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ELECTRICALLY-EVOKED RELEASE OF [^{3}H]-HISTAMINE FROM THE GUINEA-PIG HYPOTHALAMUS

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1 [^{3}H]-histamine was taken up by slices of guinea-pig hypothalamus against a concentration gradient.

2 Electrical field stimulation of the superfused slices resulted in an increased efflux of radioactivity, the major part of which was shown to be associated with histamine by paper chromatography.

3 The evoked release of histamine was dependent on calcium ions in the superfusate and was increased by 56% when the frequency of stimulation was doubled from 5 to 10 Hz.

Introduction

Histamine, in common with other hypothalamic amines does not readily cross the blood-brain barrier (Snyder, Axelrod & Bauer, 1964; Schayer & Reilly, 1973) but is believed to be formed from histidine by decarboxylation (White, 1959; 1960). Of the brain regions studied, its concentration is highest in the hypothalamus (Adam, 1961; Adam & Hye, 1966) where it appears to be associated not with mast cells (Adam & Hye, 1966) which are uncommon in brain (Kelsall & Lewis, 1964), but with small nerve terminals (Kataoka & de Robertis, 1967; Kuhar, Taylor & Snyder, 1971). Tritiated histidine is taken up into rat hypothalamic slices *in vitro* by a saturable process and labelled histamine and *N*-methylhistamine are formed (Verdière, Rose & Schwartz, 1974a, b). [^{3}H]-histamine can be released by depolarization with potassium and this release is calcium-dependent. These experiments parallel those on endogenous histamine by Taylor & Snyder (1973) who showed that whereas histamine efflux could be stimulated by potassium, the efflux of histidine could not.

Both [^{3}H]-histamine and [^{3}H]-histidine are taken up by guinea-pig hypothalamic slices *in vitro* (Biggs & Johnson, 1978) but an increase in the efflux of tritium on electrical field stimulation occurs only from slices incubated with [^{3}H]-histamine. The present experiments extend the observations on the electrically-evoked output of [^{3}H]-histamine from brain slices incubated with the amine.

Methods

Guinea-pigs were killed by a blow on the neck and the brain was rapidly removed and placed in Krebs solution at 37°C bubbled with 5% CO_2 in O_2. The hypothalamus was dissected free and cut into 0.3 mm wide slices by means of a McIlwain tissue chopper. The slices (approximate weight 5 to 15 mg) were then incubated for 90 min in 5 ml Krebs solution, at 37°C, to which was added 50 μCi [^{3}H]-histamine diluted with unlabelled histamine to give a final concentration of 1.1 μM. After incubation the slices were transferred to a heated superfusion chamber in which they were held on a 1 cm square of filter paper resting on the lower of a pair of platinum grid electrodes spaced 1 mm apart. The slices were superfused with bubbled Krebs solution at 37°C flowing at a rate of 2 ml/min (Watson Marlow MHRE flow inducer).

Spontaneous and electrically-evoked release of [^{3}H]-histamine

In the early stimulation experiments, 5 min (10 ml) samples were collected over the first 80 min after which time the slices were stimulated for 5 min with rectangular pulses of 5 or 10 Hz frequency, duration 0.5 ms and voltage 3 to 5 V, depending on the tissue resistance, to give a current of 100 mA. The potential difference and current were continually monitored by means of an oscilloscope to ensure that the stimulus parameters remained constant. One min samples of superfusate were collected 5 min before, then during stimulation and in the 5 to 10 min after stimulation before the resumption of 5 min collections for the remainder of the experiment. In more recent experiments the samples were pooled during the first hour. This was then followed by five 5 min and four 1 min

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samples before stimulation, five 1 min samples during stimulation and four 1 min and five 5 min samples thereafter.

In control experiments done to determine the spontaneous efflux of tritium from the hypothalamic slices, the superfusate was collected over the same time periods as in the stimulation experiments carried out in parallel.

At the end of the superfusion, the slices were removed from the electrodes, dried, weighed and solubilized in 2 ml Soluene-350. The amounts of tritium in 0.2 ml aliquots of the solubilized tissue and superfusate samples were determined by liquid scintillation spectrometry after the addition of scintillators and the outputs were expressed as d min^{-1} g^{-1}. The tissue content of tritium at time t was determined by adding to the tissue content at the end of the experiment all the tritium lost from time t onwards. For the fractional rate of tritium release (rate coefficient) the mean loss per min was divided by the tissue content at the appropriate time point.

Identification of [^3H]-histamine and [^3H]-N-methylhistamine released spontaneously and during stimulation

It has been suggested that methylation of the ring nitrogen by histamine-*N*-methyltransferase is a major means of inactivation of histamine in brain (Reilly & Schayer, 1972). This process probably occurs after the release of histamine (Pollard, Bischoff & Schwartz, 1974) and thus the excess amount of tritium obtained on electrical stimulation probably reflects histamine release. Nevertheless it was of interest to see in what proportions histamine and *N*-methylhistamine could be recovered during superfusion. The separation of the two amines was based on their differing solubilities in various solvents and different mobilities on paper chromatography. Samples collected during the 29 min before stimulation were pooled as were those during the 5 min stimulation period and the 29 min period after stimulation. Aliquots (10 ml) were dried in a rotary evaporator and the [^{3}H]-histamine and [^{3}H]-*N*-methylhistamine extracted from the residual smear with various solvents: methanol, ethanol, butanol or chloroform. As methanol was found to extract histamine and chloroform to extract *N*-methylhistamine more efficiently, these tended to be the solvents of choice. The samples were then dried in an air stream at 60 C and were re-dissolved in 50 µl of an appropriate solvent just before assay. After spotting with extracts and standards the chromatography paper was suspended in a mixture of chloroform, methanol and ammonia in the volume ratio of 12:7:1. The final positions of the amines on the chromatogram were located under u.v. light after treatment with *o*-phthaldehyde in acetone. The chromatogram was then cut into 1 cm pieces which were counted for tritium. Standard histamine and *N*-methylhistamine were quite distinct on the chromatogram having R_F values of 0.75 and 0.9, respectively.

Drugs and chemicals

Butanol (BDH); chloroform (BDH); disodium ethylene diamine tetraacetate (BDH); ethanol (Burroughs); histamine dihydrochloride (Sigma); [2,5-^{3}H]-histamine dihydrochloride (9.5 Ci/mmol; Radiochemical Centre, Amersham); methanol (BDH); *N*-methylhistamine (Calbiochem); *o*-phthaldehyde (Sigma). The tissues were superfused with Krebs solution of the following composition (mM) unless otherwise stated: NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.5. Aliquots of solubilized tissue or superfusate were added to scintillation fluid of the following composition: toluene 38.5%; 1,2-dioxan 38.5%; methanol 23%; 2,5-diphenyloxazole (PPO) 5 g/l; 1,4-di-2-(5-phenyloxazolyl) benzene (POPOP) 0.1 g/l and naphthalene 80 g/l.

Results

Uptake of histamine by hypothalamic slices

In all experiments [^{3}H]-histamine was taken up against a concentration gradient. After 90 min incubation in a concentration of 1.08 µM [^{3}H]-histamine the mean tissue uptake in 20 experiments was 3.19 ± 0.19 nmol/g giving a tissue:medium ratio of 2.95.

The spontaneous release of histamine

The spontaneous output of histamine fell progressively through the experiment. The means of 10 experiments are presented in Figure 1a which show that the rate of spontaneous release declined rapidly over the first 40 min and thereafter at a slower rate. The tissue content also fell during the experiment and a semilogarithmic plot against time gave a curve indicating a two compartment loss. The initial rapid phase was followed from 55 min onwards by a straight line component whose half-life was 68 min (Figure 1b). During the straight line phase all the histamine is derived from a single tisue compartment. For this reason electrical stimulation was never undertaken during the first 80 min of superfusion. The fractional rate of histamine release (rate coefficient) was calculated by dividing the output in Figure 1a by the corresponding content in Figure 1b. This resulted in a straight line parallel with the abscissa scale after the 55 min super-

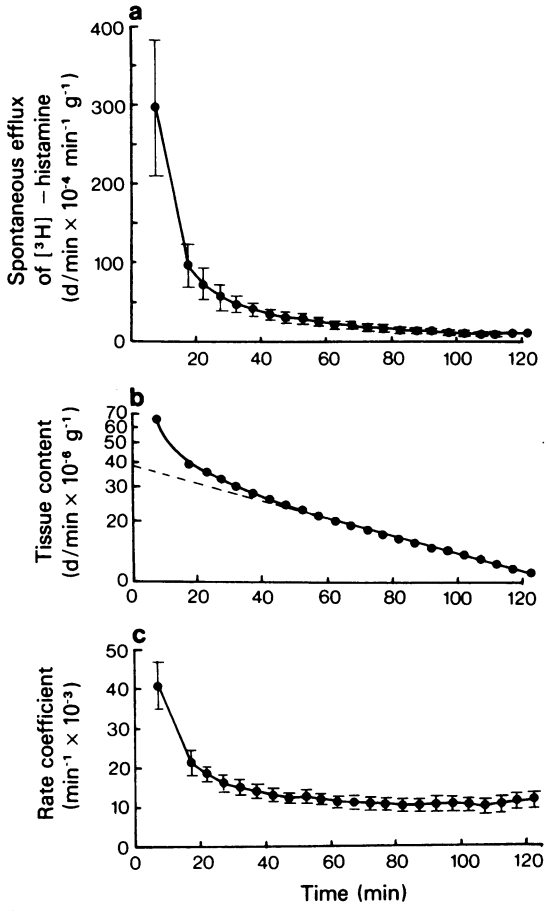

Figure 1 (a) The spontaneous efflux of [^{3}H]-histamine from guinea-pig hypothalamus (d/min × 10^{-4} min^{-1} g^{-1}, ordinate scale). (b) The hypothalamic content of [^{3}H]-histamine calculated for the same intervals throughout the experiment as in (a), (d/min × 10^{-6} g^{-1} log scale, ordinate). The tissue content half life after t = 55 min = 68 min. (c) The fractional rate of [^{3}H]-histamine release (rate coefficient, min^{-1} × 10^{-3}, ordinate scale) calculated by dividing the output in (a) by the corresponding content in (b).

fusion point (Figure 1c). This parameter was calculated from raw data for each experiment fed into a CDC 6600 computer. In all experiments which follow, the spontaneous outputs were pooled during the first 60 min and only the rate coefficients after that time are presented.

The release of histamine on electrical stimulation

When the hypothalamic slices were subjected to electrical field stimulation, the output of histamine was increased and returned to the spontaneous level before the end of the experiment (Figure 2). The increased output of histamine on stimulation was significantly greater than the spontaneous output after the first min of stimulation at 5 Hz ($P < 0.001$) and for all time points during stimulation at 10 Hz ($P < 0.001$). Furthermore, the increased output at 10 Hz was significantly greater than that at 5 Hz at all time points ($P < 0.05$; Figure 2).

When normal Krebs solution was replaced by Krebs lacking Ca^{2+} and with disodium edetate (EDTA, 5.4 mM) a significant reduction of evoked histamine release occurred on stimulation at 5 Hz ($P < 0.05$; Figure 3a). However, the spontaneous histamine release prior to electrical stimulation was higher from the no Ca^{2+}-EDTA brain slices and when the results were calculated as a percentage of the pre-stimulation spontaneous output, the reduction in the output from slices in no Ca^{2+} Krebs was highly statistically significant ($P < 0.001$; Figure 3b).

Metabolism of [^{3}H]-histamine

On paper chromatography of the superfusate the major portion of radioactivity moved identically with standard histamine having an R_F value of 0.75. The only other peak of significance corresponded to the movement of *N*-methylhistamine (R_F 0.9). The relative amounts of histamine and *N*-methylhistamine were found to be approximately 99 and 1% respectively after correction for their different solubilities in the solvents used. These proportions were the same during both spontaneous efflux and electrically stimulated release.

Discussion

Several lines of evidence suggest a possible transmitter role for histamine within the brain (Calcutt, 1976). Thus the localization of histamine in the brain follows the distribution of both histidine decarboxylase and histamine-*N*-methyltransferase (Brown, Tomchick & Axelrod, 1959; Schwartz, Lampart & Rose, 1970), its turnover is rapid (Taylor & Snyder, 1972) especially in rat hypothalamus (Dismukes & Snyder, 1974) and it alters the firing rate of hypothalamic neurones when applied by microiontophoresis (Haas, 1974). Furthermore, there is evidence based on experimental lesions at the level of the lateral hypothalamus, for a histamine-containing neuronal system in the medial forebrain bundle which innervates

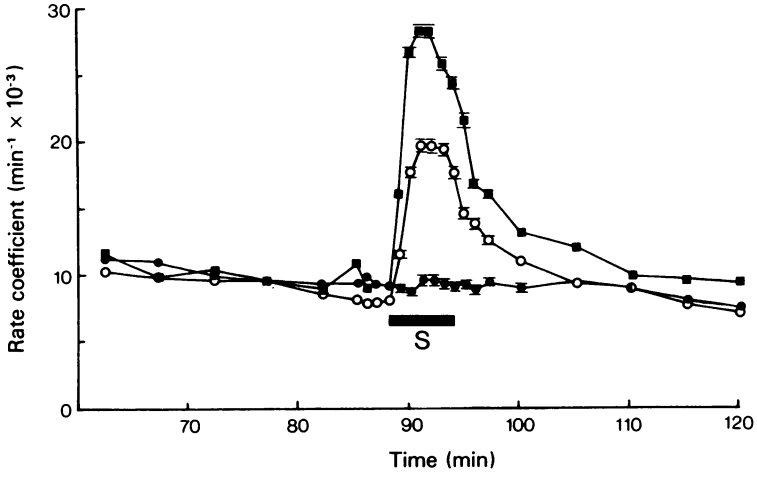

Figure 2 The release of [^{3}H]-histamine from the guinea-pig hypothalamus evoked by 5 Hz (○) and 10 Hz (■) electrical field stimulation for 5 min (horizontal bar S, n = 10). Ordinate scale as in Figure 1c. The spontaneous efflux from 10 experiments is shown by (●) for comparison. Vertical lines indicate s.e. mean.

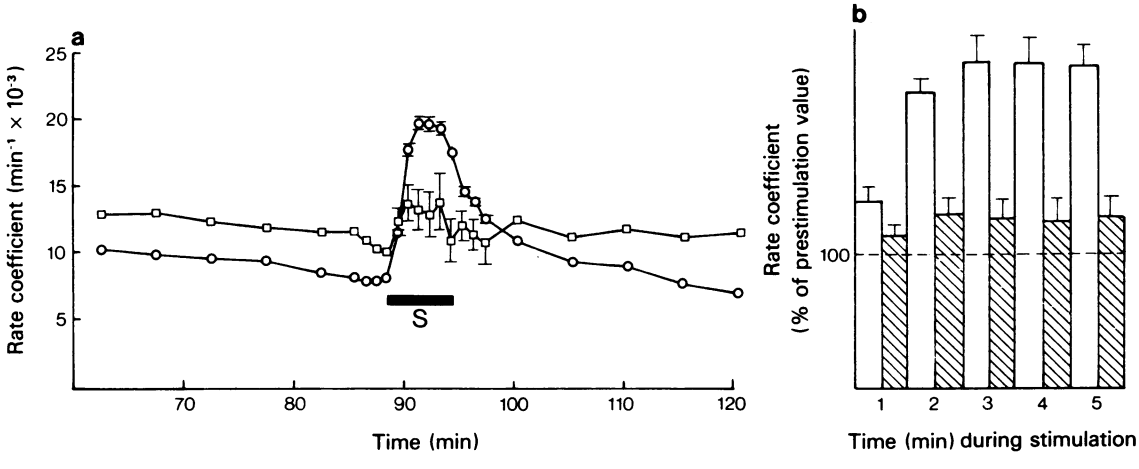

Figure 3 (a) The effect of removing Ca^{2+} from the superfusate on the output of [^{3}H]-histamine from guinea-pig hypothalamus. The [^{3}H]-histamine release by 5 Hz field stimulation (S) is shown by (○). Output in the absence of Ca^{2+} and with EDTA (5.4 mM) added is shown by (□). Axes as in Figure 2. Vertical bars indicate s.e. mean (n = 10). (b) The data in (a) during the stimulation period have been calculated as a % of the spontaneous output of [^{3}H]-histamine just before stimulation. The open columns show the control output during each minute of the 5 min stimulation period. The hatched columns show the output in the absence of calcium. Vertical bars indicate s.e. mean (n = 10). The reduction in the output from slices in no Ca^{2+} Krebs was highly statistically significant ($P < 0.001$) after the first min.

several areas rostral to the lesion (Garbarg, Barbin, Bischoff, Pollard & Schwartz, 1976).

The results of the present experiments are entirely consistent with the hypothesis that histamine has a transmitter function in the guinea-pig hypothalamus. Thus [^{3}H]-histamine was taken up by hypothalamic slices against a concentration gradient. It could be released by electrical stimulation and the release was frequency-dependent. In addition, the histamine release evoked by electrical stimulation required extracellular calcium ions.

The ratio of the [^{3}H]-histamine in the tissue slices to that in the incubation medium was 2.95 after 90 min incubation. This ratio is very close to that calculated for histamine uptake into rabbit hypothalamic slices (Tuomisto, Tuomisto & Walaszek, 1975). Tuomisto *et al.* (1975) showed that uptake continued for up to 2 h, was sodium- and temperature-dependent, could be inhibited by non-selective concentrations of desipramine, but was not affected by compound 48/80. These observations strongly suggest that histamine uptake was by neurones and not by mast cells. If true, then neuronal re-uptake of histamine after its release might suggest a second means of inactivation of this amine in addition to methylation of the ring nitrogen by histamine-*N*-methyltransferase (Dismukes & Snyder, 1974). The extent to which re-uptake played any part in the present experiments is unknown; certainly ring methylation occurred to some extent consistent with an extracellular action of the enzyme advocated by Pollard *et al.* (1974). The small extent to which ring methylation occurred in the present experiments probably reflected the rapid rate of superfusion.

These experiments on the electrically-evoked release of [^{3}H]-histamine from guinea-pig hypothalamus complement those of Verdière, Rose & Schwartz (1974a, b) in which histamine could be released by potassium depolarization. This release was also calcium-dependent. Biggs & Johnson (1978) first described the frequency-dependent release of histamine from hypothalamic slices. This has been quantified in the present study from which it can be calculated that doubling the frequency from 5 to 10 Hz leads to a less than two fold increase in the output. This is an observation that has often been made with other transmitters and in those instances it usually indicated the existence of a prejunctional inhibitory feedback mechanism.

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