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THE CONCENTRATION OF SYMPATHIN IN DIFFERENT PARTS OF THE CENTRAL NERVOUS SYSTEM UNDER NORMAL CONDITIONS AND AFTER THE ADMINISTRATION OF DRUGS

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The presence of noradrenaline and adrenaline in the brain has been demonstrated by von Euler (1946) and Holtz (1950). These substances were supposed, undoubtedly correctly, to occur in the cerebral vasomotor nerves. The present work is concerned with the question whether these sympathomimetic amines, besides their role as transmitters at vasomotor endings, play a part in the function of the central nervous tissue itself. In this paper, these amines will be referred to as 'sympathin', since they were found invariably to occur together, with noradrenaline representing the major component, as is characteristic for the transmitter of the peripheral sympathetic system.

A first approach to the problem of the function of cerebral sympathin was the determination of its distribution in different parts of the brain and spinal cord. Such an approach had proved fruitful in the investigation of the functional role of the enzyme system cholinacetylase (Feldberg & Vogt, 1948), the concentration of which was found to vary greatly in different regions. This had suggested that only certain neurones made use of acetylcholine as their transmitter substance. As briefly reported elsewhere (Vogt, 1952*a*), sympathin, too, was found to possess a specific pattern of distribution. This very fact suggests, though it does not prove, that these amines play a part in the specialized function of those regions of the brain in which their concentration is high. A detailed map of the pattern of distribution of sympathin was prepared in the dog: it forms the first part of this paper.

The second part deals with changes in the concentration of brain sympathin produced by drugs and the inferences which may be drawn from such observations.

Since it was known that the total amounts of sympathin in the central nervous system were very small (between 20 and 200 ng/g* according to

* 1 ng = 10^{-9} g.

Holtz, 1950), and useful mapping of the sympathin content of brain required the examination of small quantities of tissue, success depended on the use of sufficiently sensitive methods of amine determination. The amines were extracted from portions of fresh brain ranging in weight from 10 to 250 mg, the extracts purified, and submitted to paper chromatography. The parts of the paper which might contain any noradrenaline and adrenaline were eluted separately, the eluates evaporated and taken up in a small volume of water. This final solution was assayed biologically, the noradrenaline on the rat's blood pressure and the adrenaline on the rat's uterus. Given sensitive preparations, it was possible to detect concentrations as low as 10 ng noradrenaline and 5 ng adrenaline per g fresh tissue.

METHODS

The estimation of sympathin in different regions of the normal brain was carried out on dogs bled under chloroform anaesthesia. In a few instances intravenous pentobarbitone sodium was used instead of chloroform. The results were similar. Most of the experiments on the effects of drugs were done on cats, but a few litters of puppies were also used. The drugs were given subcutaneously, the animals anaesthetized rapidly with chloroform several hours later and killed by bleeding.

Operations

For the purpose of stimulating the cervical sympathetic trunk, dogs were anaesthetized with ether, both cervical sympathetic chains traced and cut, and both superior cervical ganglia exposed so that they could be rapidly excised later. The distal end of one of the sympathetic trunks was threaded through a fluid electrode (Collison, 1933) and stimulated with sixteen break-shocks per sec from a Lewis interrupter connected to an induction coil; 4 V were supplied to the primary coil. The responses of the pupil, lid and nictitating membrane were observed. Stimulation was carried out for periods of 5 min with intervals of 2 min for as long as good responses were obtained.

Aseptic extirpation of both superior cervical ganglia for the purpose of allowing degeneration of the postganglionic sympathetic fibres to take place was done on two cats anaesthetized with ether. Recovery from the operation was uneventful.

Denervation of the left adrenal was performed on a series of cats in an aseptic operation under ether. Through a midline abdominal incision the larger and lesser splanchnic nerves were severed and the first three lumbar sympathetic ganglia extirpated on the left side. Twice, the operation was carried out on both adrenals. The cats were injected with drugs several weeks after the operation when they had regained their preoperative weight.

Administration of drugs

Insulin was injected after an overnight fast. Solutions of β-tetrahydronaphthylamine were obtained by dissolving the carbonate in a small excess of HCl and adjusting the pH to neutrality with $NaHCO_3$. All other drugs were dissolved in 0·9 % NaCl.

Dissection

The skull or the spinal canal was opened as fast as possible, the dura removed and the brain or cord taken out. The pia mater was stripped off from the parts to be used. In order to keep the time of dissection short, no more than eight, and usually fewer, samples were taken from one brain. The order in which different parts were taken was intentionally varied. Storage of one half of a hypothalamus in the frozen state for 1 hr, while the other half was worked up without delay, did not cause any detectable loss of sympathin from the stored half.

The *cortical areas* were dissected by cutting along the boundary of grey and white matter and using the maps published by Klempin (1921).

The term '*medial thalamic nuclei*' denotes the massa intermedia and its neighbourhood, and includes part of the anterior and most of the medial thalamic nuclei.

Unless otherwise stated, the *hypothalamus* did not include the infundibular stalk, the preoptic region or the corpora mammillaria. It included the supraoptic nuclei, but the optic chiasma was always dissected off.

'*Hypothalamus, regio preoptica*' is the transitional part between hypothalamus and substantia perforata anterior.

'*Midbrain*', in the experiments on the action of drugs, denoted one half of one midbrain from which the colliculi, the basis pedunculi and the brachium colliculi inferioris had been removed.

The '*superficial part of the pons*' extended anteriorly and posteriorly to the actual borders of the pons, laterally to a distance of 4–5 mm from the midline, and dorsally to a depth of 2 mm.

The grey columns of the *cord* were obtained by freezing short pieces of the cord and punching out the requisite parts with a small silver punch fitted with a polythene plunger.

Extracts

The dissected tissue was rapidly weighed and dropped into a cooled 15 ml. centrifuge tube containing 1 ml. acidified ethanol (1 ml. conc. HCl per l. redistilled ethanol). The tissue was crushed with a glass rod and the tube kept in a mixture of dry ice and acetone till homogenization. The further steps, consisting of grinding in a glass homogenizer, and extracting successively with acid ethanol, dilute watery HCl and acetone-ethanol, have been described in detail (Vogt, 1953). The final acetone-ethanol extract was applied to a cylinder of acid-washed filter-paper and chromatographed at 26° C in phenol-HCl. Descriptions of the chromatographic procedure, the elution of the separated amines from the paper, the evaporation of the eluates, and the biological assay of noradrenaline and adrenaline in the residue from the eluates have been published (Crawford & Outschoorn, 1950; Vogt, 1952*b*).

Special precautions

Since the amounts of adrenaline and noradrenaline to be determined were often very small, the work was carried out as speedily as possible. Extracts were always prepared, purified and applied to the chromatography paper on the day of dissection, and the chromatogram allowed to develop overnight. Elutions were carried out in the morning and assays in the afternoon of the next day. If some assays had to be delayed, the residue of the evaporated eluate was kept overnight in the deep freeze (– 17° C). An inhibition of the carbachol contraction of the rat's uterus by an eluate from the adrenaline portion of the chromatogram was not considered to be due to adrenaline unless the effect disappeared on heating. To test this, the eluate was heated for 10 min at pH 8 in a stoppered tube immersed in a boiling water-bath. Inhibitions of uterine contractions were sometimes caused by eluates containing unidentified heat stable substances.

In parts of the brain which appeared to contain little or no sympathomimetic amines, it was important to make sure that the extracts were not 'masking' any adrenaline or noradrenaline owing to a content of substances which antagonized the biological effects used for the assay. Such tests were repeatedly carried out, and when 'interfering substances' were found the estimations were discarded. Except for some instances discussed later under 'Results', interference with the pressor effect of noradrenaline was largely controlled by pretreating the rats with atropine sulphate (2 mg/kg) and hexamethonium bromide (16 mg/kg).

When drugs were injected into animals before the examination of their brains, controls were carried out in order to ensure that quantities of the drugs sufficient to affect the tissues used for assay were not present in the final eluates.

In all experiments on the action of drugs on sympathin, simultaneous estimations were made on injected and control animals for the purpose of minimizing technical errors. No use of this fact has been made in drawing up the tables of results, as this would have made them very involved. It does, however, mean that the differences between treatments are often greater than the ranges shown in the tables would suggest.

Discrimination

Different biological preparations varied in their discrimination of doses; the assay was always done by 'bracketing' the unknown solutions between doses of standard differing, as a rule, by a factor of 2, in particularly good preparations by a factor of 1·5 or 1·4. This means that the errors of a single estimation were hardly ever below 20 %.

Fluorimetry

Fluorimetric estimation of noradrenaline was done according to Lund's (1949) method. A Farrand fluorimeter was used and the method slightly modified by using a little less ascorbic acid and a little more NaOH and adding the two separately (Crawford, T. B. B., unpublished).

Recovery experiments

The recovery of 0·5–1 μg noradrenaline or adrenaline added to brain homogenates was approximately 60–75 %; the main loss was incurred during the first extraction, whereas any losses due to the chromatographic separation or elution were too small to be detected by the biological assays.

Travelling speed of sympathomimetic amines in brain extracts

It is known that the R_F value of substances may be affected by the presence of other compounds; the travelling speed is usually depressed. An experiment was carried out in which a quantity of noradrenaline sufficient to give a colour reaction with ferricyanide was added to an extract of 0·25 g brain, the largest amount of tissue used in a single sample in the present work. This mixture was applied to the base-line of a paper cylinder just as if it were an ordinary brain extract. After developing the chromatogram and drying it, the whole paper was sprayed with ferricyanide. A control drop of noradrenaline in dilute HCl had been applied to another part of the base-line. The position and width of the noradrenaline spot thus produced were precisely the same as those of the band produced by the mixture of extract and noradrenaline. There was thus no interference by tissue components with the R_F value of any noradrenaline present in brain extracts.

Extracts of adrenal glands

Cats. The whole gland was ground with a little acid-washed, calcinated sand moistened with 1 ml. of 0·15 N-HCl. The mixture was washed into a 15 ml. centrifuge tube with a further 9 ml. of 0·15 N-HCl. After thorough stirring, the mixture was centrifuged and an aliquot of 1 ml. added to 7 ml. absolute ethanol contained in a 40 ml. tube. The mixture was evaporated to dryness *in vacuo* (50° C bath temperature). The residue was transferred to a 15 ml. tube by means of small quantities of ethanol; the alcoholic extract was concentrated *in vacuo* to 0·75 ml., chilled and centrifuged, and the supernatant fluid was applied to paper. The residue was washed with 0·25 ml. acetone-ethanol 1 : 1, centrifuged and this extract also applied to the paper.

Rats. Single glands were homogenized in 1 ml. acid ethanol (1 ml. conc. HCl per l. ethanol), the homogenate transferred to a centrifuge tube by means of 3 ml. acid ethanol, chilled, spun, and the supernatant poured into a wide 40 ml. tube. The residue was washed with 0·5 ml. acid ethanol, spun and the washing added to the first supernatant. Evaporation *in vacuo* and further procedure as for cat glands.

RESULTS

Identification of the sympathomimetic amines in the central nervous system

A few preliminary experiments, in which crude extracts of the dog's hypothalamus were tested in parallel biological assays on the rat's uterus and the rat's colon (see Gaddum, Peart & Vogt, 1949), had shown the presence in these extracts of some substance which inhibited the acetylcholine contractions of both organs and might have been a sympathomimetic amine. The results of quantitative assays against noradrenaline and adrenaline on the two tissues suggested that the effects were produced either exclusively or mainly by

noradrenaline. The estimations were, however, greatly interfered with by substances which stimulated the tissues used for the assay, and it was obvious that extensive purification would be required for satisfactory identification. The procedures adopted for this purpose are described in the section on methods.

The purified brain extracts prepared for paper chromatography did not contain material that interfered with the speed of travel of noradrenaline added to these extracts (see 'Methods'). With these purified extracts, evidence that one of the amines extracted from brain was indeed noradrenaline was obtained as follows: a chromatogram of brain extract was cut up into horizontal strips and the eluates of each strip were tested on the rat's blood pressure. The only eluates with pressor activity were those from strips cut at a height corresponding to the place taken up on the control strip by noradrenaline. In addition to the pressor material in the noradrenaline region, extracts of hypothalamus, but not of other parts of the brain, formed a second band of pressor activity near the front of the paper. It is due to a vasopressin-like substance which has been made the subject of a separate paper (Vogt, 1953).

Further confirmation of the identity of the pressor amine from brain extracts with noradrenaline was obtained by comparing the alkali resistance of the extracted pressor substance with that of noradrenaline. In such tests it is essential not to dissolve the noradrenaline used for comparison in physiological salt solution but in a brain extract free from noradrenaline, since the noradrenaline is destroyed more rapidly in salt solutions than in eluates of brain extracts. When this precaution was taken, no difference was found in the alkali stability of pressor amine from tissue and of noradrenaline.

Another property in which the pressor substance from brain and noradrenaline were indistinguishable was the way in which their pressor effects were inhibited by dibenamine. Apart from its R_F value, the most convincing evidence, however, for the identity of the eluate of the noradrenaline portion of a chromatographed brain extract with noradrenaline was its assay by Lund's fluorimetric method. Thus an extract from hypothalamus was found by bioassay to contain 1·5 μg, and by fluorimetry 1·4 μg noradrenaline per g of tissue.

The procedures of identification were carried out mainly with hypothalamic extracts, since these contained the largest quantities of the amine. It would appear unlikely that the eluates of the noradrenaline portion of the chromatogram of other parts of the brain did not contain the same substance, whenever they were found to be pressor. The shape of the pressor response affords some indication of the nature of the agent injected. Except for an unidentified compound found in the amygdaloid nuclei and in extracts from the cingulate gyrus (see below), there was no reason to suspect that pressor substances obtained from other parts of the brain were different from noradrenaline.

Owing to the very small quantities of material involved, the identification of the adrenaline fraction of 'sympathin' is based only on the facts that it was

being eluted from the adrenaline portion of the chromatogram, that it inhibited the carbachol contractions of the rat's uterus and that it was destroyed by 10 min heating to 100° C at pH 8.

The position taken up in the chromatogram by other biologically active substances

In addition to sympathomimetic amines, many constituents of brain tissue are soluble in acid ethanol and water and will thus be present in the extracts subjected to paper chromatography. It is therefore useful to know the place taken up in the chromatogram by those substances, usually bases, known to occur in brain tissue and to exert pharmacological actions. *Histamine* does not travel far from the base-line and would be found in and just above the noradrenaline strip (Fig. 1, *HIS*). The quantities occurring in brain are very small (Harris, Jacobsohn & Kahlson, 1952), and therefore do not represent a practical obstacle, since neither the rat's blood pressure nor the rat's uterus respond to the fraction of a microgram which might be present. *5-Hydroxytryptamine* travels faster, and part of it contaminates the adrenaline eluates. In extracts of blood it was sometimes found in eluates of the adrenaline strip, but it was not identified in eluates from brain, though it is known to occur in the central nervous system (Amin, Crawford & Gaddum, 1953). *Polypeptides* travel faster still, and thus substance P (von Euler & Gaddum, 1931) and posterior lobe hormone are found near the front of the paper above the adrenaline region. In assays on the blood pressure, they antagonize each other. Though both are found in the brain, they are well separated from the adrenaline region, and no further steps need be taken for their elimination. *Acetylcholine* may occur in eluates from brain. According to Outschoorn (personal communication), it spreads diffusely over the paper. Its effect on the blood pressure can be readily abolished by atropine.

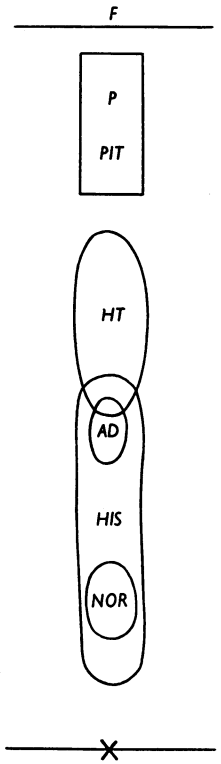

Fig. 1. Regions to which some substances occurring in brain tissue travel on paper in phenol/HCl. The solutions were applied at *X*. *NOR*, noradrenaline; *HIS*, histamine; *AD*, adrenaline; *HT*, 5-hydroxytryptamine; *PIT*, posterior lobe hormone; *P*, substance P of v. Euler & Gaddum (1931); *F*, front.

This completes the list of the known biologically active basic compounds present in brain. Frequently, however, depressor responses were elicited by

eluates of the noradrenaline strip prepared from certain parts of the brain. Since no known compound accounts for these effects, their sporadic occurrence indicates that there are more, perhaps many more, ethanol soluble and biologically highly active compounds distributed unevenly in the central nervous system.

Distribution of sympathin in the central nervous system

Tables 1–3 and Fig. 2 give a survey of the results in the dog. Table 1 lists the regions 'rich' in noradrenaline, Table 3 the parts which contain very little ($<0{\cdot}1\,\mu g/g$), and Table 2 the regions with intermediate values.

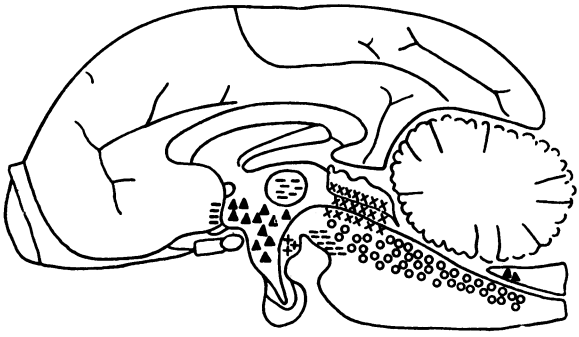

Fig. 2. Medial sagittal section of a dog's brain. Distribution of noradrenaline. ▲, $1{\cdot}0\,\mu g/g$; +, $>0{\cdot}4$ $<1{\cdot}0\,\mu g/g$; ○, $>0{\cdot}3$ $<0{\cdot}4\,\mu g/g$; —, $>0{\cdot}2$ $<0{\cdot}3\,\mu g/g$ fresh tissue.

Two regions stand out above all others by their high concentration of noradrenaline, the hypothalamus and the area postrema. The next highest concentration is recorded in the grey stratum around the aqueduct (5, Table 1) and not much less is found in other parts of the midbrain, except for the colliculi (15, 16, Table 2) and the myelinated fibre tracts. The more cellular parts of the medulla oblongata (8–12, Table 1) contain about a third of the hypothalamic concentration. The only other part of the brain in which a moderately high amount of noradrenaline is found is the medial part of the thalamus.

In the tissues shown in Table 2 and in areas **3** and **4** of the cortex (Table 5), the concentration is lower still, but higher than in all other parts of the brain which were examined and which contained less than 10% of the amount in the hypothalamus. Since every tissue supplied by vasomotor nerves will contain a little sympathin derived from the postganglionic nerve fibres, amounts below 10% of the hypothalamic concentration may well consist entirely of vasomotor sympathin. Amongst the regions lacking in appreciable amounts of sympathin are *all* myelinated fibre tracts, the cerebellum and most of the telencephalon. Conversely, the highest concentrations are found in those macroscopically translucent regions associated with autonomic activity and

TABLE 1. Noradrenaline in the dog's brain; concentrations above 0·24 μg/g

	No. of dogs	Range	Mean
Diencephalon			
1. Hypothalamus (excluding 2 and 3)	31	0·6 –1·76	1·03
2. Corpora mammillaria	2	0·32–0·50	0·41
3. Regio preoptica	4	0·18–0·32	0·28
4. Medial thalamic nuclei	12	0·12–0·40	0·24
Mesencephalon			
5. Stratum griseum centrale	11	0·27–0·80	0·42
6. 'Midbrain'*	6	0·24–0·60	0·37
7. Red nucleus and fossa interpeduncularis	7	0·15–0·36	0·26
Medulla oblongata			
8. Region of nuclei X and XII	9	0·12–0·50	0·31
9. Formatio reticularis, post. part	4	0·30–0·45	0·34
10. Formatio reticularis, ant. part	1	—	0·36
11. Area acustica	2	0·33–0·45	0·39
12. Central part of floor of fourth ventricle	5	0·10–0·40	0·27
13. Area postrema	13	0·70–2·00	1·04

* After removal of colliculi, basis pedunculi and brachium colliculi inferioris.

TABLE 2. Noradrenaline in the dog's brain; concentrations between 0·2 and 0·1 μg/g

	No. of dogs	Range	Mean
14. Pons, ventro-medial	5	0·12 –0·30	0·20
15. Superior colliculi	4	0·10 –0·21	0·16
16. Inferior colliculi	4	0·10 –0·16	0·11
17. Cortex, average*	26	0·025–0·25	0·11
18. Medial geniculate body	3	<0·09 –0·15	0·13
19. Nucleus (and funic.) gracilis and cuneatus	4	0·08 –0·22	0·11
20. Anterior horns	2	0·15 –0·20	0·18
21. 'Lateral' horns	3	0·11 –0·24	0·19
22. Posterior horns	2	0·12 –0·12	0·12

* Individual areae, see Table 5.

TABLE 3. Noradrenaline in the dog's brain; concentrations below 0·1 μg/g

Grey matter		
23. Lateral thalamic nuclei	(7)	0·08
24. Lateral geniculate body	(2)	0·07
25. Cerebellar cortex	(4)	0·07
26. Amygdaloid nuclei	(3)	0·06
27. Caudate nuclei	(4)	0·06
28. Several cortical areae*	(16)	0·05
29. Tuber olfactorium	(3)	0·05
30. Cornu ammonis, grey matter	(3)	0·04
White matter		
31. Brachium conjunctivum	(1)	0·09
32. Corpus callosum	(1)	0·08
33. Pyramid	(1)	0·06
34. Anterior roots	(1)	0·06
35. Cerebellar fibres	(1)	<0·05
36. Spinal tracts	(1)	<0·04
37. Cornu ammonis, white matter	(1)	0·03
38. Optic nerve	(1)	0·02
39. Album centrale	(1)	0·02
40. Posterior roots	(1)	0·01
41. Cerebrospinal fluid	(2)	0·005

Number of dogs in brackets. When there are several estimations, the mean is given.

* For details see Table 5.

containing predominantly ganglion cells of small diameter and many non-myelinated fibres.

Localization within the hypothalamus. Attempts were made at localizing more precisely the site of the noradrenaline in the hypothalamus itself. The tissue was divided into anterior and posterior, lateral and medial, and dorsal and ventral halves. The medial part contained more noradrenaline (Table 4) but also fewer medullated fibres, so that the concentration in the grey matter of the lateral half may not be lower. Anterior and posterior hypothalamus did not show consistent differences in sympathin content; this is of some interest

TABLE 4. Noradrenaline content (μg/g) of different parts of the dog's hypothalamus

	No. of dogs	Range	Mean
Ventral	4	0·60–1·20	0·95
Dorsal	4	0·12–0·64	0·43
Medial	3	0·8 –1·6	1·12
Lateral	3	0·5 –1·2	0·71
Anterior	3	0·54–1·15	0·86
Posterior	3	0·54–1·50	0·86

TABLE 5. Noradrenaline (μg/g) in different cortical areas of the dog

Area	No. of dogs	Range	Mean
4 (motor)	5	0·10 –0·24	0·18
3 (somaesthetic)	2	0·12 –0·25	0·19
51 (olfactory)	3	0·09 –0·15	0·12
17 (visual)	3	0·025–0·054	0·04
23 and **36*a*** (acoustic)	4	0·04 –0·06	0·05
6 (frontal)	1	—	0·08
24 (ant. cingulate gyrus)	4	0·04 –0·10	0·08
23 and **24** (medial cingulate gyrus)	1	—	0·09
26, 29 and **30** (post. cingulate gyrus)	3	0·06 –0·13	0·09

because of the controversial question whether the anterior hypothalamus is the site of parasympathetic, and the posterior hypothalamus of sympathetic centres. In this particular histochemical aspect at least, the two regions resemble each other. A definite concentration gradient, however, was found when the hypothalamus was divided into dorsal and ventral parts, the latter containing more noradrenaline than the former. Noradrenaline is not present in any large amounts in either lobe of the pituitary. It was not possible to decide whether small concentrations are present, since traces of posterior lobe hormone remained in the noradrenaline strip of the paper and interfered with the assays.

Telencephalon. All cortical areas, including those which project to the hypothalamus, contain very little noradrenaline (Table 5). Some differences between areas do, however, exist as shown by the consistently higher values found in areas **3** and **4** as compared with areas **17**, **20** and **36*a***. Another telencephalic grey mass, the caudate nucleus, contains merely traces of sympathin.

A complication arose in the assay of the amygdaloid nuclei and of the areas of the posterior cingulate gyrus. Slight pressor effects were obtained from extracts of these regions, but, being quite abrupt and short lived, they were different in shape from rises in blood pressure produced by noradrenaline. The responsible substance could not be identified, but the results leave no doubt that whatever amounts of noradrenaline were present must have been very small.

The midbrain. The grey matter around the aqueduct contained more sympathin than the remaining midbrain. Consistent differences were not found between the tegmentum and the region around the fossa interpeduncularis, but the colliculi and basis pedunculi (the latter in concordance with its high content in myelinated fibres) contained less than the tegmentum.

TABLE 6. Noradrenaline (μg/g) in the dog's thalamus

Medial nuclei	Lateral nuclei
0·12	0·03
0·29	<0·12
0·23	0·075
0·40	0·12
0·10	0·036
0·27	0·09 (dorsal part)
	0·10 (ventral part)
Mean 0·234	Mean 0·079

The thalamus. Because of their entirely different function, it seemed important, in the dissection of the thalamus, to separate the medial (and anterior) from the lateral nuclei. Table 6 shows the result of this separation in six dogs. The medial nuclei are invariably much richer in noradrenaline, whereas the lateral nuclei show only a negligible concentration. Since medial and lateral thalamic nuclei do not differ only in their functional connexions, but also in their cell density, which is less in the lateral nuclei, it was necessary to ascertain whether the sympathin content was related to cell density rather than to functional connexions. A further separation was made between the dorsal and ventral parts of the lateral nuclei. There, too, there exists a gradient in cell concentrations, this time in favour of the dorsal nuclei. The content of sympathin, however, was the same, so that there is no reason to assume that mere density of cells explains the higher sympathin content of the medial thalamic nuclei.

The bulb. This part was divided in many different ways in an attempt at localizing its sites of highest concentration of sympathin. The results were disappointing in that differences found in one experiment were difficult to reproduce. It is not likely that the difficulty of repeating exactly the same macroscopic dissection was mainly responsible: substances which interfered with the biological assays were encountered more frequently in this than in other parts of the brain ; they were mostly depressor and, of course, found in

the part of the chromatogram which contains the noradrenaline. It was, however, obvious that the whole floor of the fourth ventricle, cut about 3 mm deep, and the formatio reticularis were rich in sympathin. A single spot of very high concentration was the area postrema, a tissue devoid of ganglion cells and therefore not part of the brain proper. It had to be carefully removed when the sympathin content of the underlying nuclei of the Xth and XIIth nerves was being examined.

Spinal cord. The grey columns of the spinal cord were of some interest in view of the fact that the efferent sympathetic paths end in the lateral horns. Owing to their small width, attempts at dissecting the anterior, lateral and posterior horns separately, met with technical difficulties, particularly when dissection of the lateral horns was being attempted. Some surrounding tissue

TABLE 7. The proportion of adrenaline in hypothalamic sympathin. Normal animals and cats injected with drugs.

			Adrenaline in hypothalamus			
			ng*/g		% of total amines	
Species	Treatment	No. of animals	Range	Mean	Range	Mean
Dogs	None	14	60–240	170	6–25	13·7
Cats	None	7	35–184	94	2·5–11	6·5
Cats	Caffeine	2	80–120	100	5–7·5	6·2
Cats	Ergometrine	7	20–160	85	2–11	6·0
Cats	β-tetrahydro-naphthylamine	4	30– 60	52	4– 9	7·2
Cats	Morphine	6	20– 40	30	3– 6	4·3
Cats	Picrotoxin	3	35– 70	55	5–11	7·0

For doses of drugs refer to Table 9.

* 1 ng = 10^{-9} g.

was invariably included. There is, however, little doubt that the posterior horns contained less sympathin than the remaining spinal grey matter. All figures may have been too low owing to the long duration of the dissection.

Individual differences. Though the pattern of distribution was the same in all dogs, the absolute amount of sympathin varied considerably. Table 6 gives examples for thalamic noradrenaline. Generally speaking, low figures were more frequent among large and among very young animals. The effect of size may be an artifact due to the longer time required for anaesthesia and dissection.

Adrenaline. Table 7 represents the amount of adrenaline found in a series of extracts of hypothalamus. It is expressed in absolute figures and as percentage of the total amines. The percentage for the normal dog varies between 6 and 25% with a mean of 13·7%. A few estimations were made of the adrenaline content of a large number of regions in order to see whether the relative proportion of the two amines varied in different parts of the brain. The total range of the figures lay between 5 and 20 with a mean of 10·3%. These values

are not significantly different from those for the hypothalamus. Thus there does not appear to be a consistent regional difference in the composition of brain sympathin. The adrenaline content of the area postrema and of the cerebrospinal fluid follow the general trend, the adrenaline concentration (as percentages of total amines) amounting to 12·5 and 9·1% respectively.

Species differences. The main features of the distribution of sympathin in the dog's brain were confirmed in the cat. Here the average concentration in the hypothalamus was greater than in the dog (see Table 9, row 1), reaching not infrequently 2 μg/g fresh tissue. A single experiment on the rat's hypothalamus showed that there, too, sympathin was present. The cat's area postrema presented a particular puzzle. Extracts from this region, after elution from the chromatogram, were depressor, the fall in blood pressure

TABLE 8. Peripheral sympathetic nervous system; adrenaline and noradrenaline content (dog)

	Noradrenaline, μg/g			Adrenaline, percentage of total amines		
	No. of estimations	Range	Mean	No. of estimations	Range	Mean
Superior cervical ganglion	10	3·0 –12·0	6·81	9	0·8–7·0	2·6
Stellate ganglion	5	3·8 – 5·5	4·67	3	2·7–3·6	3·1
Nn. accelerantes	3	0·84– 2·4	1·61	1	—	6·7

being usually followed by a rise. The response did not look unlike that of a mixture of histamine and noradrenaline. Since histamine travels to the same part of the chromatogram as noradrenaline, one such extract was tested on the guinea-pig's ileum. No histamine was, however, found, and the nature of the depressor substance remains unknown. It is not substance P, since this travels to a different part of the paper (see Fig. 1).

One last difference between the sympathin in the brain of the cat and the dog is its content of adrenaline. Table 7 gives a survey of the proportion of adrenaline found in hypothalamic sympathin in fourteen normal dogs and seven normal cats. Though there is partial overlap, on the average the percentage adrenaline is higher in the dog.

Peripheral sympathetic system

The high content of sympathin of the sympathetic nerves and ganglia of horses and cattle has been demonstrated by von Euler (1946). A table of figures obtained in the dog with the technique described in this paper is shown (Table 8) in order to serve as comparison with the results on the brain. It will be seen that the sympathetic ganglia of the dog contain about 5 to 6 times more sympathin than the hypothalamus. The average percentage of adrenaline is lower than in the brain (3% of total amines).

Cervical sympathectomies

An experiment was carried out in which the sympathin in the hypothalamus was compared in a normal cat and in two cats in which the superior cervical ganglia had been extirpated and the postganglionic fibres allowed to degenerate. In the operated cats, the concentration of noradrenaline amounted to 1·2 and 1·5 μg/g, while the figure for the control cat was 1·2 μg/g. The amount of adrenaline was also the same (0·14 μg/g) in the operated and normal cats. The hypothalamic sympathin was thus not affected by the cervical sympathectomy.

TABLE 9. Effect of drugs on hypothalamic noradrenaline in cats

Drug	Total dose (mg/kg)	Duration (hr)	No. of cats	Noradrenaline μg/g Range	Noradrenaline μg/g Mean	% of normal
None	—	—	19	0·90–1·92	1·38	—
Choline *p*-tolyl ether bromide (TM 6)	50	5	3	1·44–1·80	1·66	120
Leptazol	50–60	4¾–5½	5	1·20–2·00	1·57	119
Caffeine	90–292	3½–5	5	1·30–1·80	1·52	109
Ephedrine sulphate	35–50	4¾–5½	3	1·20–1·60	1·47	106
Ergometrine maleate	3·5–7	4 –4½	5	0·80–1·90	1·34	97
	12–20	4½–5	7	1·0 –2·0	1·33	96
Apomorphine HCl	75–122	4 –4½	8	0·52–1·76	1·05	76
Ether	—	3¾–5	2	0·8 –1·2	1·00	72·5
Insulin	1·5–1·8*	4¾–5½	7	0·64–1·28	0·92†	66·6
Nicotine bitartrate	12–17	4½–6½	4	0·60–1·20	0·88	63·8
Morphine HCl	30–60	3 –5	11	0·48–1·20	0·78†	56·6
Morphine HCl with TM 6	36 50	4½–4¾	3	0·30–0·96	0·64	46·4
β-Tetrahydro-naphthylamine carbonate	40–60	3½–6	11	0·27–1·12	0·77†	55·8
Picrotoxin	1·0–1·5	4½–4½	2	0·60–0·72	0·66	47·8

Except for ether, all drugs were given by subcutaneous injection. The larger amounts of caffeine, leptazol, apomorphine, nicotine, morphine and β-tetrahydronaphthylamine were given in divided doses at intervals of 2 or 3 hr. The caffeine was injected as the sodium benzoate double salt, but the doses are given as free base.

* i.u./kg.

The means marked † are significantly ($P < 0·05$) different from the control mean of 1·38.

The action of drugs on hypothalamic sympathin

A survey of the results obtained on cats is found in Table 9; Tables 10–12 contain the results on dogs.

In the experiments of Table 9, most drugs were given in doses sufficient to cause signs of central excitation or paralysis. Choline *p*-tolyl ether caused no signs, but leptazol and caffeine were used up to convulsive doses. Ephedrine caused only pupillary dilatation, but ergometrine produced sham rage, and apomorphine excitement, sham rage and sometimes convulsions. Ether was used in a concentration producing light surgical anaesthesia, insulin up to doses leading to convulsions or coma. Nicotine caused sickness in two and convulsions in the other two experiments. Morphine elicited excitement and

often convulsions, β-tetrahydronaphthylamine sham rage and picrotoxin convulsions.

It will be seen from the last column of Table 9 that the drugs listed in the upper part of the table did not change the noradrenaline content of the hypothalamus, whereas those of the lower part of the table reduced it. The experiments with insulin, morphine and β-tetrahydronaphthylamine were done on a sufficient number of cats to obtain mean depressions of noradrenaline which were statistically significant. Ether was only tried on two cats, but there seemed no need to increase the number of ether anaesthesias, since a significant depletion of hypothalamic noradrenaline by ether was seen in four puppies (Table 10). In the experiments with apomorphine, the mean concentration of hypothalamic noradrenaline was not significantly lower than normal. Nevertheless, three cats had noradrenaline concentrations below the smallest ever encountered in the large series of nineteen control cats; it would be unreasonable to assume that these figures did not represent genuine reductions in noradrenaline: the correct conclusion appears to be that apomorphine produces these effects in some cats and not in others.

It was not possible to correlate the fall in sympathin with the severity of the clinical signs, at least not with the occurrence of convulsions. A possible correlation with the secretion of adrenaline will be discussed below.

The reason for including the tolylester of choline (TM 6) in the investigation was the demonstration by Brown & Hey (1952) that it inhibits amine oxidase. Cats given the compound by itself had hypothalamic noradrenaline concentrations lying within the normal limits. Cats given the choline ether, together with morphine, had concentrations similar to those after morphine alone. The choline ether thus did not alter the normal noradrenaline content of the hypothalamus or its reduction by morphine.

Experiments of less than 3 hr duration are not included in the table, because drugs which depleted the hypothalamic noradrenaline when allowed to act for periods of 3 hr or more, rarely did so if the animal died within the first 2 or 2½ hr of the experiment. Thus normal noradrenaline concentrations were found in experiments of short duration with picrotoxin, morphine and β-tetrahydronaphthylamine.

The effect of drugs on the relative proportions of adrenaline and noradrenaline in the hypothalamus is seen in Table 7. Of the five drugs tested, caffeine and ergometrine belong to the group which does not lower the noradrenaline content of the hypothalamus. The absolute and relative concentrations of adrenaline fall completely within the normal range after caffeine and they are not significantly lower after ergometrine. On the other hand, the last three drugs (β-tetrahydronaphthylamine, morphine and picrotoxin), all depleting noradrenaline, produce also a large fall in the absolute amounts of adrenaline. The relative proportion of the two amines, however, remains

within the normal limits. It appears, therefore, that depletion by drugs of hypothalamic sympathin affects adrenaline and noradrenaline alike.

Effect of drugs on parts other than the hypothalamus

Midbrain and pons. A first attempt at discovering whether the drugs which caused a loss in the noradrenaline from the hypothalamus also affected other parts of the brain was made by measuring the noradrenaline in the superficial medial part of the pons. The variation, however, of pontine noradrenaline was

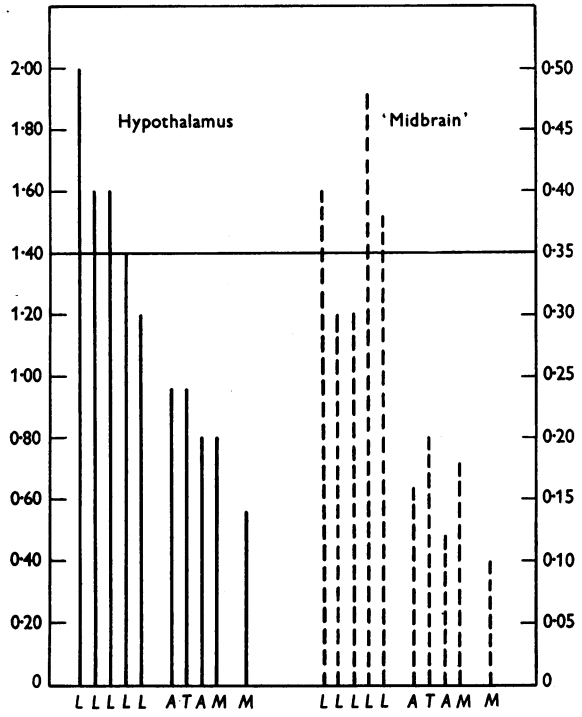

Fig. 3. The left ordinate represents hypothalamic, the right ordinate mesencephalic noradrenaline, both in $\mu g/g$ fresh tissue. Each column in the left part of the figure shows the noradrenaline of the hypothalamus of an individual cat, with the corresponding figure for the midbrain in the right part (broken columns). The cats had been injected with the drug indicated at the bottom. *L*, leptazol; *A*, apomorphine; *T*, β-tetrahydronaphthylamine, *M*, morphine. Doses as in Table 9.

so great that no consistent results were obtained. The midbrain was then used, the colliculi, the basis pedunculi and the brachium colliculi inferioris having been removed because these structures contain very little noradrenaline and were thus decreasing its concentration in the final extracts. Results with this trimmed 'midbrain' were much more consistent, and the results obtained on a group of ten cats are presented in Fig. 3.

The scales of ordinates for the hypothalamus (left) and the midbrain (right) were so chosen, that the horizontal line represents the normal average in μg/g for both tissues. The columns representing the ten noradrenaline estimations in the hypothalamus follow each other in the same order as the columns representing the assays of the corresponding midbrains. The proportionality between noradrenaline concentrations in hypothalamus and midbrain is by no means complete, but it is quite obvious that the first five cats do not show depletion in either part of the brain, also that the next four cats have suffered a moderate loss of noradrenaline in hypothalamus and midbrain, and that the unusually severe depletion of the hypothalamus in the last cat is reflected by an equally severe depletion of the midbrain.

TABLE 10. Two litters of puppies, ether for 3–4 hr; brain noradrenaline

	Normal		Ether		% of normal	
	ng*/g	Total (ng)	ng/g	Total (ng)	Concn.	Total
Area postrema	1000	17·0	1500	16·0	—	—
	880	15·0	1500	14·0	—	—
	900†	11·3	1200†	13·9	—	—
	—	—	1600†	13·9	—	—
Mean	927	14·4	1450	14·45	156·5	100·0
Hypothalamus	850	—	550	—	—	—
	1040	—	680	—	—	—
	800†	—	400†	—	—	—
	—	—	400†	—	—	—
Mean	897	—	507	—	56·6	—
Midbrain	300	—	250	—	—	—
	400	—	300	—	—	—
	380†	—	140†	—	—	—
	—	—	240†	—	—	—
Mean	360	—	233	—	64·7	—

Litter 2 (figures marked by a dagger) was 8, litter 1, 10 weeks old.
* 1 ng = 10^{-9} g.

Area postrema. The experiments had to be done on dogs, since the area postrema of the cat contains substances which interfere with the assay. In order to reduce the error, litters of three or four puppies were used and control and test animals taken from the same litter. Three drugs were tried: ether, morphine and apomorphine. It will be seen that significant depletion of hypothalamic noradrenaline was obtained by prolonged ether anaesthesia (Table 10), but that the noradrenaline content of the area postrema was not diminished. The figures for the latter are given both as concentrations and as total amounts. When dissecting this organ which weighs only 10–20 mg, the inclusion in the portion to be analysed of slightly varying amounts of neighbouring tissue of low noradrenaline content is bound to cause large variations in the apparent concentrations of noradrenaline. It was felt that the absolute figures would be more reliable because the surrounding tissue with its low

noradrenaline concentration would contribute less to the values obtained. Table 10 shows that ether anaesthesia had no effect on the absolute amount of noradrenaline in the area postrema; the concentration appears higher than in the controls, but in view of the unchanged total amounts, this is probably a dissection artifact.

In concordance with the results obtained in the cat, the noradrenaline in the midbrain is reduced along with the noradrenaline in the hypothalamus.

TABLE 11. Three litters of puppies, morphine; brain noradrenaline

	Age of litter (weeks)	Normal		Morphine HCl		% of normal	
		ng*/g	Total (ng)	ng/g	Total (ng)	Concn.	Total
Area postrema	11	1100	11·3	1200	17·3	—	—
	11	—	—	840	10·4	—	—
	10	1200	14·1	900	14·3	—	—
	10	1300	13·4	—	—	—	—
	26	800	20·5	1000	15·3	—	—
	26	1500	21·3	1400	23·5	—	—
	26	—	—	1600	26·0	—	—
Mean	—	1180	16·1	1260	17·8	107	110
Hypothalamus	11	1200	—	480	—	—	—
	11	—	—	470	—	—	—
	10	640	—	440	—	—	—
	10	640	—	720	—	—	—
	26	900	—	850	—	—	—
	26	960	—	720	—	—	—
	26	—	—	720	—	—	—
Mean	—	868	—	630	—	72·7	—

Each dog was injected with 40 mg/kg morphine HCl, and 2–3 hr later with another 13–20 mg/kg. The dogs were killed approximately 5 hr after the first injection. Litter-mates are entered in the same horizontal line.

* 1 ng = 10^{-9} g.

Table 11 summarizes the results of similar experiments carried out on three litters of puppies injected with morphine. It shows, first, that, in the dog, there was only a small and statistically non-significant ($P = 0{\cdot}1$) depletion of hypothalamic sympathin after morphine. In the cat, the same drug was seen to produce greater, and significant, depletions. It is tempting to correlate this difference with the clinical effect of morphine on the two species: cats get excited whereas dogs fall asleep, only occasionally to be roused for short periods. An experiment on a fourth litter of three puppies, not included in the table, is suggestive on the same lines. Two of these dogs had three injections of morphine HCl; they slept for 2 hr after the first injection of 30 mg/kg but were rather excited and yapped a great deal during the remaining 3 hr of the experiment in spite of a further two injections of 20 mg/kg each. The hypothalamic noradrenaline amounted to 480 ng/g in both animals, and this figure appears particularly low in view of the fact that the third litter-mate used as control had the very high noradrenaline concentration of 1920 ng/g. The dogs

entered in Table 11, on the other hand, were all asleep for most of the time of the experiment.

With regard to the area postrema, it is obvious from Table 11 that there was no depletion of noradrenaline after the injection of morphine.

Because of the suspected relation (see below) of the area postrema with the initiation of the vomiting reflex, an experiment was carried out on two litters of puppies in which the effect of apomorphine on this region was studied. There was no change in the noradrenaline content of the area postrema (Table 12). Neither was the hypothalamic noradrenaline depressed. It will be remembered that, in the cat, depletion had occurred in some of the experiments. The signs of excitement in the dog after apomorphine are less than in the cat, and the two facts may be related.

TABLE 12. Puppies, apomorphine; brain noradrenaline

		Normal		Apomorphine		% of normal	
	Litter	ng*/g	Total (ng)	ng/g	Total (ng)	Concn.	Total
Area postrema	*a*	600	9·2	300†	3·9†	—	—
	a	800	15·7	1300	19·2	—	—
	a	—	—	500	7·2	—	—
	b	500	6·3	400	5·7	—	—
	b	—	—	400	7·3	—	—
	b	—	—	740	16·9	—	—
Mean		633	10·4	670	10·0	106	96
Hypothalamus	*a*	800	—	800†	—	—	—
	a	800	—	800	—	—	—
	a	—	—	800	—	—	—
	b	640	—	640	—	—	—
	b	—	—	640	—	—	—
	b	—	—	560	—	—	—
Mean		747		707		95	

Litter '*a*' 3, litter '*b*' 2 months old.

Except for the dog marked with a dagger, which died 45 min after a single injection of 80 mg/kg, all dogs had a total dose of 43–50 mg/kg apomorphine HCl in two injections with an interval of 3 hr. The dogs were killed 4½–5 hr after the first injection.

* 1 ng = 10^{-9} g.

Depletion of hypothalamic noradrenaline and secretion from the adrenal medulla

When looking for a property common to the drugs which decrease the sympathin content of the brain, one is struck by the preponderance among such drugs of substances which cause secretion by the adrenal medulla. Examples are ether, insulin, nicotine, morphine and β-tetrahydronaphthylamine. Caffeine and ephedrine, inactive on brain sympathin, do not provoke adreno-medullary secretion. It was not known whether medullary secretion is caused by picrotoxin which lowers the concentration of brain sympathin or by ergometrine which does not lower it. Two sets of experiments, one on rats and one on cats, were carried out in order to see whether a drug which

causes loss of hypothalamic sympathin invariably elicits secretion from the adrenal medulla.

Rats. Five groups of rats were unilaterally adrenalectomized. Four groups were given subcutaneous injections of drugs and killed between 4 and 5 hr later; one group was kept as controls. Adrenal extracts were made and their content of adrenaline assayed on the rat's blood pressure.

Table 13 summarizes the results. Whereas morphine, in a dose used in the experiments on cats (60 mg/kg in divided doses), caused a loss of adrenaline, ergometrine, even in doses of 30 mg/kg (1·5–8 times the doses used in cats), did not lower the adrenaline content of the rat adrenals. Yet the rats showed exophthalmos and a ruffled coat, and were thus undoubtedly affected by the ergometrine. In a first experiment with picrotoxin, the dose used in cats (1·2 mg/kg) was injected: no effect on adrenaline secretion was seen, nor did

TABLE 13. Unilaterally adrenalectomized rats. Action of drugs on adrenaline content of the adrenal medulla.

Drug	Dose (mg/kg)	No. of rats	μg adrenaline per 100 g body weight		
			Range	Mean	% of normal
None	—	4	5·0–7·4	6·2	—
Ergometrine maleate	30	4	3·6–7·6	6·2	100
Morphine HCl	60	4	2·4–5·2	3·9	64
Picrotoxin	1·2	4	6·5–9·1	7·8	126
Picrotoxin	5·0	2	2·5–4·3	3·4	55

the rats show more than a slight tremor after the injections. Since, however, convulsive doses had been used in the cats, the amount of picrotoxin injected into a second group of rats was increased till convulsions were produced (5 mg/kg in 4 doses). This caused adrenaline secretion. It appeared, therefore, that ergometrine, which does not lower the sympathin concentration in the hypothalamus, does not cause adreno-medullary secretion, whereas picrotoxin, provided it is given in convulsive doses, produces both these effects.

Cats. The experiments were carried out on cats in which the left adrenal had been denervated. If a drug produced a release of adrenaline from the adrenal medulla by causing discharge through the splanchnic nerves, the innervated adrenal would contain less medullary hormone than the denervated gland which would act as control. If depletion of hypothalamic sympathin was also produced, it could be observed in the same cat. This procedure would allow a decision whether a correlation existed between action on the hypothalamus and on (centrally elicited) adrenal secretion even with drugs which, like apomorphine, did not have the same effect in all animals.

Fig. 4 illustrates the result of these experiments. The black columns represent noradrenaline in the hypothalamus as percentage of the figure of 1·38 μg/g, which is the mean of nineteen normal cats. The speckled columns indicate

total amines in the innervated adrenal medulla, expressed as percentage of the content of the contralateral denervated gland.

It will be seen that, in the first five cats, the hypothalamic noradrenaline did not fall below a value of 75% of the normal; in the same animals, the innervated adrenal still contained at least 80% of its amines. In the next five cats, losses in hypothalamic noradrenaline of between 31 and 59%, and of medullary amines of between 32 and 80% occurred. In the last cat, both the figures for hypothalamic noradrenaline and for medullary amines are the lowest found in this series. There is, thus, in the same animal, a correlation between the power of a drug to cause loss of noradrenaline from the hypothalamus and loss of amines from the innervated adrenal medulla.

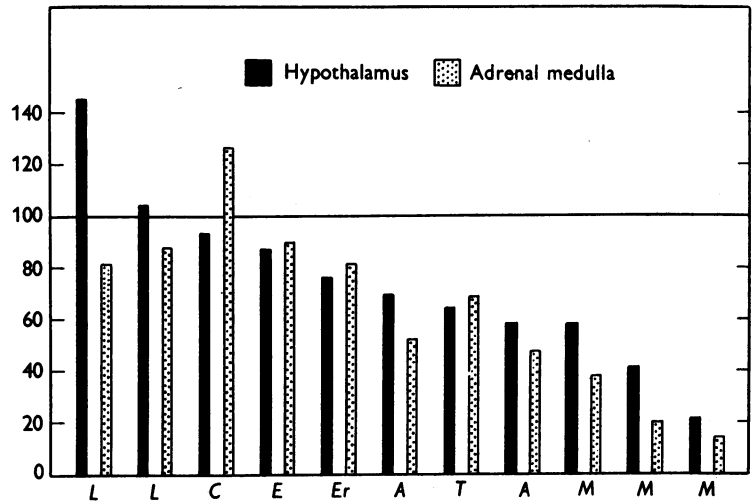

Fig. 4. Black columns: hypothalamic noradrenaline of individual cats injected with the drug indicated at the foot of the column; it is expressed as percentage of the mean of 1·38 μg/g determined for nineteen normal cats. Dotted columns: total amines of the innervated adrenal medulla of the same cats, represented as a percentage of the amines of the contralateral denervated adrenal. *L*, leptazol; *C*, caffeine; *E*, ether; *Er*, ergometrine; *A*, apomorphine; *T*, β-tetrahydronaphthylamine; *M*, morphine. Doses as in Table 9.

Whereas depletion of brain sympathin by drugs was not observed unless there was also medullary secretion from an innervated adrenal, that secretion was not the cause of the loss in sympathin. This was demonstrated in two cats injected with morphine (30 mg/kg) in which medullary secretion was prevented by previous bilateral adrenal denervation. In these cats, the noradrenaline of the hypothalamus was 38%, and that of the midbrain 34% of the normal figure; rather than being abolished, the depletion by morphine was thus larger than in the majority of intact animals.

Although no drugs were found which would produce a fall in hypothalamic

sympathin without an accompanying loss in medullary amines, in two experiments lethal doses of ergometrine caused falls in adrenal medullary amines of 54 and 69%, whilst the concentration of hypothalamic noradrenaline remained normal. As the two cats showed signs of severe asphyxia before they died, the question arose whether asphyxia causes adreno-medullary secretion without producing any effect on hypothalamic sympathin.

Four cats in which the left adrenal had been denervated 2 weeks previously were anaesthetized by intravenous pentobarbitone, care being taken not to give a dose which would depress the respiration. The trachea was cannulated and pieces of rubber tubing of variable lengths attached to the cannula. The cat was rebreathing through the rubber tubing, and this produced a controllable degree of asphyxia. Provided the duration of the experiment did not exceed 2½ hr, effects of the anaesthetic on brain sympathin could be trusted to be negligible (see p. 464).

TABLE 14. Cats, left adrenal denervated, pentobarbitone sodium anaesthesia. Effect of asphyxia caused by rebreathing on noradrenaline in hypothalamus and on difference in amine content of innervated and denervated adrenal medulla.

Cat no.	Rebreathing (min)	Hypothalamic noradrenaline (μg/g)	Hypothalamic noradrenaline % of normal	Adrenal medulla, total amines in innervated gland (% of denervated gland)
1	85	2·00	145	81
2	100	1·20	87	69*
3	140	1·12	81	75
4	100, died	0·74	54	62

* This figure is based on noradrenaline estimations only.

The experiments are listed in Table 14 in order of increasing duration or severity of the asphyxia. The greatest loss of medullary amines achieved in this series was that of 38% in Expt. 4, and this was accompanied by a significant fall in brain sympathin. Thus there was no instance, as in the experiments with ergometrine, of severe depletion of the stores of medullary hormones along with normal brain-noradrenaline. It might be argued that Expts. 2 and 3 show a greater loss of amines from the adrenal medulla than from the hypothalamus, but the normal range of the hypothalamic noradrenaline is so wide that one cannot be certain that the figures of 1·2 and 1·1 μg/g do not represent some depletion.

It has been stated (Brauner, Brücke, Kaindl & Neumayr, 1951) that central stimulation causes a preferential release of adrenaline from the adrenal medulla. In view of the fact that the difference in amine content between the innervated and the denervated adrenal measures a purely central effect, it was of some interest to see whether the drugs, when stimulating secretion on the innervated side, had caused any change in the ratio adrenaline to noradrenaline. Table 15 shows that in four of the last six experiments, in which secretion had been vigorous, both amines shared equally in the loss. In

Expts. 7 and 9, less noradrenaline than adrenaline had been lost, but this was not a regular occurrence and not typical for a particular drug. Unless it is assumed that the noradrenaline is being methylated immediately before it is secreted, this finding would suggest that stimulation of the adrenal gland by drugs acting centrally does not, as a rule, lead to preferential discharge of adrenaline.

TABLE 15. Action of drugs on the ratio between adrenaline and noradrenaline in the adrenal medulla

No. of expt.	Drug	Total amines in innervated gland (% of denervated gland)	Adrenaline (as percentage of total amines)	
			Denervated side	Innervated side
1	Leptazol	81	77·0	71·5
2	Leptazol	87·5	75·0	57·0
3	Caffeine	126	68·5	58·3
4	Ether	89	68·5	72·3
5	Ergometrine	81	70·2	65·2
6	Apomorphine	51	69·5	67·5
7	β-tetrahydronaphthylamine	68·5	62·9	50·0
8	Apomorphine	47·5	62·5	63·0
9	Morphine	38	42·3	15·1
10	Morphine	20	64·0	65·0
11	Morphine	14	66·7	74·0

Doses of drugs as in Table 9.

Stimulation of the cervical sympathetic chain

The preceding observations suggest that loss of sympathin may be incurred by the hypothalamus under conditions which cause its stimulation over a prolonged period of time. An attempt was made at finding out whether the sympathin concentration in a peripheral sympathetic ganglion would be affected by electrical stimulation of the preganglionic fibres. Both cervical sympathetic nerves were cut in the necks of three dogs, and the central end of one nerve stimulated for 2 hr in two dogs and for 2½ hr in the third. Stimulation was interrupted for 2 min after every 5 min. Pupil, eyelid, and nictitating membrane showed good responses throughout the experiment. No significant differences between the noradrenaline concentrations of the stimulated and of the control ganglion were observed.

DISCUSSION

Relation between brain sympathin and vasomotor nerves

For the interpretation of this work it is essential to be certain whether the foregoing results provide good evidence that brain sympathin is not exclusively localized in the vasomotor fibres. A first consideration deals with the quantitative aspect of the question. Hypothalamic noradrenaline in the dog has an average concentration of 1 μg/g of fresh tissue; pure peripheral postganglionic fibres (Table 8) were shown to reach concentrations about twice and sympathetic ganglia concentrations averaging 6 times that figure. Though

the hypothalamus is known to be a vascular region, it is hardly conceivable that one-half or one-sixth of its weight is made up of vasomotor fibres, particularly in view of the fact that the pia mater which contains the majority of the vessels is dissected away before assaying the sympathin. A second point concerns the attempt at removing the sympathetic supply to the cerebral vessels by extirpating the superior cervical ganglia and allowing time for degeneration of the postganglionic fibres. It has been shown (Cannon & Lissák, 1939; von Euler, 1946) that degeneration of postganglionic sympathetic fibres to an organ is followed by a substantial loss in sympathin; yet cervical sympathectomy did not affect the concentration of brain sympathin (p. 463). The value of such experiments is, however, invalidated by observations made by Chorobski & Penfield (1932), which suggest that the sympathetic supply to the cerebral vessels does not undergo complete degeneration after extirpation of the superior cervical ganglia. This is due to the existence, inside the skull, of ganglion cell stations which are inaccessible to experimental removal. Even so it would seem likely that a significant fall in hypothalamic sympathin would have been detectable after removal of the superior cervical ganglia if the vasomotor nerves were the only structures containing sympathin. Finally, the view that hypothalamic sympathin is not accounted for by the amine content of its vasomotor nerves, is supported by the observation that both the cerebral and the cerebellar cortex, which are very vascular, contain no more than 10% of the sympathin encountered in the hypothalamus. It is, however, arguable that the sympathin in the cerebral and cerebellar cortex is entirely contributed by the vasomotor fibres to these areas.

Correlation between sympathin content and central representation of sympathetic functions

With exception of the area postrema, those regions which contain the highest concentrations of sympathin—the hypothalamus, parts of the midbrain and the floor of the fourth ventricle—also contain the central representation of sympathetic activity.

Kabat, Magoun & Ranson (1935) and Ranson & Magoun (1939) mapped out the distribution of points in the brain stem from which pressor responses to electrical stimuli are elicited; the points all lie in regions with a high content of sympathin. In addition to pressor effects, it is possible to elicit from the hypothalamus practically all responses mediated by the peripheral sympathetic nerves. Karplus & Kreidl (1909, 1918) showed that hypothalamic stimulation caused dilatation of the pupil and the palpebral fissure, retraction of the nictitating membrane, and sweating. Adrenaline secretion was demonstrated by Houssay & Molinelli (1925), piloerection and inhibition of peristalsis by Kabat, Anson, Magoun & Ranson (1935), increased heart rate by Beattie, Brow & Long (1930), glycosuria by Himwich & Keller (1930), relaxation of the

bladder by Uvnäs (1947) and dilatation of skeletal blood vessels by Eliasson, Folkow, Lindgren & Uvnäs (1951). Many of these effects have subsidiary representation or pathways in the midbrain and in the floor of the 4th ventricle. Sympathin is found in gradually diminishing concentrations along these descending pathways and may be followed into the grey matter of the spinal cord.

The hypothalamic nuclei do not only connect with lower parts of the cerebro-spinal axis but also with higher centres. It is of some interest to consider the sympathin content of those higher centres which are known to give origin to fibre tracts leading to or from the hypothalamus. These are, first, the anterior and medial thalamic nuclei, considered to be relay centres in the transmission of hypothalamic stimuli to cortical areas. It was shown (Table 6 and Fig. 2) that this part of the thalamus has a sympathin content approximating that of the floor of the fourth ventricle, and four times as high as that of the lateral thalamic nuclei which lack hypothalamic connexions. This concentration of sympathin is not just a function of cell density in the thalamus. It is true that the medial thalamus is more cellular than the lateral thalamus, but there is no difference in sympathin concentration between the dorsal and the ventral parts of the lateral thalamus, though the dorsal part has the greater cell density.

Secondly, there are cortical areas, especially the frontal area **6** and the areae of the cingulate gyrus, which project into the medial and anterior thalamic nuclei and are also reputed to have direct fibre connexions with the hypothalamus (Le Gros Clark & Meyer, 1950; Ward & McCulloch, 1947). These areas do not, however, contain more than the minimal amounts of sympathin characteristic of all parts of the cerebral cortex. The same holds for the amygdaloid nuclei which are credited with a close relation to the hypothalamus on account of the observation that their destruction leads to the release of hypothalamic sham rage (Bard & Mountcastle, 1948). Low concentrations are also found in the hippocampus which is connected by fibres of the fornix system to the mamillary bodies. Generally speaking, no part of the telencephalon was found to contain more than a minimal quantity of sympathin. The medial thalamic nuclei are the highest parts of the cerebro-spinal axis to show a relatively large amount of brain sympathin.

The action of drugs on brain sympathin

Attempts at influencing the sympathin concentration in the hypothalamus by means of drugs have shown that prolonged action of some drugs may cause a fall in the concentration of sympathin. A drug producing such a fall need not have a convulsive action, as demonstrated by the fact that ether causes, whereas leptazol fails to cause, a loss in hypothalamic sympathin. When simultaneous measurements were made of the ability of a drug to deplete the

level of hypothalamic sympathin and to stimulate the sympathetic centres which control adreno-medullary secretion by way of impulses through the splanchnic nerves, the two actions were found to be correlated. More particularly, when loss of sympathin had occurred from drug action (or from prolonged asphyxia), the innervated adrenal had invariably lost a large proportion of its stores of hormones. Contrariwise, centrally evoked secretion of the adrenal gland was occasionally found unaccompanied by loss of hypothalamic sympathin. It is suggested that it is the stimulation of the diencephalic and mesencephalic sympathetic centres produced by the drug which leads to the loss of sympathin from these regions. If this interpretation is correct, it provides strong support for the view that brain sympathin plays a functional role in the activity of those regions in which its concentration is high. It explains the fact that a great variety of drugs which seem otherwise to have little in common—like ether, insulin, and morphine—produce the same effect on hypothalamic sympathin because they all stimulate the sympathetic centres, as shown by the secretion of adreno-medullary hormones. It may be noted that in the experiments on morphine poisoning, depletion of hypothalamic sympathin was found to be most severe in those cats in which pupillary dilatation had been most conspicuous and thus presumably the level of blood adrenaline particularly high.

The influence of drugs on mesencephalic sympathin resembled that on hypothalamic sympathin. It would have been interesting to carry out, in parallel with the observations on the midbrain, measurements of drug action on the sympathin in the medulla oblongata, but this was prevented by insuperable technical difficulties.

There is, as yet, no indication as to where the sympathin is lost to when drugs cause a decrease in its concentration. If sympathin plays a part in the metabolism of the active cells, it may be that its depletion indicates a lag of resynthesis behind utilization when excessive demands are made by the stimulated tissue. Such a view is supported by the observation that a drug action of several hours' duration is usually required for any significant change in the concentration of hypothalamic sympathin to occur.

The finding of a high concentration of sympathin in the area postrema was a surprise. This organ, known to anatomists by its unusual permeability to dyes (Wislocki & Putnam, 1924), has recently been credited with the role of a chemoreceptor for drugs, which, like apomorphine and oubain, cause vomiting through stimulation of the bulb (Wang & Borison, 1950; Borison & Brizzee, 1951). In the cat and the dog, this tissue is devoid of ganglion cells. Considering the small amount of tissue available for investigation, technical errors are liable to be more serious in observations on the area postrema than in those on the hypothalamus. Great changes in sympathin content, however, would not have escaped detection, yet neither ether, while causing depletion

of hypothalamic sympathin, nor apomorphine, presumed to stimulate certain receptors in the area postrema, had any demonstrable effect on its sympathin content. Whatever the function of sympathin in this non-nervous tissue may be, its concentration appears not to be affected by drugs depleting hypothalamic sympathin. In this connexion the observations by Bülbring, Philpot & Bosanquet (1953), showing variable, and often high, concentrations of noradrenaline in gliomas may be recalled. Since, like the gliomas, the area postrema contains astrocytes and astroblasts, the occurrence of noradrenaline in the two tissues may be related to the metabolism of a tissue element common to both.

Comparison with other substances

It is of interest to compare the distribution of sympathin with that of other substances occurring in the central nervous system. Raab's encephalin, described as a sympathomimetic amine differing in its chemical and biological properties from all known catechol derivatives, bears no resemblance to sympathin in its distribution (Raab & Gigee, 1951): high concentrations are reported in the cortex, the basal ganglia, white matter and cerebrospinal fluid, regions containing only minimal amounts of sympathin. Among other substances for which distribution patterns have been described, some are compounds, the concentration of which appears to be correlated with the cell density of the region. Certain enzymes belong to this group: acetyl phosphatase, carbonic anhydrase, and succinic dehydrogenase (Ashby, Garzoli & Schuster, 1952; Burgen & Chipman, 1951). Their action may be related to ganglion cell metabolism in general. Phosphamidase, on the other hand, when examined histochemically, follows the distribution of myelinated fibres (Sinden & Scharrer, 1949; Scharrer, personal communication). Provided the reaction really indicates the presence of enzyme in the fibres, this might be an example of a substance required for the special metabolism of fibre tracts.

The distribution of amine oxidase is different again; its activity is fairly homogeneous throughout the brain, and certainly does not follow the density in ganglion cells (Thompson & Tickner, unpublished, see Bülbring *et al.* 1953). Neither does amine oxidase occur only in regions where sympathin is abundant, so that its distribution does not suggest that its main role might be the destruction of sympathin. The observation that poisoning of amine oxidase with the *p*-tolyl-ether of choline had no effect on either the normal level of hypothalamic noradrenaline or on its depletion by morphine, also fails to demonstrate any action of amine oxidase on brain sympathin.

Of all substances with a characteristic pattern of distribution, those exhibiting large differences between maximal and minimal concentrations are by far the most interesting. It is this group which one would expect to play a part in the specialized function of those regions which contain them in unusually high concentrations. It comprises acetylcholine and the enzymes

concerned with acetylcholine formation and hydrolysis (Nachmansohn, 1939; MacIntosh, 1941; Feldberg & Vogt, 1948), histamine, reported to be concentrated in the tuber cinereum (Harris, *et al.* 1952), substance P (Pernow, 1953; Amin *et al.* 1953), and 5-hydroxytryptamine (Amin *et al.* 1953). In contrast to sympathin, substance P and acetylcholine both occur in the caudate nuclei and in medullated fibre tracts, though they are apparently never found together in the same tract. In contrast, 5-hydroxytryptamine, like sympathin, is not found in medullated fibres. Its distribution closely mimics that of sympathin (Amin *et al.* 1953). However, this rule has a notable exception in the peripheral sympathetic system, which contains amounts of sympathin several times as high as the hypothalamus, whereas it is completely lacking in 5-hydroxytryptamine (Amin & Gaddum, unpublished). One further fact is worth emphasizing: sympathin, histamine, substance P and 5-hydroxytryptamine have their highest intracerebral concentration in the hypothalamus; this region also contains acetylcholine and vasopressin. From the point of view of the manufacture of pharmacologically active compounds, it is the most versatile part of the central nervous system so far recognized.

The function of cerebral sympathin

The foregoing experiments did not answer the question of the function of sympathin in the central nervous system. They have, however, by demonstrating the uneven distribution of sympathin, its predilection for certain centres, particularly the central representation of the sympathetic system, and its depletion when these very centres were stimulated by drugs, made a case for a specialized functional role of sympathin in the brain distinct from its ubiquitous action at sympathetic vasomotor endings. It might be tempting to assign to the cerebral sympathin a transmitter role like that which we assign to the sympathin found in the sympathetic ganglia and their postganglionic fibres. There are, however, a number of facts which call for caution: The occurrence of sympathin in gliomas (Bülbring *et al.* 1953), and its high concentration in the area postrema which is devoid of ganglion cells, show that synthesis of noradrenaline may occur in tissue which plays no part in the transmission of impulses. Further, the depletion of hypothalamic sympathin when the diencephalic representation of adrenomedullary secretion was stimulated by drugs has no known counterpart in the peripheral sympathetic system: prolonged electrical stimulation of preganglionic nerves did not cause a fall in the sympathin content of the stimulated ganglia. It is, of course, possible that in the brain the processes of resynthesis are less efficient in keeping up with utilization than they are in the periphery, but we have at present no evidence that this is so.

Finally, if sympathin were concerned in the transmission of impulses in the diencephalic sympathetic centres, signs of stimulation of these centres would

be expected to follow parenteral administration of sympathin. After the injection of adrenaline both excitation and anaesthesia have been reported, and modifications of the electroencephalogram have been recorded in the posterior hypothalamus and other parts of the brain (Porter, 1952). None of these observations distinguish clearly between secondary effects due to vasomotor reactions and direct effects of the amines; neither do any of the observations throw emphasis on the sympathetic centres; in fact, the somatic sensory areas of the cortex, for example, were also stimulated in the experiments of Porter and other investigators. Even less is known about central actions of noradrenaline, except that it appears less active than the methylated compound (Goldenberg, 1951). It is possible to interpret these failures by assuming that parenterally injected sympathomimetic amines do not penetrate to the sites at which they are normally produced. This view would agree with the observations by Leimdorfer, Arana & Hack (1947), and Leimdorfer & Metzner (1949), who obtained surgical anaesthesia and rises in blood sugar by intrathecal adrenaline administration. The interpretation of such experiments is not unequivocal, but it would appear that a central action was responsible for the effects. Noradrenaline, though less active, produced qualitatively the same results (Leimdorfer, 1949).

A second hypothesis concerning the role of cerebral sympathin is that it may modify transmission by other substances, for instance acetylcholine. This possibility is considered by Duke & Pickford (1951) in their work on the interference of adrenaline with the stimulation of the supraoptic nuclei by acetylcholine. A modifying influence of adrenaline on transmission at spinal cholinergic synapses has been convincingly demonstrated (Bülbring & Burn, 1941; Stavraky, 1947). Little is known of the role of noradrenaline in this respect, a great drawback unless one is prepared to consider noradrenaline as a mere precursor of the 'fully active' methylated compound.

The distribution of sympathin in the brain has also to be considered in relation to the theory that either adrenaline or noradrenaline (or both) might act as humoral transmitters between the hypothalamus and the anterior lobe of the pituitary (Sawyer, Markee & Everett, 1950). The high concentration of sympathin in the hypothalamus appears at first sight to support this theory. When, however, it is remembered that fairly large amounts of sympathin are also found in the midbrain which sustains no functional connexions with the anterior lobe, there seems to be little justification for singling out the hypothalamic sympathin as a humoral stimulus for the secretion of trophic hormones and to attribute a totally different role to the mesencephalic sympathin. This would seem particularly unreasonable in view of the fact that certain drugs deplete the hypothalamic and mesencephalic sympathin simultaneously. Nevertheless, the findings are compatible with the view of an adrenergic activator of anterior lobe secretion.

SUMMARY

1. A method is described which permits the estimation of adrenaline and noradrenaline in small pieces of brain tissue.

2. Noradrenaline with an admixture of adrenaline, described as 'sympathin', was identified in the brain by a number of biological and chemical tests. It was found in all parts of the central nervous system, but its distribution was quite uneven, some parts containing at least 20 times as much as others.

3. The average percentage of adrenaline in sympathin was 14 in the dog and 7 in the cat.

4. A detailed map of the distribution of noradrenaline is given for the dog's brain.

5. The highest concentrations of sympathin are in the regions which contain the diencephalic, mesencephalic and bulbar representations of sympathetic activities. There was also a high concentration in the area postrema, which is not nervous tissue proper.

6. The average amount of hypothalamic noradrenaline was 1 μg/g fresh tissue in the normal dog and 1·4 μg/g in the normal cat.

7. Some drugs, when acting over a period of at least 3 hr, caused a fall in the concentration of the cat's hypothalamic sympathin. Such a fall was not found unless the drugs had also produced a stimulation of adreno-medullary secretion by their central action. The reverse does not hold; there may occasionally be adrenal secretion without depletion of hypothalamic sympathin. Asphyxia, too, lowered hypothalamic sympathin provided it had been sufficiently severe to cause considerable medullary secretion. Preventing medullary secretion by adrenal denervation did not interfere with the depleting action of drugs on the sympathin in the hypothalmus.

8. When a drug lowers the concentration of noradrenaline in the hypothalamus, it also lowers it in the midbrain.

9. In the dog, ether reduced the concentration of noradrenaline in hypothalamus and midbrain, but neither ether, nor morphine or apomorphine, changed the noradrenaline content of the area postrema.

10. When drugs caused the secretion of adrenaline by way of central stimulation, the relative proportions of adrenaline and noradrenaline in the depleted gland were usually the same as in the contralateral resting (denervated) gland.

11. The amount of sympathin in the hypothalamus was not affected by cervical sympathectomy carried out 18 days before the assay.

12. A substance which inhibits the action of amine oxidase (*p*-tolyl-choline ether) did not affect the normal level of hypothalamic noradrenaline nor its depletion by morphine.

13. The results are compared with our knowledge of the distribution of other pharmacologically active substances in the brain. Different hypotheses concerning the function of brain sympathin are discussed.

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