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VASOPRESSOR, ANTIDIURETIC, AND OXYTOCIC ACTIVITIES OF EXTRACTS OF THE DOG'S HYPOTHALAMUS

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Abel (1924) has shown that pressor and oxytocic activities are present in extracts of the hypothalamus of the dog after careful removal of the pituitary stalk and median eminence. He had no doubt that he was dealing with the same substance which he had described in extracts of the posterior lobe. Though similar results were obtained by Trendelenburg (1928) and Sato (1928), later workers came to doubt the accuracy of these findings (Rioch, 1938), till the reinvestigation of this problem by Bargmann and his school confirmed and extended the old observations (Bargmann, 1949; Hild and Zetler, 1951; Hild, 1951).

In the course of some work on the distribution of sympathin in the brain of the dog, acid alcoholic extracts of the hypothalamus were submitted to paper chromatography in phenol HCl, and eluates of the different regions of the chromatogram tested biologically. When eluting a strip adjacent to but not including the solvent front of the paper, a strong pressor activity of the eluate was noticed when the extracts had been prepared from hypothalamus, whereas this activity was lacking in extracts of other parts of the central nervous system. The position on the chromatogram excluded noradrenaline and adrenaline as substances responsible for the effect, and it was possible that it was due to a vasopressin-like substance. Abel (1924) had shown that posterior lobe hormones are soluble in 95% alcohol, so that the unorthodox method of extraction did not preclude this possibility. The paper deals with the properties of these hypothalamic extracts.

METHODS

Dogs and cats were anaesthetized with chloroform and bled to death.

Preparation of Extracts.—0.1–0.25 g. brain tissue, which had been stripped of its pia mater, was homogenized in a glass grinder containing 1 ml. chilled absolute ethanol acidified with HCl (1 ml. conc. HCl per l. ethanol). The homogenate was transferred to a centrifuge tube with more (3.5 ml.) acid ethanol, chilled and spun; the supernatant was poured into a wide tube, and the residue washed with 1 ml. acid ethanol and spun. The combined supernatants were evaporated *in vacuo* (bath temperature 50° C.), the residue detached from the tube and transferred into a microtube with the aid of 0.5 ml. H_2O and four portions of 0.25 ml. 0.001 N HCl. The aqueous suspension was chilled and centrifuged; the supernatant was poured off and evaporated *in vacuo*. The new residue was detached from the tube with 0.5 ml. ethanol-acetone mixture 1:1, centrifuged, and the supernatant applied to paper for chromatography. The residue was treated with a further 0.25 ml. of acetone-alcohol mixture and centrifuged, and this supernatant was also applied to the paper. The chromatography, elution, and preparation of extracts for biological assay is described in a previous paper (Vogt, 1952a). The purpose of this procedure is to separate sympathomimetic amines from other constituents of the brain tissue and is not intended for quantitative extraction of posterior lobe principles.

Assays.—Commercial posterior lobe extract (Parke, Davis) served as a standard. All eluates to be assayed were prepared from the top 5 cm. of the chromatograms (excluding the actual front).

Pressor activity was assayed on the blood pressure of the hexamethonium-treated rat as described by Outschoorn (1952).

Oxytocic activity was assayed on the rat's uterus. The organ was suspended at 30° C. in a modified Ringer's solution containing only 0.006% $CaCl_2$ (see Gaddum, Peart, and Vogt, 1949) in order to suppress the spontaneous activity.

Antidiuretic activity was assayed by intravenous injection either into the trained, conscious, hydrated dog, or into rats anaesthetized with 10% ethanol following the procedure of Ames and van Dyke (1952). Comparison was always made with posterior lobe extract injected intravenously during the course of the same experiment.

The *inactivation* of posterior lobe activity was carried out by adding N-sodium thioglycollate, freshly prepared from the acid with H_2O and $NaHCO_3$, in a proportion of 1:100 to the extracts, adjusting the *p*H to not less than 7.4 and allowing the mixture to stand at room temperature for 20–40 min.

The 5-hydroxytryptamine salt used was the creatinine sulphate, the strength being stated in terms of the base.

Results

Several observations were made which demonstrated that the pressor substance in hypothalamic extracts did, in fact, greatly resemble posterior lobe hormone.

1. The extracts showed the same biological activity when their pressor and when their antidiuretic potencies were assayed against commercial pituitrin.

2. Dog posterior lobe tissue was extracted, chromatographed, and eluted in exactly the same manner as hypothalamic tissue. The biological activity was recovered from the same strip of the chromatogram (next to the solvent front) which had contained the activity in hypothalamic extracts.

3. Incubation, at room temperature and for periods of about 30 minutes, of hypothalamic extracts with 0.01 M sodium thioglycollate at *p*H 7.4 completely destroyed their pressor, antidiuretic, and oxytocic activity. This test is considered by van Dyke, Chow, Greep, and Rothen (1942) to be specific for posterior lobe hormone.

There was, however, one point in which hypothalamic extracts differed from posterior lobe extracts, and that was their oxytocic activity. Table I shows a series of parallel assays of the different biological activities of hypothalamic extracts in terms of international units of posterior lobe powder. It will be seen that, though agreement between pressor and antidiuretic activity is satisfactory, the oxytocic activity is invariably far below the expected value, the ratio, pressor over oxytocic activity (" P/O "), having a mean of 14.6.

TABLE I

EXTRACTS OF HYPOTHALAMUS OF THE DOG

Activity in m.u./g. fresh tissue

Part of Hypothalamus Extracted	Pressor	Anti-diuretic	Oxytocic	P/O	Age
Whole	1,040		150	7	Adult
Ventral	4,800		120	40	Adult
	4,000	5,000	240	17	Adult
Ventral, no median eminence	2,400	2,400	200	12	Adult
	4,000		240	17	Adult
	800		80	10	10–11 weeks
	300*		30	10	10–11 weeks
	520		40	13	10–11 weeks
	320		16	20	10–11 weeks
	400*		24	17	10–11 weeks
				M=14·6	

* Injected with morphine hydrochloride 5 and 2 hr. before bleeding.
M = Geometric mean.

The possibility was then investigated that the oxytocic assays gave too low values owing to inhibitory substances present in the eluates. Standard oxytocin was added to eluates in which the hypothalamic hormone had been inactivated by incubation with 0.01 M thioglycollate, and the mixture immediately assayed: no inhibition of the activity of the standard was observed. The conclusion was, then, that hypothalamic extracts contain a substance akin in its properties to posterior lobe hormone, except for a much weaker oxytocic activity.

There was one more point which required checking. Amin, Crawford, and Gaddum (1952, personal communication) found that 5-hydroxytryptamine is present in the hypothalamus. Since this substance stimulates the rat's uterus at low concentrations, and since it travels in the chromatogram to a region which is adjacent to that from which the posterior lobe-like activity is recovered, it was essential to rule out the possibility that the oxytocic activity found in the eluates was due to 5-hydroxytryptamine.

As also shown by Amin *et al.*, dihydroergotamine abolishes the oxytocic action of 5-hydroxytryptamine in doses which leave posterior lobe activity unaffected. A rat's uterus was suspended in a 2 ml. bath and contractions of comparable height were elicited by 0.35 m.u. pituitrin (" P "), 0.005 ml. hypothalamic extract (" X "), and 12.5 μg. 5-hydroxytryptamine (" H_2 "). The contractions are seen in the top part of Fig. 1. Then 10 μg. dihydroergotamine methane sulphonate was added to the bath, left in for 11 minutes, washed out, and H_2, P, and X again added to the bath. The lower part of Fig. 1 shows that the effect of 5-hydroxytryptamine had been suppressed by the dihydroergotamine, whereas the effects of pituitrin and hypothalamic extract were precisely the same as before.

There is thus no reason to doubt that what oxytocic activity is exerted by the hypothalamic extracts is due to a substance which has biological and chemical properties similar to those of posterior lobe hormone.

In order to be certain that the chemical procedure adopted was in no way responsible for the low oxytocic activities obtained, extracts were made of the posterior pituitary of three dogs, treated by the same procedure as hypothalamic extracts and assayed for pressor, antidiuretic, and oxytocic activity. Table II shows the agreement between the different assays and an average pressor/oxytocic ratio of 1.13 which does not significantly differ from 1. Since the acid alcohol

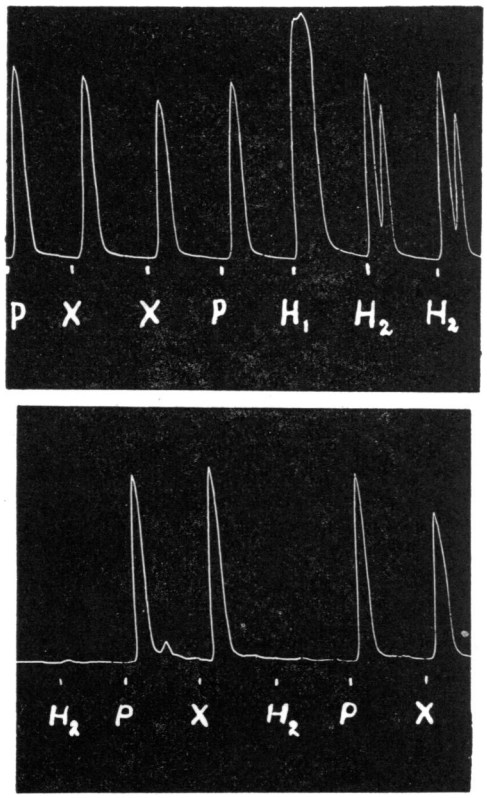

FIG. 1.—Uterus of oestrous rat, suspended in 2 ml. bath, stimulated by drugs every 4 minutes. P=0.35 m.u. pituitrin P.D. X= 0.005 ml. hypothalamic extract, corresponding to 0.0012 g. fresh tissue. The extract was administered as a 1 : 5 dilution in 0.9% NaCl. $H_1 = 50$ μg., $H_2 = 12.5$ μg. 5-hydroxytryptamine. Between the upper and the lower tracing interval of 15 minutes, during which 10 μg. dihydroergotamine methane sulphonate was added to the bath and washed out 11 minutes later.

TABLE II
EXTRACTS OF POSTERIOR LOBE OF THE DOG
Activity in m.u./g. fresh tissue

Dog No.	Pressor	Antidiuretic	Oxytocic
1	74,000		120,000
2	160,000		80,000
3	120,000	80,000	104,000

used in these experiments cannot be relied on to extract the whole activity, the figures in Table I may be underestimates, but they show, seeing that the weight of tissue employed ranged from 150 to 200 mg., that the amount of vasopressin in the adult brain is not less than about 1 i.u. Most of this activity was present in the ventral hypothalamus, from which the median eminence had been carefully removed in order to prevent contamination with pituitary tissue. The hormone was also found in the cat's hypothalamus. No activity (<2.5 m.u./ml.) was detected in dog's cerebrospinal fluid. Table I (last 5 figures) also indicates that young dogs have a lower hypothalamic hormone concentration than adult ones. The lowest figures ever observed, however, were obtained in puppies injected with morphine; some of these figures are not entered in the table because the activities were too small for an accurate assessment of the oxytocic effect. The action of drugs is under investigation with methods which ensure quantitative extraction of the hormone.

DISCUSSION

It is apparent from this work that the posterior lobe-like activity found in the hypothalamus of the dog is due to a substance differing in its properties from the hormone stored in the pituitary gland. Hild and Zetler (1951) also report a ratio of vasopressor/oxytocic potency which is greater than 1, but, as their ratio for the pituitary showed the same trend, they came to the conclusion that this was a general property of dog's posterior lobe hormone, irrespective of its localization. Since the authors were unaware of the presence of rather high concentrations of noradrenaline (1–2 μg./g. fresh tissue, Vogt, 1952b) in the hypothalamus, their estimation of hypothalamic pressor activities may have been subject to some inaccuracy; and in the important work of Zetler (1952), demonstrating the depletion of hypothalamic posterior lobe-like activity by thirst, the error introduced into the pressor assays by sympathin might, indeed, not have been negligible, since any contamination would have a greater influence on the results when the vasopressin present was only a fraction of the normal. If this were the correct explanation of the relatively high values reported by Zetler for vasopressor activity, there would be no need to postulate a dissociation of vasopressor from antidiuretic activity in the hypothalamus. This would certainly bring Zetler's results more in line with the view that these two activities are due to the same substance.

Kovács and Bachrach (1951) report that in experiments still in progress carried out by Kovács, Oláh, and Varró hypothalamic extracts of dogs had a greater antidiuretic than oxytocic potency. In contrast to the findings in the dog, this ratio was found to be unity in human material (Hild and Zetler, 1952). If these species differences are confirmed, they introduce a further complication into the problem.

Bargmann (1949), and Bargmann and Scharrer (1951), have put forward the theory that the

"posterior lobe" hormone is synthesized in the supraoptic and paraventricular nuclei and travels down the supraoptico-hypophyseal tract into the pituitary where it is stored. If we accept this theory, it would appear from the present work that the hormone, as synthesized in the ganglion cells, undergoes some chemical modification within the posterior lobe which enhances its relative oxytocic activity. Van Dyke, Chow, Greep, and Rothen (1942) have shown that a homogeneous protein can be prepared from posterior lobe tissue which has the same ratio of vasopressor to oxytocic activity as posterior lobe extracts. If the corresponding activities of hypothalamic extracts were also attached to a single molecule, it is this molecule which should undergo modification on its journey into the posterior lobe.

Recent experiments by du Vigneaud (1952) and Popenoe, however, suggest another interpretation. These authors prepared a highly purified vasopressin which did not contain any oxytocin, as shown by the complete absence from the preparation of leucine and isoleucine, two essential constituents of the oxytocin molecule. This vasopressin, when assayed on the guinea-pig's uterus, exhibited oxytocic activity of the order of 5% of the vasopressor potency. The authors therefore conclude that pure vasopressin has some intrinsic oxytocic activity. Comparison of the figure of approximately 5% for the intrinsic oxytocic activity of pure vasopressin and of that of 7% for hypothalamic hormone suggests that the substance extracted from the hypothalamus may be pure vasopressin.

It would be of great interest to know whether a secretion which is essentially vasopressor and very little oxytocic is ever given off into the blood stream. The only available evidence on the properties of the hormone circulating in the blood is that obtained by Harris (1947, 1948) in the rabbit. When the infundibular stem was stimulated electrically, and the effects on uterine activity and antidiuresis or vasoconstriction compared, the ratio vasopressor/oxytocic activity was not greater but smaller than 1 and amounted to 0.2–0.4. Unless we are here confronted with species differences, or with transformations of the hormone in the blood itself, Harris's results would therefore suggest that the circulating hormone was not identical either with the substance present in the hypothalamus or with that stored in the posterior lobe.

Summary

Extracts of the hypothalamus of the dog were assayed for pressor, antidiuretic, and oxytocic activity against pituitary (posterior lobe) extracts. The oxytocic activity was only about 7% of the pressor and antidiuretic activities.

Extracts of dog's posterior lobe, prepared in exactly the same way as the hypothalamic extracts, showed no such discrepancy between the various biological activities.

Before testing, all extracts were purified by paper chromatography so that the eluates used for biological assay were free of noradrenaline, adrenaline, and 5-hydroxytryptamine.

If one accepts the theory that the posterior lobe hormone is manufactured in the ganglion cells of the hypothalamus, these findings suggest that a modifying influence is exerted by the posterior lobe tissue on the molecule as it travels down to its site of storage in the pituitary. An alternative explanation would be that the hypothalamic nuclei synthesize vasopressin, whereas oxytocin is manufactured in the posterior lobe itself.

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