug treatment of Parkinson's disease. Our original 5-HT hypothesis has been further elaborated by many investigators, leading to the monoamine hypothesis of mental disorders and psychotropic drug action. The demonstration that reserpine, benzoquinolizines, and MAO-inhibitors interfere with cerebral neurotransmitters other than 5-HT, e.g., noradrenaline and dopamine, and the elucidation of the action mechanism of reserpine-like compounds (interference with the vesicular monoamine transporters), were essential contributions which opened up progress in the field. The discovery of antidepressants other than the MAOIs (e.g., the tricyclics in the late fifties) which act on brain monoamines by mechanisms different from those of reserpine and MAO-inhibitors (e.g., by interference with the uptake of monoamines at the cytoplasmic membrane), added evidence for the validity of the monoamine hypothesis.

Our knowledge about the significance of monoamines in brain function and drug action has been considerably refined by the discovery of a wealth of monoamine receptors and subtypes, the elucidation of their molecular structure, signalling pathways, and functional role, and so on. In the light of these and other developments, our original 5-HT hypothesis looks quite

primitive. Nevertheless, it contributed to the birth of biological psychiatry, which has now been widely accepted, even by many of those psychiatrists who initially had difficulty adopting the new paradigm.

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