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ABSORPTION OF HISTAMINE INTO THE BLOOD STREAM ON PERFUSION OF THE CEREBRAL VENTRICLES, AND ITS UPTAKE BY BRAIN TISSUE

By M. DRAŠKOCI,* W. FELDBERG, K. FLEISCHHAUER† AND P. S. R. K. HARANATH*

From the National Institute for Medical Research, Mill Hill, London, N.W. 7

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Bhawe (1958) has shown that when histamine is injected into the lateral cerebral ventricle of an anaesthetized cat acid gastric secretion occurs. This secretion was attributed to passage of histamine into the blood stream and it was assumed that the histamine had passed from the ventricles into the subarachnoid space and then into the endocranial venous sinuses. The present experiments show that histamine also passes into the blood stream from the cerebral ventricles without entering the subarachnoid space. In addition, it was found that histamine perfused through the cerebral ventricles entered the brain tissue surrounding the ventricular cavities and that this uptake of histamine showed differences dependent on the structures of the ventricular wall.

METHODS

The experiments were carried out in cats anaesthetized by intraperitoneal injection of pentobarbitone sodium 35 mg/kg; additional pentobarbitone was injected when it became necessary in the course of the experiment. The trachea was cannulated. Histamine was usually perfused through the lateral and third ventricles. The fourth ventricle was not included in the perfusion since, in order to prevent the perfusion fluid from entering into the subarachnoid space, the effluent was collected from a cannula inserted into the aqueduct, as described by Bhattacharya & Feldberg (1956). The effluent was assayed for histamine after its volume had been measured. The absorption of histamine into the blood stream was shown by estimating the acid gastric secretion.

Perfusion of cerebral ventricles. The fluid used for perfusion was that introduced by Merlis (1940) and later used by Leusen (1949). Its composition, which is approximately that of normal cerebrospinal fluid, is as follows (g/l.): NaCl 8·1; KCl 0·25; $CaCl_2$ 0·14; $MgCl_2$ 0·11; $NaHCO_3$ 1·76; NaH_2PO_4 0·07; $CO(NH_2)_2$ 0·13; and glucose 0·61. This solution will be referred to as artificial cerebrospinal fluid (artificial c.s.f.). A Collison cannula was implanted into the left lateral ventricle as described by Feldberg & Sherwood (1953). The perfusion was maintained by a continuous slow injector (C. F. Palmer, Ltd.) at a rate of 0·1 ml./min. In order to insert the outflow cannula into the aqueduct the muscle layers covering the atlanto-occipital membrane and the lower part of the occipital bone were dissected away, and the

* W.H.O. Fellow. † British Council Scholar.

membrane was opened. The lower part of the vermis of the cerebellum was exposed by nibbling away the margins of the occipital bone at the border of the foramen magnum. This made it possible to lift the cerebellum gently with a small curved spatula from the medulla oblongata and to insert a fine polythene tube along the floor of the fourth ventricle into the middle of the aqueduct. Perfusion was always begun with artificial c.s.f. without histamine and was continued for more than 1 hr before the ventricles were perfused with histamine 1:1000 or 1:10,000 at a rate of 0·1 ml./min.

Injections of histamine into the lateral ventricle and into the cisterna. In a few experiments the effect of an intraventricular injection of 500 μg histamine in 0·2 ml. volume on acid gastric secretion was examined under conditions which prevented the histamine from passing into the subarachnoid space. The ventricular cavities were first perfused for some time in the usual manner with artificial c.s.f. Perfusion was then stopped but the outflow was allowed to drain off from the aqueductal cannula; 0·2–0·3 ml. effluent was collected before the flow ceased. The aqueductal cannula was then closed by inserting a small glass stopper and the histamine was slowly injected through the ventricular cannula.

In order to find out whether histamine was absorbed from the subarachnoid space it was, in one experiment, infused into the cisterna under conditions which prevented its entrance into the cerebral ventricles. The cisterna was cannulated by puncturing the atlanto-occipital membrane and c.s.f. fluid (0·8 ml.) was allowed to flow out; the perfusion pump was then connected to the cisternal cannula through a polythene tube and a 1:1000 histamine solution was infused into the cisterna at a rate of 0·1 ml./min for 5½ min. The dead space of the cannula was about 0·05 ml. so that the histamine was in fact infused for 5 min only. The infusion was stopped, the cisternal cannula was closed and kept closed during the following 2 hr, during which acid gastric secretion was observed. In order to prevent any histamine from entering the cerebral ventricles, a counterflow of histamine-free fluid at half the rate was maintained from the implanted cannula during the 5½ min period of histamine infusion. The evidence that histamine had not entered the cerebral ventricles was obtained when, at the end of the experiment, the procedure was repeated, but with dye instead of histamine in the fluid used for cisternal infusion. Post mortem the outside surface and meninges of the spinal cord and the base of the brain stem and hypothalamus were found to be deeply stained but there was no staining in the fourth, third or lateral ventricles, nor of the surface of the cerebral hemispheres.

Acid gastric secretion was estimated by the method of Edkins (1906). The details of the procedure were essentially those described by Bhawe (1958). The cats were kept without food for 24 hr. A polythene tube was inserted into the stomach through a small cut on the anti-mesenteric border below the pyloro-duodenal junction, and after washing out the stomach it was filled with 15 ml. warm NaCl solution 0·9 % (w/v) introduced through the polythene tube and allowed to remain in the stomach for 20 min. The solution was then removed and replaced by another 15 ml. NaCl solution 0·9 %, and the procedure was repeated every 20 min. The volume of the 20 min samples was measured and their free HCl content titrated against 0·01 N-NaOH, with Töpfer's reagent as indicator. In several experiments the acid gastric secretion was compared with that produced by a slow infusion of histamine into the femoral vein through a continuous slow injector.

Histamine uptake by brain tissue

In order to determine the amounts of histamine taken up by various parts of the brain, samples of tissue were removed from different regions lining the ventricles and also from parts away from the perfused area. After perfusion of the cerebral ventricles with histamine 1:1000 for 1 hr and then with artificial c.s.f. for 20 min the cat was killed by an intravenous overdose of pentobarbitone sodium. The brain was quickly removed and cut in the mid line. Unless otherwise stated the tissue samples were taken from the left half of the brain, the side of cannulation of the lateral ventricle. Small blocks of tissue, usually weighing 20–40 mg

but sometimes as much as 70 mg, were cut out with fine scissors, dipped into a few millilitres of artificial c.s.f., gently dried on filter paper, weighed in small tin-foil cups on a torsion balance and then at once ground in a mortar with a small volume of N/3-HCl, boiled for 1–2 min, neutralized with N/3-NaOH after cooling, filtered and assayed for histamine. The following parts of the brain were taken:

Hypothalamus. First the hypophysis was cut off and discarded, then a nearly square tissue sample of about 2 mm depth was cut out of the hypothalamus. Its position and shape are shown in the inset of Fig. 6. The upper side of the square runs through the lower part of the massa intermedia; the lower side is formed by the floor of the third ventricle; anteriorly the square extends to a line passing through the optic chiasma and the anterior commissure, and posteriorly to a line just in front of the mamillary bodies and the beginning of the aqueduct. This tissue sample will be referred to as superficial hypothalamus. From the same site a further, deeper sample of tissue was cut out, also about 2 mm in thickness. This sample will be referred to as deep hypothalamus. In three experiments 125–150 sagittal sections of 40 μ thickness were cut out from a frozen block of the same region of the hypothalamus shown in the inset of Fig. 6. The first 40–52 sections were extracted and assayed in groups of four, the later ones in groups of eight.

Caudate nucleus. From the body of the caudate nucleus in the floor of the lateral ventricle, near the interventricular foramen, two tissue samples, a superficial and a deep, each about 2 mm thick, were removed from the same site. They will be referred to as caudate nucleus, superficial and deep.

Septum pellucidum. The medial wall of the lateral ventricle anterior to the interventricular foramen was cut out.

Massa intermedia. The massa was transected when the brain was divided in half. By cutting along the margin of the massa intermedia of the left half, a layer of tissue, about 2 mm thick, was removed.

Lateral wall of lateral ventricle. A square block of about 7 mm $\times$ 2 mm was cut from the central part of the ventricle; it contained white matter of the corona radiata.

Fimbria. The medial wall of the lateral ventricle was folded back and the exposed white tissue along the hippocampus was cut off.

Whole brain. After removal of the cerebellum and brain stem by cutting the mid-brain at the intercollicular level, the brain was divided in the mid line and each half was ground with sand in N/3-HCl about 1 ml./g. The further procedure of extraction was the same as for the tissue blocks.

Assay of histamine. The extracts of brain tissue as well as the effluent collected during and after perfusion of the cerebral ventricles were assayed for histamine on the guinea-pig ileum preparation suspended in 15 ml. oxygenated Mg-free Tyrode solution at 34° C containing atropine $1/3 \times 10^{-7}$. When extracts of brain tissue were assayed no precautions were taken to avoid interference by other substances in the extracts which either cause contraction or sensitize the preparation to histamine. When extracts yielding high histamine values per gram tissue were assayed, only small amounts of tissue (1 mg or less) needed to be added to the bath; the values so obtained can be taken as representing the true histamine content, since these amounts of tissue from a brain not perfused with histamine did not stimulate the guinea-pig gut and did not potentiate the action of histamine. On the other hand, when the tissue contained less than 5 μg histamine per gram larger amounts of extract were required for testing; the actual histamine content of the extract may therefore have been somewhat lower than the values recorded. In some of these assays giving low histamine values it was in fact found that a residual contraction remained when the extract was tested after the addition of mepyramine (0·003 μg/ml.), which abolished equivalent histamine contractions. However, since we were not interested in the exact histamine content of samples which yielded only 5 μg/g tissue or less, purification of the extracts was not attempted. All values given for histamine in this paper refer to the base.

RESULTS

Acid gastric secretion

Perfusion with histamine 1:1000 from the lateral ventricle to the aqueduct resulted in acid gastric secretion. A central effect of histamine, mediated either via the vagi or by release of adrenocorticotrophic hormone, which in its turn would release adrenocortical hormones capable of producing acid gastric secretion (French, Longmire, Porter & Movius, 1953; Porter, Movius & French, 1953), could be excluded, since the secretion occurred also after section of the vagi and after removal of the suprarenals.

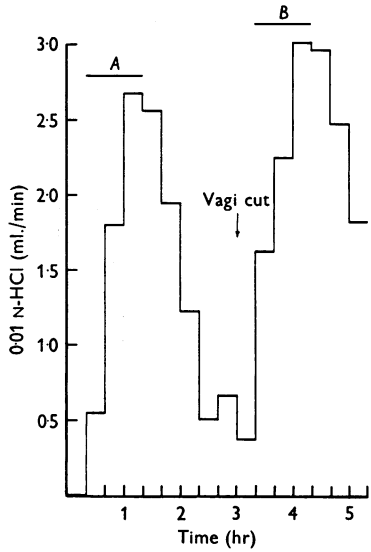

Fig. 1. Acid gastric secretion (ordinate) produced in a 2·6 kg cat under pentobarbitone sodium anaesthesia by two 1 hr periods of perfusion of the cerebral ventricles with histamine 1:1000 at a rate of 0·1 ml./min. The solid lines on top give the periods of histamine perfusion (*A*) before and (*B*) after cutting the vagi in the neck (at arrow).

Figure 1 illustrates the acid gastric secretion produced by perfusion of the cerebral ventricles with histamine, and the fact that the secretory response persists after dividing the vagi. A similar result was obtained by Bhawe (1958) with intraventricular injections of histamine.

The maximal acid gastric secretion, produced during the perfusion with histamine 1:1000, amounted in some cats to less than 0·5 ml., in others to more than 5 ml. N/100-HCl per minute. These great variations result, not from differences in the rate of absorption of histamine into the blood stream, but from differences in the sensitivity of the oxyntic cells. The acid gastric secretion, whether it was weak or strong, corresponded always to

that produced by an intravenous infusion of 1·2 μg histamine per minute. This is illustrated in Fig. 2, which gives the results obtained in three cats. In cat *A* the secretory response to perfusion of the cerebral ventricles with histamine 1:1000 for 1 hr was weak, in cat *B* it was intermediate, and in cat *C* it was strong. But the response was always of the same order as that obtained with intravenous infusion of histamine at 1·2 μg/min for 1 hr. The figure shows also that when histamine was applied by the same route for a second time the response was greater. This was usually observed.

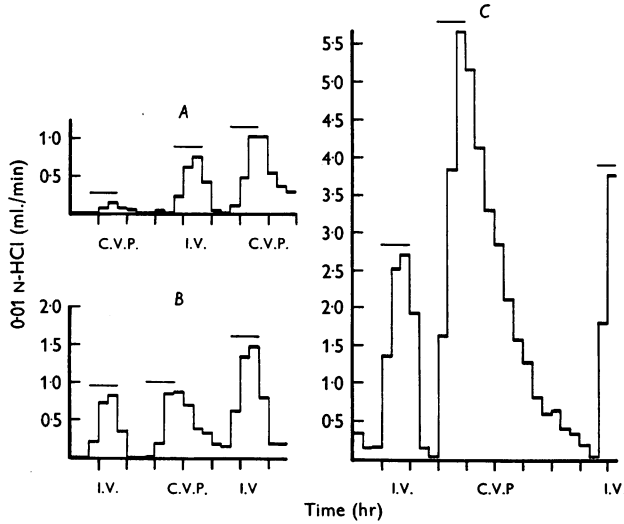

Fig. 2. Acid gastric secretion (ordinate) produced in three cats under pentobarbitone sodium anaesthesia. Comparison of the secretion produced by perfusion of the cerebral ventricles with histamine 1:1000 at a rate of 0·1 ml./min with that produced by intravenous infusion of 1·2 μg histamine per minute. (*A*) 2·6 kg cat showing a weak, (*B*) 2·6 kg cat showing an intermediate, and (*C*) 2·5 kg cat showing a strong secretory response. The solid horizontal lines give the periods of cerebral ventricular perfusion (C.V.P.) and of intravenous infusion (I.V.) of histamine.

The onset of secretion and its rise during the 1 hr periods were similar whether the hisatmine was infused intravenously or perfused through the cerebral ventricles. This was particularly striking in those experiments in which the secretory responses were small and delayed. If acid secretion failed to appear during the first 20 min period, it did not occur after histamine was administered by either route. However, since the periods of sampling of fluid from the stomach were always 20 min, differences of a few minutes in the onset of secretion would scarcely have been revealed.

There was one difference in the time course of secretion. When the intravenous infusion of histamine was stopped, the secretion decreased relatively quickly and often came to an end within 1 hr. On the other hand, on perfusion of the cerebral ventricles the maximal acid secretion was

sometimes obtained in the 20 min sample collected after the end of the histamine perfusion (see cat *B*, Fig. 2); in some experiments this sample contained as much acid as the sample collected during the last 20 min of histamine perfusion (see cat *A*, Fig. 2). Further, the acid gastric secretion continued at a slowly diminishing rate but on a low level for several hours (see cat *C*, Fig. 2).

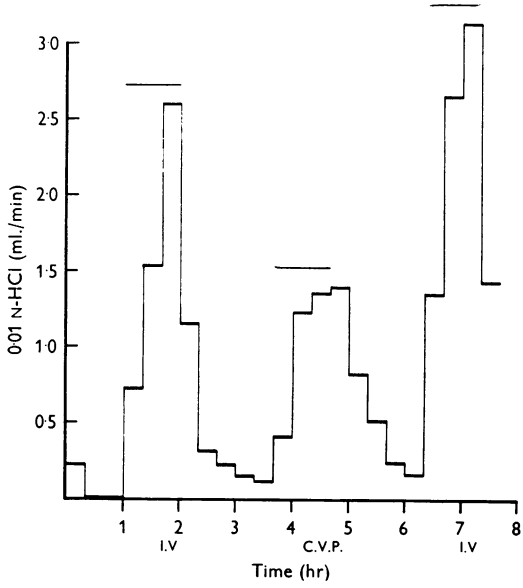

Fig. 3. Acid gastric secretion (ordinate) produced in a 2·4 kg cat under pentobarbitone sodium anaesthesia. Comparison of the secretion produced by perfusion of the cerebral ventricles with histamine 1:10,000 at a rate of 0·1 ml./min with that produced by intravenous infusion of 1·2 μg histamine per minute. The solid horizontal lines give the periods of cerebral ventricular perfusion (C.V.P.) and of intravenous infusion (I.V.) of histamine.

From the fact that the perfusion of the cerebral ventricles with histamine 1:1000 produced an acid gastric secretion similar to that obtained on intravenous infusion of 1·2 μg/min, we may conclude that during the 1 hr perfusion with 6000 μg histamine about 72 μg passed into the circulation, and even when we take into account the small additional amounts of histamine which must have entered the circulation during the following hours whilst acid gastric secretion continued at a low level, the total amount which entered the circulation cannot have been more than 100 μg histamine and was probably less.

Acid gastric secretion occurred also when the cerebral ventricles were perfused with histamine 1:10,000 but the effect was less than that produced by intravenous histamine 1·2 μg/min, as is shown in Fig. 3. Thus less histamine is absorbed than during perfusion with histamine 1:1000.

The amount absorbed, as shown by the three experiments of Fig. 4, appeared to be about 0·15 μg/min. In each experiment the maximal rate of acid gastric secretion was about 0·5 ml. N/100-HCl per minute, and this secretion was less than that produced by intravenous histamine 0·6 and 0·3 μg/min and more than that produced by 0·075 μg/min; it was about

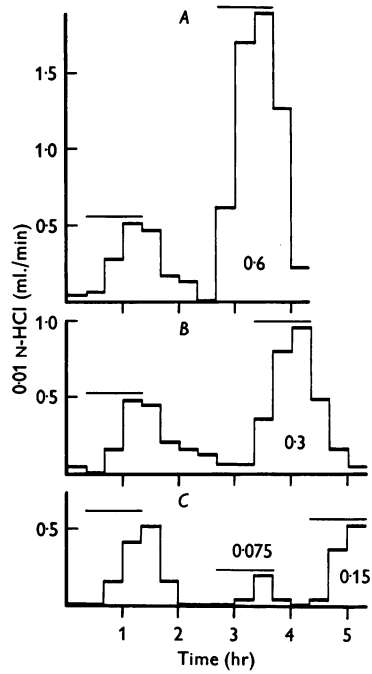

Fig. 4. Acid gastric secretion (ordinate) produced in three cats under pentobarbitone sodium anaesthesia. Comparison of secretion produced by 1 hr perfusion of the cerebral ventricles with histamine 1:10,000 at a rate of 0·1 ml./min with that of intravenous infusion. The amounts infused per minute intravenously are given by the figures in the histograms. Cat *A* (2·3 kg) 0·6 μg/min; cat *B* (2·4 kg) 0·3 μg/min; cat *C* (2·1 kg) 0·075 and 0·15 μg/min. The unmarked first response in all three cats is due to the perfusion of the ventricles with the histamine. The horizontal lines give the periods of ventricular perfusion or intravenous infusion of histamine.

equal to, or slightly greater than, that produced by 0·15 μg/min. This would correspond to a passage into the blood stream of about 10 μg histamine during the one hour from a total of 600 μg perfused through the cerebral ventricles.

Histamine is also absorbed from the subarachnoid space, since it produced acid gastric secretion when infused into the cisterna under conditions in which its entrance into the cerebral ventricles was prevented. This is shown in the experiment Fig. 5.

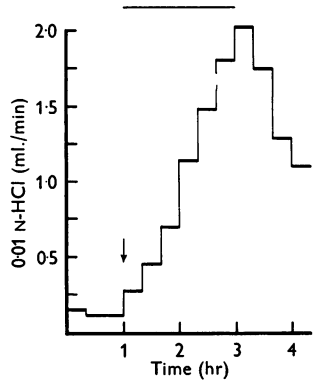

Fig. 5. Acid gastric secretion (ordinate) produced in a 2·5 kg cat under pentobarbitone sodium anaesthesia by introduction of 500 μg histamine into the cisterna. At the arrow 0·5 ml. histamine 1:1000 infused intracisternally at a rate of 0·1 ml./min. Cisternal cannula closed for 2 hr, as indicated by the horizontal line.

Recovery of histamine in aqueductal effluent

During the perfusion with histamine 1:1000 or 1:10,000 at a rate of 0·1 ml./min for 1 hr, the amount of fluid collected from the aqueduct was 6 ml. or a little more. There was therefore no net fluid loss. Nor was there any fluid loss when perfusion was continued without histamine. On the other hand, histamine could not be fully recovered from the effluent. This is shown in Tables 1 and 2.

Table 1 summarizes the results of seven experiments in which histamine 1:1000 was perfused, and gives for each experiment the histamine recovered in successive 20 min samples of aqueductal effluent. The first three 20 min samples collected whilst the 6 ml. histamine 1:1000 was perfused through the ventricles contained large amounts of histamine but, with the exception of one sample, less than 2000 μg. When perfusion was continued without histamine there was a rapid fall of histamine in the effluent. The first 20 min sample still contained between 100 and 200 μg, and in one experiment even more, but the second and particularly the third sample following the histamine perfusion usually contained less than 10 μg. Small amounts of histamine continued to appear in the effluent for several hours, as is shown in the last three experiments in the Table. By adding up these amounts it was found that the prolonged perfusion had added only another 25–45 μg to the recovery. Thus when determining the recovery in the effluent the error introduced by stopping the perfusion 1 hr after the histamine perfusion is negligible.

The recovery in the seven experiments of the Table varied between 4785 and 5761 μg (mean 5167 μg). This is a mean loss of 833 μg or of 14 %. When

considering this loss one should bear in mind the 5 % error involved in the assay of histamine. For the later samples, which contain relatively small amounts of histamine, this error would amount to a few μg only, but for the first three samples collected during the histamine perfusion, in which most of the histamine is recovered, this error could add up to nearly 300 μg.

The recovery of histamine in the aqueductal effluent during perfusion with histamine 1:10,000 is shown for four experiments in Table 2. The duration of perfusion with histamine was 1 hr in three experiments and

TABLE 1. Histamine recovered (μg) in successive 20 min samples of aqueductal effluent during perfusion for 1 hr with histamine 1:1000; total amount perfused 6000 μg

	Expt. no.						
Sample	1	2	3	4	5	6	7
1	1820	1740	1270	1670	1680	1450	1450
2	2000	1800	1670	1700	1680	1600	1670
3	1820	1600	1700	1670	1520	1600	1600
4	110	150	130	104	164	168	366
5	7	7	10	9	12	19	20
6	4	3	5	6·4	10	16	8
7	—	—	—	—	10	9	7
8	—	—	—	—	7	9·6	5
9	—	—	—	—	5	5·3	3
10	—	—	—	—	2·5	4·8	2·4
11	—	—	—	—	2·5	3·2	1·7
12	—	—	—	—	—	2·5	1·5
13	—	—	—	—	—	2·4	1·2
14	—	—	—	—	—	1·8	0·9
15	—	—	—	—	—	1·6	0·7
16	—	—	—	—	—	1·6	0·5
17	—	—	—	—	—	0·27	0·3
18	—	—	—	—	—	0·21	0·2
19	—	—	—	—	—	0·16	0·12
20	—	—	—	—	—	—	0·075
Total recovery	5761	5300	4785	5159	5094	4895	5139

TABLE 2. Histamine recovery during perfusion with histamine 1:10,000; histamine content (μg) in successive 20 min samples of aqueductal effluent. In Expts. 1–3, perfusion with histamine was continued for 1 hr, in Expt. 4 for 2 hr. Total amount of histamine perfused, 600 and 1200 μg respectively

	Expt. no.			
Sample	1	2	3	4
1	145	148	145	145
2	170	167	145	160
3	175	167	150	160
4	14	14·8	17	145
5	3	1·5	3	145
6	1·4	0·64	—	160
7	0·8	0·34	—	11·6
8	0·44	0·21	—	2·2
9	0·28	0·11	—	—
10	0·14	—	—	—
Total recovery	510	500	460	929

2 hr in one. The time course of recovery was similar to that found in the perfusions with histamine 1:1000. The loss in the three experiments, in which 600 μg histamine was perfused during 1 hr, was between 90 and 140 μg, and in the one experiment in which 1200 μg was perfused during 2 hr, the loss was 271 μg. The mean loss in all four experiments was 19 %.

Protein content. When an equal amount of concentrated nitric acid was added to a sample of c.s.f. collected from the cisterna a faint but definite opalescence was obtained, indicating the presence of small amounts of protein in the c.s.f. This test was always negative when applied to samples of aqueductal effluent collected during perfusion of the cerebral ventricles,

TABLE 3. Histamine content (μg/g fresh tissue) of various parts of the left half of the brain and of the choroid plexus after perfusion for 1 hr of the cerebral ventricles with histamine 1:1000 from the cannulated left lateral ventricle

Tissue samples	Expt. no. 1	2	3	4	5	6	7	8	9	10	Mean
Hypothalamus (superficial)	104	114	111	137	111	—	160	173	333	148	155
Hypothalamus (deep)	—	50	59	44	23	—	—	—	—	—	44
Massa intermedia	—	—	—	—	—	57	142	—	55	52	77
Septum pellucidum	320	266	125	242	182	200	363	470	250	200	262
Caudate nucleus (superficial)	—	200	172	250	211	143	363	190	333	225	232
Caudate nucleus (deep)	—	—	30	50	32	—	—	—	—	—	37
Lateral wall of lateral ventricle	16	50	11	80	—	—	—	—	76	40	46
Fimbria	—	—	45	235	27	32	222	47	31	111	94
Choroid plexus	33	15	25	57	32	—	—	—	22	57	34

whether with or without histamine. A different result was obtained in the experiments in which 500 μg histamine in 0·2 ml. artificial c.s.f. was injected, either into the ventricle or into the cisterna. The fluid was kept in these spaces so as to allow any protein to accumulate. When the aqueductal or cisternal cannula was opened 2 hr later, between 0·4 and 1·2 ml. of fluid flowed out under pressure. This fluid contained large amounts of protein and gave a heavy precipitate when treated with concentrated nitric acid. Such an effect did not occur when 0·2 ml. artificial c.s.f. without histamine was injected intraventricularly, although there was also a similar rapid outflow of fluid when the aqueductal cannula was opened 2 hr later; this fluid gave a just-perceptible opalescence when treated with concentrated nitric acid. This shows that histamine leads to the appearance of large amounts of protein in the c.s.f.

Uptake of histamine by brain tissue

The histamine content of the various samples of brain tissue examined after 1 hr perfusion of the cerebral ventricles with histamine 1:1000 is shown in Table 3. The content is expressed in μg/g fresh tissue.

High histamine values (between 104 and 470 μg/g) were regularly found in the samples of tissue taken from regions of grey matter lining the ventricles; i.e. hypothalamus (superficial), caudate nucleus (superficial) and septum pellucidum. In all but one experiment the values for the caudate nucleus and septum pellucidum were higher than for the hypothalamus. But there were no great differences between caudate nucleus and septum pellucidum, sometimes the one and sometimes the other giving the higher value.

The deeper layers of the hypothalamus and caudate nucleus contained 23–59 μg histamine per gram. Although these values are lower than those of the corresponding superficial tissue layers, they provide evidence that histamine had passed from the ventricular surface into these distant layers of grey matter, since their normal histamine content was 5 μg/g or less.

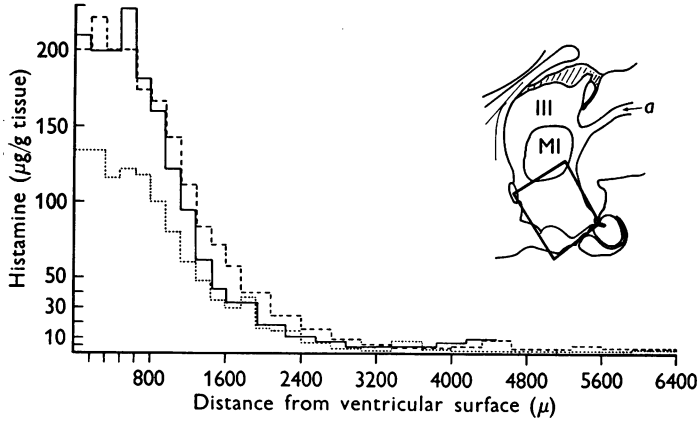

Fig. 6. Penetration of histamine into the wall of the hypothalamus after perfusion of the lateral ventricles with histamine 1:1000 for 1 hr. Three different experiments: —— Expt. *A*, – – – Expt. *B*, · · · Expt. *C*. Histamine content in μg/g of frozen sections cut parallel to the ventricular surface. Inset: diagram of the medial aspect of the diencephalon; the block for tissue sections was taken from the area indicated by the square.

In three experiments the penetration of histamine was studied in more detail in frozen sections of the hypothalamus cut parallel to its ventricular surface. The results are given as histograms in Fig. 6. In experiments *A* and *B* the histamine content of the first 16 sections was 200 μg/g or more; thereafter the histamine values fell rapidly with each group of four sections, and values of less than 5 μg/g were obtained after about 80 sections. In experiment *C* the histamine content of the first sections was only just over 130 μg/g, but in the subsequent sections it declined about as rapidly as in the other two experiments. From these results it is evident that histamine penetrates into the hypothalamus for at least 2·5 mm.

In experiment *A*, after the last frozen section was cut, the remainder of the block was fixed in formalin for histological examination and the first section cut from this block was stained for cells and fibres with Luxol Fast Blue according to the procedure of Klüver & Barrera (1953). It corresponded in Hess's atlas of the cat's brain (Hess, 1932, 1956), to the sagittal section in plane 180, which is over 4 mm from the mid line. This agrees well with the number of frozen sections cut, since 112 sections of 40 μg had been removed before the block was fixed in formalin.

In experiment *A*, after the histamine value of the frozen sections had fallen to 4·4 μg/g, it started to rise again and reached a value of 9·5 μg/g in the last 8 frozen sections. In the other two experiments there were also small transient increases in the histamine values after they had fallen to a low level. These rises cannot be fully explained. They may reflect a penetration of histamine from the lateral ventricle; for instance, in experiment *A*, in which the remainder of the block was fixed in formalin, the last section had actually passed through the tip of the inferior horn. Or they may reflect the presence in these regions of the brain of larger amounts of naturally occurring gut-stimulating substances other than histamine, such as substance P or 5-HT. The problem has not been further examined.

The massa intermedia was examined in four experiments. In three the histamine values were just over 50 μg/g, which corresponds to the values obtained for the deeper layers of the hypothalamus and caudate nucleus. In one experiment the value was 142 μg/g. This high value could be the result of a more effective penetration of histamine in this particular experiment, but it could just as well be due to the fact that the piece of tissue removed contained a larger proportion of tissue from near the ventricular surface.

The white matter of the lateral wall of the central part of the lateral ventricle yielded in each experiment considerably lower histamine values than the grey matter lining either the lateral ventricle (septum pellucidum, caudate nucleus) or the third ventricle (hypothalamus). This finding suggests that histamine passes less readily into the white matter than into the grey. The values showed great individual variations, i.e., between 11 and 80 μg/g. The lowest value, of 11 μg/g, was obtained in an experiment in which the values for other parts of tissue lining the lateral ventricle were also low in comparison with other experiments. Perfusion of the ventricle may thus have been less thorough in this experiment than usual. This explanation would not apply to the second-lowest value of 16 μg/g obtained for the white matter of the lateral wall. If the histamine were to pass only a very short distance into the white matter, small differences in thickness of the tissue samples would greatly affect their histamine value and differences in thickness of the tissue samples taken may therefore have contributed to the wide individual variations observed.

The greatest individual variations were obtained with fimbria which consists of white matter. The values varied between 27 and 237 μg/g. On

the assumption that the histamine passes less readily into white than into grey matter, low values would have been expected, and were in fact obtained in five out of eight experiments. An explanation for the two exceptionally high values may be the thinness of the fimbria.

No histamine had apparently reached the floor of the fourth ventricle, the cerebellum and the suprasylvian or lateral gyrus of the cerebral cortex, since the histamine equivalents obtained with tissue samples from these regions, which are far removed from the perfused ventricular surface, were between 1 and 6 μg/g. Values of the same order were also obtained in corresponding tissue samples obtained in control experiments in which the ventricles had been perfused with artificial c.s.f. not containing histamine. Since relatively large amounts of tissue extracts had to be used for the assay of these tissue samples their actual histamine content may have been even less than these low values (see Methods).

TABLE 4. Histamine content (μg/g fresh tissue) of various parts of the right half of the brain and of the choroid plexus after perfusion of the cerebral ventricles with histamine 1:1000 from the cannulated left lateral ventricle. The experiment numbers refer to those in Table 3

Tissue samples	Expt. no. 3	4	5	9	10	Mean
Septum pellucidum	45	73	21	30	22	38·0
Caudate nucleus (superficial)	—	—	—	17	5	11·0
Lateral wall of lateral ventricle	—	—	—	8	5	6·5
Fimbria	—	29	—	2	—	15·5
Choroid plexus	—	—	—	9	5	7·0

In a few experiments tissue samples were taken from the non-cannulated, right half of the brain. The histamine values are given in Table 4 and are lower than those of the corresponding tissue samples from the cannulated side. This is not surprising, since experiments in which a 0·2 % solution of Evans Blue was perfused through the cerebral ventricles had shown that the lateral ventricle was less stained on the non-cannulated than on the cannulated side; the staining was often slight and sometimes even absent. The uneven perfusion did not extend to the third ventricle and accordingly the values for the superficial hypothalamus were also high when taken from the right half of the brain. In fact, the values for experiments 7 and 8 in Table 3 were obtained from the right side and the value for experiment 2 from both sides of the hypothalamus.

In several experiments the histamine content of the choroid plexus of the left lateral ventricle was determined; sometimes the samples included the plexus of the third ventricle. They contained between 15 and 57 μg/g (mean 34 μg/g). These values indicate an uptake of histamine from the perfusion fluid, since the histamine equivalents for choroid plexus in two experiments in which the ventricles were perfused with artificial c.s.f. not

containing histamine were 8 and 7 μg/g only. There was no appreciable uptake of histamine by the choroid plexus of the non-cannulated ventricle, for in two histamine perfusion experiments in which the choroid plexus of the right lateral ventricle was also examined its histamine values were 9 and 5 μg/g.

In three experiments the histamine content of both halves of the brain was determined without the cerebellum, the brain being cut at the intercollicular level just caudal to the tip of the aqueductal cannula. The results are shown in Table 5. The mean histamine content of both halves together was 116 μg. The actual histamine uptake by the brain is likely to be lower, since no corrections were made for the normal histamine content of the brain or for sensitizing substances present in brain extracts.

TABLE 5. Histamine content of brain after perfusion of the cerebral ventricles from the cannulated left ventricle with histamine 1:1000 for 1 hr

	Histamine in brain (μg)			Deficit of histamine in aqueductal effluent (μg)
Expt. no.	Left half	Right half	Total brain	
1	60	49	109	620
2	71	27	98	840
3	121	20	141	653

In the three experiments of Table 5 the histamine was also assayed in the aqueductal effluent. As in other experiments of this kind, the 6000 μg histamine infused during the 1 hr period was not fully recovered in the effluent and the last column gives the loss for each experiment (averaging 704 μg). Since the histamine recovered from the brain was of the order of 100 μg and the absorption into the blood stream, as measured by the acid gastric secretion, accounted for at most another 100 μg, there remains an unaccounted loss of about 500 μg histamine. A few preliminary experiments were carried out to see whether this loss could be explained by enzymic destruction of histamine. However, when histamine was added to homogenized brain tissue or tissue of the choroid plexus and incubated for 1 hr at 37° C it was recovered almost quantitatively.

DISCUSSION

The present experiments show that histamine can be absorbed into the blood stream from the cerebral ventricles without entering the subarachnoid spaces. In addition, on its passage from the lateral ventricle to the aqueduct, histamine is taken up by the brain tissue and by the choroid plexus.

Histamine could have been absorbed into the blood stream by capillaries of either the choroid plexus or of the brain tissue itself. If the absorption

took place via the choroid plexus the histamine would have to pass through a layer of epithelial cells and then through a layer of connective tissue before reaching the capillary structures. If the absorption were through the brain capillaries, the histamine would also have to pass first through an epithelial layer, the ependyma, and then through a layer of nervous tissue. Since histamine was shown to pass into the choroid plexus as well as into the brain substance surrounding the ventricles it is not possible to state whether the passage into the blood is through the vessels of the choroid plexus or of the brain tissue or of both.

Histamine can also be absorbed from the subarachnoid space. This is shown by the fact that acid gastric secretion occurs also on injection of histamine into the cisterna under conditions which prevent its entrance into the cerebral cavities. The histamine may either enter directly into the small vessels which are so abundant in the subarachnoid spaces, or it may be absorbed by special structures such as the arachnoid villi. Some absorption may also occur via those parts of the choroid plexus which protrude through the lateral recesses into the subarachnoid space. It is, however, not necessary to assume that histamine reaches the epicerebral cranial spaces and is then absorbed via the endocranial sinuses, since we found that dye injected intracisternally under similar conditions did not reach the upper surface of the cerebral hemispheres.

The uptake of histamine from the ventricles into the brain tissue lining these cavities and its deep penetration into the grey matter within one hour is of interest in connexion with the fact that substances injected into the ventricular system of conscious cats produce profound changes in behaviour. These changes resemble in many respects those which Hess (1932, 1956) obtained in his classical experiments on electrical stimulation of diencephalic structures. In Hess's experiments the stimulating electrodes were placed at different points of the diencephalon, but responses were obtained predominantly from points within 3 mm of the mid line, i.e., from a region which can be easily reached by substances penetrating from the cerebral ventricles, as is shown by the present experiments with histamine.

The finding that the uptake of histamine by brain tissue occurs more readily in grey than in white matter, and the observation that there are also differences in histamine uptake between different regions of grey matter lining the ventricles, are at variance with conclusions reached by other authors. For instance, Spatz (1933) concludes that the penetration of dyes from the outside or inside of the brain surface progresses on a broad front independent of any structural differences. The brain behaves, as he says, like a colloidal mass. More recently Bakay (1956) states that 'we have no reason to believe that an isotope follows any particular path-

way of absorption within the brain. The process starts with a diffuse inhibition of the superficial layers. In microscopic radio-autographs P^{32} seems to invade the brain from the cerebrospinal fluid diffusely.'

A different result, however, was recently obtained by Roth, Schoolar & Barlow (1959), who injected labelled acetazoleamide (Diamox) intravenously into cats and found that it is first secreted into the cerebral ventricles and then passes into the brain tissue. This uptake was found to be more pronounced in the grey matter lining the ventricles and the aqueduct than in the white matter. Similarly when the Na-salt of the dye bromophenol blue is perfused through the cerebral ventricles, it is also taken up predominantly by grey matter (Feldberg & Fleischhauer, 1959). The fact that not only histamine but other substances as well pass more readily from the ventricular cavities into the grey matter than into the white suggests, therefore, that it is not the ability of histamine to increase capillary permeability which is the cause of its deep penetration into the grey matter. Nevertheless, histamine increases capillary permeability when injected into the cerebral cavities or into the subarachnoid space. This is evident from the appearance of protein in the ventricular and subarachnoid fluid after such injections. Histamine may also increase the permeability of ependymal cells, which might facilitate its passage into the brain tissue, but this could not explain the differences in penetration of grey and white matter.

SUMMARY

1. In cats anaesthetized with pentobarbitone sodium the cerebral ventricles were perfused from a cannula implanted into the left lateral ventricle to the cannulated aqueduct with solutions of histamine 1:1000 or 1:10,000 at a rate of 0·1 ml./min and for periods of 1 hr.

2. The histamine perfusion caused acid gastric secretion. This secretion was not a central effect of histamine mediated either via the vagi or by a liberation of adrenocorticotrophic hormone and subsequent release of adrenal cortical hormones. The secretion is attributed to absorption of histamine from the ventricles into the blood stream.

3. The amounts of histamine absorbed from the ventricles into the blood stream were assessed by comparing the secretion with that produced by intravenous infusions of histamine. On perfusion of the ventricles with histamine 1:1000 the absorption corresponded to about 1·2 μg/min, or during perfusion for 1 hr to less than 100 μg. On perfusion with histamine 1:10,000 the absorption corresponded to about 0·15 μg/min, or during perfusion for 1 hr to about 10 μg.

4. Histamine was also absorbed from the subarachnoid space, as is shown by the fact that a cisternal injection of 500 μg histamine, under

conditions which prevented its entrance into the cerebral ventricles, produced acid gastric secretion.

5. After perfusion of the cerebral ventricles for 1 hr with histamine 1:1000 histamine was found to have penetrated into the brain tissue from the ventricular surfaces. More histamine was taken up by grey than by white matter.

6. The penetration of histamine into grey matter was determined in greater detail by assaying the histamine content of frozen sections of the wall of the hypothalamus cut parallel to the ventricular surface. Histamine penetrated to a distance of at least 2·5 mm from the surface.

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