Biochemical Pharmacology, Vol. 28, pp. 2799–2805.

0006-2952/79/0915-2799 $02.00/0

IN VITRO EXPERIMENTS ON THE ACCUMULATION AND RELEASE OF ^{14}C-HISTAMINE BY SNAIL (*HELIX POMATIA*) NERVOUS TISSUE

N. N. OSBORNE,* K.-D. WOLTER and V. NEUHOFF

Max-Planck-Institut für experimentelle Medizin, Forschungsstelle Neurochemie, Hermann-Rein-Str. 3, 3400 Göttingen, West Germany

(*Received* 31 *January* 1979; *accepted* 10 *April* 1979)

Abstract—When isolated snail ganglia were incubated at 25° in a medium containing ^{14}C-histamine, tissue:medium ratios of about 4 were obtained after only 5 min. Metabolism of the accumulated amine is rapid, for even within this short incubation period, 20 per cent of the substance was metabolised. The process responsible for this accumulation showed properties of an active transport system: it was temperature-sensitive, sodium dependent, and particularly inhibited by ouabain, phenoxybenzamine, chlorpromazine and desimipramine. The accumulation of ^{14}C-histamine showed saturation kinetics typical of a carrier-mediated process and can be divided into sodium-sensitive and -insensitive components. Kinetic analysis of the data revealed similar K_m values for both sodium-sensitive and sodium-insensitive plus -sensitive components, i.e. 10^{-5} M.

A rapid efflux of radioactivity from tissue loaded with ^{14}C-histamine was observed when exposed to 70 mM KCl. This release was inhibited when the calcium content in the medium was replaced by sucrose. Moreover, cobalt ions added to the incubation medium counteracted the effect of KCl.

The discussion of the present results is based on the hypothesis that histamine has a transmitter function in the snail CNS.

Despite the existence of a vast amount of biochemical and physiological data on histamine in the vertebrate central nervous system, the actual function of this substance is not understood, although a transmitter role is suspected (see ref. [1]). Recent studies on certain invertebrate preparations (viz. gastropod molluscs) containing large neurones have now fortified the supposition that histamine can function as a transmitter. For example, it has been shown that certain neurones in the gastropods contain this specific amine [2–4] and the enzyme which is required to synthesise histamine from histidine, histidine-decarboxylase, exists only in these neurones [5, 6]. Furthermore, it has been demonstrated that neurones receiving monosynaptic connections from a defined histamine-containing neurone respond to exogenously applied histamine in a manner qualitatively similar to the effect produced by histamine stimulation [7]. These data thus provide convincing arguments in favour of histamine functioning as a transmitter in the gastropods (e.g. the snail *Helix pomatia*) and therefore probably in the vertebrate CNS as well.

One requisite of a transmitter substance is that it is rapidly inactivated after delivery of its message. Acetylcholine is inactivated by the enzyme acetylcholinesterase at cholinergic synapses [8, 9] whereas dopamine and 5-hydroxytryptamine are inactivated by specific high affinity re-uptake mechanisms in both vertebrate and invertebrate (i.e. *Helix*) nervous tissue [10–12]. The aim of the present work was to see whether an active high affinity uptake system for histamine exists in the snail CNS. Turner and Cottrell's report [3] suggests that such a mechanism may exist. By means of autoradiography they demonstrated that a certain neurone in the pond snail *Lymnea* specifically accumulated histamine. Experiments have also been undertaken on the KCl-induced release of radioactivity from snail tissue loaded with ^{14}C-histamine in order to gain more information on the functional role of the amine.

METHODS

Helix pomatia were obtained from Alfred Koch, 3450 Holzminden, G.F.R., and kept at room temperature in a moist atmosphere for 24 hr before use. Suboesophageal ganglia from a number of animals were rapidly dissected and placed in a beaker containing cold snail saline. The snail saline [13] consisted of NaCl (3.45 g/l), KCl (0.43 g/l), $CaCl_2$ (1.17 g/l), $NaHCO_3$ (1.0 g/l) and $MgCl_2$ (1.55 g/l). Each ganglion was subsequently blotted dry on filter paper, weighed (4–6 mg) and placed in a vial containing 2 ml ice-cold snail saline. After a preincubation of 5 min at 25° in a shaking water bath, various amounts of [2-^{14}C]histamine (Radiochemical, Amersham, 59.7 mCi/mmole) were added to the incubation medium and the incubation was continued for varying periods of time. At the end of the incubation the ganglia were recovered with forceps and rapidly rinsed in 20 ml ice-cold saline. It had been previously established that no significant release of radioactivity from the tissues occurred during the washing process. Individual ganglia were then placed in vials containing 0.5 ml tissue solubilizer (Soluene-350 Packard) for at least 2 hr at room temperature before adding 10 ml Dimilume (Packard). Radioactivity was measured in a Packard liquid scintillation spectrometer. A small amount (50–100 μl) of radioactive incubation mixture dissolved in 10 ml Dimilume

* Present address: Nuffield Laboratory of Ophthalmology, The University of Oxford, Walton Street, Oxford, England.

was also counted. The counting efficiency was monitored by internal standards of ^{14}C-toluene and the appropriate correction applied. The counting efficiency was 80%.

Tissue/medium (T/M) ratios were calculated as c.p.m. in g of tissue per c.p.m. in 1 ml medium. The amount of amine accumulated by the tissues was calculated from values obtained from the T/M ratios at 0° and 25° [14]. Unlabelled histamine was added to the labelled amine to produce the solutions containing higher concentrations of histamine required for the kinetic studies. Kinetic constants were determined by computer, where an iterative method was used to fit data directly to rate equations for a two or single carrier model.

Ganglia were also incubated in [2-^{14}C]histamine for varying periods at 25°, the tissue was homogenised in 0.01 N HCl/acetone (1:1, v/v) and the supernatants, after centrifugation at 1500 g, applied to silica precoated plates (Merck). Chromatography was in an ascending way using either *n*-butanol/water/acetic acid (12:5:3, by vol) or choloroform/methanol/ammonia (12:7:1, by vol). Standard histamine on the chromatograms was identified by spraying with ninhydrin solution. Autoradiograms of the chromatograms were then prepared using Kodax X-ray film (exposure time from 2 to 4 days). Substances were eluted from the silica with methanol/water (1:1, v/v), and the eluates dried and counted in a scintillation spectrometer. From the results the percentage of metabolised ^{14}C-histamine was calculated.

In order to study the efflux of radioactivity from nervous tissue, five to eight suboesophageal ganglia were incubated in 2 ml snail saline containing 10^{-7} ^{14}C-histamine for 5 min at 25°. The ganglia were recovered, rinsed rapidly and transferred for a definite duration to vials, each containing 1 ml snail saline or a modified form thereof. Bray's scintillation solution was then added to the 1 ml saline samples and the radioactivity counted.

RESULTS

When individual suboesophageal ganglia were incubated at 25° in a medium containing 10^{-7}M ^{14}C-histamine, the amine was rapidly metabolised. After an incubation time of 5 min only, chromatographic analysis showed that approximately 20 per cent of the radioactivity associated with the tissue was no longer histamine but a single other substance. Figure 1 is an autoradiogram of a chromatogram showing the metabolism of ^{14}C-histamine by snail nervous tissue incubated with radioactive amine for 20 min. It is clear that after this period of incubation, the single major product formed from ^{14}C-histamine occurs in greater concentrations (ca. 65%) than the amine itself. The nature of the histamine product was not identified but is not methylhistamine.

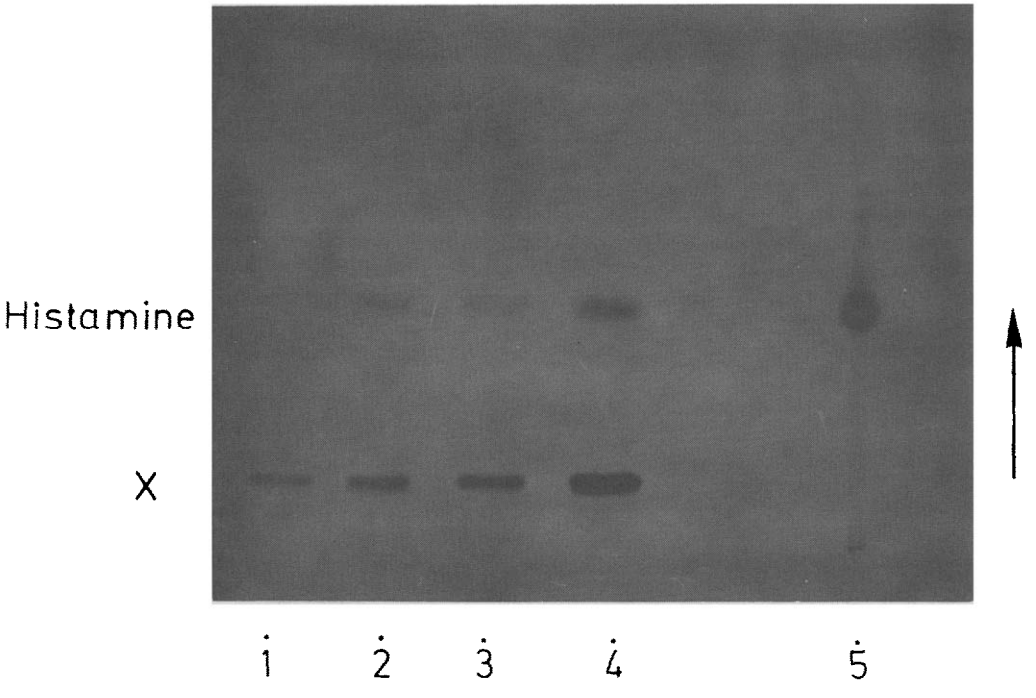

Fig. 1. Photograph of an autoradiogram showing the metabolism of ^{14}C-histamine by snail nervous tissue. Tissue was incubated with 10^{-7} M ^{14}C-histamine for a period of 20 min. Thereafter the amines and amino acids were extracted and chromatographed (see Methods). The direction of chromatographs is shown by the arrow. Autoradiograms were then made from the chromatograms. Spots 1–4 show different amounts of the same extract applied to the chromatogram while spot 5 represents pure ^{14}C-histamine. It can be seen that the radioactive histamine accumulated by the tissue is rapidly metabolised to form a single substance *x* and by comparison of the different amounts applied to the chromatogram it can be seen that within the period of incubation more than 50 per cent of the accumulated histamine is metabolised.

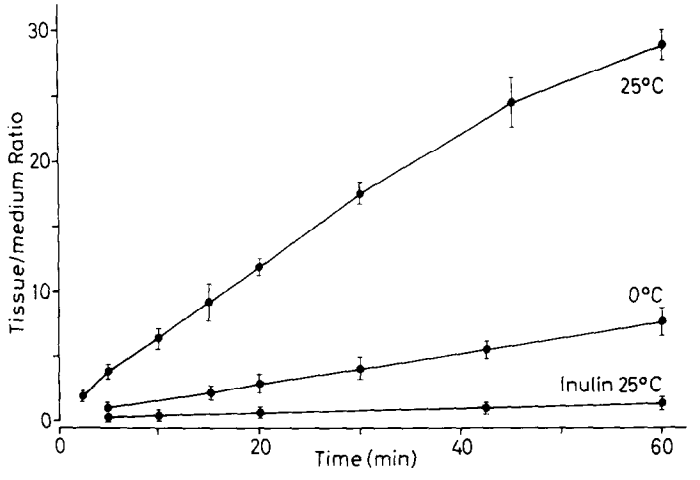

Fig. 2. Time course of ^{14}C-histamine and ^{3}H-inulin influx into ganglia incubated in 2 ml medium with 10^{-7} M radioactive substance. Vertical bars indicate the S.E.M.s. where n is at least 5.

Time course of ^{14}C-histamine accumulation and the effect of temperature.

The time course of ^{14}C-histamine accumulation in snail nervous tissue incubated with 10^{-7} M radioactive amine at 25° and 0° is illustrated in Fig. 2. The amount of radioactivity in the tissue increases almost linearly with time for the first 30 min. At 0° the accumulation of radioactivity is linear between 5 and 60 min and much slower than at 25°. The extracellular space of the tissue was determined by incubating ganglia for various periods with ^{3}H-inulin as shown in Fig. 1. After 5 min a T/M ratio of approximately 0.1 was obtained, about 40 times less than the amount of radioactivity associated with the ganglia when incubated in ^{14}C-histamine at 25°C. It is thus obvious that the amount of ^{14}C-histamine accumulated into the extracellular space is small compared with the total accumulation at 25°. The results presented in this paper have therefore not been corrected for radioactivity accumulated into extracellular spaces.

Table 1. ^{14}C-Histamine accumulation by snail ganglia *in vitro*

Exogenous ^{14}C-histamine (μM)	^{14}C-Histamine accumulation due to Na sensitive and insensitive components (pmole/mg/min)		^{14}C-Histamine accumulation due to Na sensitive component (pmole/mg/min)	
	Observed	Predicted	Observed	Predicted
0.1	0.11 ± 0.005	0.09	0.08 ± 0.007	0.06
0.2	0.22 ± 0.037	0.18	0.163 ± 0.004	0.12
0.5	0.48 ± 0.045	0.44	0.36 ± 0.04	0.31
1	0.96 ± 0.07	0.89	0.74 ± 0.07	0.61
2	1.94 ± 0.08	1.75	1.3 ± 0.13	1.20
5	4.65 ± 0.34	4.16	3.45 ± 0.36	2.83
10	6.58 ± 0.66	7.69	4.48 ± 0.65	5.12
25	13.33 ± 0.88	15.7	8.83 ± 0.79	9.98
50	26.7 ± 0.22	24.02	17.13 ± 1.37	14.58
100	40.1 ± 3.3	32.69	24.3 + 2	18.96

Ganglia were preincubated at 25° for 5 min and incubated at either 25° or 0° for a further 5 min with radioactive histamine. The influx of histamine was then calculated (observed values) by subtracting the value of 0° from that of 25° in either normal physiological solution (i.e. sodium-sensitive plus -insensitive components, i.e. total) or physiological solution free of sodium (sodium-sensitive component). The predicted values are those for single carrier systems with following kinetic values:
$K_{m\,\text{total}} = 5.6 \times 10^{-5}$ M, $V_{\max\,\text{total}} = 0.5$ pmole/mg/min.
$K_{m\,\text{sodium}} = 4.2 \times 10^{-5}$ M, $V_{\max\,\text{sodium}} = 0.27$ pmole/mg/min.
Standard deviations around 24%. The observed values for the total influx of histamine could also be made to fit a two carrier system with the following kinetic parameters: K_{m1} 9.4×10^{-6} M. $V_{\max 1}$ 5.6 pmole/mg/min. K_{m2} 6×10^{-4} M, $V_{\max 2}$ 0.024 pmole/mg/min with a standard deviation of 36%. There is no mathematical reason for suggesting that the data for the two carrier system fits better than the single system.

Kinetic analysis of ^{14}C-histamine accumulation

For the kinetic analysis ganglia were incubated at 0° or 25° for 5 min with 12 different concentrations of ^{14}C-histamine, ranging from 10^{-7} M to 10^{-4} M in either normal physiological snail solution or physiological solution where the sodium ions were replaced with sucrose. The incubation lasted only 5 min because the tissue rapidly metabolised ^{14}C-histamine. A shorter incubation period, where more than 80 per cent of radioactivity would remain histamine, was, for practical reasons, not possible. Since the accumulated radioactivity at 0° was the same in both normal physiological or sodium-free solution, it was considered that only a diffusible component was operating at this temperature. The diffusible component was therefore subtracted from the values obtained at 25°. An iterative method was then used to fit the corrected data for the accumulation of ^{14}C-histamine at 25° (Table 1) directly into rate equations with a digital computer. Models consisting of a single or two carrier system were examined. Assessed in terms of goodness of fit of data into the iterated model parameters, a single carrier model system with kinetic contrasts of approximately the same variations was found for both total and sodium-sensitive influx of histamine accumulation at 25° (see Table 1). Table 1 shows the predicted and observed values for single carrier systems for the total accumulation and the sodium-sensitive influx (i.e. total accumulation minus the accumulation of histamine in sodium-free solution) of ^{14}C-histamine. The results show that a sodium-sensitive component (V_{max} 0.27 pmole/mg tissue/min) contributes about 50 per cent to the total accumulation (V_{max} 0.51 pmole/mg/tissue/min) of ^{14}C-histamine by the tissue. The affinity constants, i.e. K_m values for the sodium-sensitive and sodium-insensitive plus sensitive components, i.e. total, could not be separated. Moreover, for the total accumulation of histamine at 25° the data could also be made to fit a two carrier system (see Table 1).

Effect of sodium and other ions

There was a linear relationship between the amount of ^{14}C-histamine accumulated by snail ganglia and the sodium concentrations in the medium at 25° (Fig. 3). The molarity of the incubation medium was kept constant by substitution of sucrose. In the complete absence of sodium, the influx of histamine was reduced by about 80% of that in the normal medium (Table 2). In contrast, when potassium or magnesium ions were omitted from the incubation medium, the influx was not significantly altered. However, in calcium-free solution

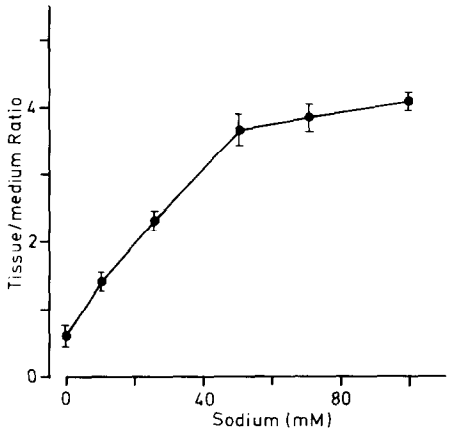

Fig. 3. Effect of sodium concentration on the accumulation of ^{14}C-histamine by snail ganglia *in vitro*. Ganglia were incubated for 5 min with ^{14}C-histamine in medium containing various concentrations of sodium ions. Each point is the mean with S.E.M.s of at least 5 estimations.

the accumulation of radioactivity in tissue was stimulated by about 20 per cent (see Table 2).

Specificity of ^{14}C-histamine accumulation

Of the seven substances tested as competitors for histamine influx, it was found that 1-methyl-4-imidozole, L-histidinol and methylhistamine were the strongest, with ID_{50} values of 5×10^{-4} M, 2×10^{-4} M and 2×10^{-4} M respectively (see Table 3). Approximately a ten-fold concentration of 5-HT, octopamine and L-methylhistidine was required for an equivalent effect, while L-carnosine at the concentration of 5×10^{-3} M had little influence. In all instances, the sodium-insensitive component for ^{14}C-histamine accumulation remained almost totally unaffected by the substances at concentrations which had a 50% effect on the total histamine influx (except L-carnosine) (see Table 3).

Effect of inhibitors

Twelve different inhibitors were tested for their effects on the total accumulation of ^{14}C-histamine by snail nervous tissue at 25° (see Table 3). The α-blocker phenoxybenzamine was the most potent inhibitor with an ID value of 7×10^{-6} M. Chlorpromazine, benzotropine, desimipramine and tubocurarine were also fairly potent antagonists of histamine accumulation, while atropine and *N*-ethylmaleimide were the least effective of the substances tested.

Table 2. The influence of ions on the accumulation of 10^{-7} M histamine

Incubation conditions	^{14}C-histamine accumulation (as per cent of controls)
Sodium replaced by lithium	34 ± 6
Sodium replaced by sucrose	22 ± 6
Calcium replaced by sucrose	128 ± 4
Magnesium replaced by sucrose	101 ± 2
Potassium replaced by sucrose	110 ± 10

Values are mean ± S.E.M. for 4 determinations. Tissues were incubated in the same way as described in Table 3.

Table 3. Effect of various substances including metabolic inhibitors on the influx of histamine

Substance and concentration		Influx of histamine (per cent of control)	
		Normal saline ID_{50} values	Na^+-free saline
Inhibitors			
Phenoxybenzamine	7×10^{-6} M	50	79
Ouabain	5×10^{-5} M	50	83
Chlorpromazine	2×10^{-5} M	50	98
Benzotropine	1.5×10^{-4} M	50	97
Desimipramine	8×10^{-5} M	50	90
N-Ethylmaleimide	1.5×10^{-3} M	50	93
Tubocurarine	2.5×10^{-3} M	50	95
Imipramine	5×10^{-4} M	50	89
Atropine	1.1×10^{-3} M	50	92
Other substances			
5-Hydroxytryptamine	2×10^{-3} M	50	84
Octopamine	5×10^{-3} M	50	91
1-Methyl-4-imidozole	5×10^{-4} M	50	85
L-Histidinol (β-Amino-imidazole-4(5)-propanol)	2×10^{-4} M	50	99
L-Methyl histidine (1-methyl-histidine)	2×10^{-3} M	50	106
L-Carnosine	5×10^{-3} M	90	119
1-Methyl histamine	2×10^{-4} M	50	90

Ganglia were preincubated at 25° for 5 min and then incubated for a further 5 min with ^{14}C-histamine (10^{-7} M) in the presence of the test substance (also present during preincubation). Values for per cent control influx are mean (approx. 9 per cent error) for 4 or more independent determinations.

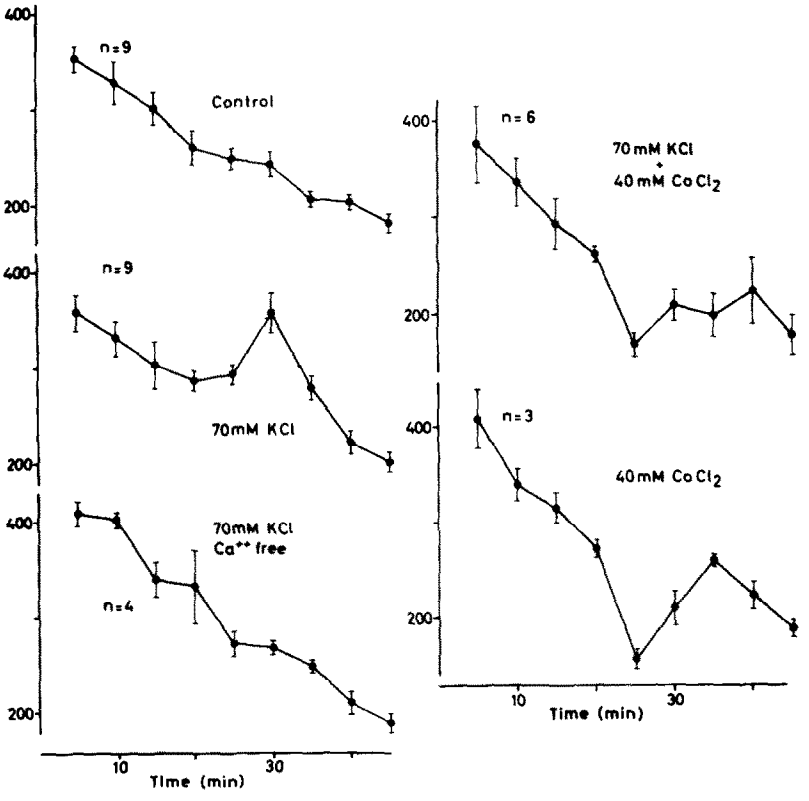

Fig. 4. Release of previously accumulated ^{14}C-histamine from snail nervous tissue (see results and methods). Substances, as shown above, were either added to or eliminated from the normal medium between the 20th and 25th minute. S.E.M.s indicated by the vertical bars. The vertical axis represents the radioactivity (cpm) in 50 mg nervous tissue.

Efflux of radioactivity from ganglia

The protocol of these experiments was as follows: 5–8 suboesophageal ganglia were incubated for 5 min with 0.1 μM ^{14}C-histamine, then rinsed in physiological solution and thereafter placed for 5 min periods in 1 ml physiological solution. In some experiments, KCl (70 mM) and/or $CoCl_2$ (40 mM) were added to the physiological solution between the 20th and 25th minutes. The whole of the 1 ml solutions were than analysed for their radioactivity, as described in the methods.

The results shown in Fig. 4a demonstrate the passive efflux of radioactivity from tissues loaded with ^{14}C-histamine, where it can be seen that there is a constant and fairly slow release of radioactivity. The radioactivity associated with tissue at 0 min was found to be approximately 0.09 pmole/mg/min ($n = 8$) and after 45 min was of the order of 0.044 pmole/mg/min ($n = 8$), which means that about 50 per cent of the total radioactivity is passively released within 45 min. The efflux of radioactivity was not increased by adding non-radioactive histamine (2 mM) to the medium after 20 min.

When 70 mM KCl is added to the medium after 20 min it can be seen from Fig. 4b that the amount of radioactivity released from the tissue increases. This increase is calcium-dependent (see Fig. 4c) since elimination of Ca^{2+} from the medium (substitution of sucrose) has the effect of counteracting the KCl influence. Moreover, Co^{2+} ions, which, according to Weakly [15], interfere with the entry of calcium ions inside the synaptic endings and cause a reduction in the transmitter release, not only abolished the stimulatory effect of KCl in the release of radioactivity from the tissue (Fig. 4d), but also increased the normal passive release (Fig. 4e).

DISCUSSION

The present data demonstrate that snail ganglia possess the ability to concentrate ^{14}C-histamine from an external medium by a combination of a sodium-sensitive and insensitive transport process. The accumulation of ^{14}C-histamine is saturable, temperature-sensitive, inhibited by ouabain and shows some specificity for imidazoles. Examination of the data by kinetics shows that the influx conforms to a single carrier system for both the sodium-sensitive and the total influx of histamine with about the same K_m values. The K_m values of both components were approximately the same (i.e. $K_m = 10^{-5}$ M) but with different V_{max}. The actual rate of accumulation of histamine in snail nervous tissue is fairly fast with T/M ratios of about 4 after incubation in solution for 5 min at 25°. This rate compares with the influx of 5-hydroxytryptamine and dopamine by snail nervous tissue [12] and is faster than the influx of either choline [16] or glutamate [17]. Desimipramine, phenoxybenzamine and chlorpromazine also powerfully inhibited the influx of histamine, and these substances are supposed to block the membrane transport mechanism of most or all biogenic amines in different tissues [18–21]. The influx of histamine is also specific. As shown in Table 3, 5-HT at a concentration of 2×10^{-3} M inhibited histamine influx by 50 per cent and since the affinity constant for the amine is in the μM range [12] it is clear that the inhibition of histamine influx by 5-HT is not due to competition for the same sites but probably due to unspecific effects. Clearly any attempt to talk about inhibitory constants would therefore be inappropriate. The same argument can be applied to dopamine which like 5-HT is thought to have transmitter functions in the snail CNS. This substance effected histamine influx at even higher concentrations (date not shown in Table 3) than 5-HT.

A question posed by these results is: what is the function of the sodium-dependent active influx of histamine in snail nervous tissue? From recent studies on the biochemistry, physiology and localisation of histamine in the snail it would appear that histamine may function as a transmitter (see introduction). The active influx mechanism for histamine described in this paper could therefore be interpreted as showing that a re-uptake mechanism exists for terminating the action of histamine at the synapse (see introduction). It is thought that such mechanisms occur for other amines, e.g. 5-hydroxytryptamine and dopamine [10, 12]. Furthermore, the active sodium-dependent uptake mechanism for histamine has been demonstrated in vertebrate nervous tissue, e.g. rabbit hypothalamus [22]. However, one peculiarity of the histamine influx into snail nervous tissue is that the accumulated amine is rapidly metabolised. For this reason, the shortest practical incubation times (5 min) were used in this study, and even within this period circa 20 per cent of the accumulated histamine was metabolised. It could therefore be argued that with such a rapid rate of metabolism, histamine, should it be a transmitter, could be inactivated by enzymatic degradation, as in the case of acetylcholine. This is certainly a possibility, though at this stage nothing is known about the specificity and nature of enzyme(s) involved in the catabolism and whether the enzyme(s) is associated with synaptic endings of histamine neurones.

The demonstration of potassium-induced release of radioactivity from snail nervous tissue previously loaded with ^{14}C-histamine and, which is calcium-dependent supports the idea that histamine may be a transmitter in the snail. This hypothesis is further strengthened by the influence of cobalt ions, which were found to counteract the release of radioactivity caused by potassium. It is thought that cobalt ions interfere with calcium entry into the synaptic terminals [15]. It is known that potassium can induce release of radioactive histamine from rat brain slices and that this effect is calcium-dependent [23]. However, before comparing the present data with those reported for rat brain, a cautionary note is necessary. It will be recalled that histamine is rapidly metabolised in snail nervous tissue, and what was actually measured in the present release experiments was in fact radioactivity and not histamine. Further experiments are therefore necessary to prove that histamine is indeed the major substance released by potassium.

Taken as a whole, the results do corroborate the idea that histamine in the gastropod CNS may function as a transmitter substance. However, they also raise a number of questions. Is the metabolism of histamine a specific process? What is the function of the sodium-dependent uptake mechanism described in this study? Could it be that the uptake mechanism has another

function? Perhaps it does not represent a specific uptake process into neurones but rather a general uptake mechanism into glial tissue. Moreover, the autoradiographical study by Turner and Cottrell [3] must be reassessed. These authors demonstrated that a specific neurone accumulates radioactivity after the ganglion has been incubated in radioactive histamine for 2 hr. As this study has shown, such a period of incubation would result in substantial amounts of the radioactive histamine being metabolised.

APPENDIX

While this paper was being considered for publication a report by Weinreich appeared in *J. Neurochem.* (Vol. 32, pp. 363–369) which is consistent with the data represented. It was shown that *Aplysia* nervous tissue rapidly metabolised glutamate to form a single product identified to be γ-glutamylhistamine. It was suggested that this may be the physiological mechanism used by molluscan nervous tissue to catabolise histamine.

REFERENCES

1. K. M. Taylor, in *Handbook of Psychopharmacology* (Eds. L. L. Iversen, S. D. Iversen & S. H. Snyder), Vol. 3, pp. 327–329. Plenum Press New York (1975).
2. M. J. Brownstein, J. M. Saavedra, J. Axelrod, G. H. Zeman and D. O. Carpenter, *Proc. natn. Acad. Sci. U.S.A.* **7**, 4662 (1974).
3. J. D. Turner and G. A. Cottrell, *Nature, Lond.* **267**, 447 (1977).
4. D. Weinreich, C. Weiner and R. McCaman, *Brain Res.* **84**, 341 (1975).
5. D. Weinreich, in *Biochemistry of Characterised Neurones* (Ed. N. N. Osborne), pp. 153–175 Pergamon Press, Oxford. (1978).
6. D. Weinreich and Y. -T. Yu, *J. Neurochem.* **28**, 361 (1977).
7. D. Weinreich, *Nature, Lond.* **267**, 854 (1977).
8. J. C. Eccles, B. Katz and S. W. Kuffler, *J. Neurophysiol.* **5**, 211 (1942).
9. B. Katz and S. Thesleff, *Br. J. Pharmac. Chemother.* **12**, 260 (1957).
10. L. L. Iversen, *Br. J. Pharmac. Chemother.* **21**, 523 (1963).
11. L. L. Iversen, *Br. J. Pharmac. Chemother.* **41**, 571 (1971).
12. N. N. Osborne, L. Hiripi and V. Neuhoff, *Biochem. Pharmac.* **24**, 2141 (1975).
13. K. Meng, *Zool. Jahr* **68**, 539 (1960).
14. E. G. Shaskan and S. H. Snyder, *J. Pharmac. exp. Ther.* **175**, 404 (1970).
15. J. N. Weakly, *J. Physiol.* **234**, 577 (1973).
16. N. N. Osborne, *J. Neurochem.* **27**, 517 (1976).
17. N. N. Osborne, H. U. Schröder and V. Neuhoff, *Brain Res.* **152**, 543 (1978).
18. F. Berti and P. A. Shore, *Biochem. Pharmac.* **16**, 2091 (1967).
19. E. A. Carlini and J. P. Green, *Br. J. Pharmac. Chemother.* **20**, 264 (1963).
20. L. L. Iversen, P. J. Salt and H. A. Wilson, *Br. J. Pharmac. Chemother.* **46**, 386 (1974).
21. D. N. Teller, T. DeGuzman and A. Lajtha, *Biochem. Pharmac.* **25**, 1271 (1976).
22. L. Tuomisto, J. Tuomisto and E. J. Walaszek, *Med. Biol.* **53**, 40 (1975).
23. N. Subramanian and A. H. Mulder, *Europ. J. Pharmacol.* **35**, 203 (1976).