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THE *IN VITRO* UPTAKE OF BIOGENIC AMINES BY SNAIL (*HELIX POMATIA*) NERVOUS TISSUE

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Abstract—The uptake of [^{3}H]5-HT, [^{3}H]dopamine, [^{3}H]noradrenaline and [^{3}H]octopamine into the suboesophageal ganglia of the snail, *Helix pomatia*, was studied. When tissues were incubated at 25° in mediums containing radioactive amines, tissue : medium ratios of about 30 : 1, 18 : 1, 4 : 1 and 5 : 1 for 5-HT, dopamine, noradrenaline and octopamine respectively were obtained after 20–30 min incubation. Tissues incubated at 25° in mediums containing radioactive amines for 20–30 min showed that 90% of the radioactivity was present as unchanged [^{3}H]5-HT, [^{3}H]dopamine, [^{3}H]noradrenaline or [^{3}H]octopamine. The high tissue : medium ratios for 5-HT and dopamine, but not for noradrenaline and octopamine, demonstrated saturation kinetics which were dependent upon temperature and sodium ions. From the Lineweaver–Burk plots, two uptake mechanisms for 5-HT at 25° were resolved, the high affinity uptake process having a K_{m_1} value of $8{\cdot}48 \times 10^{-8}$ M and V_{max_1} value of 0·077 nmole/g per min, while the lower affinity process had a K_{m_2} value of $1{\cdot}8 \times 10^{-6}$ M and a V_{max_2} value of 0·66 mole/g per min. At 0° a single uptake mechanism for 5-HT occurred which gave a K_m value of $0{\cdot}152 \times 10^{-8}$ M and a V_m value of 0·0203 nmole/g per min. In the case of dopamine, the Lineweaver–Burk plot of 25° showed a single uptake process with values for K_m and V_{max} of $1{\cdot}02 \times 10^{-7}$ M and 0·0673 nmole/g per min respectively. This process did not function at 0°. The effects of various agents and ions upon the accumulation processes for all amines were also studied, and the findings indicate that the same neurons may well accumulate more than one amine type. It is concluded that 5-HT and dopamine uptake in the snail ganglia is a mechanism for inactivating these substances at 25° and that an uptake mechanism for 5-HT also functions at 0°. The present results are discussed from the point of view of the monoamines having transmitter functions in the snail CNS.

Substantial amounts of 5-HT (5-hydroxytryptamine) and dopamine occur in the snail nervous system and the available data suggest that both amines function as neurotransmitters in this situation[1–7]. Small amounts of octopamine[8] and noradrenaline[9] also exist in the snail brain and there is some evidence that they may have transmitter functions too, since the former has been shown to occur only in defined molluscan neurons[10] and electrophysiological studies have disclosed that certain receptors are particularly sensitive to octopamine[11] and noradrenaline[12].

In order to support further the opinion that a substance has a transmitter function in a certain situation it is important to show that a mechanism exists for the termination of the action of the compound on the postsynaptic membrane[13]. From the work on vertebrate brain tissue it is well known that the enzyme monoamine oxidase is involved in the degradation of biogenic monoamines[14–15]. However, although small amounts of monoamine oxidase exist in the snail brain[16], all the data available show that the biogenic amines are degraded very slowly in this situation[17–18]. Another inactivation method which has been proven for several transmitters is a re-uptake mechanism[19–21]. Autoradiography studies have shown that certain nerves in the snail brain are capable of accumulating radioactive 5-HT[22] and studies by Osborne and Neuhoff[23] have also demonstrated the same process by biochemical procedures.

The aim of the present work was to continue the biochemical studies on the accumulation of 5-HT into the snail brain by investigating the mechanism by which the amine enters the nervous tissue. Experiments were also designed to see whether the snail brain accumulates dopamine, noradrenaline and octopamine, and if so, to compare the method of entry with that of 5-HT.

MATERIALS AND METHODS

Helix pomatia were obtained from Alfred Koch, 345 Holzminden, West Germany 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 saline (24). The snail saline 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. Vials containing the ganglia were placed in a shaking water bath and ascorbic acid (0·2 mg/ml), glucose (5 mM) and pargyline (0·01 mM) was added to each sample followed by a preincubation for either 15 min (in the cases of 5-HT and octopamine) or 10 min (in the cases of dopamine and noradrenaline); then various amounts of radioactive amines were added to the medium and the incubation was continued for varying periods of time. Unless otherwise stated,

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preincubations and incubations were performed at either 0° or 25°. At the end of the incubation ganglia were recovered with forceps and rinsed twice in 20 ml ice-cold snail saline. Preliminary experiments had established that there was no significant release of radioactivity from the tissues during the washing process. The individual ganglia were then placed in vials containing 0·5 ml tissue solubiliser (Soluene-350 Packard) for at least 1 hr at room temperature before adding 10 ml Dimilume® (Packard). Radioactivity was measured in a Packard liquid scintillation spectrometer. A small amount (20–100 μl) of the radioactive incubation mixture was dissolved in 10 ml Dimilume® and also counted. The counting efficiency was monitored by the addition of internal standards of [^{3}H]toluene and the appropriate corrections applied. The counting efficiency for tritium varied between 20 and 23%.

Tissue/medium ratios (T/M) were calculated as cpm of tritium in 1 g of tissue per counts of tritium in 1 ml medium. The amount of amine accumulated by the tissues (v = nmoles amine/g per incubation time) was calculated from values obtained from the T/M ratios at 0° and from the values from the T/M ratios at 25°[25]. Unlabelled amines were added to the labelled amines to produce the higher concentration of solutions required for the kinetic studies. Linear regressions for determining kinetic constants were determined by computer. Six to ten independent experiments were carried out for each value.

In order to analyse the metabolism of amines during the uptake studies, ganglia were incubated in different radioactive amines for 30 min at 25°; the tissues homogenised in 0·01 N HCl and the supernatants, following centrifugation at 1500 *g*, then applied to Silica gel precoated plates (Merck −60). Small amounts of carrier amines were routinely added to the chromatograms which were then developed in an ascending way using either *n*-butanol–pyridine–acetic acid–water (15:2:3:5 by vol.) or the organic phase from a mixture of chloroform–acetic acid–water (2:2:1 by vol). Substances were identified by spraying the chromatograms with 1% potassium ferricyanide in ammonium hydroxide solution. Individual substances were eluted from the Silica gel with methanol, the eluates were dried under a stream of nitrogen and then counted in a scintillation spectrometer. From the results the percentage of amines metabolised was calculated.

The chemicals used were as follows: 5-hydroxy [G-^{3}H]tryptamine creatine sulphate 17·3 Ci/m-mole (Amersham), [^{3}H]dopamine HCl 2·3 Ci/m-mole (Amersham), D.L-[7-^{3}H]noradrenaline HCl 15 Ci/m-mole (Amersham), D.L.-(2-^{3}H]octopamine HCl 3·7 Ci/m-mole (New England Nuclear), acetylcholine chloride (Roche), 5-hydroxytryptamine creatine sulphate (Merck), dopamine (Aldrich), noradrenaline (Serva), octopamine (Aldrich), chlorimipramine (Geigy), chlorpromazine (Bayer), imipramine (Geigy), pargyline HCl (Abbott), reserpine (Ciba), tetrabenazine (Roche), lysergic acid diethylamide (Sandoz), 5,7-dihydroxytryptamine (Regis), 6-hydroxydopamine (Sigma), ouabain (Merck).

RESULTS

Time course of [^{3}H]*amine uptake.* The time course of [^{3}H]5-HT, [^{3}H]dopamine, [^{3}H]noradrenaline and [^{3}H]octopamine accumulation in the snail suboesophageal ganglia incubated with 0·05 μM [^{3}H]amine is illustrated in Fig. 1. Between 0 and 30 min there is a rapid linear uptake of [^{3}H]5-HT followed by a somewhat slower phase (30–50 min). In the case of dopamine, the uptake of radioactive amine is linear only for the first 20 min, followed by a slower uptake phase (20–30 min). The initial accumulation phases for [^{3}H]noradrenaline and [^{3}H]octopamine (0–20 min) are much slower than those for the other two amines. In these instances the uptake of [^{3}H]octopamine shows a slow linear process (0–50 min), while in the case of [^{3}H]noradrenaline uptake a linear accumulation procedure occurs for the first 20 min, succeeded by a slower process (20–30 min), which eventually levels off (30–50 min).

Metabolism of [^{3}H]*amines.* The chromatographic results demonstrated that 93% 5-HT, 92% dopamine, 94% [^{3}H]noradrenaline and 91% octopamine remained unmetabolised in the ganglia after incubation at 25° for 30 min in 0·05 μM of either [^{3}H]5-HT, [^{3}H]dopamine, [^{3}H]dopamine, [^{3}H]noradrenaline or [^{3}H]octopamine. Less than 4% of the individual amines was incorporated into protein from the suboesophageal ganglia.

Effect of temperature on [^{3}H]*amine uptake.* Incubations of ganglia were carried out at 0–35° for 20–30 min in saline containing 0·05 μM of [^{3}H]5-HT, [^{3}H]dopamine or [^{3}H]noradrenaline (see Fig. 2). In all instances the uptake of amine was dependent upon temperature, and two linear uptakes were able to be resolved for dopamine and 5-HT. The Q10 values at the different temperature ranges for each amine were as follows: $5\text{-HT}_{0\text{-}25°} = 1{\cdot}86$, $5\text{-HT}_{25\text{-}35°} = 2{\cdot}00$, $\text{dopamine}_{5\text{-}35°} = 1{\cdot}43$, $\text{noradrenaline}_{5\text{-}35°} = 1{\cdot}35$.

Kinetic analysis of amine uptake into snail suboesophageal ganglia. In order to evaluate the kinetics of amine uptake in the suboesophageal ganglia, tissues were incubated in various concentrations of [^{3}H]amine, ranging from 0·01 to 100 μM

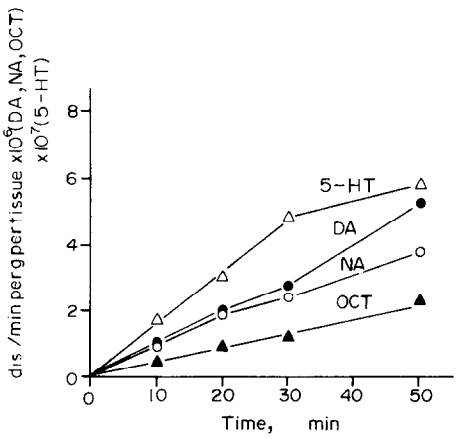

Fig. 1. Time-course of [^{3}H]5-HT, [^{3}H]DA (dopamine), [^{3}H]NA (noradrenaline) and [^{3}H]OCT (octopamine) uptake in snail supra oesophageal ganglia incubated at 25° with [^{3}H]-amine (0·05 μM). Each point is the mean value of 6–10 experiments.

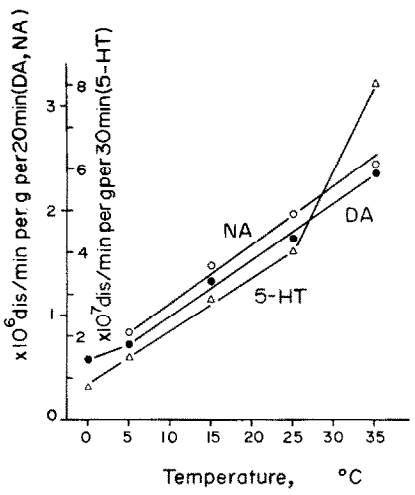

Fig. 2. Effect of temperature on uptake of [^{3}H]NA, [^{3}H]DA, and [^{3}H]5-HT by snail supra oesophageal ganglia incubated with 0·05 μM [^{3}H]-amine for either 30 min (in case of 5-HT) or 20 min (in case for DA and NA). Each point is a mean value for at least six experiments.

(Table 1). In the instances of 5-HT and octopamine the incubation time was 30 min, while for dopamine and noradrenaline it was 20 min, since at these time intervals the accumulation process of the individual amines is linear as shown in Fig. 1. At 25°, T/M ratios of 5-HT and dopamine decrease with increasing amine concentrations in the medium, showing that a saturation process occurs in each case. As illustrated in Table 2, this also takes place for 5-HT and dopamine at 0°, though the T/M ratio for each concentration of amine in the medium is much lower than that for the same amine concentration at 25°. In contrast, the T/M ratios for noradrenaline and octopamine do not saturate but remain more or less constant with increasing concentrations of amines (0·01–10 μM) in the mediums. Moreover, the T/M ratios for noradrenaline and octopamine, especially at lower amine concentrations (e.g. 0·01–1 μM) at 25° are very much less than the T/M values for 5-HT or dopamine.

The relatively high T/M ratios for 5-HT and dopamine at 25° and 0° for low amine concentrations in the incubation mechanism, which gradually decrease and saturate at higher amine concentrations (see Tables 1 and 2), were further analysed by Lineweaver–Burk plots (see Figs. 3–7). In the instance of 5-HT at 25° the Lineweaver–Burk plot[26] can be resolved into two components (see Figs. 3 and 4): a higher affinity component (i.e. uptake 1) with K_m and V_{max} values referred to as K_{m_1} and V_{max_1}, and a lower affinity component (i.e. uptake 2) with K_{m_2} and V_{max_2} values. From Figs. 3 and 4 it can be seen that at 25° the K_{m_1} and V_{max_1} values for 5-HT are $8{\cdot}48\times10^{-8}$ M and 0·077 nmoles/g per min respectively, and those for K_{m_2} and V_{max_2} are $1{\cdot}8\times10^{-6}$ M and 0·66 nmoles/g per min. The Lineweaver–Burk plot for dopamine at 25° can also be resolved into two components, though in this case one of the components is considered to go practically through the zero point (since the values determined by computer in relation to the variation corresponded with this idea), thus having no significant K_m or V_{max} values (see Fig. 6). The other component for dopamine at 25° has K_m and V_{max} values of $1{\cdot}02\times10^{-7}$ M and 0·0673 nmoles/g per min. The Lineweaver–Burk plot for 5-HT at 0° is similar to that of dopamine at 25° as shown in Fig. 5, with one component considered to go through the zero point and the other having K_m and V_{max} values of $9{\cdot}152\times10^{-8}$ M and 0·0203 nmoles/g per min. In contrast, the plot for dopamine uptake at 0°C is a single component

Table 2. T/M ratios for [^{3}H]5-HT and [^{3}H]dopamine accumulation into the suboesophageal ganglia over a wide range of amine concentrations at 0°

Amine concn (μM)	5-HT	Dopamine
0·01	6·0 ± 1·2	6·34 ± 0·67
0·02	5·31 ± 0·12	6·01 ± 0·705
0·05	4·2 ± 0·5	5·46 ± 0·38
0·1	3·29 ± 0·11	5·02 ± 0·29
0·2	2·58 ± 0·19	5·48 ± 0·33
0·5	3·34 ± 0·33	4·74 ± 0·69
1·0	3·48 ± 0·70	2·78 ± 0·36
2·0	3·07 ± 0·47	2·11 ± 0·11
5·0	3·02 ± 0·44	1·68 ± 0·09
10·0	2·61 ± 0·07	1·43 ± 0·02
50·0	0·951 ± 0·0021	0·63 ± 0·02
100	0·92 ± 0·05	0·41 ± 0·01

Table 1. T/M ratios for [^{3}H]amine accumulation into the suboesophageal ganglia over a wide range of amine concentrations at 25°

Amine concn (μM)	5-HT	Dopamine	Noradrenaline	Octopamine
0·01	30·3 ± 1·62	18·43 ± 2·44	4·18 ± 0·09	5·73 ± 0·63
0·02	26·58 ± 1·64	16·76 ± 1·71	4·41 ± 0·69	5·52 ± 0·24
0·05	21·9 ± 1·66	14·27 ± 2·36	5·09 ± 0·75	4·78 ± 0·31
0·1	17·3 ± 1·89	13·79 ± 1·46	4·91 ± 0·44	6·16 ± 0·95
0·2	12·38 ± 1·58	11·56 ± 1·17	4·45 ± 0·39	4·96 ± 0·40
0·5	12·55 ± 1·09	10·42 ± 1·86	4·20 ± 0·31	4·42 ± 0·31
1·0	9·66 ± 0·47	11·11 ± 1·06	3·71 ± 0·05	5·05 ± 0·19
2·0	8·97 ± 0·71	7·88 ± 0·36	4·49 ± 1·06	3·93 ± 0·34
5·0	8·22 ± 0·78	6·95 ± 0·70	4·22 ± 0·18	4·43 ± 0·29
10·0	7·23 ± 1·28	5·45 ± 0·58	3·89 ± 0·65	—
50·0	5·35 ± 0·76	3·37 ± 0·13	—	—
100	3·57 ± 0·12	—	—	—

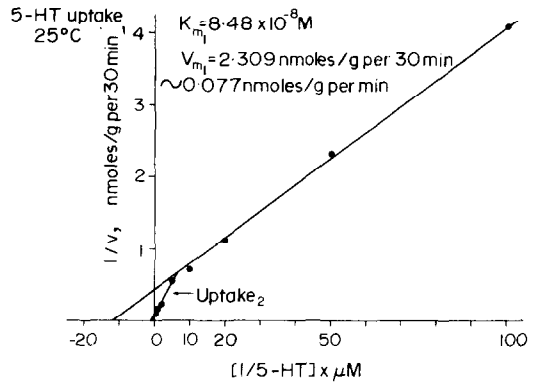

Fig. 3. Graphic analysis (computer determined: regression line $y = A.x + B$) of the reciprocals of [^{3}H]5-HT concentration and its accumulation in the snail supra oesophageal ganglia by method of Lineweaver–Burk (1934). Ganglia were incubated for 30 min at 25° with [3H]5-HT concentrations varying from 0·01–100 μM. Amine accumulation (v) is expressed as nmoles of ^{3}H/30 min per g and was calculated by multiplying the T/M ratio by the medium 5-HT concentration. The points represent mean values for 6 to 10 experiments. It can be seen that two components can be resolved, one referred to as uptake 1 (K_1 and V_1 value) the other uptake 2 (K_2 and V_2) values). The graphic analysis for uptake 2 is more clearly demonstrated in Fig. 4. From the computer analysis the constant B (for determination of V_1) was 0·433 + 0·057 and the constant B/A (for K_1) was 11·781 + 1·202.

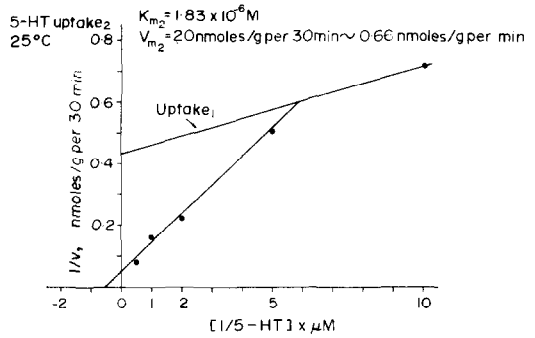

Fig. 4. Graphic analysis (computer determined: regression line $y = A.x + B$) for 5-HT uptake by snail ganglia incubated in high amounts of 5-HT (i.e. uptake 2) at 25°. The upper line corresponds to the uptake 1 component (Fig. 3). The points represent mean values for more than 6 experiments. Constant B (for V_2) was 0·05 ± 0·014, constant B/A (for K_2) was 0·547 ± 0·022.

and to all purposes also goes through the zero point (see Fig. 7).

Since the Lineweaver–Burk plots show that 5-HT alone has two uptake mechanisms which occur only at 25°, the Michaelis–Menten equation was used to calculate the relative contributions of uptake 1 and 2 to the total accumulation of 5-HT in the suboesophageal ganglia at different 5-HT concentrations as shown in Fig. 8. It can be seen from the figure that at low concentrations the high affinity uptake system (uptake 1) contributes a greater proportion of 5-HT accumulation, so that at 0·01 μM the velocity of uptake 1 is three times that of uptake 2 (low affinity uptake system). At about 0·4 μM the contribution of uptake 1 and 2 occurs at equal velocities, whereas at higher concentrations

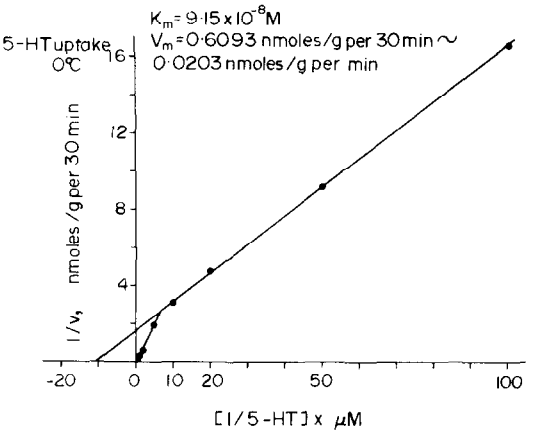

Fig. 5. Graphic analysis (computer determined: regression line $y = A.x + B$) of reciprocals of [^{3}H]5-HT concentration and its accumulation in the snail ganglia. Ganglia were incubated for 30 min at 0° with [^{3}H]5-HT concentration varying from 0·01 to 100 μM. It can be seen that the Lineweaver–Burk plot can be resolved into two components but since one of the components was determined to go through the zero point (B = 0·095 ± 1·4; B/A = 0·176 ± 0·43) thus giving single K (B/A = 10·92 ± 0·43) and V (B = 1·64 ± 0·08) values. The points represent mean values for more than 6 experiments.

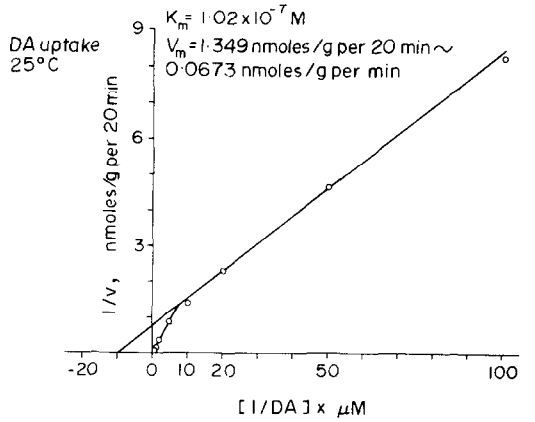

Fig. 6. Graphic analysis (computer determined: regression line $y = A.x + B$) of reciprocals of [^{3}H]DA concentration and its accumulation in the snail ganglia. Ganglia were incubated for 20 min at 25° with [^{3}H]DA concentration varying from 0·01 to 100 μM. It can be seen that the Lineweaver–Burk plot can be resolved into two components one of which goes through the zero point (B = 0·015 ± 0·048; B/A = 0·320 ± 0·321) thus giving single K (B/A = 9·764 ± 1·049) and V (B = 0·74 ± 0·096) values. The points represent mean values for more than 7 experiments.

uptake 2 becomes the major contributor. At 100 μM the velocity of uptake 2 is about six times that of uptake 1.

The effect of alteration of incubation medium on amine uptake. The effects of altering the incubation medium on the accumulation of amines are shown in Table 3. Omitting either pargyline or glucose from the medium did not have a drastic effect on any of the four amines, though the accumulation of 5-HT was the most affected by the absence of pargyline. Ouabain in the incubation medium slightly inhibited the 5-HT uptake but had little

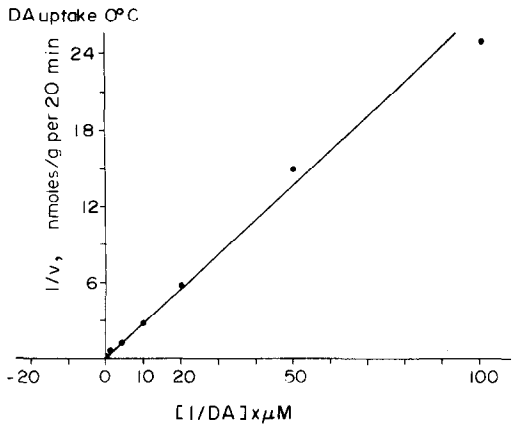

Fig. 7. Graphic analysis (computer determined: regression line $y = A.x + B$) of reciprocals of [^{3}H]DA concentration and its accumulation in the snail ganglia at 0°. It can be seen that the Lineweaver–Burk plot is a single component and goes through the zero point ($B = 0{\cdot}033 \pm 0{\cdot}027$; $B/A = 0{\cdot}099 \pm 0{\cdot}121$).

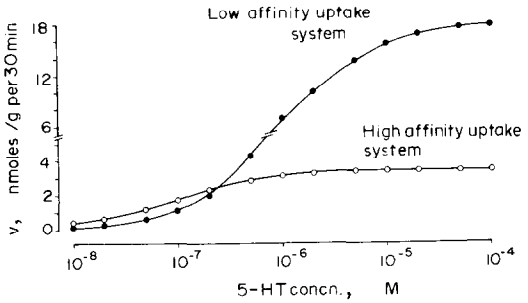

Fig. 8. Velocity of 5-HT accumulation by uptake$_1$ and uptake$_2$ at varying 5-HT concentrations at 25°. Kinetic constants for the two 5-HT uptake systems were substituted in the Michaelis–Menten equation, $v = V_{max}/(1 + K_m/S)$, K_m and V_{max} values were determined from Figs. 3 and 4. 5-HT concjentrations (S) were selected from 0·01 to 100 μ.

influence on the other monoamines. Variations in the ionic composition of the medium greatly affected the accumulation of the various amines (see Table 3). Ganglia incubated in mediums in which sodium was replaced by sucrose at either 25° or 0° resulted in a drastic inhibition of the uptake of

Table 3. Effect of alteration of the incubation medium on the uptake of amines at 25° (in percentage of control)

	5-HT	Dop-amine	Noradren-aline	Octop-amine
Omitting glucose	86·5	102·3	98·7	88·5
Omitting pargyline	77·9	99·5	119·8	—
Omitting glucose but adding 10^{-3} M ouabain	50·5	98·5	—	—
Na^+ free (25°)	13·05	9·45	13·7	12·2
Na^+ free (0°)	18·4	11·3	—	—
Ca^{2+} free	107·6	104·5	110·8	70·8
Mg^{2+} free	96·2	105·7	97·8	—
K^+ free	88·2	68·7	70·9	—

Sucrose was substituted for the ions omitted from the incubation medium. The composition of the incubation medium is described in Methods.

all amines studied. The absence of calcium had little effect on the uptake of 5-HT, dopamine or noradrenaline, but diminished the accumulation of amines, while the omission of potassium slightly inhibited the uptake of all amines into the ganglia.

Effect of amines and pharmacological agents on amine uptake. The effect of amines and pharmacological agents on the uptake of [^{3}H]5-HT and [^{3}H]dopamine is given in Table 4. In some cases the ID_{50} values, a 50% inhibition derived from log probit plots of percentage change against concentration of substance used, could not be determined because of the non-linear form of the plot. From the ID_{50} values determined, it is clear that both dopamine and 5,7-dihydroxytryptamine inhibit the uptake of 5-HT to a greater extent than noradrenaline. However, 3-chlorimipramine, imipramine and chlorpromazine are all more potent inhibitors of the 5-HT uptake, the effect of each agent being similar. ID_{50} values for the effects of 6-hydroxydopamine tetrabenazine, reserpine and LSD-25 on the uptake of 5-HT were not obtained for a number of reasons. In the case of 6-hydroxydopamine no influence was exerted on the uptake of 5-HT between 5×10^{-5} and 10^{-4} M, but at 5×10^{-4} M a 55% inhibition occurred. Similar results were achieved for tetrabenazine; there was no effect at all from concentrations below 10^{-5} M, but 5×10^{-5} M inhibited the uptake by 90%. In the instances of reserpine and LSD-25 no effect upon the accumulation of 5-HT into the ganglia was recorded for the concentrations used (10^{-5} to 2×10^{-4} M).

The inhibition of dopamine uptake by 5-HT is approximately the same as the inverse (see Table 4). ID_{50} values were also determined for the influence of noradrenaline, 6-hydroxydopamine, chlorimipramine, imipramine and chlorpromazine on the uptake of dopamine, showing that chlorpromazine was the most effective, being about 3 times more potent than imipramine, about 50 times more potent than either noradrenaline or 6-hydroxydopamine and about 600 times more potent than 3-chlorimipramine. ID_{50} values were not obtainable for 5,7-dihydroxytryptamine, tetrabenazine, reserpine or LSD-25 for a variety of reasons. Between 5×10^{-5} and 5×10^{-4} M, 5,7-dihydroxytryptamine inhibited the uptake of dopamine by 50%. In contrast, no effect was recorded for tetrabenazine at concentrations below 10^{-4} M, though this amount of drug inhibited the uptake of dopamine by 80%. For reserpine and LSD-25 no effect was recorded for doses varying between 10^{-5} and 2×10^{-4} M.

DISCUSSION

The present experiments clearly demonstrate that the suboesophageal ganglia of *Helix* possess an active uptake mechanism for 5-HT at 25°. Previous reports [17, 23, 27] showed that gastropod nervous tissue had the ability to accumulate 5-HT even though it was known that connective tissues surrounding the ganglia also take up 5-HT [28], but detailed kinetic data were not reported. Moreover, autoradiography studies had shown that nervous elements in snail ganglia are the sites which take up the radioactive 5-HT [22]. Under the conditions of

Table 4. Effect of amines and pharmacological agents on the uptake of [^{3}H]5-HT and [^{3}H]dopamine by the suboesophageal ganglia

	[^{3}H]5-HT uptake	[^{3}H]dopamine uptake
5-HT	—	$2{\cdot}3 \times 10^{-5}$ M
Dopamine	3×10^{-5} M (ID_{50} value)	(ID_{50} value)
Noradrenaline	6×10^{-4} M (ID_{50} value)	$2{\cdot}3 \times 10^{-5}$ M (ID_{50} value)
5,7-Dihydroxytryptamine	5×10^{-5} M (ID_{50} value)	inhibits, but not linearly, uptake by 50% at 5×10^{-5}–5×10^{-4} M
6-Hydroxydopamine	no effect at 5×10^{-5}–10^{-4} M but at 5×10^{-4} M inhibits 55%	3×10^{-5} M (ID_{50} value)
3-Chlorimipramine	$4{\cdot}5 \times 10^{-6}$ M (ID_{50} value)	10^{-5} M (ID_{50} value)
Imipramine	$5{\cdot}02 \times 10^{-6}$ M (ID_{50} value)	$2{\cdot}5 \times 10^{-6}$ M (ID_{50} value)
Chlorpromazine	4×10^{-6} M (ID_{50} value)	6×10^{-6} M (ID_{50} value)
Tetrabenazine	no effect at 5×10^{-7}–10^{-5} M but at 5×10^{-5} M inhibits 90%	no effect at 5×10^{-7}–5×10^{-5} but at 10^{-4} M inhibits 90%
Rerspine	no effect at 10^{-5}–2×10^{-4} M	no effect at 10^{-5}–2×10^{-4} M
LSD-25	no effect at 10^{-5}–2×10^{-4} M	no effect at 10^{-5}–2×10^{-4} M

ID_{50} values determined between the concentration range of 5×10^{-7}–5×10^{-4}.

these experiments, ganglia rapidly accumulated [^{3}H]5-HT from mediums containing low concentrations of the amine. This uptake of substrate appears to be an active transport process, since after 20 min incubation at 25° the T/M ratio was 30:1 for 5-HT and the rate of accumulation shows saturation kinetics, sodium-ion dependency, temperature sensitivity and partial inhibition by ouabain. Furthermore, the amine does not undergo appreciable metabolism once accumulated by the ganglia, a prerequisite for the kinetic analysis of the transport mechanism. It is of interest to note that the values of the high affinity uptake process, i.e. K_{m_1}, for 5-HT in the snail nervous tissue are lower than those analysed for the uptake of the amine in the vertebrate brain, though the V_{max} values for the vertebrates are considerably higher than for the snail [19, 21, 25, 29–31]. The values of the low affinity process, i.e. K_{m_2}, for 5-HT accumulation in the snail nervous tissue and vertebrate brain tissue (see e.g. [21]) are however similar. Furthermore, uptake of 5-HT in the snail ganglia exhibits a number of other distinctive features. Unlike the vertebrates, where the rank order of potency for inhibiting the accumulation of 5-HT is generally chlorimipramine > imipramine > chlorpromazine [21, 25], no differences in the potency of the three drugs in the uptake of 5-HT were found to occur in either the ganglia or snail auricle [32]. In addition, neither reserpine inhibited the uptake of 5-HT into the ganglia, contrary to the case in the vertebrates [25]: nor did LSD-25. Another distinctive feature of 5-HT uptake is the ability of the ganglia to accumulate large amounts of the indoleamine at 0°, and this accumulation is linear between 0–40 min (diagram not shown) and, like the procedure at 25° seems to be an active transport process, since it shows saturation kinetics and apparent sodium ion dependency. However, more experiments are needed in order to analyse critically whether glucose is needed as a source of energy and whether sodium is required in the uptake of 5-HT at 0°. In order to investigate the need for sodium, tissues should perhaps be given preincubations with drugs like 2,4-dinitrophenol, ouabain, tetrodotoxin or gramicidin D before analysing whether these substances affect the uptake of 5-HT. It may also be that a new type of APAase functions at 0°. In any event, examination of the Lineweaver-Burk plot for 5-HT uptake at 0° reveals that although two components can be restored, one of them projects through the zero point, so that only single K_m and V_{max} values can be determined. From the nature of the component through the zero point it may be tentatively concluded that it represents passive accumulation (and/or unspecific binding) of the indoleamine into the ganglia, while the low K_m value for the other component suggests that this is a high affinity uptake mechanism, i.e. K_{m_1}. It may thus perhaps be deduced that at 0° a single high affinity uptake mechanism (into postsynaptic endings [19]) exists for 5-HT, while at 25° this mechanism occurs together with a low affinity uptake mechanism [19]. The lower affinity uptake mechanism, plus passive diffusion (and/or unspecific binding) at 25° thus results in giving K_{m_2} and V_{max_2} values, while at 0° the passive diffusion (and/or unspecific binding) component goes through the zero point of a Lineweaver–Burk plot. The results as a whole thus demonstrate that a high affinity uptake mechanism for 5-HT occurs in the ganglia and functions at 25° or 0°. A lower affinity uptake mechanism also occurs, but only at 25°.

The present findings reveal, too, that dopamine is actively taken up by the ganglia at 25°. Previous reports [18, 27] showed that gastropod nervous tissue could accumulate dopamine, though detailed kinetic results were not reported. Moreover, autoradiographic studies have disclosed that nervous elements in the molluscan nervous system are the sites which take up radioactive dopamine [33]. Under the conditions of these experiments, ganglia rapidly accumulated radioactive dopamine from mediums containing low concentrations of the catecholamine. The mechanism of uptake appears to be an active transport process, as after 30 min incubation at 25° the T/M ratio was 18:1 for dopamine, and the rate of accumulation showed saturation kinetics, sodium-ion dependency and

temperature sensitivity, though it remained unaffected by ouabain. In addition, negligible amounts of the accumulated dopamine were metabolised. However, examination of the Lineweaver–Burk plot of dopamine uptake at 25° shows the curves to be similar to that of 5-HT uptake at 0°, i.e. two components, one of which goes through the zero point. In this respect, the uptake of dopamine into the ganglia of the snail differs from vertebrate studies, where it has been proven that both uptake 1 and uptake 2 mechanisms exist for dopamine uptake in nervous tissue preparations[19, 29, 34, 35]. The single K_m value for dopamine uptake in the ganglia at 25° suggests that this is a K_{m_1} value (i.e. high affinity uptake), and that the other component belongs to passive diffusion and/or unspecific binding. Unlike the uptake of 5-HT at 0°, examination of the Lineweaver–Burk plot for dopamine uptake at this temperature shows only a single component which projects through the zero point. Hence the accumulation of dopamine at 0° would appear to be merely passive (and/or unspecific binding), though the quite high T/M ratios for low concentrations of amine (about 6) suggests something more complex. Moreover the accumulation of dopamine at 0° is linear between 0 and 40 min (diagram not shown) and for inexplicable reasons this accumulation seems also dependent upon sodium ions. Like the case of 5-HT uptake at 0° further critical studies are clearly required in order to determine whether dopamine accumulation at 0° is energy and sodium dependent. The uptake of dopamine at 25° is dependent upon the potassium ion but independent of the calcium ion content, as is the uptake of 5-HT. Moreover, as in the vertebrates, imipramine and chlorpromazine are more potent in inhibiting the uptake of dopamine than 3-chlorimipramine, while the effect of tetrabenazine is inconclusive. The uptake of dopamine was not influenced by LSD-25 or reserpine, contrary to findings in vertebrate studies with respect to the latter substance[35]. As a whole, the results thus show that a single high affinity uptake mechanism for dopamine occurs in the suboesophageal ganglia of *Helix*, and functions at 25° but not at 0°. Furthermore, a distinctive lower affinity uptake mechanism, i.e. uptake 2, could not be determined for either temperature.

Though the ganglia accumulated both noradrenaline and octopamine at 25°, the T/M ratios for high and low concentrations of substrate were low and did not show saturation kinetics. These accumulation processes were not studied any further, for such results indicated that neither amine was involved in high affinity uptake processes in the ganglia. The absence of a high affinity uptake process for octopamine and noradrenaline does not necessarily mean that these two amines do not serve as transmitter functions in the snail ganglia, for it may well be that only a minute proportion of the cells in the suboesophageal ganglia actually utilises these amines, with the result that any specific uptake processes will not be noticed because of the 'background noise'. This idea receives support from the fact that only minute amounts of noradrenaline[9] and octopamine[8] occur in the snail brain, in comparison to the 5-HT and dopamine content[2–4]. Moreover, some other evidence suggests that both amines have transmitter functions in the snail CNS, since octopamine only occurs in particular gastropod neurons[10] and electrophysiological studies have shown receptors especially receptive to octopamine[11] and noradrenaline[12]. The absence of a specific uptake process for substances may also simply mean that the 'transmitter' is inactivated by other procedures, e.g. enzyme degradation or simple diffusion. Nevertheless, it was interesting to note that the accumulation of noradrenaline and octopamine into the ganglia was dependent upon sodium ions and temperature, resembling in this respect the accumulation process of dopamine at 0°. This indicates that these processes are not purely passive diffusion and/or unspecific interaction, but something more complicated. One possibility could be that an ion exchange process occurs not only for octopamine and noradrenaline, but also for 5-HT and dopamine (which may involve energy), so that certain amounts of noradrenaline, for example, can be accumulated by neurons which do not contain endogenous amounts of the catecholamine. There is much evidence supporting the idea that defined neurons can accumulate other transmitter substances[21, 36, 37], which is further supported by the present study which clearly reveals that the uptake of dopamine and 5-HT is influenced by the presence of other amines in the incubation medium. Unfortunately, the detailed influence of the various amines on the uptake of specific substances was not studied kinetically by applying the method of Dixon[38] to see how the individual substances compete with one another. It is of interest to note that 5,7-dihydroxytryptamine, a close analogue of 5-HT, inhibits the uptake of 5-HT as has been shown in vertebrate tissues[39] and the snail brain[40] and also affects the uptake of dopamine. Similarly, the close analogue of dopamine, 6-hydroxydopamine, which blocks the uptake of catecholamines in vertebrates[41], inhibits the uptake of dopamine but also influences the uptake of 5-HT.

Taken as a whole, the present results show that high affinity uptake mechanisms exist for 5-HT and dopamine at 25°, but not for octopamine and noradrenaline. That 5-HT and dopamine are inactivated by reuptake is further supported by the fact that negligible amounts of accumulated amines are broken down and only trace amounts of monoamine oxidase activity exist in the snail brain[16]. Moreover, there is impressive evidence that 5-HT and dopamine have transmitter roles in the snail CNS[1–7]. The absence of a high affinity uptake mechanism for noradrenaline and octopamine suggests that should these two substances have transmitter functions, they may either be inactivated by some other means, i.e. breakdown, or that the proportion of neurons in the ganglia utilising these amines is very small. The possession of a high affinity uptake mechanism for 5-HT at 0° is unusually interesting, though it would seem a logical mechanism for these animals, since snails often tolerate temperatures lower than 0° in their natural habitat. However, by the same argument, the absence of a dopamine uptake mechanism at 0°

is difficult to understand, though it may simply be that certain neurons function optimally at different temperatures.

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