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Release from brain tissue of compounds with possible transmitter function: interaction of drugs with these substances *

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The task I have set myself is to discuss release, and interaction with drugs, of substances which might act as transmitters of impulses in the central nervous system (CNS). In effect, I shall restrict myself to the first transmitter ever discovered, acetylcholine, and the substances now known as cerebral monoamines—the catecholamines dopamine and noradrenaline, and the indole derivative 5-hydroxytryptamine. First, I shall very briefly discuss a few other candidates for impulse transmission and give the reasons why I shall only mention them in passing.

Among the basic substances there is the diamine histamine, shown by Adam & Hye (1966) to have a characteristic distribution in the dog brain, somewhat similar to that of noradrenaline; this histamine is not, like, for example, that of the pituitary gland, situated in mast cells; its tissue concentration falls after reserpine and rises after an inhibitor of monoamine oxidase (MAO). Michaelson, Coffman & Vedral (1968) have confirmed this distribution in the monkey. Yet release on nerve stimulation has not been shown, neither have correlations been found between functional states of the brain and changes in brain histamine, so that it is premature to assume that it must be a transmitter.

Among polypeptides occurring in the brain " substance P ", discovered many years ago by von Euler & Gaddum (1931), is thought by some to act as a transmitter substance; there is, however, to date, no good evidence for this view and the suspicion that it may act as a local vasodilator is perhaps a little more likely.

Many amino-acids have the property of either stimulating or inhibiting firing of neurones; thus glutamic acid excites many cells when it is applied locally, and γ-aminobutyric acid (GABA) and glycine inhibit firing. Krnjević & Phillis (1963), Galindo, Krnjević & Schwartz (1967) and Krnjević & Schwartz (1967) have suggested that the first two acids may be excitatory and inhibitory transmitters. Attempts at showing release of endogenous GABA were first successful in brain slices (Mitchell, Neal & Srinivasan, 1968; 1969). Quite recently, Mitchell & Srinivasan (1969) applied [^{3}H]-GABA to the surface of the cat's brain for 90 min; the brain surface was then washed till efflux of radioactivity had levelled off; electrical stimuli of a kind which produces synaptic inhibition released [^{3}H]-GABA at greatly enhanced speed. Furthermore, Obata & Takeda (1969) obtained GABA in perfusates of the fourth ventricle after stimulation of the Purkinje cells of the cerebellar cortex. The axons of these cells end in the cerebellar nuclei adjacent to the fourth ventricle, where they exert an inhibitory action.

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In spite of these spectacular successes the possibility that GABA exerts some of its actions by being formed in the course of brain metabolism rather than by being released as a transmitter has to be borne in mind.

Recently glycine has been emphatically proposed as a likely inhibitory transmitter in the cord. Aprison & Werman (1965) based their views on the distribution of glycine in different parts of the cord, and Curtis *et al.* (1967) on the strychnine-sensitive hyperpolarization seen in spinal motoneurones and interneurones with this amino-acid. Release has not yet been demonstrated.

Finally, some acids, belonging to the so-called prostaglandins, have indeed been shown by Ramwell & Shaw (1966) to be released into a cup applied to the surface of the somatosensory cortex of the cat when afferent nerves or the contralateral cortex are stimulated ; yet the fact that prostaglandins are also set free when many peripheral nerves are stimulated, and that prostaglandins might be transformed breakdown products of phospholipid membranes, suggests a general metabolic role in cell activity rather than a transmitter role for these compounds.

Acetylcholine

The mapping of cholinergic neurones has been carried out on a macroscopic scale (Feldberg & Vogt, 1948) using the distribution of the synthesizing enzyme choline acetyltransferase ; nowhere in the brain was the concentration as high as in the anterior roots, although anterior horns and caudate nucleus were very rich in enzyme compared with other parts of the brain from which the enzyme was nearly absent. This showed that the brain was not just a mass of cholinergic neurones, but contained regions with many and regions with few such neurones.

The more recent work of Shute & Lewis (1961a, b ; 1967) traced cholinergic neurones histologically ; the method was the combination of a cholinesterase stain with lesions indicating the direction in which the fibres travel ; this is possible because a severed cholinergic axon accumulates the enzyme central to the cut. Among other conclusions drawn, the studies are interpreted as demonstrating that the ascending reticular activating system consists mainly of cholinergic neurones. Because of its bearing on what I will discuss later, I want only to mention another detail, namely, the finding of cholinergic fibres travelling from cells in the *substantia nigra compacta* to many regions of di- and telencephalon, including the pallidum. From there, and from other nuclei, cholinergic fibres enter the striatum, where they contribute to this region's dense neuropil.

Release of acetylcholine from brain tissue was first shown by MacIntosh & Oborin (1953), who fixed small cups to the brain surface, filled them with eserinized Ringer and assayed the fluid for acetylcholine. The authors also showed that anaesthesia depressed release. Two more recent methods have shown release of acetylcholine from brain tissue: first, the local perfusion of a small area of tissue by what is called Gaddum's push-pull cannula (Gaddum, 1961), a pair of concentric needles placed into the tissue. Fluid is pumped in through the inner and recovered by suction from the outer needle. Mitchell & Szerb (1962) observed the release of acetylcholine from the caudate nucleus on stimulation of a small area of the frontal cortex of the cat ; with the same method, McLennan (1964) found release of acetylcholine from the caudate nucleus when he stimulated one of the thalamic nuclei (*nucleus ventralis anterior*). The other method was devised by

Carmichael, Feldberg & Fleischhauer (1964) and is in effect a " superfusion " of the caudate nucleus. It was used by Portig & Vogt (1966) to study the release of acetylcholine from this nucleus when a number of stimuli were applied which elicit evoked potentials in the caudate nucleus.

The perfusion technique is illustrated in the first figure. When prostigmine is added to the artificial CSF, acetylcholine appears in the perfusate, as shown earlier by Bhattacharya & Feldberg (1958). In our work, this release amounted to a mean of 1·7 ng/min, and rose to a mean of 3·2 ng/min when the cats had been injected with atropine, which has been known for a long time to increase release of acetylcholine from brain tissue. In the course of an experiment, as the anaesthesia gradually became less deep, the concentration of acetylcholine tended to rise, a fact also observed by Bhattacharya & Feldberg (1958). Figure 2 shows the result of an experiment in which a variety of stimuli were applied. There is a gradual rise in the basal secretion, but superimposed is a release caused by each of four stimuli: electrical stimulation of the paws, of the sciatic nerves, of the contralateral caudate nucleus and noise. A similar rise occurs if stimulating electrodes are placed into the *substantia nigra*. It would therefore appear, in good agreement with the anatomical findings, that activation of the caudate nucleus along a multitude of pathways converges on cholinergic synapses; some of the liberated acetylcholine can be found in the ventricular fluid if cholinesterase is inhibited.

There are several drug actions which are compatible with our present views of the gross distribution of cholinergic neurones. Anticholinesterases, if not given in excess, cause arousal (Freedman, Bales, Willis & Himwich, 1949); at least one of the sites of this action is likely to be the cholinergic neurones of the ascending

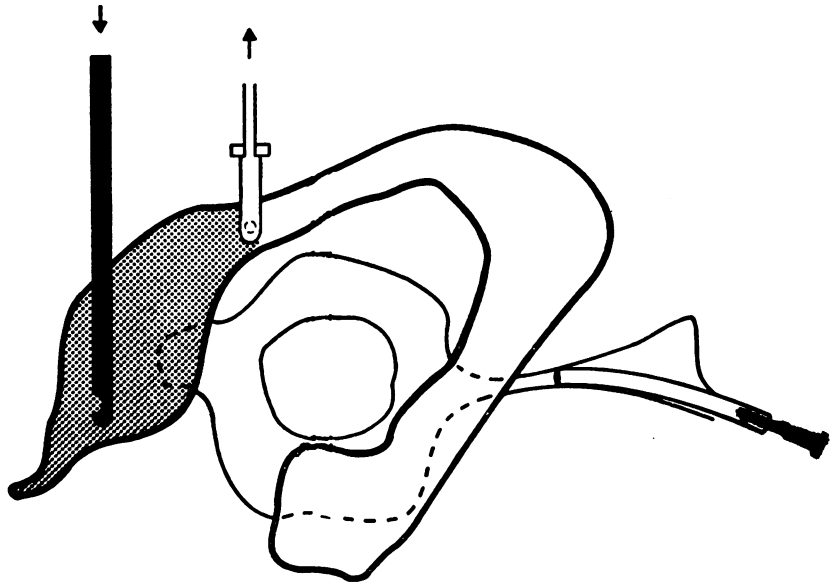

FIG. 1. Position of cannulae for perfusion of the anterior horn of the lateral cerebral ventricle of the cat. Perfused area shaded. (Modified from Carmichael, Feldberg & Fleischhauer (1964), by permission of *J. Physiol., Lond.*)

reticular formation. This effect is antagonized by atropine ; on its own, atropine causes a slowing of the waves of the electrocorticogram. Atropine is also known to be useful in the treatment of Parkinsonism. As will be discussed a little later, there is good reason to attribute some, at least, of the Parkinsonian signs to unopposed activity of cholinergic neurones in the corpus striatum. Atropine, by checking this activity, restores motility to some degree of normalcy.

Noradrenaline

The next possible transmitter substance I wish to discuss is noradrenaline (NA). Its macroscopic distribution was shown (Vogt, 1954) to be preferentially in the brain stem, with highest concentration in hypothalamus and somewhat lower values in midbrain and medulla. It does not occur in white matter. Fluorescence microscopy has shown that the NA is present in neurones, that its cells of origin are mainly in the midbrain, and its terminals, though scattered throughout the brain, are highly concentrated in the hypothalamus (Carlsson, Falck & Hillarp, 1962 ; Dahlström & Fuxe, 1964).

Experiments on release like those described for acetylcholine have not yet been carried out. Drug action, on the other hand, has been extensively investigated for the last 15 years. The first link with function was found by the use of drugs which stimulated the sympathetic centres in the hypothalamus and midbrain (Vogt, 1954) ; their administration led to a fall in noradrenaline content of these centres which was explained by increased utilization in the active regions, a utilization with which resynthesis was not keeping pace: the last step of noradrenaline synthesis,

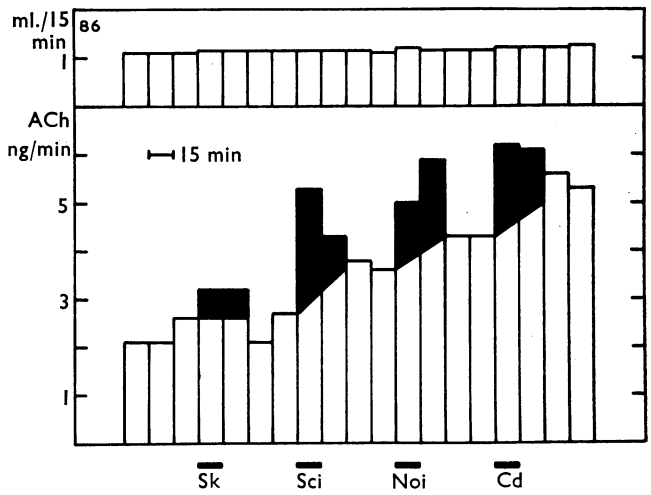

FIG. 2. Lower columns: acetylcholine (in ng/min) found in 15 min effluent from perfused anterior horn of cerebral ventricle of anaesthetized cat. SK, Electrical stimulation of skin of paws ; Sci, stimulation of central ends of sciatic nerves ; Noi, loud noise ; Cd, stimulation of contralateral caudate nucleus. Upper columns: volume of perfusate (ml./15 min). (By permission of *J. Physiol., Lond.*)

the β-oxidation of dopamine to noradrenaline is a slow process. Conditions of emotional stress, accompanied by enhanced sympathetic activity, equally lead to loss of noradrenaline from the hypothalamus. It also occurs when sham rage is produced by electrical stimulation of the amygdala (Reis & Gunne, 1965). Another link with function has been suggested by Feldberg & Myers (1964), who saw changes in body temperature on injecting noradrenaline into the hypothalamus, and suggested that the noradrenergic neurones there play a role in regulation of body temperature.

Dopamine

Whereas the role of dopamine (DA) as a precursor of noradrenaline has long been known, Carlsson's finding in 1959 that a part of the brain which contains practically no noradrenaline, the corpus striatum, is extremely rich in dopamine, suggested that dopamine may have a function of its own. Apart from small nuclei which contain much dopamine, the striatum is the only large brain region with really high concentrations.

A structure analogous to the dopamine-rich striatum of the mammal is found in the brain of birds (Juorio & Vogt, 1967) and of reptiles (Juorio, 1969). Whereas it was comparatively easy to accept acetylcholine and noradrenaline as transmitters in the brain because such a role for these substances was known in the periphery, it was more difficult for DA, peripheral dopaminergic nerves being unknown in mammals. It was, therefore, particularly important to seek, in different directions, for clues to the function of striatal DA.

The first indication came from findings in human pathology. Ehringer & Hornykiewicz (1960) estimated the DA content of the caudate nucleus and putamen of Parkinsonian patients and found it reduced to values often as low as 10% of normal. The same was true of the main acidic metabolite of DA, homovanillic acid (HVA) (Bernheimer & Hornykiewicz, 1965). Interestingly enough, the histological changes in the striatum of such patients are slight (Vogt & Vogt, 1920); furthermore, in patients who had suffered from Huntington's chorea the striatum, though very atrophic, showed a normal concentration of dopamine (Ehringer & Hornykiewicz, 1960).

These findings have been interpreted in two different ways: some authors consider Parkinson's disease to be a "biochemical lesion", and postulate that in these patients dopamine metabolism is disturbed throughout the body; others, and their view is gaining ground all the time, assume that the loss of dopamine is caused by the degeneration of "dopaminergic" fibres which originate outside the striatum. Human pathology suggests that the cells giving rise to these fibres lie in the *substantia nigra*; Hassler (1938) found a good correlation between destruction of the *substantia nigra compacta* and signs of Parkinson's disease, thus confirming the view first put forward by Trétiakoff (1919) that the crucial lesion in Parkinsonian patients is in the *substantia nigra.* If a dopaminergic *nigro-striatal* pathway is not a figment of the imagination, it should be possible to have histological confirmation of the intraneuronal localization of striatal dopamine. This, however, has proved difficult to obtain. Fluorescence microscopy, carried out in a way which easily demonstrates noradrenaline-containing terminals, only discloses a diffuse fluorescence of the striatum. However, by first incubating sections of rat caudate nucleus with α-methylnoradrenaline (α-CH_3NA), an amine which is not attacked by the

DA destroying enzymes monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT), then embedding the slices in araldite, cutting sections 2 μ thick, and exposing them to formaldehyde vapour, a fine network of terminals carrying varicosities was observed by Andén, Fuxe, Hamberger & Hökfelt (1966). Furthermore, Hökfelt (1968) succeeded in preparing similarly incubated slices of striatum for the electron microscope, using permanganate fixation (Richardson, 1966). He saw that many fine axons contained so-called dark-core vesicles, which are the electron microscopic equivalent of synaptic vesicles which contain either NA or the artificial α-CH_3NA. On the assumption that it is the axons which normally contain DA which take up the α-CH_3NA, both the histological and electron microscope findings suggest that there are fine, dopaminergic fibre terminals in the striatum and that they constitute 16% of all axons.

Confirmation about the origin of these fibres has also been obtained experimentally. Thus a loss of dopamine from the striatum, akin to that in the Parkinsonian patient, was seen after making lesions in animals in the region of the midbrain which included the *substantia nigra*; in the rat, a diminution in catecholamine fluorescence of striatal tissue was seen microscopically (Andén, Carlsson, Dahlström, Fuxe, Hillarp & Larsson, 1964), in the monkey a lowered content of dopamine was found chemically (Poirier & Sourkes, 1965).

Finally, Hökfelt & Ungerstedt (1969) looked for the dark-core vesicles in electron microscope preparations of rat brain slices, incubated in α-CH_3NA and taken from animals in which unilateral lesions of the midbrain had been made at a site at which the nigro-striatal fibres are thought to converge on to the striatum. A few days after the operation, the number of dark-core vesicles was greatly reduced on the side of the lesions.

Release

McLennan (1964, 1965) used the push-pull cannula mentioned earlier to try and obtain dopamine from different parts of the striatum. With stimulating electrodes in the thalamic nucleus *centrum medianum*, he obtained DA release when the cannula had been inserted into the caudate, and with electrodes placed in the *substantia nigra* he saw a release from a cannula in the putamen. These experiments suggest that dopamine may be released from the striatum as a transmitter, but they do not prove it beyond any doubt. The cannula is bound to cause some local leakage of substances present in the tissue. This leakage might increase as a result of unspecific changes in permeability or blood supply caused by an inflow of impulses, and more dopamine may come out for this reason, and not because it is released as a transmitter from activated nerve endings. Evidence for such a possibility has been obtained by showing that stimulation may release into the effluent of a push-pull cannula inert compounds such as labelled inulin, urea or α-aminoisobutyric acid which had been allowed to equilibrate with brain tissue (Chase & Kopin, 1968).

Portig and I (1966, 1968) tried to obtain a release of DA from the caudate nucleus while avoiding tissue lesions by using the ventricle perfusion described earlier for the experiments on acetylcholine. Electrodes were placed into the *substantia nigra* of anaesthetized cats and the perfusate was tested for DA. Release of DA was, indeed, sometimes obtained, but quantities were small (between 0·1 and 0·3 ng/min), very variable, and so were the latent periods between stimulation and emergence of the

amine. There were two obvious reasons for the difficulties: any dopamine which might be released had to diffuse to the ventricles to be detected, and would be waylaid and transformed into metabolites by the enzymes MAO and COMT within the tissue ; the second problem was an anatomical one: one could only hope for DA to reach the ventricles if it was released by axons ending near the ventricular surface of the caudate, not if the nigral axons ended in the putamen, or in parts of the caudate remote from the ventricles. Yet there was no knowledge of the localization of cells within the *substantia nigra* which might send out axons impinging on ventricle-near cells of the caudate nucleus. Whereas this difficulty has not been solved, there were two obvious ways out of the first difficulty: to inhibit the DA metabolizing enzymes, or to make use of the metabolites themselves to detect release of amines.

The administration of inhibitors of metabolizing enzymes did not improve the release of dopamine, but the alternative of making use of its main metabolite, HVA, for the detection of release of the amine met with a measure of success (Portig & Vogt, 1969a, b). Some of the HVA which forms in the striatum finds its way into the cerebrospinal fluid or ventricular perfusate, where it can be estimated fluorimetrically (Portig, Sharman & Vogt, 1968). Control samples of perfusate collected for, say, 30 min from the resting anaesthetized cat contained 60 to 240 ng HVA ; this is very different from the amount of dopamine found in such samples, which lay between zero and 1 ng. Other things being equal, the resting concentration of HVA fell as anaesthesia was deepened ; this in itself suggests that HVA is formed and enters the ventricles as a result of nervous activity, presumably of dopaminergic neurones.

When several samples of perfusate had been collected to establish a baseline, the *substantia nigra* was stimulated electrically, the duration of stimulation being from 4 to 30 min. There was, in most experiments, an increase of HVA in the effluent collected during the stimulation period ; it was of the order of 30 to 100 ng per 30 min, and it often lasted for over an hour.

Results obtained by varying the duration of stimulation suggested that the first few minutes were responsible for most of the release of HVA, the yield after 8 min being little greater than after 4 min, and after 20 min no better than after 10. It was also shown that mere arousal did not produce release of HVA. The most serious technical pitfall was the effect which obstruction to flow (leading to changes in perfusion pressure) had on the basal release before stimulation: any small impediment of flow reduced the movement of HVA from the tissue fluid into the ventricular space and interfered with the baseline from which the increments had to be determined. This greatly reduced the number of experiments in which the effect of two different stimuli could be compared in the same animal. The long duration of the increment in HVA content of the perfusates after short stimulation periods is considered to be the result of slow movement of the acid from the depth of the tissue and not of prolonged activation of dopaminergic neurones.

Drug action

It is the dream of any neuropharmacologist to find a drug which produces only one biochemical change in the brain and one visible functional disturbance, so that the two can be brought into a causal relationship. There probably is no such drug, but in the context being discussed here there is the next best to this: a group of

drugs which affect only the metabolism of dopamine, and not that of any other monoamine or of acetylcholine. The effect observed is an increased turnover of DA. Since dopamine synthesis is a rapid process, increased turnover is manifested by a fall in tissue concentration of the amine only in rather extreme conditions, but is readily shown by an accumulation in the tissue of the main DA metabolite, HVA.

However, in some species—rat, mouse, pigeon—accumulation of HVA can be caused by another process, namely inhibition of a specific transport mechanism, which removes the acid metabolites from brain tissue. If one wants to use increase in HVA in brain tissue as an index of increased turnover of DA in these species, control experiments are needed to rule out interference with transport mechanism by the drug in question.

The drugs shown to accelerate dopamine turnover in animal brain are those derivatives of phenothiazine and certain butyrophenones having in common that they are useful in schizophrenia and are liable to produce Parkinsonian side-effects in a proportion of patients. Andén, Roos & Werdinius (1964) were the first to show accumulation of HVA in the rabbit corpus striatum after two such drugs, chlorpromazine and haloperidol. To see whether there was consistent correlation between drug-induced Parkinsonism and altered dopamine metabolism, Laverty & Sharman (1965) compared, in the cat, the biochemical action of three phenothiazines, known to produce a high incidence of Parkinsonian side-effects, with that of thioridazine, the administration of which hardly ever leads to Parkinsonism.

Treatment of cats by daily oral administration over a period of 2 weeks caused increased homovanillic acid content of the caudate nucleus when the three " toxic " phenothiazines, but not when thioridazine, was used (Fig. 3). The doses had been chosen to produce a comparable degree of sedation and were therefore different for each drug. Since chlorpromazine, 20 mg/kg, had been found to raise, and thioridazine, 15 mg/kg, not to affect the tissue concentration of homovanillic acid, it was decided to repeat the experiment with equal doses of the two compounds (O'Keeffe, to be published). The results were the same as before, an increase in HVA following the administration of chlorpromazine, but not of thioridazine.

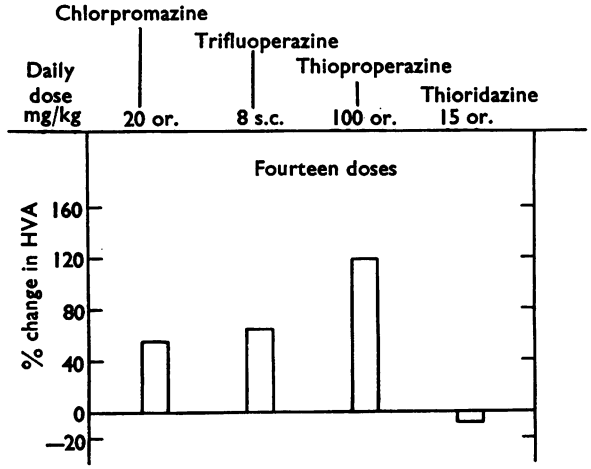

FIG. 3. Change in homovanillic acid content of the caudate nucleus of cats treated for 2 weeks with the phenothiazines indicated. (Data from Laverty & Sharman, 1965.)

A quantitative comparison of the actions of chlorpromazine and haloperidol was then carried out by O'Keeffe in the mouse. In man, haloperidol is about 50 times as potent as chlorpromazine in the treatment of psychoses and in the production of drug-induced Parkinsonism. The same potency ratio was seen to hold for the effect on turnover of DA (Fig. 4). DA concentration in the brain was slightly up with chlorpromazine 1 mg/kg and down with chlorpromazine 100 mg/kg, and the same was true of haloperidol 0·01 and 1 mg/kg; HVA was slightly elevated with the smallest doses used and then reached a plateau whatever the amount of either drug given.

Thus, to obtain the same biochemical effects, the dose of chlorpromazine had to be somewhere between 10 and 100 times that of haloperidol. Yet, in other respects the two compounds hardly differed in their activity: for example, the doses required to sedate the mice were nearly equal; so are, according to Janssen (1961), the lethal doses for mice. This author also showed that the adrenolytic potency is even reversed in favour of chlorpromazine. Hence some effects of haloperidol—neuroleptic effect in man, tendency to cause Parkinsonism, acceleration of turnover of cerebral dopamine in animals—which are produced by chlorpromazine only with doses about 50 times larger than those required of haloperidol, can be dissociated from other effects, such as acute toxicity or sedation in mice, in which these compounds are equiactive. In contrast to " sedation " of mice, which is obviously no valid index of therapeutic efficacy in man, there are some behavioural tests on animals, such as conditioned avoidance reflexes in the dog, which give a potency ratio for haloperidol versus chlorpromazine which is similar to that seen in clinical trials (Janssen, 1961).

The experiments with haloperidol have confirmed the correlation, found earlier with different phenothiazines, between the tendency of a drug to cause extra-

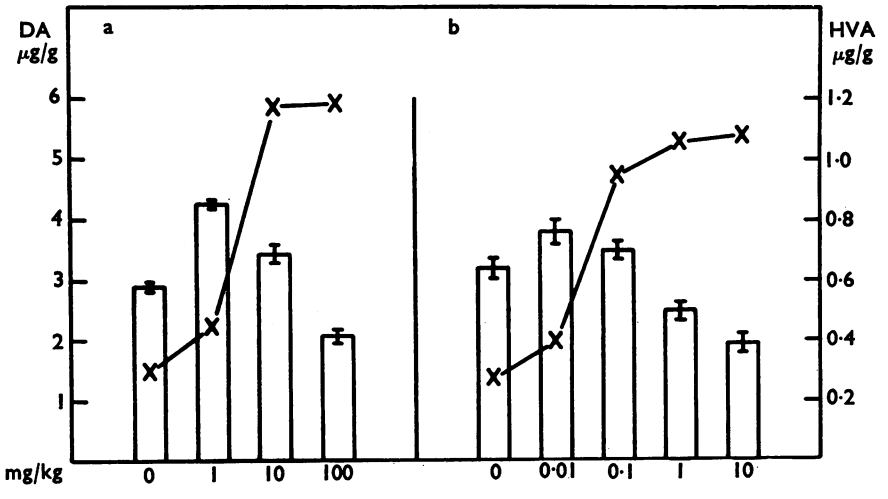

FIG. 4. Columns: concentration of DA (μg/g, mean ± S.E.) in mouse striatum (left scale). x——x, Concentration of HVA (mean, μg/g; right scale). Abscissa: dose of (a) chlorpromazine, (b) haloperidol, injected intraperitoneally. Tissue analysed 2 hr after injection (O'Keeffe, Sharman & Vogt, to be published).

pyramidal side-effects in man and to produce an acceleration of dopamine turnover in the brain of animals. If this acceleration also takes place in man, one would have to explain why Parkinson's disease occurs when dopamine is lost from the human striatum by midbrain lesions, whereas the drugs which produce Parkinsonism appear to accelerate dopamine turnover without necessarily causing a reduction in tissue dopamine. A widely held theory is that the drugs which induce Parkinsonian signs act by antagonizing the action of dopamine rather than by removing it. Evidence for this view is at present only by analogy. Thus antagonism between noradrenaline and chlorpromazine is seen in the brain on electrophoretic application of the drugs to cells of the reticular formation (Bradley, Wolstencroft, Hösli & Avanzino, 1966). Furthermore, peripheral dopamine receptors which are resistant to the action of α- and β-blockers can be inactivated by haloperidol, as first shown by v. Rossum (1966). However, since large doses of tranquillizers lower the concentration of DA in the brain of animals, there is also another possibility. If, in man, resynthesis of DA is not as efficient as it is in animals, there might be actual loss of DA from brain tissue after quite low doses of chlorpromazine or haloperidol. We shall not know the answer till chemical analyses become available of brains of patients who suffered from drug-induced Parkinsonism.

5-Hydroxytryptamine

5-Hydroxytryptamine (5-HT) like NA, does not occur in medullated fibres ; its distribution resembles that of NA, but it is also found in the caudate nucleus and part of the limbic system (Paasonen, MacLean & Giarman, 1957), both of which lack NA. In the anterior medulla and pons, the 5-HT is mainly found in the cells of midline-nuclei, or nuclei raphes, whereas the terminals are scattered throughout brainstem, hypothalamus and even cerebral cortex. In the vertebrates, there is no evidence for the occurrence of 5-HT containing peripheral nerves, but fluorescence microscopy has shown the 5-HT of the brain to be localized in neurones.

Release

(*a*) *Cortex.* By placing cups on the sigmoid gyrus of the cat's brain and stimulating electrodes in the raphe nuclei, Eccleston, Randić, Roberts & Straughan (1968) obtained an increase in 5-HT content of the cups during stimulation ; the amounts were very small in spite of the use of an inhibitor of MAO.

(*b*) *Caudate nucleus.* Though the concentration of 5-HT in the striatum is quite high, there is no histological evidence to show whether it lies in nerve terminals, or in fibres which pass through the tissue, or whether it might even be situated in cells. We perfused (Portig & Vogt, 1969b) the anterior horn of a lateral ventricle and tested the effluent for 5-HT ; the quantities of 5-HT found, in confirmation of the findings by Feldberg & Myers (1966), were extremely small. It was just possible to detect them on the rat fundus strip. A twofold increase in concentration (to 1 ng/ml. or 2·1 ng/30 min) was seen after nialamide 10 mg/kg. In contrast to acetylcholine, the concentration of which increases as perfusion proceeds, the concentration of 5-HT tended to fall in the course of an experiment. Stimuli causing evoked potentials in the caudate nucleus such as electrical stimulation of the paws, of the central ends of the sciatic nerves, or of the *substantia nigra* failed to release any 5-HT. Nor did the production of shivering by perfusing the ventricles

with D-tubocurarine. It remains to be seen whether stimulation of the midbrain raphe nuclei will produce a different result.

It should be mentioned here that Poirier's experiments on monkeys suggested to him that the occurrence of tremor after midbrain lesions was related to a fall in the concentration of striatal 5-HT and a destruction of pathways which lie medial to the dopaminergic *nigro-striatal* pathway discussed earlier (Poirier, Sourkes, Bouvier, Boucher & Carabini, 1966).

Action of drugs

The most interesting results are those obtained with *p*-chlorophenylalanine, a substance which interferes with synthesis of 5-HT by inhibiting the tryptophane hydroxylase. Jouvet & Renault (1966) and Renault (1967) showed that this drug inhibits sleep in cats and rats; it affects predominantly slow-wave sleep and, to some extent, paradoxical sleep. The result is the same as that produced by surgical lesions of the raphe nuclei. Other functions connected with 5-HT containing neurones are revealed by the use of this drug; hyperalgesia develops in rats treated with this substance, and greatly enhanced doses of analgesics of the morphine family are required to produce analgesia (Tenen, 1968). That the reverse may also be true is suggested by the recent observation (Rogers & Thornton, 1969) that toxicity of pethidine increases in the mouse in parallel with increased brain concentrations of 5-HT. The explanation of Tenen's observations might be the fact that the raphe nuclei send fibres into the posterior columns which normally act by reducing sensory impulses.

In our laboratory, Shillito (1969) has recently found another action of *p*-chlorophenylalanine, perhaps caused by the removal of normal inhibitions; the drug greatly increased social grooming in young male rats, to such an extent that the rats became bald if kept together in groups; in adult males social interactions also much increased, but took the form of frequent mounting. The effect on female rats was negligible. The effect on males is completely abolished by atropine in doses which do not alter the motility of the animals. That the strange behaviour is indeed due to lack in 5-HT is shown by the fact that a dose of the precursor 5-hydroxytryptophan given shortly before the observations restores behaviour to normal.

These last examples illustrate that, in spite of the complexity of drug action, it is sometimes possible to gain information about the physiological role of naturally occurring substances from their interaction with drugs, information which is often unobtainable in any other way.

REFERENCES

ADAM, H. M. & HYE, H. K. (1966). Concentration of histamine in different parts of brain and hypophysis of cat and its modification by drugs. *Br. J. Pharmac. Chemother.*, **28**, 137–152.

ANDÉN, N.-E., CARLSSON, A., DAHLSTRÖM, A., FUXE, K., HILLARP, N.-Å. & LARSSON, K. (1964). Demonstration and mapping out of nigro-neostriatal dopamine neurons. *Life Sci., Oxford*, **3**, 523–530.

ANDÉN, N.-E., FUXE, K., HAMBERGER, B. & HÖKFELT, T. (1966). A quantitative study on the nigro-neostriatal dopamine neuron system in the rat. *Acta physiol. scand.*, **67**, 306–312.

ANDÉN, N.-E., ROOS, B.-E. & WERDINIUS, B. (1964). Effect of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. *Life Sci., Oxford*, **3**, 149–158.

APRISON, M. H. & WERMAN, R. (1965). The distribution of glycine in cat spinal cord and roots. *Life Sci., Oxford*, **4**, 2075–2083.

BERNHEIMER, H. & HORNYKIEWICZ, O. (1965). Herabgesetzte Konzentration der Homovanillinsäure im Gehirn von parkinsonkranken Menschen als Ausdruck der Störung des zentralen Dopaminstoffwechsels. *Klin. Wschr.*, **43**, 711–715.

BHATTACHARYA, B. K. & FELDBERG, W. (1958). Perfusion of cerebral ventricles: assay of pharmacologically active substances in the effluent from cisterna and aqueduct. *Br. J. Pharmac. Chemother.*, **13**, 163–174.

BRADLEY, P. B., WOLSTENCROFT, J. H., HÖSLI, L. & AVANZINO, G. L. (1966). Neuronal basis for the central action of chlorpromazine. *Nature, Lond.*, **212**, 1425–1427.

CARLSSON, A. (1959). The occurrence, distribution and physiological role of catecholamines in the nervous system. *Pharmac. Rev.*, **11**, 490–493.

CARLSSON, A., FALCK, B. & HILLARP, N.-Å. (1962). Cellular localization of brain monoamines. *Acta physiol. scand.*, **56**, suppl. 196.

CARMICHAEL, E. A., FELDBERG, W. & FLEISCHHAUER, K. (1964). Methods for perfusing different parts of the cat's cerebral ventricles with drugs. *J. Physiol., Lond.*, **173**, 354–367.

CHASE, T. N. & KOPIN, I. J. (1968). Stimulus-induced release of substances from olfactory bulb using the push-pull cannula. *Nature, Lond.*, **217**, 466–467.

CURTIS, D. R., HÖSLI, L., JOHNSTON, G. A. R. & JOHNSTON, I. H. (1967). Glycine and spinal inhibition. *Brain Res.*, **5**, 112–114.

DAHLSTRÖM, A. & FUXE, K. (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. *Acta physiol. scand.*, **62**, suppl. 232, 5–55.

ECCLESTON, D., RANDIĆ, M., ROBERTS, M. H. T. & STRAUGHAN, D. W. (1969). In *Metabolism of Amines in the Brain*, ed. Hooper, G., pp. 29–33. London: Macmillan.

EHRINGER, H. & HORNYKIEWICZ, O. (1960). Verteilung von Noradrenalin und Dopamin (3-Hydroxytyramin) im Gehirn des Menschen und ihr Verhalten bei Erkrankungen des extrapyramidalen Systems. *Klin. Wschr.*, **38**, 1236–1239.

EULER, U. S. VON & GADDUM, J. H. (1931). An unidentified depressor substance in certain tissue extracts. *J. Physiol., Lond.*, **72**, 74–87.

FELDBERG, W. & MYERS, R. D. (1964). Effects on temperature of 5-hydroxytryptamine, adrenaline and noradrenaline injected into the cerebral ventricles or the hypothalamus of cats. *J. Physiol., Lond.*, **173**, 25–26*P*.

FELDBERG, W. & MYERS, R. D. (1966). Appearance of 5-hydroxytryptamine and an unidentified pharmacologically active lipid acid in effluent from perfused cerebral ventricles. *J. Physiol., Lond.*, **184**, 837–855.

FELDBERG, W. & VOGT, M. (1948). Acetylcholine synthesis in different regions of the central nervous system. *J. Physiol., Lond.*, **107**, 372–381.

FREEDMAN, A. M., BALES, P. D., WILLIS, A. & HIMWICH, H. E. (1949). Experimental production of electrical major convulsive patterns. *Am. J. Physiol.*, **156**, 117–124.

GADDUM, J. H. (1961). Push-pull cannulae. *J. Physiol., Lond.*, **155**, 1–2*P*.

GALINDO, A., KRNJEVIĆ, K. & SCHWARTZ, S. (1967). Micro-iontophoretic studies on neurones in the cuneate nucleus. *J. Physiol., Lond.*, **192**, 359–377.

HASSLER, R. (1938). Zur Pathologie der Paralysis agitans und des postenzephalitischen Parkinsonismus. *J. Psychol. Neurol. Lpz.*, **48**, 387–486.

HÖKFELT, T. (1968). *In vitro* studies on central and peripheral monoamine neurons at the ultrastructural level. *Z. Zellforsch.*, **91**, 1–74.

HÖKFELT, T. & UNGERSTEDT, U. (1969). Electron and fluorescence microscopical studies on the nucleus caudatus putamen of the rat after unilateral lesions of ascending nigro-neostriatal dopamine neurons. *Acta physiol. scand.*, in the Press.

JANSSEN, P. A. J. (1961). Vergleichende pharmakologische Daten über sechs neue basische 4'-Fluorobutyrophenon-Derivate. *Arzneim. Forsch.*, **11**, 819–824; 932–938.

JOUVET, M. & RENAULT, J. (1966). Insomnie persistante après lésions des noyaux du raphé chez le chat. *C.r. Séanc. Soc. Biol.*, **160**, 1461–1465.

JUORIO, A. V. (1969). The distribution of dopamine in the brain of the tortoise, *Geochelone chilensis* (Gray). *J. Physiol., Lond.*, in the Press.

JUORIO, A. V. & VOGT, M. (1967). Monoamines and their metabolites in the avian brain. *J. Physiol., Lond.*, **189**, 489–518.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963). Iontophoretic studies of neurones in the mammalian cerebral cortex. *J. Physiol., Lond.*, **165**, 274–304.

KRNJEVIĆ, K. & SCHWARTZ, S. (1967). The action of γ-aminobutyric acid on cortical neurones. *Exp. Brain Res.*, **3**, 320–336.

LAVERTY, R. & SHARMAN, D. F. (1965). Modification by drugs of the metabolism of 3,4-dihydroxyphenylethylamine, noradrenaline and 5-hydroxytryptamine in the brain. *Br. J. Pharmac. Chemother.*, **24**, 759–772.

MACINTOSH, F. C. & OBORIN, P. E. (1953). Release of acetylcholine from intact cerebral cortex. *Abstr. Int. physiol. Congr.* XIX, pp. 580–581.

MCLENNAN, H. (1964). The release of acetylcholine and of 3-hydroxytyramine from the caudate nucleus. *J. Physiol., Lond.*, **174**, 152–161.

McLennan, H. (1965). The release of dopamine from the putamen. *Experientia*, **21**, 725–726.

Michaelson, I. A., Coffman, P. Z. & Vedral, D. F. (1968). The regional distribution of histamine in brain of the Rhesus monkey (*Macaca mulatta*). *Biochem. Pharmac.*, **17**, 2435–2441.

Mitchell, J. F., Neal, M. J. & Srinivasan, V. (1968). The release of ^{3}H-gamma-aminobutyric acid (GABA) from rat cerebral cortex. *Br. J. Pharmac. Chemother.*, **34**, 661–662*P*.

Mitchell, J. F., Neal, M. J. & Srinivasan, V. (1969). The release of amino-acids from electrically stimulated rat cerebral cortex slices. *Br. J. Pharmac.*, **36**, 201–202*P*.

Mitchell, J. F. & Srinivasan, V. (1969). The release of ^{3}H γ-aminobutyric acid from the surface of the brain by electrical stimuli which produce synaptic inhibition. *J. Physiol., Lond.*, in the Press.

Mitchell, J. F. & Szerb, J. C. (1962). The spontaneous and evoked release of acetylcholine from the caudate nucleus. *Excerpta Medica*, Series No. 48, Leiden, Abstract No. 819.

Obata, K. & Takeda, K. (1969). Release of γ-aminobutyric acid into the fourth ventricle induced by stimulation of the cat's cerebellum. *J. Neurochem.*, **16**, 1043–1047.

Paasonen, M. K., MacLean, P. D. & Giarman, N. J. (1957). 5-Hydroxytryptamine (serotonin, enteramine) content of structures of the limbic system. *J. Neurochem.*, **1**, 326–333.

Poirier, L. J. & Sourkes, T. L. (1965). Influence of the substantia nigra on the catecholamine content of the striatum. *Brain*, **88**, 181–192.

Poirier, L. J., Sourkes, T. L., Bouvier, G., Boucher, R. & Carabini, S. (1966). Striatal amines, experimental tremor and the effect of harmaline in the monkey. *Brain*, **89**, 37–52.

Portig, P. J., Sharman, D. F. & Vogt, M. (1968). Release by tubocurarine of dopamine and homovanillic acid from the superfused caudate nucleus. *J. Physiol., Lond.*, **194**, 565–572.

Portig, P. J. & Vogt, M. (1966). Search for substances released on stimulation of the caudate nucleus in the cat. *J. Physiol., Lond.*, **186**, 131–132*P*.

Portig, P. J. & Vogt, M. (1968). Activation of a dopaminergic nigro-striatal pathway. *J. Physiol., Lond.*, **197**, 20–21*P*.

Portig, P. J. & Vogt, M. (1969a). In *Third Symposium on Parkinson's Disease*, ed. Gillingham, J. & Donaldson, I. M. L., pp. 22–24. Edinburgh: Livingstone.

Portig, P. J. & Vogt, M. (1969b). Release into the cerebral ventricles of substances with possible transmitter function in the caudate nucleus. *J. Physiol., Lond.*, **205**, in the Press.

Ramwell, P. W. & Shaw, J. E. (1966). Spontaneous and evoked release of prostaglandins from cerebral cortex of anaesthetized cats. *Am. J. Physiol.*, **211**, 125–134.

Reis, D. J. & Gunne, L.-M. (1965). Brain catecholamines: relation to the defense reaction evoked by amygdaloid stimulation in cat. *Science, N.Y.*, **149**, 450–451.

Renault, J. (1967). *Monoamines et sommeils, III.* Thèse. Lyon.

Richardson, K. C. (1966). Electron microscopic identification of autonomic nerve endings. *Nature, Lond.*, **210**, 756.

Rogers, K. J. & Thornton, J. A. (1969). The interaction between monoamine oxidase inhibitors and narcotic analgesics in mice. *Br. J. Pharmac.*, **36**, 470–480.

Rossum, v. J. M. (1966). The significance of dopamine-receptor blockade for the mechanism of action of neuroleptic drugs. *Archs int. Pharmacodyn. Thér.*, **160**, 492–494.

Shillito, E. (1969). The effect of p-chlorophenylalanine on social interactions of male rats. *Br. J. Pharmac.*, **36**, 193*P*.

Shute, C. C. D. & Lewis, P. R. (1961a). Cholinergic nervous pathways in the forebrain. *Nature, Lond.*, **189**, 332–333.

Shute, C. C. D. & Lewis, P. R. (1961b). The use of cholinesterase techniques combined with operative procedures to follow nervous pathways in the brain. *Bibl. anat. Basel*, **2**, 34–49.

Shute, C. C. D. & Lewis, P. R. (1967). The ascending cholinergic reticular system: neocortical, olfactory and subcortical projections. *Brain*, **90**, 497–520.

Tenen, S. S. (1968). Antagonism of the analgesic effect of morphine and other drugs by p-chlorophenylalanine, a serotonin depletor. *Psychopharmacologia (Berl.)*, **12**, 278–285.

Trétiakoff, C. (1919). *Contribution à l'étude de l'anatomie pathologique du locus niger de Soemmering.* Thèse. Paris.

Vogt, C. & Vogt, O. (1920). Zur Lehre der Erkrankungen des striären Systems. *J. Psychol. Neurol. Lpz.*, **25**, 627–846.

Vogt, M. (1954). The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. *J. Physiol., Lond.*, **123**, 451–481.

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