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RELEASE OF TRANSMITTERS INTO THE PERFUSED THIRD CEREBRAL VENTRICAL OF THE CAT

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SUMMARY

1. The third cerebral ventricle of cats treated with nialamide and anaesthetized with chloralose was perfused, and the effluent was tested for 5-hydroxytryptamine (5-HT) and also for acetylcholine (ACh) when the perfusion fluid contained neostigmine.

2. Under 'resting' conditions a 25 min sample of effluent contained from < 1 to 6 ng 5-HT; the release remained steady during many hours of perfusion. It was necessary to watch out for traces of blood which might contribute to the 5-HT content and which were only visible after centrifugation.

3. A number of regions in the ventral mid-brain and hind-brain were stimulated, including the two most anterior nuclei of the raphe, nucleus linearis rostralis and intermedius. Release of 5-HT (rarely more than 2 ng) was only obtained on stimulation of these two nuclei, whereas ACh was released by stimulating many points, such as the reticular formation or the decussation of the superior cerebellar peduncles, but not the two raphe nuclei.

4. Low frequencies of stimulation were more effective at releasing 5-HT, and high frequencies at releasing ACh.

5. Since the amount of 5-HT released on stimulation was rarely more than 2 ng, a powerful re-uptake process was suspected and confirmed by the use of chlorimipramine. Intravenous, intraperitoneal and intraventricular use of this drug temporarily increased the basal release to values ranging from 20 to 50 ng in 25 min samples, and about trebled the release on stimulation of either of the linear nuclei.

6. Intravenous administration of chlorimipramine (10 mg/kg) caused the disappearance of electrical responses evoked in the brain stem by afferent sensory stimuli.

INTRODUCTION

When the anterior horn of the lateral ventricle of a cat's brain is perfused with artificial cerebrospinal fluid, a very small amount of 5-hydroxytryptamine (5-HT) appears in the effluent. The amount is increased (Holman & Vogt, 1972) by electrical stimulation of the two most anterior nuclei of the raphe, the nucleus linearis rostralis or the linearis intermedius, sites of 5-HT-containing cells (for nomenclature see Taber, Brodal & Walberg, 1960). No such increase is seen on stimulation of the substantia nigra, which causes a release of dopamine and ACh, or during the activation of other pathways which release ACh (Portig & Vogt, 1969). The release of 5-HT pointed to the existence of tryptaminergic terminals in caudate nucleus and septum, two 5-HT-rich structures bordering on the anterior horn. The present work deals with release of transmitters from the surface of the perfused third ventricle. The adjoining hypothalamus contains, in addition to catecholamines, 5-HT and ACh, and the experiments were carried out in order to see whether a fraction of these substances was situated in ventricle-near terminals and to examine from where such terminals could be activated. A preliminary account has been published (Ashkenazi, Holman, & Vogt 1973).

METHODS

Adult cats of either sex were injected s.c. with nialamide 25 mg/kg 15 hr before the experiment. Longer intervals had sometimes proved fatal, but there were no casualties when no more than 15 hr elapsed between injection and the start of the experiment. Signs of sham rage or apprehension were common. The cats were anaesthetized with ether and then given chloralose I.V. (60–80 mg/kg). The trachea, and a femoral vein and artery, were cannulated; blood pressure was recorded by transducer and the cats were usually given artificial respiration since the combination of nialamide and an anaesthetic depressed respiration. The head was fixed in a stereotaxic instrument and body temperature was kept at 38–38·5° C.

Perfusions and assays. The method of perfusion was that of Carmichael, Feldberg & Fleischhauer (1964). After cannulation of the aqueduct, a vertical infusion needle was lowered at co-ordinates A 10–11, LO and H -1; the needle either bypassed or cut through the anterior part of the massa intermedia, ending in the anteroventral part of the ventricle. If the perfusion pressure did not fall to or below zero at H -1, the needle was lowered further till the pressure fell and the volume of fluid escaping from the aqueduct was the same as the inflow. Perfusion was by a proportioning (autoanalyser) pump, using a Technicon tubing supplying about 5 ml./hr. Occasionally, the flow rate was halved. The composition of the perfusion fluid and the arrangement for monitoring ventricular pressure have been reported (Portig & Vogt, 1969). Neostigmine (6×10^{-5} M) was present in the perfusion fluid when the effluent was tested for ACh. This was assayed on the eserinized dorsal muscle of the leech, and 5-HT on the rat fundus strip (Vane, 1957; see also Holman & Vogt, 1972). Methysergide bimaleate (5 μg added for 20 min to the 5 ml. bath) was used to identify the 5-HT in the perfusate. All values for 5-HT are in terms of the base.

Special checks were made to see whether the presence of chlorimipramine in the artificial cerebrospinal fluid affected the bio-assay of 5-HT. No evidence for interference was found, but as a safeguard the standards were made up in perfusion fluid containing chlorimipramine whenever the brain had been perfused with fluid containing the drug. All perfusates were collected in tubes standing in ice water and the experiment was started when no blood was visible to the naked eye. The samples were centrifuged at 4° C. If there was a deposit of red cells, its diameter was measured, and the supernatant pipetted off and frozen till it could be assayed.

A 1% solution of chlorimipramine hydrochloride was prepared in 0·9% sodium chloride solution for I.P. use, and diluted further with 4–6 volumes of 0·9% sodium chloride for I.V. infusion. When it was added to the perfusion fluid, it was added in doses varying from 10 to 50 mg/l.

Electrodes. Co-axial electrodes were positioned stereotaxically with the help of evoked responses as previously described (Holman & Vogt, 1972). The stimulus parameters for the raphe nuclei were biphasic 0·5 msec pulses of 4V strength, usually in trains of 3 stimuli (45 Hz) repeated 5 times/sec for 15 min. The parameters for stimulating the reticular formation will be discussed in the text. A difficulty which had not been present in perfusions of the lateral ventricles was the greater likelihood of contamination of the effluent with blood, the 5-HT content of which vitiated the assays. The reason was that the perfusate was collected from the very part of the ventricular system where bleeding might have been occasioned: in and near the midplane, vessels were in the paths of both the perfusion needle and the electrodes. An attempt to avoid bleeding from injury due to the electrode in frontal plane A4 was made by approaching the nucleus linearis rostralis (co-ordinates A4, L 0·4, H −3·5 to −4·5) at an angle of 25° in plane A4; theoretically, with this approach, the nucleus should be hit twice, once in its dorsal and once in its ventral region, the first time ipsilateral and the second time contralateral to the side of entry. In practice we found that the electrode did not reach the contralateral side as the stereotaxic map suggested, but we did avoid haemorrhages which had been particularly severe when approaching the same nucleus vertically. The intermediate linear nucleus was aimed at by a vertical electrode at A1, L0·4 and H −3·5 to −4·5, and all points of the reticular formation were reached by vertical electrodes. All placements were checked histologically.

RESULTS

5-Hydroxytryptamine

Fig. 1 gives one example each of the effect of electrical stimulation of the nucleus linearis rostralis (left) and nucleus linearis intermedius (right). The clear columns represent the amount of 5-HT in the absence of stimulation: in one cat it was less than 1 ng in a 25 min sample, in the other it was about 3·5 ng, and remained so for about 5 hr. Stimulation was during the first 15 min of the 25 min collection period, and the parameters of stimulation (described under Methods) were the same in both cats; in each it caused a clear increment in the release of 5-HT. Another stimulation of the intermediate linear nucleus, using a faster frequency (trains of 12 pulses at 60 Hz repeated twice a second), preceded the one illustrated in Fig. 1*b*: it elicited no response.

Whereas collection of perfusate for the purpose of estimating 5-HT was

never attempted when traces of blood could be seen suspended in the samples, it was only by centrifuging the perfusates that the presence of the smallest amounts of blood which interfere with the assays could be detected. As a rule, samples containing blood spots of more than a millimetre in diameter were discarded; waiting for several hours or interruption of the perfusion for 10 or 15 min often allowed the bleeding to stop. On rare occasions the increment in 5-HT release on stimulation was so

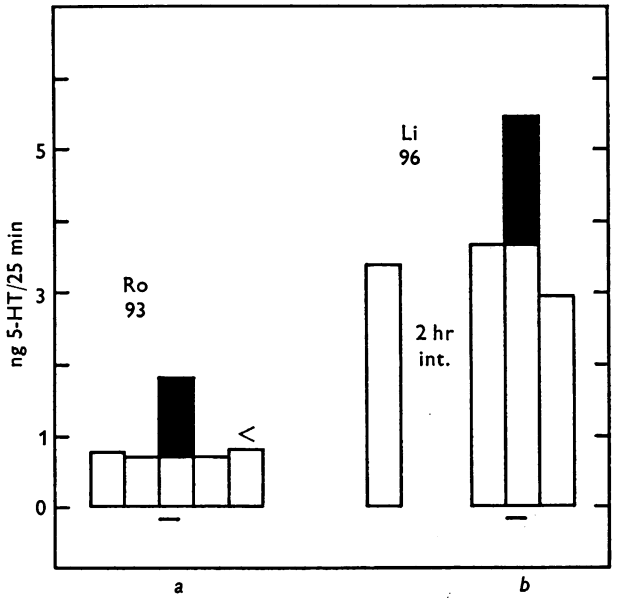

Fig. 1. Columns represent ng of 5-HT in consecutive 25 min samples of perfusate of 3rd ventricle of two cats anaesthetized with chloralose (60 mg/kg I.V.) and given nialamide (25 mg/kg S.C.) 15 hr before the experiment. No blood in any sample. Electrical stimulation of two raphe nuclei with the same parameters (trains of three biphasic pulses of 4 V repeated 5 times/sec). Increment produced by stimulation shaded. *a* Black bar indicates stimulation of nucleus linearis rostralis (*Ro*) during the first 15 min of the collection period. *b* Higher basal release of 5-HT than in *a*, which, however, kept constant for 5 hr. Black bar, stimulation of nucleus linearis intermedius (Li). 2 hr int. = interval of 2 hr.

great that it could be detected in spite of a contamination of the samples with blood. This is illustrated in Fig. 2, in which hatching indicates the diameter of the blood spots; it was 2·5 mm in the first and 0·2 mm in the last sample. The difference between the 5-HT content of the first and the last (control) samples was 2·5 ng, which was thus the amount probably contributed by a blood spot of 2·5 mm; since stimulation produced an

increase of 5 ng 5-HT while the blood spot was getting smaller, there was little doubt about the releasing effect of stimulation.

In ten of the twelve experiments in which the electrode was found to be in the rostral nucleus, stimulation caused an increase in the 5-HT content of the effluent. Similarly, in ten of fourteen correct placements in the intermediate nucleus, a release of 5-HT was observed. In three of the failures, the electrode, on entering or passing through the nucleus, had caused much cell damage. The 15 min stimulation period of either nucleus rarely released more than 1–2 ng 5-HT. There was no or little spill-over into the next 25 min sample.

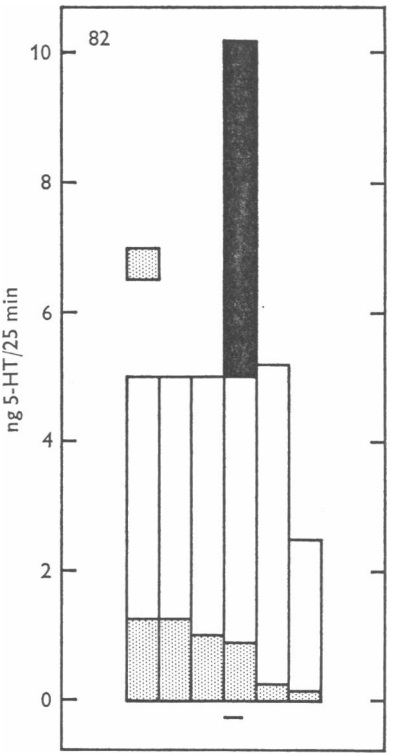

Fig. 2. Columns represent ng of 5-HT in consecutive 25 min samples of perfusate of 3rd ventricle of cat anaesthetized with chloralose (80 mg/kg) and pretreated with nialamide (25 mg/kg s.c.) 18 hr before experiment. There was blood in the perfusate, and the diameter of the blood spot obtained by centrifuging is indicated by stippling; the scale for the diameter of the spots is shown by the height of the square drawn in the upper part of the figure which represents a spot of 1 mm. At the bar, stimulation of nucleus linearis rostralis for 15 min (same parameters as Fig. 1). The difference in resting release of 2·5 ng 5-HT between first and last sample indicates that the blood in the first sample contributed about 50 % of the 5-HT present.

Whenever the electrodes were in places other than the linear nuclei, release of 5-HT was not observed. Thus there was no release on stimulation of the interpeduncular nucleus, the nucleus ruber and its vicinity, and the decussation of the superior cerebellar peduncles, sites which were frequently tested since they are near the raphe nuclei; other regions giving negative results, and stimulated only occasionally, were the third nucleus, the nucleus reticularis pontis, the decussatio tegmenti dorsalis, the habenulo-interpeduncular bundle, the nucleus pedunculi mammillaris and other parts of the hypothalamus.

The very low basal release of 5-HT into the lateral as well as into the third ventricle in the presence of an inhibitor of monoamine oxidase suggested an efficient uptake mechanism for the 5-HT liberated during nervous activity. This possibility was tested by the administration of chlorimipramine. This compound was chosen because it had been found to be the most potent antagonist of 4-methyl-α-ethyl-meta-tyramine, a drug causing a loss of cerebral 5-HT, the antagonism being attributed to inhibition of uptake of 5-HT (Carlsson, Corrodi, Fuxe & Hökfelt, 1969).

Fig. 3 shows an experiment in which electrode 1 was later found to be in the nucleus pedunculi mammillaris. After a control perfusion lasting more than 2 hr, during which stimulation of electrode 1 produced no effect, chlorimipramine hydrochloride 10 mg/kg was infused I.V. over a period of 20 min. Collection of perfusate was resumed 20 min later. The blood pressure was 15 mm lower than before the infusion, but slowly returned to the original level. In spite of this, it was impossible to obtain evoked responses by recording from the electrode during electrical stimulation of the four paws. These evoked responses had been of normal size before the infusion of chlorimipramine and are used as a guide to the depth of the electrode tips (Holman & Vogt, 1972). Electrode placement had now to rely exclusively on the stereotaxic co-ordinates, of which the depth is the least reliable.

The first perfusate (Fig. 3), collected 20 min after the end of the chlorimipramine infusion, contained 14 times the amount of 5-HT found before giving the drug. This increase lasted for 100 min, when the output levelled off to about 7 times the original amount. Stimulation of electrode 1 again produced no increment in 5-HT. A second electrode was then lowered (co-ordinates A1, L0·7, H $-4{\cdot}0$); there was still complete absence of any evoked responses to sensory stimuli. This electrode was later found to be in the nucleus linearis intermedius and its stimulation produced a release of 6 ng of 5-HT.

Other experiments, in which chlorimipramine 10 mg/kg was infused slowly intravenously, showed that the onset of the increase in 5-HT was very rapid, reaching half its maximal value during the first 30 min after

the end of the infusion, and its maximum (about 30–37 ng in 25 min) during the next 30 min. Different ways were tried to avoid damage to the heart and the loss of cerebral evoked responses while retaining the long-lasting increase of the 5-HT content of the effluent. Reduction of the intravenous dose shortened the duration of the inhibition of uptake but still interfered wih evoked responses. Addition of the drug to the perfusion fluid in doses ranging from 10 to 50 mg/l. produced rises of up to 25 ng in

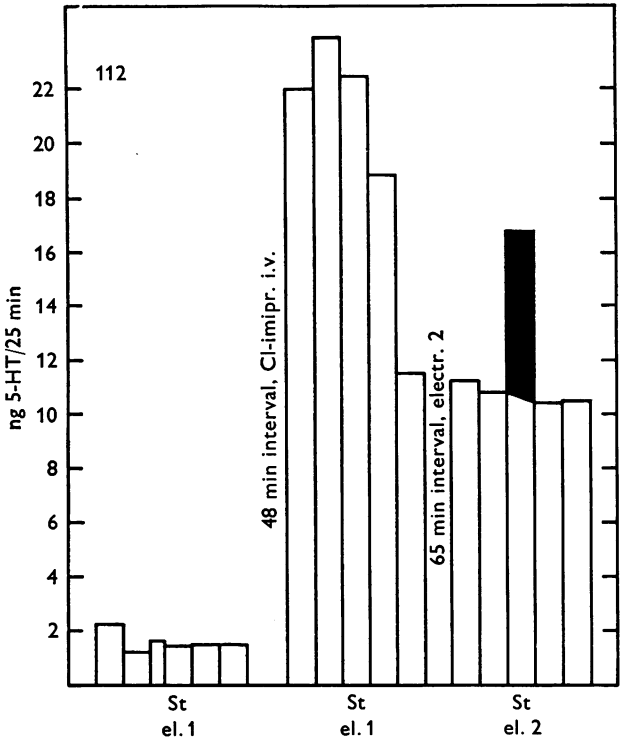

Fig. 3. Columns represent ng of 5-HT released in 25 min into consecutive samples of perfusate of third ventricle of cat anaesthetized with chloralose (70 mg/kg) and given nialamide (25 mg/kg s.c.) 15 hr before the experiment (the perfusion fluid contained neostigmine and the cat was injected s.c. with atropine sulphate 3 mg/kg in order to be able to carry out estimations of ACh). Electrode 1 (angled) was aimed at the nucleus linearis rostralis but deviated anteriorly and was found in the nucleus pedunculi mammillaris. Its first stimulation (St el. 1) did not release any 5-HT. Chlorimipramine, 10 mg/kg, was then slowly infused I.V., and 20 min later the collection of perfusate was resumed. Basal secretion rose from 1·5 to 22 ng, and stimulation of electrode 1 again produced no effect. During a second interval electrode 2 was lowered and placed just laterally to the nucleus linearis intermedius. Its stimulation (St el. 2) elicited a release of 6 ng 5-HT.

the resting 5-HT content of 25 min samples of perfusate and hardly any interference with evoked responses. The efficacy did not appear to increase with the dose beyond about 20 mg/l. The method, however, had a slight practical disadvantage: the chlorimipramine is taken up by the plastic perfusion tubing used in the pump; the stored drug is not readily removed by rinsing, but is slowly given off by the tubing if this is used for a second perfusion.

An example of the effect of adding chlorimipramine to the artificial cerebrospinal fluid is shown in Fig. 4. Both electrodes were positioned

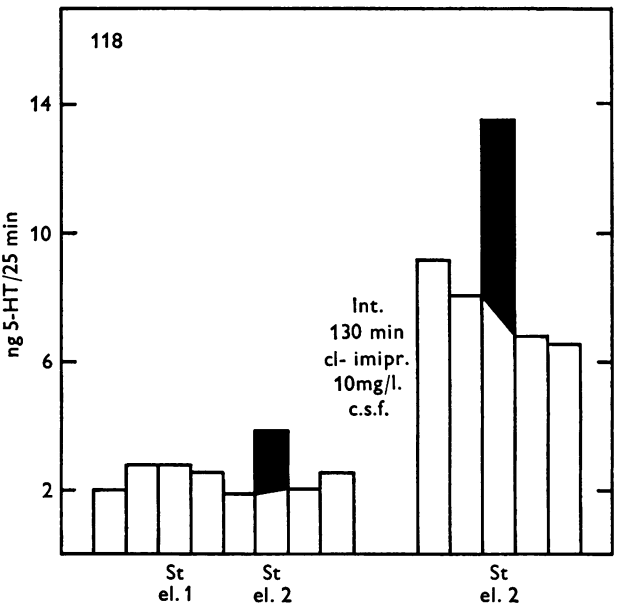

Fig. 4. Columns represent 5-HT content of consecutive samples of perfusate from the 3rd ventricle of a cat anaesthetized with chloralose (60 mg/kg) and injected s.c. with nialamide (25 mg/kg) 15 hr before the experiment (the perfusion fluid contained neostigmine but ACh estimations were not carried out). Samples before the interval were collected for 25 min, and after the interval for 20 min. For the sake of clarity, the release is expressed everywhere for periods of 25 min. At the start of the experiment, two electrodes had been inserted, electrode 1 was later found to be in the interpeduncular nucleus, electrode 2 in the nucleus linearis rostralis. Stimulus parameters as in Fig. 1. Stimulation of electrode 1 had no effect, but that of electrode 2 released 2 ng of 5-HT. After an interval of 75 min, the perfusion fluid was changed to fluid containing chlorimipramine 10 mg/l. Because of the dead space of the perfusion system, the chlorimipramine reached the ventricle 15 min later. The first control sample shown in the right part of the Figure was started when the ventricles had been perfused with chlorimipramine for 40 min. The basal release had risen about fourfold, and stimulation of electrode 2 now released 5·5 ng of 5-HT.

before starting the collection of perfusate; electrode 1 was later found to be in the interpeduncular nucleus, and electrode 2 in the rostral linear nucleus. Stimulation of electrode 1 (left side of Fig. 4) did not release any 5-HT, whereas stimuli of the same parameters applied to electrode 2 released 2 ng (100% increase over resting level). During the interval of 130 min between the two parts of the Figure, chlorimipramine (10 mg/l.) was added to the perfusion fluid 55 min before, and it reached the brain 40 min before the collection period which is represented by the first column on the right part of Fig. 4. The increase in resting release was modest compared with the effect of I.V. infusion of 10 mg/kg, but stimulation of electrode 2 now released 5·5 ng 5-HT which represented a 68% increase over the preceding resting period, and 2·7 times the release obtained before the drug had been given.

Finally, in five experiments, the chlorimipramine was injected intraperitoneally; collection of samples was resumed 10–15 min after the injection. Their 5-HT content had reached its maximum by that time; with 10 mg/kg the highest output of 5-HT for 25 min lay between 20 and 40 ng, and in one experiment with 20 mg/kg it was about 50 ng. Evoked responses were usually unaffected or only slightly reduced, but in one cat blood pressure started to fall precipitously 1 hr after the I.P. injection of 10 mg/kg, and when it had fallen below 50 mm Hg the evoked responses disappeared.

Whichever mode of administration of chlorimipramine had been chosen, the increment in the 5-HT content of perfusate during stimulation of either of the linear nuclei was usually quite high, and frequently reached 6–8 ng; however, expressed as percentage of control release, it tended to become smaller as the resting values rose. Since in a bioassay the response increases with the logarithm of the dose, an increment representing a smaller rise over control was less easy to estimate in spite of the larger absolute amounts involved.

A number of perfusates, obtained both before and after having given chlorimipramine, were tested on the rat stomach strip which had been exposed to methysergide (bath concentration 10^{-6} g/ml.). The contraction was always abolished. The sensitivity of the preparation to the addition of 5-HT-free cerebro-spinal fluid was, however, exaggerated by the methysergide, so that the tests had to include a cerebrospinal fluid blank to ascertain whether or not the response to 5-HT had disappeared. A similar hypersensitivity to arresting the flow of Krebs solution to the organ bath after the use of bromolysergic acid diethylamide has been reported (Feldberg & Myers, 1966).

Acetylcholine

In twenty-one experiments, the perfusion fluid contained neostigmine and the release of ACh was studied. Basal release was nearly always lower than from the anterior horn of the lateral ventricle, and frequently did not exceed 5 ng in 20 min.

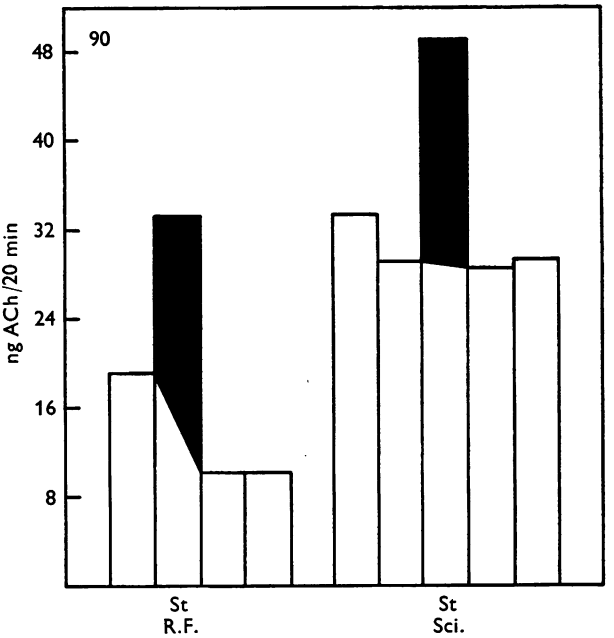

Fig. 5. The columns represent the ACh content of consecutive 20 min samples of perfusate of the third ventricle of a cat anaesthetized with chloralose 60 mg/kg (nialamide (25 mg/kg) had been injected s.c. 15 hr before the experiment, but as the perfusate contained some blood, no estimations of 5-HT were made). Neostigmine was present in the perfusion fluid, and before anaesthesia the cat was injected s.c. with atropine sulphate 2 mg/kg. The left part of the Figure illustrates the effect of stimulating the reticular formation (in frontal plane A6) for 10 min with trains of twelve 0·5 msec stimuli (60 Hz) repeated every 2 sec. The right part shows the ACh release during a 10 min stimulation of the central ends of the severed sciatic nerves (biphasic pulses at 2/sec). 40 min interval between the two parts of the Figure.

The electrodes were either aimed at the reticular formation (co-ordinates between A4 and A6, usually L3, H -1) or at the raphe nuclei. If the intended placement was one of the raphe nuclei, the same electrode position was used, whenever possible, to test also for the release of 5-HT. However, the number of cats in which the same placement could be used

to study the release of both transmitters was limited by the frequent presence of blood in the perfusate. A valid estimation of both transmitters with the electrode in the same position was obtained in twelve experiments. In no instance was there a simultaneous release of 5-HT and ACh. Though the stimulation of many sites gave rise to release of ACh, release failed to occur when the electrode was in the nucleus linearis rostralis (four tests) or intermedius (two tests). In contrast, ACh was liberated 5 out of 6 times when different parts of the reticular formation were stimulated, 6 out of 7 times when the electrode was in the decussation of the superior cerebellar peduncles, and from a number of sites explored only once or twice, such as the medial geniculate body, the capsule around the red nucleus, the third nucleus and the region immediately ventral to it, or the nucleus interstitialis. On stimulation of two regions, the interpeduncular nucleus and the nucleus pedunculi mammillaris, ACh was released in some cats and not in others; the structure responsible for the effect was possibly in the vicinity of these sites and not the nucleus itself. Release was also seen when the central ends of the severed sciatic nerves were stimulated.

Fig. 5 gives examples of release of acetylcholine. The cat had been injected with atropine sulphate (2 mg/kg) and the perfusion fluid contained neostigmine. Each column represents the ACh content of a 20 min collection period; at St R.F., the reticular formation was stimulated for 10 min with trains of 12 pulses (60 Hz) repeated once every 2 sec; St Sci. indicates a 10 min period of stimulation of the central ends of the severed sciatic nerves with biphasic pulses of 2 Hz. The release of ACh by both stimuli is unmistakable.

The magnitude of the release was dependent on the frequency of stimulation. Since 5-HT release from the raphe nuclei is optimal when low frequencies are used (Holman & Vogt, 1972), relatively low frequencies (trains of 3 pulses at 45 Hz, repeated 5 times a second) suitable for 5-HT release from the raphe nuclei were frequently employed when testing for ACh release. However, higher frequencies of stimulation, such as trains of 12 pulses at 60 or, better still, 120 Hz, repeated 4 times a second, released more acetylcholine, and, at some sites, were necessary to obtain any release at all. The reverse was true of 5-HT release: when tested in two cats, in which the electrodes were situated in the nucleus linearis intermedius, trains of 3 pulses (45 Hz) released 5-HT, but trains of 12 pulses (60 Hz) did not.

DISCUSSION

Perfusates of the third ventricle of anaesthetized cats given an inhibitor of monoamine oxidase contained 5-HT; the quantities were so small that it was necessary to ascertain, by centrifuging the effluent, whether there were traces of blood which might have contributed to the 5-HT content. In the absence of blood, 5-HT release 'at rest', though varying from cat to cat, was quite steady. Stimulation of either part of the nucleus linearis raphe led to an increment in the 5-HT content of the perfusate, indicating that tryptaminergic terminals which had their cells of origin in the nucleus linearis raphe existed in ventricle-near parts of the hypothalamus.

The increment did not outlast the duration of stimulation provided the dead space of the system was included in the sample by collecting for another 10 min after the end of stimulation. This is in contrast to observations on the release into the lateral ventricle of homovanillic acid, a metabolite of dopamine, after stimulation of the substantia nigra. The acid diffused into the lateral ventricles for periods of 1 hr or more, even if the stimulation lasted only 4 min. The metabolite is stable in nervous tissue and can obviously reach the ventricles from sites deep in the caudate nucleus, so that the material liberated from superficial terminals is found in the first, and that released at greater depth in subsequent samples of perfusate. Its parent transmitter amine, dopamine, appears, like 5-HT, only during the stimulation period (Portig & Vogt, 1969).

It had been shown previously (Holman & Vogt, 1972) that low frequencies of stimulation of the raphe nuclei were more effective than high frequencies in releasing 5-HT into the anterior horn of the lateral ventricle. This was confirmed for release into the third ventricle, two trains per sec of 12 pulses at 60 Hz twice failing to release 5-HT, whereas subsequent stimulation of the same site with five trains per sec of 3 pulses at 45 Hz was effective.

The presence of tryptaminergic terminals in the cat's hypothalamus is also suggested by observations (Myers, Veale & Beleslin, 1970) of an enhanced release of 5-HT into a push-pull cannula when fluid containing a high concentration of calcium ions was perfused through certain sites.

In confirmation of the observations by Feldberg & Myers (1966) we found that perfusates of the third ventricle did not contain detectable amounts of the organic acid, presumably a prostaglandin, which the authors found in perfusates of the lateral ventricles, and the presence of which would be shown up by a methysergide resistant contraction of the rat's stomach.

The low basal and evoked release of 5-HT into perfusates of both the lateral (Holman & Vogt, 1972) and the third ventricle needed explanation

in view of the fact that 5-HT content of several structures bordering on the ventricles is quite high. The inhibitor of monoamine oxidase used might have inhibited release by a feed-back mechanism, but previous experiments (Holman & Vogt, 1972) had shown that the mean basal secretion rises in the presence of the doses of nialamide used in this work. The alternative was a very efficient neuronal re-uptake of released transmitter. This was confirmed by the use of chlorimipramine. It had been shown that this substance reduces uptake of exogenous 5-HT into synaptosomes, the brain of reserpinized rats, and brain slices (Lidbrink, Jonsson & Fuxe, 1971; Ross, Renyi & Ögren, 1972). The fact that chlorimipramine reduces the loss of brain 5-HT produced by 4-methyl-α-ethyl-meta-tyramine (Carlsson *et al.* 1969) suggested that it would also act on endogenous transmitter, but the magnitude of the amount of transmitter normally handled by the uptake process was unknown. It proved to be very large indeed, allowing the basal release to increase by a factor of 10 or more, so that up to 50 ng 5-HT might appear in a 25 min sample instead of 1–3 ng in the absence of chlorimipramine. Release by electrical stimulation of the raphe nuclei was also much greater.

The rise in basal release of 5-HT after intravenous or intraperitoneal injection developed within minutes but soon declined, first rapidly and later more slowly. The time course is reminiscent of that of the imipramine concentrations in the brain of rabbits given two I.V. doses of 5 mg/kg with an interval of 15 min (Häfliger & Burckhardt, 1964). Half-an-hour after the second dose, the brain concentration was very high, falling rapidly at first and more gradually later, while throughout the whole period the plasma concentration remained below the threshold of the method. The avidity of brain tissue for the drug was only rivalled by that of the kidney.

The disruptive effect of intravenous chlorimipramine on responses in the brain stem evoked by sensory stimuli was in striking contrast to the negligible effect of adding the drug to the perfusion fluid. In spite of using concentrations in the cerebrospinal fluid of up to 50 mg/l., the chlorimipramine cannot have penetrated deep enough into the brain to inactivate the pathways responsible for evoked responses. The fall in blood pressure caused by intravenous chlorimipramine did not account for the loss of evoked responses since this occurred also when the effect on the blood pressure was small or even absent.

In conscious cats, chlorimipramine (10 mg/kg S.C. or I.P.) produced very slight ataxia and a tendency to sleep which lasted for a few hours. The cats remained arousable and there were no after-effects. The severe disturbance of brain function indicated by the loss of evoked responses obviously required the high cerebral concentrations of the drug attainable by I.V. infusion.

When neostigmine was added to the perfusion fluid, small amounts of ACh appeared in the effluent. The low basal release is in agreement with anatomical findings (Shute & Lewis, 1966). These authors found that the main sites of cholinergic terminals in the vicinity of the third ventricle were the dorsal and anterior hypothalamic areas, and the paraventricular and posterior hypothalamic nuclei. In all these regions the density of fibres and terminals was low.

Stimulation of the central ends of the sciatic nerves, as well as of a number of regions in the brain, caused increments in the release of ACh into the third ventricle. The increments from several sites were larger with higher frequency of stimulation, but effects were also obtained with the lower frequencies suitable for stimulation of the raphe nuclei. When the electrode was in one of the linear nuclei, no ACh was released on stimulation, although in each instance high frequencies as well as low frequencies were tried. This finding agrees well with the observation (Ashkenazi, Holman & Vogt, 1972) that perfusates of the lateral ventricle, collected during stimulation of the linear nuclei, contained 5-HT but not ACh. Activation of tryptaminergic neurones thus takes place independently of that of cholinergic neurones, and both types of neurones have terminals in the hypothalamus as well as in the caudate nucleus and septum.

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