J. Physiol. (1966), **184**, *pp.* 837–855
With 9 text-figures
Printed in Great Britain

APPEARANCE OF 5-HYDROXYTRYPTAMINE AND AN UNIDENTIFIED PHARMACOLOGICALLY ACTIVE LIPID ACID IN EFFLUENT FROM PERFUSED CEREBRAL VENTRICLES

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(*Received* 27 *August* 1965)

SUMMARY

1. In cats anaesthetized with intraperitoneal pentobarbitone sodium, three regions of the cerebral ventricles, the third ventricle, the inferior or the anterior horn, were perfused with artificial c.s.f. and the effluent was tested on the fundus strip of the rat's stomach.

2. Effluent from all three regions contracted the fundus strip. The contractions were due to at least two substances as revealed by treatment of the strip with 2-bromolysergic acid diethylamide (BOL). The contractions that were sensitive to BOL are attributed to 5-hydroxytryptamine (5-HT) whereas the BOL resistant contractions appear to be due to an unknown hydroxy acid related to irin or the prostaglandins.

3. The contractions produced by effluent collected from the third ventricle were due wholly or mainly to 5-HT, those from the inferior horn to the unknown hydroxy acid, and those from the anterior horn to both substances in varying proportions. In addition, some samples of effluent from the third ventricle seemed to contain catecholamines as well.

4. The 5-HT in the effluent from the third ventricle is thought to be derived from the hypothalamus. The amounts assayed in 1 ml. effluent—the volume collected during 10 or 20 min perfusion—varied between 0·4 and 12 ng 5-HT. Output of 5-HT was initially high, then usually decreased but sometimes increased again during prolonged perfusion when temperature began to rise as anaesthesia lightened or when additional pentobarbitone sodium was given intravenously.

5. When perfusion of the third ventricle was continued after death the 5-HT content in the effluent increased 3 to 24-fold during the first hour and then gradually declined. This post mortem rise in 5-HT output

* Supported by U.S.A. Office of Naval Research Contract N 62558–4263, NR 101–640.
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suggests an abnormal state of release of 5-HT from the hypothalamus. The theory is discussed that the same may happen in certain cases of brain injury and that the abnormal release of 5-HT would explain the pyrexia and shivering seen in such cases.

6. The intraperitoneal injection of 5-hydroxytryptophan greatly increased the output of 5-HT in the effluent from the perfused third ventricle but only when this precursor of 5-HT was injected in large doses which caused respiratory arrest thus necessitating artificial ventilation. Upon the injection of 150 mg/kg the output of 5-HT rose to 90 ng/ml. and a further rise to 180 ng/ml. occurred when perfusion was continued after death.

7. It was not possible to establish a relation between the presence of the hydroxy acid in the effluent from the inferior horn and neuronal activity.

8. The 5-HT detected in the effluent from the anterior horn is assumed to have been released from the caudate nucleus.

INTRODUCTION

Recently the theory was put forward (Feldberg & Myers, 1964*a*, *b*) that the mechanism whereby temperature is controlled in the hypothalamus is the release of the amines 5-HT, adrenaline and noradrenaline, which are natural constituents of this part of the brain (Amin, Crawford & Gaddum, 1954; Vogt, 1954). This view was the outcome of pharmacological experiments which showed that these amines affect body temperature when injected into the cerebral ventricles of the cat or into its anterior hypothalamus. Further evidence in favour of this theory would be provided by demonstrating the release of these amines and, if they were to enter the c.s.f. from the hypothalamus, they may be detected in the effluent from perfused cerebral ventricles.

In the anaesthetized cat, methods have been evolved for perfusing the cerebral ventricles, or selected parts, such as the third ventricle or the inferior or anterior horn of a lateral ventricle (Carmichael, Feldberg & Fleischhauer, 1964). The effluent can then be tested for the presence of the amines by bioassay. A sensitive test for one of them, 5-HT, is the contraction it produces in the fundus strip of the rat's stomach (Vane, 1957), and the present experiments are confined to the effect on this preparation of effluent collected from the different parts of the perfused cerebral ventricles.

METHODS

Cats of both sexes weighing between 2·1 and 3·1 kg were anaesthetized with intraperitoneal pentobarbitone sodium (33 mg/kg) or with intravenous chloralose (45 mg/kg). For the injection of chloralose the right femoral vein was cannulated under anaesthesia induced with ethyl chloride and ether. Following the injection of the anaesthetic, rectal temperature was measured by a thermistor probe and recorded on a Kent multichannel recorder as

described previously (Feldberg & Myers, 1964*a*). After cannulation of the trachea the head was fixed in a stereotaxic instrument whilst the cat was lying on its belly insulated from the table by a cotton-wool pad. No external heat was applied. Room temperature varied between 22 and 25° C.

Perfusion of third ventricle, inferior and anterior horn of the left lateral ventricle. The arrangements of the cannulae for perfusing separately the three parts of the cerebral ventricles are shown diagrammatically in Fig. 1. The cannulae and the procedures used for their insertion are the same as described by Carmichael *et al.* (1964). If not otherwise stated the perfusion fluid was the artificial c.s.f. of Merlis (1940); its composition was (g/l.): NaCl 8·1; KCl 0·25; $CaCl_2$ 0·14; $MgCl_2$ 0·11; $NaHCO_3$ 1·76; Na_2HPO_4 0·07; urea 0·13; and glucose 0·61.

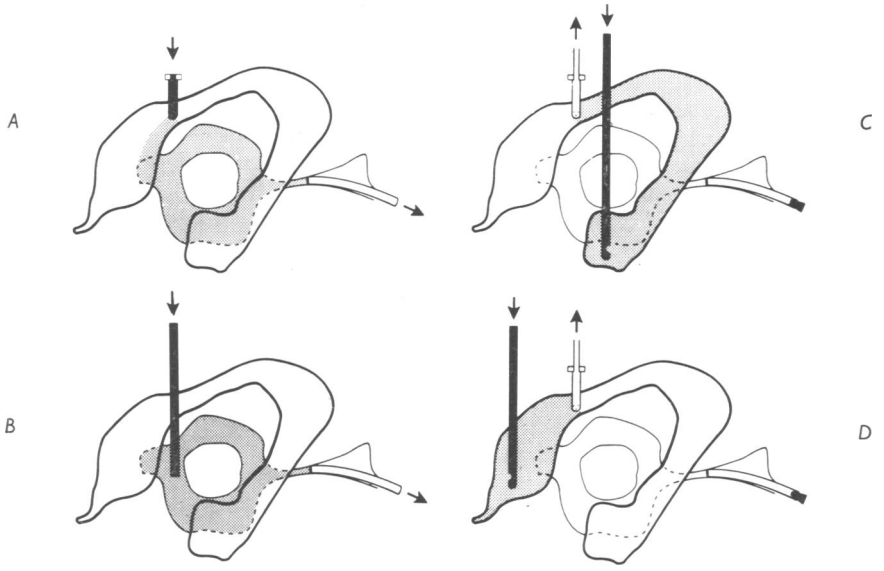

Fig. 1. Diagrams of the arrangements for perfusing the third ventricle (*A* and *B*) the inferior (*C*) or anterior (*D*) horn of the left lateral ventricle of the anaesthetized cat. (For details see text.)

For perfusion of the third ventricle, two methods were used. Cannulation of the body of the left, or of both lateral ventricles near the foramina of Monro (diagram *A*); or of the third ventricle (diagram *B*). In each instance the outflow was collected from the cannulated aqueduct. When both lateral ventricles were cannulated the rate of inflow through each cannula was 0·05 ml./min; when one lateral or the third ventricle was cannulated, the rate was either 0·05 or 0·1 ml./min.

When perfusing the inferior or anterior horn (diagrams *C* and *D*) a cannula was inserted into either horn, and the inflow was collected through a modified Collison cannula implanted into the body of the lateral ventricle. The aqueduct was cannulated as well, but once flow had been established the cannula was stoppered as indicated in the diagrams. The rate of inflow was 0·1 ml./min.

With these methods perfusion was not entirely restricted to the area between inflow and outflow cannulae, although passage of perfusion fluid into regions beyond the two cannulae could only be slight. Admixture of c.s.f. which might be secreted into the non-perfused region and then combined with the perfusion fluid could also only be slight. In a number of experiments in which the third ventricle was cannulated and perfused, the spread of the perfusing fluid into the lateral ventricles was ascertained by adding bromophenol blue to

the perfusing fluid 15–20 min before the cat was killed. The head was then perfused from the thoracic aorta with 30% formaline. On examination of the brain, faint staining of the walls of the lateral ventricles was sometimes seen, but usually the staining was limited to the walls of the third ventricle, which were stained deeply; in some experiments the staining was even restricted to those parts of the walls of the third ventricle which lie ventral to the massa intermedia, indicating that the perfusing fluid had passed through only the ventral half of the ventricle.

Assay of effluent for 5-HT. Effluent was collected in 15–60 min samples which were kept on ice until assayed for 5-HT on the rat's stomach fundus strip, according to the method described by Vane (1957). The strip was suspended in a 5 ml. bath of Krebs solution through which a mixture of 95% O_2 and 5% CO_2 was bubbled. The bath fluid was continuously changed by a slow flow of Krebs solution from the bottom to the top of the bath and removed by overflow. Effluent or 5-HT was added to the bath every 4·5–5 min, and kept in the bath for 90 sec during which time the inflow of Krebs solution was turned off. The contractions of the strip were recorded on a smoked drum by a light isotonic lever.

Drugs used for testing on the rat fundus strip. 5-Hydroxytryptophan kindly supplied by Dr A. L. Morrison, Roche Products Ltd., Welwyn Garden City; 5-hydroxytryptamine creatinine sulphate; (−)-adrenaline bitartrate; (−)-noradrenaline bitartrate; acetylcholine chloride; histamine acid phosphate; and L-ergothioneine HCl, kindly supplied by Dr J. Crossland (all values for these salts are expressed in terms of the base); bradykinin synthesized by Parke Davis and Co., Ann Arbor, kindly supplied by Dr E. D. Nicolaides; angiotensin (synthetic val^5-hypertensin II-asp-β-amide, Hypertensin, Ciba) kindly supplied by Ciba Laboratories Ltd., Horsham; substance P, preparation RO 1-9256/3 made by Hoffmann La Roche, Bâle, 1958, containing 75 u./mg and kindly supplied by Sir John Gaddum; prostaglandin E1, kindly supplied by Dr E. W. Horton; irin, kindly supplied by Dr N. Ambache; γ-amino-butyric acid (GABA); and 2-bromolysergic acid diethylamide (BOL) (Sandoz).

RESULTS

Effluent from the perfused cerebral ventricles contracted the fundus strip of the rat's stomach. The contractions were due to 5-HT or to an unknown lipid acid, or to both, depending upon the area perfused. The activity in effluent from the third ventricle appeared to be mainly, or wholly due to 5-HT, that from the inferior horn of the lateral ventricle to the lipid acid, and that from the anterior horn to a combination in varying proportions of both substances.

Third ventricle perfusion

In most experiments the contractions of the fundus strip produced by samples of effluent from the third ventricle resembled those caused by 5-HT, but in some they were preceded by a transient relaxation which did not occur with 5-HT; in still other experiments the contractions ceased or were even followed by slight relaxation before the sample was washed out, and again this did not occur with 5-HT. With several preparations the sensitivity of the strip to 5-HT changed after a sample had been tested; it either increased or decreased.

The contractions in response to the samples were often as sensitive to BOL as those produced by 5-HT, and could thus be fully accounted for by

this amine. Contractions produced by some samples, however, were not reduced by BOL to the same extent as contractions after equipotent doses of 5-HT. This suggested that the samples contained not only 5-HT but another substance as well which contracted the fundus strip, and which may have been the same as the hydroxy-acid responsible for the contractions produced by effluent from the inferior horn (see page 850). The contribution made by this unknown substance to the response of the fundus strip was never great.

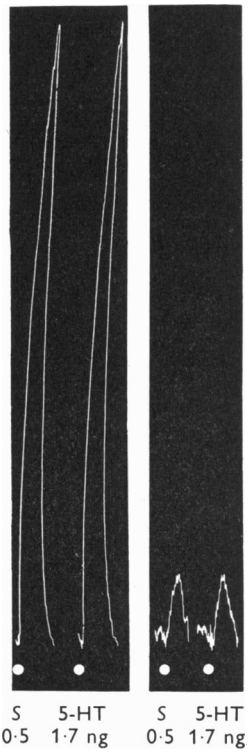

Fig. 2. Fundus strip of rat's stomach suspended in 5 ml. Krebs solution. At the white spots, responses to 0·5 ml. effluent (*S*) from perfused ventral half of the third ventricle and to 1·7 ng 5-HT. BOL (10 μg for 10 min) added to the bath in the interval between the two panels.

Figure 2 illustrates part of an assay of a sample, the activity of which could be fully accounted for by 5-HT. The sample is from an experiment in which only the ventral half of the third ventricle was perfused, as verified at the end of the perfusion with bromophenol blue. The first panel shows the strong and equal contractions produced by 0·5 ml. sample and 1·7 ng 5-HT, and the second panel the remnants of these contractions after BOL.

The small contractions which remained after treatment with BOL were not always a residual effect of the 5-HT or the sample. Similar contractions were often produced simply by turning off the overflow for 90 sec, the time for which 5-HT or sample was kept in the bath when tested. This is illustrated in Fig. 3 which gives part of an assay of two samples of effluent from Expt. 5 of Table 1. The contractions in the first panel were obtained

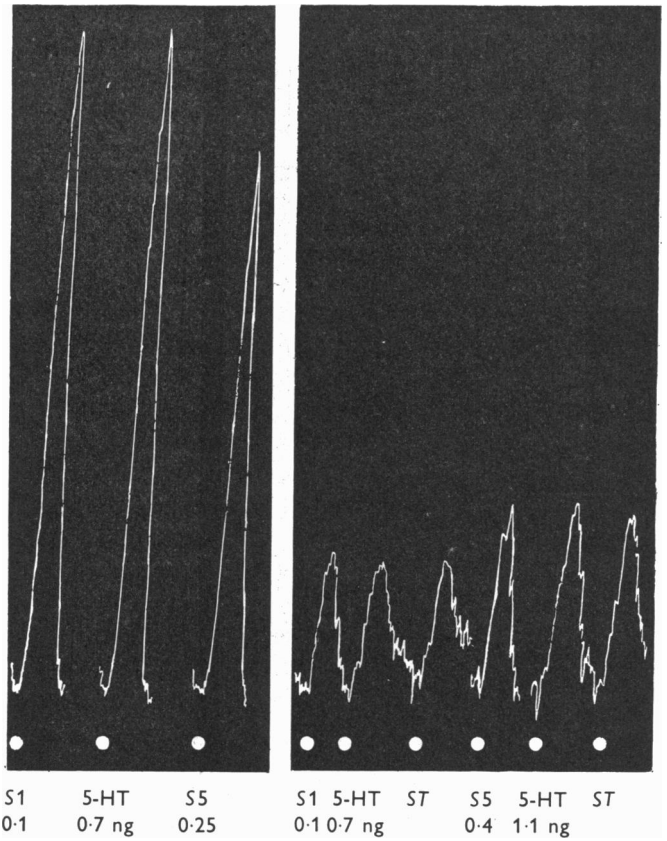

Fig. 3. Fundus strip of rat's stomach suspended in 5 ml. Krebs solution. At the white spots, responses to two samples (*S* 1 and *S* 5) of effluent from perfused third ventricle of Expt. 5, Table 1, to 5-HT (0·7 and 1·1 ng) and to turning off (*ST*) the over-flow to the bath for 90 sec. The amounts of sample in ml. tested are given below *S* 1 and *S* 5. BOL (10 μg for 10 min) added to the bath in the interval between the two panels.

before, those in the second panel after BOL. If the effect of stopping the overflow (at *ST*) had not been tested in this assay, it would have appeared as though the BOL had reduced, to the same extent, the effect of 0·1 ml. sample 1, and of 0·7 ng 5-HT, and further that the somewhat larger con-

traction produced by the 0·4 ml. of sample 4 was due to its containing 1·1 ng of 5-HT. However, all the contractions produced after BOL are accounted for by stopping the over-flow to the bath. This resulted in a contraction which increased in size as the strip became more sensitive to this procedure.

Another feature occasionally encountered, and illustrated in Fig. 4, was the stepwise return of sensitivity of the fundus strip that had been treated with BOL. Each time the sample was tested, the sensitivity of the strip

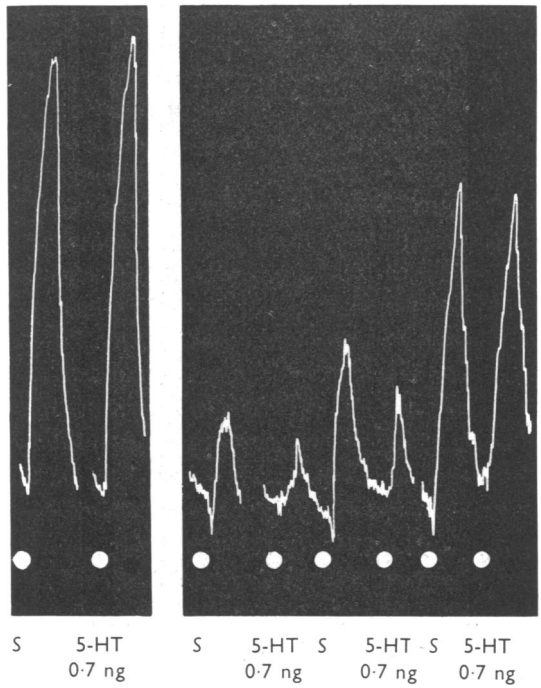

Fig. 4. Fundus strip of rat's stomach suspended in 5 ml. Krebs solution. At the white spots, responses to 0·35 ml. effluent (*S*) from the perfused ventral half of the third ventricle and to 0·7 ng 5-HT. BOL (10 μg for 10 min) added to the bath in the interval between the two panels.

increased; 5-HT itself did not have this effect. The first panel shows contractions before, and the second after BOL had been in the bath for 10 min when sample and 5-HT were re-tested 3 times.

The relaxations which preceded or followed the contractions produced by some samples could often be duplicated by testing 5-HT together with adrenaline. Further, the initial relaxations, like those produced by adrenaline, were resistant to BOL, which made them more distinct. Figure 5 shows responses of a strip treated with BOL which was exceptionally sensitive to the relaxing action of adrenaline. The effluent was

from an experiment in which the third ventricle was perfused with 0·9% NaCl solution and not with artificial c.s.f., and in which temperature did not show any sign of rising in the course of several hours of perfusion. Additions of 1·4 ng adrenaline (*A*), of 0·4 ml. effluent (*S*), or of 1·4 ng adrenaline plus 1·0 ng 5-HT (*A* + 5-HT), produced relaxation followed by contraction, whereas 1·0 ng 5-HT alone (5-HT) caused contraction only.

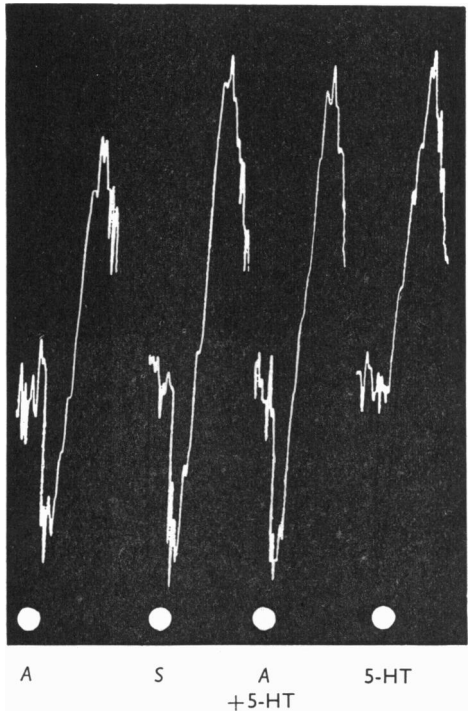

Fig. 5. Fundus strip of rat's stomach suspended in 5 ml. Krebs solution and treated with BOL (10 μg for 10 min). At the white spots, responses to 1·4 μg adrenaline (*A*), to 0·4 ml. effluent (*S*) from third ventricle perfused with 0·9% NaCl solution, to 1·4 ng adrenaline plus 1·0 ng 5-HT (*A* + 5-HT) and to 1·0 ng 5-HT (5-HT).

A similar contraction was produced by adding 0·4 ml. 0·9% NaCl solution and was probably a response to turning off the overflow; thus no significance can be attached to any of the contractions. On the other hand, the initial relaxation produced by the effluent may signify the presence of adrenaline in this fluid, particularly since noradrenaline was effective only when added to the bath in doses of several ng.

Whether the contractions of the rat fundus strip produced by effluent were as sensitive to BOL as those produced by 5-HT, or whether they were found to be somewhat less resistant to this blocking agent for 5-HT, they

were assayed against 5-HT and the activity of the samples was expressed in terms of ng 5-HT/ml.

The results of such assays for nine experiments are summarized in Table 1. The highest activity was usually obtained with the first sample; it then declined for one, two or even more samples, but in some experiments, activity rose again. This rise could be correlated to the rising phase of temperature as anaesthesia lightened. It happened in the first three experiments as well as in the Expts. 8 and 9 of the Table. In Expts. 2, 8 and 9, the rise to 3·0, 4·7 and 3·7 ng/ml. was obtained in those samples

TABLE 1. Activity expressed as ng 5-HT/ml. of successive samples of effluent collected from the perfused third ventricle in cats anaesthetized with pentobarbitone sodium (Expts. 1–6) or chloralose (Expts. 7–9). In Expts. 2–5 and 7, inflow was from the cannulated third ventricle, in Expt. 1 from the left, and in Expts. 6 and 8 from both lateral ventricles. In Expts. 1 and 2, rate of inflow was 0·05 ml./min, in all others 0·1 ml./min. Samples were collected for 15 or 30 min with the perfusion stopped for the remainder of the hour between each sample, with the exception of Expts. 5 and 6, where collection was continuous and each sample was collected for 30 min

Samples	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Expt. 6	Expt. 7	Expt. 8	Expt. 9
1	1·4	1·6	2·1	2·3	7·1	11·9	2·4	2·6	10·7
2	1·0	1·1	0·1	1·4	3·6	4·4	1·1	4·7	2·0
3	1·1	3·0	0·4	0·9	1·6	1·9	0·4	3·9	3·7
4	2·0	1·4	0·4	0·9	2·9	1·4	0·4	3·4	2·1
5	—	2·1	0·6	1·0	3·1	1·4	—	—	—
6	—	1·7	0·6	—	3·7	1·3	—	—	—
7	—	—	—	—	2·1	0·9	—	—	—
8	—	—		—	2·1	0·9	—	—	—
9	—	—	—	—	—	0·7	—	—	—
10	—	—	—	—	—	0·7	—	—	—

collected at the beginning of the rising phase of temperature and whilst the cat was shivering. On the other hand, a rise in temperature was not invariably associated with an increase in 5-HT output. For instance, in the Expts. 4, 6 and 7, temperature rose during the collection of the last samples, but their 5-HT content diminished or remained at about the same level.

In two experiments additional pentobarbitone sodium (24 mg in Expt. 2, and 30 mg in Expt. 5) was given intravenously after collection of the third sample. This resulted in a transient fall of temperature. In Expt. 2, the fall was associated with a decrease in 5-HT activity in the fourth sample from 3·0 to 1·4 ng/ml., followed by an increase to 2·1 ng/ml. in the fifth sample as the temperature was rising again. In Expt. 5, the 5-HT activity rose from 1·6 to 2·9 ng/ml. in the first sample collected after the injection, and then continued to rise to 3·1 and 3·7 ng/ml. in the following two samples.

Release of 5-HT after death. When perfusion was continued after the cat had been killed either by an overdose of pentobarbitone sodium injected

intravenously, or by opening the thorax and clamping the heart, the activity in the effluent increased greatly. The results of eight experiments are summarized in Table 2. The activity expressed in terms of ng 5-HT/ml. is given for the last two samples collected before, and for two to six samples collected after the cat was killed. The activity of the first, second or third sample collected post mortem increased 3 to 24-fold and then gradually decreased.

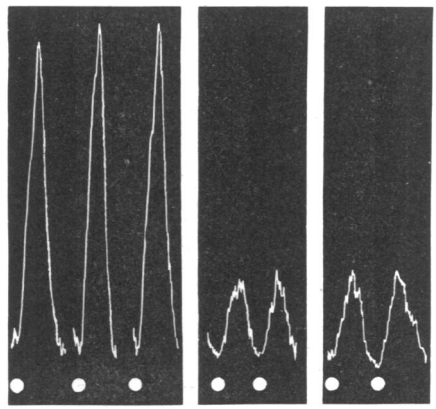

Fig. 6. Fundus strip of rat's stomach suspended in 5 ml. Krebs solution. At the white spots, responses to 5-HT (1·1 and 2·2 ng) and to the post-mortem samples *S*4 and *S*8 of Expt. 8 of Table 2. The amounts of sample in ml. tested are given below *S*4 and *S*8. BOL added to the bath in the interval between the first and second (2 μg for 10 min) and between the second and third panel (10 μg for 10 min).

The responses of the fundus strip to the samples collected post mortem were as sensitive to BOL as were those to 5-HT. This is illustrated in Fig. 6 for the second (*S*4) and last (*S*8) post-mortem samples of Expt. 8 of Table 2. The first panel shows that 0·2 ml. *S*8, and 0·05 ml. *S*4 produced strong contractions, slightly greater than that caused by 1·1 ng 5-HT. The second panel shows the contractions elicited by 1·1 ng 5-HT and 0·2 ml *S*8 after 2 μg BOL; and the last panel those produced by 0·1 ml. of *S*4 and by 2·2 ng 5-HT after 10 μg BOL.

Figure 7 illustrates the contractions produced by different amounts of 5-HT and of the post-mortem effluent before and after BOL. The effluent tested was the last post-mortem sample of Expt. 2 of Table 2. The first panel shows not only that the contractions produced by 1·1 ng 5-HT and by 0·2 ml. sample (*S*5) were equal, but that they increased to the same degree when either 5-HT or sample was added to the bath in double the amount. The second panel shows the reduced contractions in response to

the same amounts of 5-HT or sample after the strip had been treated with 1 μg BOL for a few minutes. The contractions caused by 5-HT and by the sample were equally reduced. The third panel was obtained some time later when the preparation appeared to have partially recovered from the

TABLE 2. Activity expressed as ng 5-HT/ml. of successive 15–30 min samples of effluent collected from the perfused third ventricle in cats anaesthetized with pentobarbitone sodium. The first two samples were collected before, the others after the cats were killed by an overdose of pentobarbitone sodium, or, as in Expt. 6, by clamping the ascending aorta

Samples	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Expt. 6	Expt. 7	Expt. 8
1	0·9	0·7	0·9	3·0	2·9	0·7	1·1	1·1
2	0·7	0·6	0·4	3·1	1·7	0·7	2·0	1·0
Post-mortem samples								
3	4·9	0·6	0·6	4·7	7·1	11·4	12·1	5·7
4	4·4	5·9	4·3	9·0	9·6	10·1	18·9	23·9
5	—	5·7	7·9	7·6	3·9	6·3	14·3	15·7
6	—	—	6·4	7·4	—	7·1	—	10·7
7	—	—	5·7	—	—	7·1	—	6·1
8	—	—	5·3	—	—	—	—	6·6

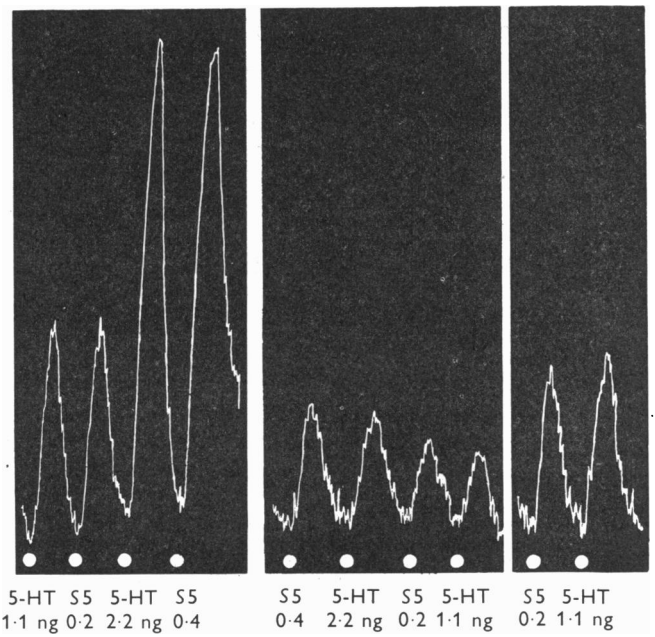

Fig. 7. Fundus strip of rat's stomach in 5 ml. Krebs solution. At the white spots, responses to 5-HT (1·1 and 2·2 ng) and to the post-mortem sample (*S* 5) from perfused third ventricle of Expt. 2 of Table 2. The amounts of sample tested are given in ml. below *S* 5. BOL (1 μg for 10 min) added to the bath in the interval between first and second panel. Interval of about 30 min between the second and third panel.

action of BOL. The responses to both 5-HT and sample had recovered to the same degree.

Intraperitoneal injection of 5-hydroxytryptophan (5-HTP). Intraperitoneal injections of this precursor of 5-HT greatly increased the output of 5-HT in the effluent from the perfused third ventricle, but only when injected in large doses; these led to central depression, as indicated by respiratory arrest.

The 5-HTP injections were given shortly after the cats had been anaesthetized with pentobarbitone sodium but the first samples of effluent from the perfused third ventricle were not collected until 1·5–2 hr later. In one experiment an injection of 20 mg/kg 5-HTP had no detectable effect on the output of 5-HT; in another experiment 75 mg/kg increased the 5-HT activity in several successive half-hour samples to 7–11 ng/ml. After still larger doses of 5-HTP the 5-HT activity in the effluent increased greatly and remained high for several hours, but the respiratory arrest which occurred required artificial ventilation. In spite of the rise in 5-HT output, rectal temperature continued to fall. In one experiment with 100 mg/kg 5-HTP, activity of the effluent rose to 30 ng/ml. 5-HT, and in another, with 150 mg/kg, to 90 ng/ml. with a further rise to 180 ng/ml. 5-HT when perfusion was continued after the cat was killed. This post-mortem rise occurred in the second half-hour sample collected after death; activity in the effluent then diminished.

Inferior horn perfusion

Samples of effluent contracted the rat stomach strip. The activity varied in different experiments and always decreased after the first or second sample. Table 3 gives for two experiments the activity expressed in terms of ng 5-HT/ml. The first four samples were collected before the cat was killed. In these two experiments the activity was particularly strong, in others the initial activity corresponded to only 3 ng/ml. 5-HT and decreased to 0·4 ng/ml.

Although expressed in terms of 5-HT, the activity was not due to this amine, since the contractions produced by effluent were not abolished by BOL, as illustrated in Fig. 8. The first panel shows the contractions produced by 0·9 ng 5-HT and by 0·12 ml. effluent (*S* 1). The strip was then exposed for 5 min to the action of 2 μg BOL with the result, shown in the second panel, that 0·9 ng 5-HT no longer contracted the strip, whereas the activity of the effluent was not affected. Such a result does not, however, exclude the possibility that the effluent from the inferior horn was entirely devoid of 5-HT. It may have contained this amine in concentrations too weak to be detectable in the presence of the other active principle which produced BOL-resistant contractions.

TABLE 3. Activity expressed as ng 5–HT/ml. of successive samples of effluent collected from the perfused inferior horn of the left lateral ventricle in two cats anaesthetized with pentobarbitone sodium. The samples were collected for periods of 30 min (Expt. 1) and 20 min (Expt. 2). Until the cats were killed by an over-dose of intravenous pentobarbitone sodium, perfusion was stopped between each sample for the remainder of the hour. After death, collection was continuous. The last sample* in both columns refers to effluent collected from the aqueduct

Sample	Expt. 1	Expt. 2
1	7·1	16·4
2	4·9	17·1
3	2·4	9·4
4	1·9	3·3
Post-mortem samples		
5	0·7	2·1
6	0·6	2·1
7	4·7*	1·1
8	—	1·1
9	—	0·9
10	—	1·1
11	—	9·6*

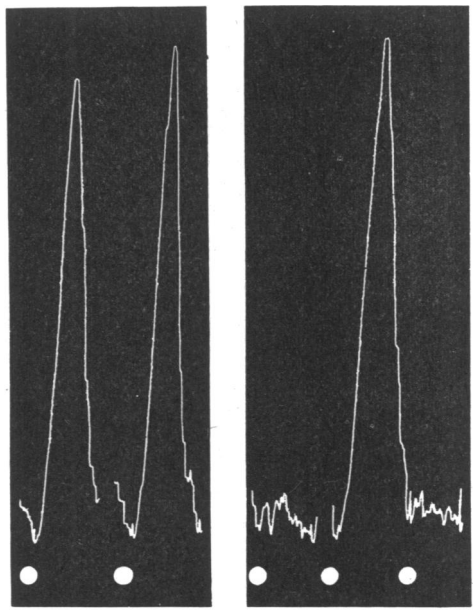

Fig. 8. Fundus strip of rat's stomach suspended in 5 ml. Krebs solution. At the white spots, responses to 0·9 ng 5-HT and 0·12 ml. of a sample (*S* 1) of effluent from perfused inferior horn of the left lateral ventricle of Expt. 1 of Table 3. BOL (2 μg for 10 min) added to the bath in the interval between the first and second panel.

Neither GABA, L-ergothioneine, histamine, acetylcholine, substance P, bradykinin or angiotensin were responsible for the BOL-resistant contractions produced by the effluent from the inferior horn. GABA did not contract the fundus strip but produced some relaxation when added to the bath in concentrations of up to 100 μg/ml. L-ergothioneine produced only a slight contraction when tested in a concentration of 2 μg/ml. and the effect did not increase when the concentration was raised 10-fold. Histamine, acetylcholine and substance P, were excluded by comparison of the activities on the rat fundus strip with those on the guinea-pig ileum preparation. In contrast to the guinea-pig ileum, the fundus strip contracted only to relatively large doses of histamine and acetylcholine. However, the guinea-pig ileum did not contract in response to effluent when tested in amounts which elicited strong contractions on the fundus strip. Substance P caused a contraction of the fundus strip, again only in relatively large concentrations (8 u./ml.), but was somewhat more potent in contracting the guinea-pig ileum. Substance P was further eliminated by the chymotrypsin test, which excluded bradykinin and angiotensin as well. The activity of effluent was compared on the rat fundus strip to that of either bradykinin or angiotensin; then equipotent amounts of effluent, bradykinin and angiotensin were incubated separately with chymotrypsin for 10 min. The incubated solutions of bradykinin and angiotensin became inactive, but there was no diminution in potency of the incubated effluent.

The active principle in the effluent appears to be a lipid soluble acid. Dr N. Ambache kindly extracted an active sample of the effluent with ether partition at neutral and acid pH. Most of the activity went into the acid ether phase. The active principle appears therefore to be a lipid acid resembling the hydroxy acid in extracts of rabbit brain (Ambache & Reynolds, 1960; Ambache, Reynolds & Whiting, 1963), i.e. an acid related to irin and the prostaglandins, which in relatively low concentrations caused contraction of the rat fundus preparation.

In one experiment the activity in the effluent from the inferior horn increased 5-fold when the hippocampus, which lines this part of the ventricular cavity, was activated, but in three subsequent experiments no increase was obtained. Hippocampal activation was brought about by prolonged perfusion of the inferior horn with tubocurarine 1/500, which produced in the electrocorticogram a rhythmic discharge of high voltage spikes which originate in the hippocampus (Feldberg & Fleischhauer, 1963).

Perfusate collected after death. When perfusion of the inferior horn was continued after the cat was killed, the activity in the effluent gradually declined. This is shown in Table 3. In Expt 1, the activity, expressed in terms of 5-HT, decreased from 1·9 to 0·6 ng/ml. within 60 min of death,

and in Expt. 2 from 3·3 to 1·1 ng/ml. within 1 hr and remained at about this level during the subsequent three samples. The last sample from each experiment was obtained by collecting the effluent from the aqueduct instead of from the body of the lateral ventricle so that the perfusing fluid passed through the third ventricle; the high values obtained with these samples indicate the post-mortem rise in 5-HT release from the walls of the third ventricle.

Anterior horn perfusion

Samples of effluent from the anterior horn also caused contraction of the fundus strip. BOL affected the contractions produced by different samples to a varying degree. Contractions in response to some samples were nearly as sensitive to BOL as those produced by equipotent doses of 5-HT, but contractions in response to other samples were much less sensitive and were reduced only partially whilst those to equipotent doses of 5-HT were abolished by BOL. This is illustrated in Fig. 9 for two samples obtained

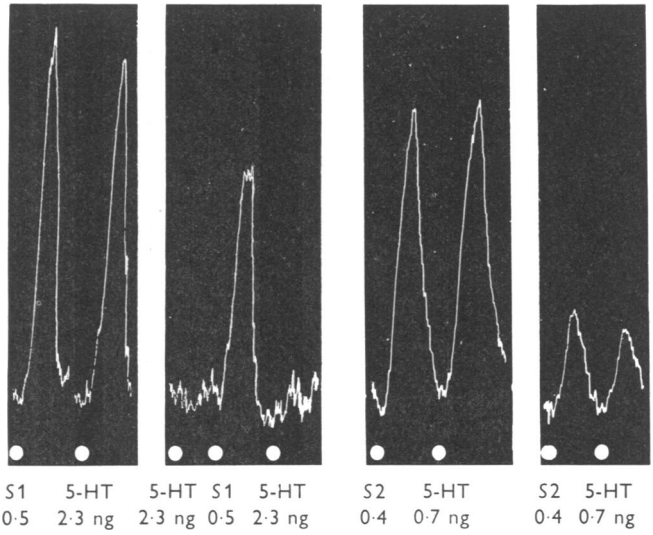

Fig. 9. Fundus strips of two rat's stomachs, each suspended in 5 ml. Krebs solution. At the white spots, responses to 5-HT (2·3 and 0·7 ng) and to two samples (*S*1 and *S*2) of effluent from the perfused anterior horn of different experiments. The amounts of sample in ml. tested are given below *S*1 and *S*2. Panels 1 and 2, responses of one strip; panels 3 and 4, of the other. Panels 2 and 4 after BOL (10 μg for 10 min) was added to the bath.

from different experiments. In the one, the contraction produced by 0·5 ml. of sample corresponded approximately to that in response to 2·3 ng 5-HT, and was reduced by BOL to about one half, whereas the 5-HT contraction was abolished. In the other experiment contractions pro-

duced by 0·4 ml. of sample and 0·7 ng 5-HT were similar, and both were reduced about equally by BOL. The activity of the samples therefore appears to result from the presence, in varying proportions, of 5-HT and of an unknown substance which may well be identical to that of the lipid acid found in the effluent from the inferior horn. No experiments have been done to characterize this unknown substance as in the experiments with effluent from the inferior horn.

The activity of effluent from the perfused anterior horn diminished in successive samples and did not rise after the cats were killed, except in one experiment in which there was a small post-mortem rise.

DISCUSSION

Perfusion of the cerebral ventricles provides a method for detection by bioassay of substances released from structures within the ventricular walls. This method has hitherto been used for measuring the release of acetylcholine when its destruction was prevented by an anticholinesterase (Bhattacharya & Feldberg, 1958; Beleslin, Carmichael & Feldberg, 1964; Beleslin & Polak, 1965; Polak, 1965). The present experiments in which no anticholinesterase was used demonstrate that acetylcholine is not the only pharmacologically active substance which enters the fluid perfusing the ventricular cavities. Effluent from three areas, the third ventricle, the inferior and the anterior horn of the lateral ventricle produced contractions of the fundus strip of the rat's stomach. These contractions were due to at least two substances as revealed by their differing sensitivity to BOL.

It is reasonable to assume that the contractions which were abolished by the action of BOL, and which showed the same dose–response relation as 5-HT were due to this amine, although no other tests towards its identification, pharmacological or chemical, were carried out. The contractions resistant to BOL and thus not due to 5-HT apparently result from the action of an hydroxy acid akin to irin or the prostaglandins. Evidence in favour of this view has so far been obtained only with effluent from the inferior horn, but when such a contraction was elicited by effluent from the other perfused areas, the same lipid acid was probably responsible.

The 5-HT in the effluent from the third ventricle which accounted wholly or mainly for the contractions produced by this effluent is thought to be released from the hypothalamic structures in the walls of the ventral half of the third ventricle. The 5-HT in the effluent from the anterior horn would appear to originate from the caudate nucleus which, like the hypothalamus, is known to contain a relatively large amount of this amine. The fact that its release can be detected in the effluent from the perfused anterior horn may open up a new approach to the function of this nucleus about which so little is known.

The main purpose of the present experiments was to find out whether 5-HT released within the hypothalamus escapes into the fluid passing through the third ventricle. The results obtained can be taken as evidence that at least part of the released 5-HT does so. The question then arises as to whether the changes observed in the 5-HT content of the effluent are related to changes in body temperature, since temperature is most likely only one of the many functions influenced by the amines present in the hypothalamus. The output of 5-HT was initially high in all experiments. This may have been due to a washing-out of 5-HT, since a similar initial high output was also found with regard to the unknown hydroxy acid in the effluent from the inferior horn. Alternatively it may have been an effect of the anaesthetic. It has been suggested that anaesthetics, although they lower body temperature, release not only the catecholamines but also the 5-HT in the hypothalamus (Feldberg & Myers, 1964*b*). This would also explain the rise in 5-HT output which occurred in several instances when a small dose of pentobarbitone sodium was given intravenously midway through an experiment. The other condition in which an increase in 5-HT output was encountered during prolonged perfusion was when the temperature began to rise as anaesthesia lightened. As this rise was not invariably associated with an increase in the 5-HT output a correlation between a rise in temperature and an increased 5-HT release has not yet been obtained. The manifold increase in 5-HT output following the intraperitoneal injections of large doses of 5-HTP also was not associated with a rise in rectal temperature. Since the large doses of 5-HTP, however, had a strong central depressant effect, as evidenced by respiratory failure, the 5-HTP may have affected the responsiveness of the anterior hypothalamus to 5-HT as well, or it may have exerted a paralysing effect on central mechanisms activated by the anterior hypothalamus and involved in the control of temperature.

The rise in 5-HT activity of effluent from the third ventricle which occurred after death may perhaps explain a phenomenon which has puzzled neurologists for a long time: the high fever and bouts of shivering that occur in certain cases of brain injury. If body temperature is the outcome of a fine balance in the release of the catecholamines and of 5-HT in the hypothalamus, then the basal mechanism for keeping up temperature would be the release of 5-HT. The present experiments show that after death this release proceeds at an abnormally high rate, but any interference during life with the blood supply to the hypothalamus may have the same effect, and if this were to happen in brain injuries after accidents the pyrexia and shivering would be explained.

The present experiments were not designed to detect the release of the catecholamines into the perfused ventricles. In fact, any adrenaline or noradrenaline escaping into the artificial c.s.f. used for perfusion would

quickly be destroyed since this fluid is slightly alkaline. Nevertheless, in several experiments the responses of the fundus strip to the effluent from the third ventricle suggested the presence of catecholamines as well as of 5-HT. So far, the strongest indications for the release of adrenaline was obtained in an experiment in which the perfusion fluid was 0·9% NaCl instead of artificial c.s.f. and the effluent was tested on the fundus strip rendered insensitive to 5-HT by treatment with BOL. It would thus appear that not only 5-HT but catcholamines as well escape from the hypothalamus into the fluid passing through the third ventricle.

Any consideration of the origin of the unknown hydroxy acid resembling irin or the prostaglandins, detected in the effluent from the inferior horn, must take into account the appearance of apparently the same substance in effluent from the other areas perfused. The only difference was that in the effluent from the inferior horn the hydroxy acid alone was responsible for the contractions of the fundus strip, whereas the contractions caused by effluent from the anterior horn were in part and in varying proportions due to 5-HT which was mainly and sometimes entirely responsible for the contractions produced by effluent from the third ventricle. It is possible that the unknown hydroxy acid in this effluent is not derived from structures lining the third ventricle but represents an admixture of c.s.f. from the inferior horn, a possibility not excluded by the methods of perfusion used in the present experiments. For anatomical reasons such an admixture is even more likely to occur on perfusion of the anterior horn. On the other hand, it may well be that the hydroxy acid has a ubiquitous origin in many structures of the brain, and in this connexion it is interesting to note that Ambache *et al.* (1963) found an hydroxy acid resembling irin in extracts of the brain stem and cerebral hemisphere of the rabbit.

Whether the hydroxy acid detected in the effluent from the cerebral ventricles and particularly from the inferior horn is associated with neuronal activity has still to be shown. The present experiments do not give a definite answer to this problem. The hippocampus lines the inferior horn and may be the main source of the hydroxy acid. Its activation by tubocurarine, however, increased the activity in the effluent from the inferior horn in only one of four experiments.

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