CATECHOLAMINES IN BRAIN

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Norepinephrine is present in all postsynaptic sympathetic neurones; it is located both in the adrenergic nerve fibres and in the ganglia from which these fibres originate. All mammalian organs supplied by sympathetic vasoconstrictor nerves therefore contain some norepinephrine, and the brain is no exception to this rule. Detailed study of the distribution of norepinephrine in the brain of the dog has, however, shown that the concentration of this amine is quite uneven and does not run parallel with brain vascularity (19). The highest concentration was found in the hypothalamus, somewhat less in the grey stratum around the aqueduct, a little less still in the tegmentum of the mid-brain, in the medullary reticular formation and in the medial part of the thalamus. Throughout the brain, medullated fibres do not contain more than traces of norepinephrine, about 1 % of the hypothalamic concentration. Many grey regions, however, such as the lateral parts of the thalamus, the entire grey matter of the telencephalon, and the cerebellum contain only slightly more norepinephrine than does the white matter, about 5 % of the amount found in the hypothalamus.

The histological site of the norepinephrine is unknown; it may be situated in nerve cells, non-medullated fibres, glia, or at several of these sites.

Macroscopically, there is a high concentration of norepinephrine in all parts which contain the central representation of the sympathetic system; we will see later that this may not be mere coincidence. Many of these norepinephrine-rich regions, but not all, belong to the so-called reticular formation.

The uneven distribution of a pharmacologically active substance in the brain strongly suggests that the agent has a role to play in the specialized function of those regions where its concentration is high. With regard to norepinephrine, some pharmacological evidence for this hypothesis will be given later.

In peripheral sympathetic nerves, the norepinephrine is accompanied by small quantities of epinephrine; the same holds for brain; here, the percentage of the methylated amine is somewhat higher than it is in the paravertebral, and lower than in the prevertebral sympathetic ganglia. Whether or not epinephrine serves the same purpose as norepinephrine remains to be determined. From recent work of Weil-Malherbe and Bone (20) and Carlsson *et al.* (3), we know that dopamine is also present in brain. Its distribution in relation to that of norepinephrine and epinephrine has not yet been mapped out. Whether it is both a precursor of norepinephrine and an active agent in its own right is also uncertain.

Many different approaches have been used to try to obtain information on the function of the catecholamines in brain.

1) The parenteral administration of exogenous epinephrine has been studied extensively. Dr. Rothballer will review this work in detail, and I wish only to make brief reference to such facts as may help to discriminate between the actions of different catecholamines.

The work of Bonvallet, Dell and Hiebel (2) has shown that small intravenous doses of epinephrine cause arousal by stimulation of the ascending activating system of the reticular formation. Rothballer (17) has shown the same to hold for norepinephrine, and the two amines to be of equal potency.

It is a well-known fact that parenteral administration of epinephrine causes anxiety in man, whereas norepinephrine has no such effect. This apprehension is usually attributed to the cardiac action of epinephrine and not to any direct cerebral effect. Except for the greater activation of the carotid sinus reflexes, the cardiac action of norepinephrine is, however, sufficiently similar to that of epinephrine to make this interpretation somewhat uncertain. If, on the other hand, we were to consider the anxiety caused by epinephrine to be of cerebral origin, it would follow that there should exist cerebral actions of epinephrine which are not shared by norepinephrine.

Large intravenous (8), intracisternal (9) or intraventricular (5) doses of epinephrine cause lethargy and anaesthesia. It is not certain whether these effects have a vascular component, but, in this action, epinephrine is more powerful than norepinephrine. Isoprenaline, which is a vasodilator drug, has qualitatively the same effect, and this is of importance in assessing the possible contribution of vasoconstriction in the anaesthetic effect of epinephrine; it can obviously not be fundamental.

2) The effect of catecholamines on synaptic transmission has been extensively studied; the subject will be reviewed by Dr. Rothballer.

3) A more direct approach to the problem of the function of the cerebral catecholamines was attempted by studying the effect of drugs with pronounced cerebral actions on the norepinephrine content of brain (6, 7, 19). The experiments were carried out on cats or dogs subjected to the action of different drugs for periods of at least 3 hours, since preliminary experiments had shown that shorter duration failed to cause any change. Most drugs were given subcutaneously, ether by inhalation and reserpine intraperitoneally. Control and experimental animals were killed by bleeding under chloroform. The hypothalamus and the midbrain were examined. The following results were obtained:

Of the drugs tested, one group, let us call them the "active" drugs, lowered the hypothalamic norepinephrine; the remaining drugs had no effect on the catecholamine content. The question arising from this observation was whether a property could be found common to the "active" drugs and absent from the drugs of the inactive group. It was easy to rule out the possibility that the difference was due to inadequate dosage of the inactive substances, since they were tried in the highest tolerated and sometimes in lethal doses. They included two convulsants (caffeine and leptazol), the central depressant chlorpromazine, and ephedrine. Depressant and convulsive drugs were also represented among the active substances, *e.g.*, ether, nicotine and picrotoxin. These properties were obviously not decisive for the effect on hypothalamic norepinephrine. Insulin, morphine and β-tetrahydronaphthylamine were found to be highly active, and this suggested that activity and stimulation of the sympathetic centres might be correlated. Experiments were therefore devised, in which depletion of hy-

pothalamic norepinephrine and activation of the sympathetic centres were measured in the same cat. This can be done by denervating one adrenal in a preliminary operation. When the animal has recovered, the drug is given and the difference estimated between the catecholamines of innervated and denervated adrenal; this difference is produced by the action of the drug on the autonomic centres, which leads to reflex release of adrenal medullary amines only on the intact side. In these experiments it was found that there was excellent correlation between loss of hypothalamic norepinephrine and stimulation of the sympathetic centres measured in this way: all drugs of the inactive group failed to cause a difference between catecholamines of innervated and denervated gland, whereas large depletion of amines of the innervated adrenal followed the use of drugs which seriously lowered hypothalamic norepinephrine. In the experiments with combinations of drugs, the same correlation was found: when nalorphine was employed to suppress the signs of excitation by morphine, there was no loss in hypothalamic norepinephrine or in medullary amines. On the other hand, on combining morphine with chlorpromazine, which is not a morphine antagonist, loss in hypothalamic norepinephrine and secretion of amines from the innervated adrenal were just as large as with morphine alone (6). It follows, that norepinephrine is lost from the hypothalamus whenever the sympathetic centres situated there are vigorously stimulated. This is circumstantial evidence for the view that the norepinephrine of the hypothalamus plays an important role in certain of its functions. It does not help us to find out what these functions are.

Another correlation was invariably found, namely that between the action of a drug on the norepinephrine of the hypothalamus and on the norepinephrine of the midbrain. When one is depleted the other is also low and *vice versa*. Whatever the function of the mesencephalic norepinephrine, it appears to be of the same nature as that of its hypothalamic counterpart. In contrast, the norepinephrine in the area postrema, which is not nervous tissue proper, is not changed by any of the active drugs tested. The function of the catecholamines which are stored in the area postrema is therefore unlikely to be the same as that of the amines in diencephalon and mesencephalon.

The proportion of epinephrine in cerebral catecholamines has a wide range in normal animals. When drugs were given, this proportion remained in the same range; in other words, losses of norepinephrine were probably always accompanied by losses of epinephrine.

Another drug which causes lowering of the catecholamine concentration of brain and which has recently been much in the limelight is reserpine. Let us examine the question whether the action of reserpine sheds light on the physiological role of these amines.

Soon after the observation by Pletscher *et al.* (15) that reserpine causes the loss of 5-hydroxytryptamine from the gastrointestinal tract, it was found that it also produces a disappearance of 5-hydroxytryptamine from brain tissue (13, 16). When the norepinephrine of the hypothalamus was examined after an injection of reserpine (7), it was found equally depleted. According to Weil-

Malherbe and Bone (21) and to Carlsson *et al.* (3), dopamine also disappears from brain after an injection of reserpine.

In trying to correlate the loss of cerebral catecholamines caused by reserpine with the signs produced by the drug, it is important to keep in mind the conclusion arrived at in the preceding section: this was that drugs which stimulate the sympathetic centres, thus causing discharge in the peripheral sympathetic nerves and such central effects as excitement and rage, deplete these very centres of their norepinephrine; the concentration of 5-hydroxytryptamine, incidentally, is not affected by these drugs (13). Signs of sympathetic discharge, as indicated by a difference in the catecholamine content of innervated and denervated adrenal or by transient piloerection, hyperglycaemia and hypertension, are in fact produced after reserpine. When, however, these effects were compared on a quantitative basis (7), there was no parallelism between the central sympathetic activity and the loss in catecholamines from the brain as had been found with other drugs: whereas in a series of 12 experiments hypothalamic losses of norepinephrine were uniformly severe, never amounting to less than 60 % and going up to 97 %, amine losses from the innervated adrenal were often no more than 20 to 40 % and only twice greater than 70 %. It follows that the two effects are to a certain extent independent of each other: although activity of the sympathetic centres may contribute to, it does not fully explain the disappearance of norepinephrine from the hypothalamus caused by reserpine. The main factor would appear to be the interference by the drug with the binding capacity of tissues for certain amines. How this action affects peripheral tissues will be discussed below.

Since many drugs which lower brain norepinephrine cause excitement, it is also evident that the loss in cerebral catecholamines by itself does not explain the sedation produced by reserpine. The fact, however, that reserpine causes 5-hydroxytryptamine and catecholamines to disappear from the hypothalamus, and thus from the sympathetic centres, should provide an opportunity to test whether the presence of these amines is essential for central sympathetic activity. Experiments are in progress with Dr. Iggo to examine this question; their aim is to establish whether the spontaneous or induced sympathetic activity, as reflected in impulses led off from preganglionic fibres, is prevented by reserpine. Only a few experiments have so far been carried out, but it is perfectly clear already that a course of 4 to 6 large daily doses of reserpine does not reduce the electrical activity travelling down the preganglionic fibres of the cervical sympathetic of the cat. If there is any change at all, it lies in the direction of more frequent impulses. On the other hand, it has been known for some time that signs of peripheral sympathetic activity are rarely seen and difficult to elicit in the reserpinized animal. This is due to the fact that reserpine, in addition to its effects on brain, causes the transmitter substance norepinephrine to disappear from all sympathetic ganglia and fibres (1, 10, 11, 12). This loss can be so severe as to render electrical stimulation of postganglionic fibres completely ineffective: this has been shown for the cervical sympathetic and the hypogastric nerves by Muscholl and Vogt (10), and for the splenic and the splanchnic nerves by Carls-

son *et al.* (4). Except for substances of the reserpine family, other drugs capable of depleting hypothalamic norepinephrine have not been found to reduce the amount of transmitter in the superior cervical ganglia. This has been tested for morphine (11) and for the nicotine-like substance dimethylphenylpiperazine (Sanan, to be published), both of which are very active in depleting hypothalamic norepinephrine in doses which do not affect the ganglia.

A completely different way of trying to determine whether the sedation due to reserpine is the result of a lack of essential amines is the use of antagonists. A cat sedated by reserpine can be instantaneously aroused by many drugs which are central stimulants in the normal animal, such as morphine, methadone, amphetamine, or lysergic acid diethylamide; this arousal takes place without restoring to the brain the norepinephrine the reserpine has caused it to lose; if the dose of reserpine is not too large, signs of increased sympathetic activity may accompany the action of these drugs. It would appear that the initiation rather than the performance of brain stem activity is impaired by reserpine; perhaps this is the process in which one or several of the cerebral amines are normally involved.

Whereas there are several ways of raising the cerebral 5-hydroxytryptamine, no drugs have been discovered which increase the norepinephrine content of cat brain beyond levels which may normally occur. Among the drugs tested were two inhibitors of amine oxidase and two precursors of norepinephrine. Of the amine oxidase inhibitors tried, choline-*p*-tolyl-ether (50 mg/kg) led to concentrations of hypothalamic norepinephrine which were in the upper range of normal, whereas iproniazid (100 mg/kg) decreased it significantly by 35%. There was usually some degree of excitement with iproniazid, and in the light of the conclusions reached earlier these two facts are probably causally related.

Of possible precursors of norepinephrine, dopamine and the corresponding amino acid dihydroxyphenylalanine (DOPA) were examined. Both substances were used alone in repeated doses and also, following the example of Udenfriend *et al.* (18) in their studies of the metabolism of 5-hydroxytryptamine, combined with iproniazid to inhibit the destruction of any newly-formed amine by amine oxidase. Only three experiments were done with dopamine, and no conspicuous change in the concentration of norepinephrine in either brain or sympathetic ganglia was observed. As the penetration of these amines into the brain is known to be less complete than that of the corresponding amino acids, the matter was not pursued, and the experiments were continued with DOPA. It was found that DOPA alone caused no significant change in hypothalamic or ganglionic norepinephrine; the cats showed sometimes, but not invariably, a moderate degree of excitement. When DOPA was combined with iproniazid, excitement was much more pronounced. The norepinephrine content of the hypothalamus, which is reduced with iproniazid alone, was restored to normal but did not rise above that level. In contrast, the norepinephrine in the sympathetic ganglia, which is normally 3.5 μg/g, was greatly increased; in one cat, a concentration of 12.8 μg/g tissue was obtained by giving four injections of 35 mg DOPA/kg each, starting 24 hours after the iproniazid. The difference in response between brain and

ganglia may be due to the fact that it is only in the brain that iproniazid alone causes a fall in norepinephrine, which has first to be compensated for before the content can rise above normal; in addition, there may be better penetration of the drug into peripheral sympathetic tissue than across the blood-brain barrier. Other species appear to behave differently; Pletscher (14) found a rise in "total" catecholamines in the brain of rats injected with iproniazid, and also some increase when DOPA was combined with iproniazid. It is not clear whether the divergence in the results is due to the use of another species or to the fact that total catecholamines rather than norepinephrine was estimated.

The observation that iproniazid does not raise the norepinephrine content of cat brain made it rather doubtful whether it would protect this amine from removal by reserpine. In contrast to observations by Carlsson *et al.* (4) that such protection takes place in the rabbit, and in contradistinction to the protective action of iproniazid on the 5-hydroxytryptamine of rabbit and rat brain, Table 1 shows no significant difference between the norepinephrine concentrations in brain and in sympathetic ganglia of cats injected with reserpine alone and with iproniazid followed (6 to 22 hours later) by reserpine.

The species difference in the response to the combination of iproniazid and reserpine is not restricted to the effects on hypothalamic norepinephrine: it is even more striking in the signs produced in the animals. Whereas in rabbits, the sedation caused by reserpine is transformed by iproniazid into a picture of excitement with pupillary dilatation, hyperventilation and tremor, in the cat, the only change made by iproniazid in the reserpine syndrome is the prevention of the extreme miosis; no excitement, tremor, panting or pupillary dilatation is produced. It is a pity that the only conclusion one can draw from these findings is that the miosis cannot be related to low cerebral catecholamines. Figures on the 5-hydroxytryptamine content of cat brains after reserpine and iproniazid are not published, but Dr. Brodie informs me that they are high. This would suggest that the peculiar form of excitement seen in rabbits after iproniazid and reserpine is not a consequence of the elevated 5-hydroxytryptamine in brain. The fact that, in the rabbit, the catecholamines are not as low as after reserpine alone may be related to those signs, but the mechanism of such a relationship is difficult to

TABLE 1

Effect of intraperitoneal injections of reserpine alone and of reserpine preceded by iproniazid on the norepinephrine content of the hypothalamus and the superior cervical ganglion of the cat

No. of Cats	Reserpine (mg/kg)	Iproniazid (mg/kg)	Norepinephrine (μg/g Fresh Tissue)	
			Hypothalamus	Superior cervical ganglia
4	1.0–1.5	0	0.15 ± 0.07	0.62 ± 0.20
4	1.0–1.5	100	0.23 ± 0.08	0.37 ± 0.04
15	0	0	1.40 ± 0.07	3.50 ± 0.30
6	0	100	0.91 ± 0.08	4.06 ± 0.65

Norepinephrine concentration: mean ± S.E. of the mean.

visualize. It is possible that, after the administration of these drugs, the site of the amines differs from normal. Obviously, most of the work required to clarify these drug actions has still to be done.

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