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THE EFFECTS OF DRUGS ON THE UPTAKE OF AMINES BY MAST CELLS

BY

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Neoplastic mast cells, taken from an ascitic tumour in mice and incubated *in vitro*, took up ^{14}C-labelled 5-hydroxytryptamine and histamine from the medium. Uptake during the first hour gave an approximate measure of the initial rate. The amount of each amine taken up in this time was determined by bioassay and by radioactivity, the two methods giving similar results. The curves obtained by plotting initial rate of uptake against concentration in the medium suggested that the uptake of 5-hydroxytryptamine was by an active process and also by diffusion, whereas uptake of histamine was by diffusion only. The cells also took up ^{14}C-labelled (±)-noradrenaline and tryptamine, apparently by diffusion. The active uptake of 5-hydroxytryptamine was inhibited by lowering the temperature to 25° C or by increasing the *p*H to 8.9, procedures which had little effect on histamine uptake. The effects of cocaine, imipramine, chlorpromazine, mepyramine, promethazine, phenoxybenzamine, lysergic acid diethylamide, bromolysergic acid diethylamide, methysergide, guanethidine, dichloroisoprenaline and pronethalol on the uptake of amines were examined. In general, any antagonist which inhibited uptake of 5-hydroxytryptamine had little effect on uptake of histamine, and *vice versa*. Possible ways in which these antagonists produce their effects on amine uptake are discussed. A high concentration of 5-hydroxytryptamine, of tryptamine or of noradrenaline inhibited uptake of histamine, but only tryptamine decreased uptake of 5-hydroxytryptamine. These results, together with those from experiments with antagonists, suggest that there are specific binding sites for 5-hydroxtryptamine in these cells.

The mast cell tumour P-815 has been grown in culture and maintained as an ascitic tumour in mice for a number of years. Its cells contain 5-hydroxytryptamine, histamine and heparin which they are able to synthesize (Schindler, 1958; Schindler, Day & Fischer, 1959; Green & Day, 1960; Day & Green, 1962a). They are also able to take up 5-hydroxtryptamine and histamine (Day & Green, 1962b). A type of cell which can both synthesize and take up amines appeared to be a useful model for studying factors which influence uptake, binding and synthesis of amines by cells in general and for investigating the effects of antagonists on these processes. This paper describes the effects of such drugs on the uptake and binding of amines by these mast cells.

METHODS

Neoplastic mast cells were obtained from P-815-X-I tumours in mice (Green & Day, 1960). The tumours were maintained in the ascitic form in DBA/2 mice and transferred at weekly intervals. Cells were removed from mice by aspiration of peritoneal fluid, collected by centri-

fugation and washed twice in the solution used for incubating the cells. This was the medium previously used to grow the cells in culture (Schindler *et al.*, 1959) but with the vitamins, amino acids and antibiotics omitted. After washing, the cells were resuspended in a large volume of solution to give a concentration of 5 to 6 $\times$ 10^5 cells/ml.; earlier work had shown that this concentration is suitable for maintaining the cells in culture in good condition. The suspension of cells was then divided between an appropriate number of flasks; the volume of a flask was always at least thirty-times that of the suspension in it. The flasks were held in a water bath at 37° C; each flask was tilted to an almost horizontal position so that the suspension formed a thin layer. A stream of 95% oxygen and 5% carbon dioxide was blown over the surface of the suspension; this maintained the *p*H at 7.5 to 7.6. In some experiments a solution of different *p*H was used. A low *p*H was obtained by decreasing the sodium bicarbonate in the medium to 50 mg/l. and making up the molarity with sodium chloride; the *p*H of this solution was 6.9 when gassed with 95% oxygen and 5% carbon dioxide. A *p*H of 8.9 was obtained by gassing the normal solution with oxygen instead of with 95% oxygen and 5% carbon dioxide.

In all experiments the cells were incubated for 30 min before the addition of any substance. Histamine or 5-hydroxytryptamine was added and the cells were then incubated for several hours in the first experiments, but for only 1 hr in later experiments. When the effects of antagonists on the uptake of amines were studied, the antagonist was incubated with the cells for 30 min before the addition of the amine.

At the end of all experiments a cell count was taken and the cells were collected by centrifugation, washed three-times in ice-cold saline, and the amine was extracted. Histamine and noradrenaline were extracted by the method described previously for histamine (Schindler *et al.*, 1959). 5-Hydroxytryptamine and tryptamine were extracted by adding distilled water to the cells and freezing and thawing them; this was found to be as efficient as the acetone-extraction used previously (Schindler *et al.*, 1959). All the amines were labelled with ^{14}C so that uptake could be determined from the radioactivity of the cell extract, using a liquid scintillation counter. Histamine and 5-hydroxytryptamine were also determined by bioassay; histamine was assayed on the isolated ileum of the guinea-pig and 5-hydroxytryptamine on the rat stomach strip (Vane, 1957).

Radioactive histamine, 5-hydroxytryptamine and noradrenaline were obtained from the Radiochemical Centre, Amersham. One μC was equivalent to 5.2 μg of 2-[^{14}C]-histamine, 15.7 μg of 3'-[^{14}C]-5-hydroxytryptamine and 53.1 μg of carbinol-[^{14}C]-(±)-noradrenaline.

2'-[^{14}C]-Tryptamine (1 μC equivalent to 120 μg) was obtained from the New England Nuclear Corporation. The amounts of all amines are expressed as weights of base. The amounts of the following drugs are expressed as weights of salts: phenoxybenzamine hydrochloride (Dibenzyline), cocaine hydrochloride, methysergide, lysergic acid diethylamide, bromolysergic acid diethylamide, mepyramine maleate, promethazine hydrochloride, chlorpromazine hydrochloride, dischloroisoprenaline, pronethalol (Nethalide), guanethidine sulphate and imipramine.

To estimate the concentration ratio between cells and medium, the counts/min of radioactive material in 1 g of cells (approximately 10^9 cells) were divided by the counts/min of radioactive material in 1 ml. of medium.

RESULTS

Uptake of amines by mast cells taken from the mouse

Most of the previous work on the uptake of amines by these mast cells had been done with cells in culture (Day & Green, 1962b). It was therefore necessary to find out whether cells taken from the mouse and incubated *in vitro* would maintain their stores of endogenous amines and would show a similar uptake of exogenous amines to those in culture. When the cells were incubated without added amines for 1 and 2 hr there was no loss of 5-hydroxytryptamine or histamine.

In the first series of experiments, cell suspensions were incubated with either [^{14}C]-histamine or [^{14}C]-5-hydroxytryptamine (0.3μg/ml.) for several hours. Samples were removed at various intervals for extraction, bioassay and counting of radioactivity. The amounts of both amines taken up varied considerably from one experiment to another. In most experiments, the cells took up more *in vitro* than they had done in culture. The amount of histamine taken up was less than the amount of 5-hydroxytryptamine (Table 1) as was found with the same strain of cells

TABLE 1

UPTAKE OF 5-HYDROXYTRYPTAMINE AND HISTAMINE BY MAST CELLS

Uptake was measured after several hours incubation at 37° C with the amines (0·3 μg/ml.)

5-Hydroxytryptamine				Histamine			
Amount in cells before incubation	Period of incubation (hr)	Uptake of [^{14}C]-amine (ng/10^6 cells)	Increase by bioassay (ng/10^6 cells)	Amount in cells before incubation	Period of incubation (hr)	Uptake of [^{14}C]-amine (ng/10^6 cells)	Increase by bioassay (ng/10^6 cells)
52	2	58	36	34	4	10	11
42	2	26	22	18	5	6	2
65	3	50	13	58	5	21	8
76	3	36	3	62	5	7	0
40	4	72	10	58	5	7	0

in culture (Day & Green, 1962b). After an incubation period of several hours, uptake as measured by radioactivity was usually higher than uptake as determined by bioassay (Table 1); either the radioactive amines had exchanged with the endogenous stores, or some destruction of the amines had occurred. Similar results were obtained with cells in culture (Day & Green, 1962b).

Fig. 1 shows the increase in uptake of 5-hydroxytryptamine and of histamine with time; uptake during the first hour gave an approximate measure of the initial rate.

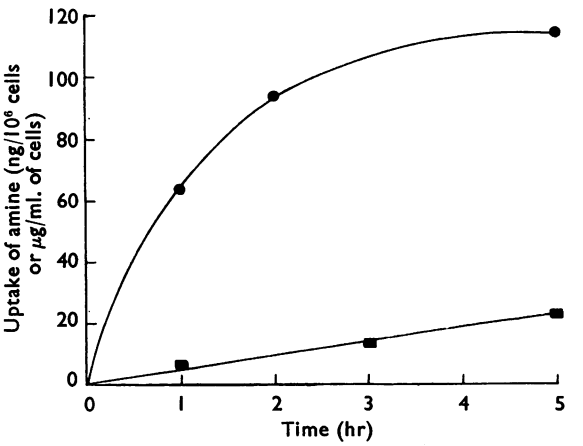

Fig. 1. Uptake of [^{14}C]-5-hydroxytryptamine (●—●) and of [^{14}C]-histamine (■— ■) by neoplastic mast cells during a 5 hr period of incubation at 37° C with the amines at a concentration of 0·3 μg/ml.

In experiments in which cells were incubated with different concentrations of the amines for 1 hr only, the uptake as measured by bioassay agreed, within the limits of experimental error, with uptake as calculated from radioactivity (Table 2). Therefore, in all subsequent experiments the cells were incubated with the amines for 1 hr only. Shorter times could not be used because too little amine was taken up for accurate bioassay.

TABLE 2

UPTAKE OF [^{14}C]-5-HYDROXYTRYPTAMINE AND [^{14}C]-HISTAMINE BY MAST CELLS

Uptake was measured after incubation at 37° C for 1 hr with various concentrations of the amines. Each group of three incubations represents a single experiment

5-Hydroxytryptamine			Histamine		
In medium (μg/ml.)	Uptake of [^{14}C]-amine (ng/10^6 cells)	Increase by bioassay (ng/10^6 cells)	In medium (μg/ml.)	Uptake of [^{14}C]-amine (ng/10^6 cells)	Increase by bioassay (ng/10^6 cells)
0·03	25	24	0·03	0·4	0
0·3	47	47	0·3	2	9
3·0	90	96	3·0	19	21
0·03	33	31	0·5	4	3
0·3	68	79	2·0	13	10
3·0	105	97	8·0	37	33

The uptake of amines in the first hour was measured in the presence of different concentrations of 5-hydroxytryptamine and histamine (Fig. 2). The points shown on Fig. 2 are the means of five experiments for both 5-hydroxytryptamine and histamine; the standard deviations of the means are given in Table 3. The uptake of 5-hydroxytryptamine differed remarkably from that of histamine. With very low concentrations of amine in the medium, the initial rate of uptake of 5-hydroxy-

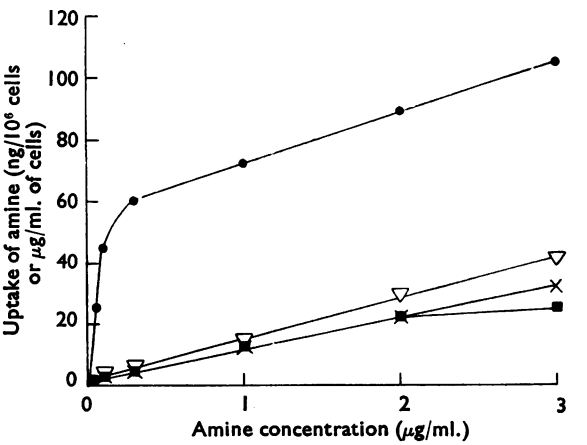

Fig. 2. Uptake of ^{14}C-labelled 5-hydroxytryptamine (●—●), histamine (■—■), noradrenaline (X—X) and tryptamine (▽—▽) by neoplastic mast cells after incubation at 37° C for 1 hr with the amines. The figures for 5-hydroxytryptamine and for histamine are the averages of five experiments, for noradrenaline of three experiments and for tryptamine of two experiments.

TABLE 3

UPTAKE OF [^{14}C]-5-HYDROXYTRYPTAMINE AND [^{14}C]-HISTAMINE BY MAST CELLS INCUBATED AT 37° C WITH DIFFERENT CONCENTRATIONS OF THE AMINES

Figures are the means and standard deviations of five experiments for each amine

Concentration of amine (μg/ml.)	Uptake of [^{14}C]-5-Hydroxytryptamine (ng/10^6 cells)	Uptake of [^{14}2]-Histamine (ng/10^6 cells)
0·03	23·7 ± 8·3	0·5 ± 0·13
0·1	45·0 ± 16·5	2·0 ± 0·8
0·3	59·7 ± 23·2	4·4 ± 1·8
1·0	71·8 ± 20·5	13·3 ± 3·6
2·0	89·3 ± 32·4	22·9 ± 7·3
3·0	105·2 ± 26·9	25·1 ± 5·8

tryptamine was much greater than that of histamine. With concentrations of 0.03 μg/ml. the concentration ratio was 833 for 5-hydroxytryptamine and only 18 for histamine. The uptake of 5-hydroxytryptamine continued to increase with increases in concentration in the medium but, at concentrations greater than 1.0 μg/ml., the slope of the curve was similar to that for histamine. When the results were plotted in the form, C_o/C_i against C_o, where C_o=concentration of the amine in the solution and C_i=initial rate of uptake with concentrations up to 1 μg/ml. the points fell on a straight line (Lineweaver & Burk, 1934). The uptake of histamine increased

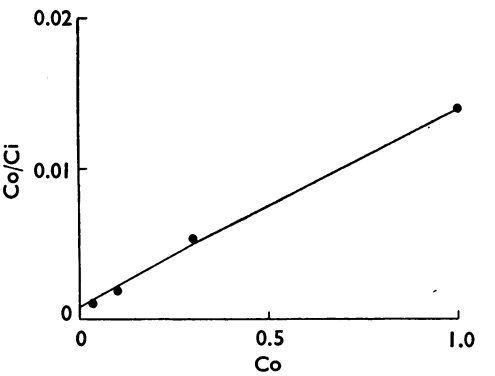

Fig. 3. Relation between 5-hydroxytryptamine in the medium (C_o) and the initial rate of uptake of 5-hydroxytryptamine by neoplastic mast cells. Initial rate of uptake is taken as the increase in concentration of 5-hydroxytryptamine found in mast cells (C_i) after incubation at 37° C for 1 hr. C_o/C_i (ordinate) is plotted against C_o (abscissa).

linearly with its concentration in the solution up to 2 μg/ml. (Fig. 2). These results suggest that 5-hydroxytryptamine is taken up by an active process and also by a process similar to that for the uptake of histamine, presumably by diffusion.

Having obtained such different results for 5-hydroxytryptamine and histamine, similar experiments were done with tryptamine and noradrenaline to see whether their uptake was like that of 5-hydroxytryptamine or like that of histamine. The

curves obtained with tryptamine (average of two experiments) and with noradrenaline (average of three experiments) were similar to the curve obtained for histamine (Fig. 2).

Effects of pH

The uptakes of 5-hydroxytryptamine and of histamine by the cells at *p*H 6.9 and 8.9 were compared with the uptake at *p*H 7.6. The uptake of 5-hydroxytryptamine was measured at two concentrations, 2 and 0.1 μg/ml.; the uptake of histamine was measured at a concentration of 2 μg/ml. The results are shown in Table 4. A low

TABLE 4

EFFECT OF *p*H ON THE UPTAKE AT 37° C OF [^{14}C]-5-HYDROXYTRYPTAMINE AND [^{14}C]-HISTAMINE BY MAST CELLS

Results are expressed as percentages of the uptake at *p*H 7·6

	Uptake of		
	5-Hydroxytryptamine		Histamine
*p*H	0·1 μg/ml.	2 μg/ml.	2 μg/ml.
6·9	97	81	100
7·6	100	100	100
8·9	39	77	72

*p*H had relatively little effect on uptake. At *p*H 8.9, however, the uptake of 5-hydroxytryptamine at 0.1 μg/ml. was reduced by 61%, whilst the uptakes of 5-hydroxytryptamine and histamine at 2 μg/ml. were reduced by 23% and 28% respectively.

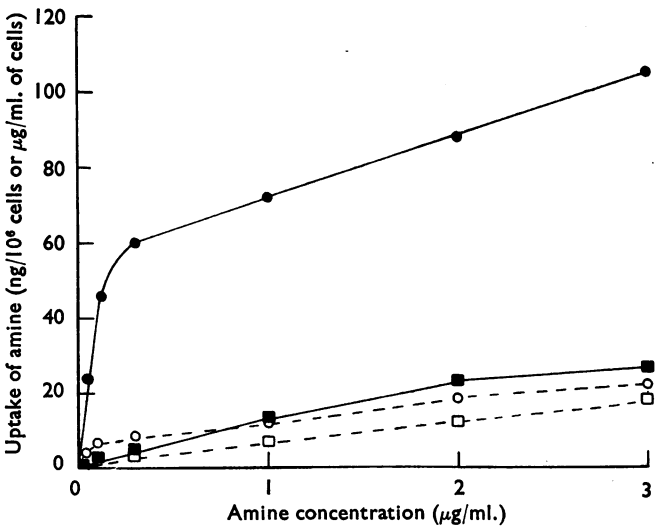

Fig. 4. Uptake of [^{14}C]-5-hydroxytryptamine at 37° C (●—●) and at 25° C (○---○) and uptake of [^{14}C]-histamine at 37° C (■—■) and at 25° C (□---□) by neoplastic mast cells after incubation for 1 hr with the amines. Each figure is the average of five experiments.

Effects of temperature

The temperature at which the mast cells were incubated was reduced from 37 to 25° C and the uptake was measured again with various concentrations of 5-hydroxytryptamine and histamine. The results are shown in Fig. 4. At 25° C the initial rate of uptake of 5-hydroxytryptamine was reduced by 80%, giving a curve similar to that for histamine. The uptake of histamine was reduced to a much smaller extent. Table 5 shows that the rate of uptake of 5-hydroxytryptamine by the cells was five- to eight-times faster at 37 than at 25° C. A similar comparison for histamine showed that the uptake was at most only about twice as fast at 37 as at 25° C.

TABLE 5

RATIO OF THE UPTAKE OF AMINE AT 37° C TO THAT AT 25° C BY MAST CELLS INCUBATED WITH DIFFERENT CONCENTRATIONS OF [^{14}C]-5-HYDROXYTRYPTAMINE AND [^{14}C]-HISTAMINE

Concentration of amine (μg/ml.)	Ratio for	
	5-Hydroxytryptamine	Histamine
0·03	6·9	1·6
0·1	7·8	1·1
0·3	7·1	1·2
1·0	5·7	1·9
2·0	5·0	1·9
3·0	5·3	1·3

When effects of antagonists were studied, some were found to depress uptake of histamine but not of 5-hydroxytryptamine at 37° C. Since uptake of 5-hydroxytryptamine at 25° C resembled that of histamine, a number of these antagonists were also tested on 5-hydroxytryptamine uptake at 25° C.

Effects of antagonists

Much of our present information on receptors for pharmacologically active amines is based on the interpretation of the effects of antagonists (Furchgott, 1954; Gaddum & Picarelli, 1957; Day & Vane, 1963). The effects of substances known to antagonize the actions of 5-hydroxytryptamine and histamine on smooth muscle and of substances known to prevent uptake of amines by other tissues were examined for effects on the uptake of amines by mast cells. Except where otherwise stated, the concentrations of 5-hydroxytryptamine or histamine in the solution were such as to give similar rates of uptake by the cells; [^{14}C]-5-hydroxytryptamine was added at a concentration of 0.03 μg/ml. and [^{14}C]-histamine at a concentration of 2 μg/ml. (see Fig 2).

Antagonists of 5-hydroxytryptamine. Methysergide, bromolysergic acid diethylamide and lysergic acid diethylamide, which antagonize the effects of 5-hydroxytryptamine on smooth muscle, had very little effect on the uptake of 5-hydroxytryptamine by mast cells (Table 6). Rather surprisingly, they inhibited the uptake of histamine more than that of 5-hydroxytryptamine. Bromolysergic acid diethylamide reduced the uptake of histamine most. The effect of bromolysergic acid diethylamide was tested on the uptake of 5-hydroxytryptamine at 25° C. The

concentration of 5-hydroxytryptamine was 2 μg/ml. which gave a rate of uptake similar to that with 0.03 μg/ml. at 37° C (see Fig. 4); the effect of bromolysergic acid diethylamide on the uptake of the same concentration of 5-hydroxytryptamine at 37° C was also measured. Bromolysergic acid diethylamide at 10^{-6} g/ml. was without effect but, at 10^{-5} g/ml., it inhibited uptake of 5-hydroxytryptamine by 45% at both 25 and 37° C.

Antihistamines. The results obtained by incubating cells with either promethazine or mepyramine before adding 5-hydroxytryptamine or histamine are shown in Tables 6 and 7. Both antihistamines were more effective in inhibiting the uptake of 5-hydroxytryptamine than that of histamine with concentrations of the amines which gave equal rates of uptake in control cells (Table 6). The effects of the antihistamines were also tested with different concentrations of the amines (Table 7). With both antihistamines the degree of inhibition decreased with increasing amine concentration. Promethazine (10^{-6} g/ml.) inhibited the uptake of low concentrations

TABLE 6

COMPARISON OF THE EFFECTS OF VARIOUS ANTAGONISTS ON THE UPTAKE AT 37° C OF [^{14}C]-5-HYDROXYTRYPTAMINE, [^{14}C]-HISTAMINE AND [^{14}C]-NORADRENALINE BY MAST CELLS

The amines were present at concentrations which gave similar initial rates of uptake in control cells. Figures show percentage inhibitions of control uptake. *Average of two or three experiments

Antagonist		Uptake of		
Drug	Concentration (g/ml.)	5-Hydroxytryptamine at 0·03 μg/ml.	Histamine at 2 μg/ml.	Noradrenaline at 2 μg/ml.
Methysergide	10^{-6}	0	0	—
	10^{-5}	19	47	—
Lysergic acid diethylamide*	10^{-6}	0	0	—
	10^{-5}	0	46	—
Bromolysergic acid diethylamide*	10^{-6}	0	32	—
	10^{-5}	16	74	—
Mepyramine*	10^{-6}	91	32	—
	10^{-5}	98	—	—
Promethazine*	10^{-6}	0	0	—
	10^{-5}	81	36	—
Cocaine*	10^{-6}	67	0	0
	10^{-5}	91	0	0
Phenoxybenzamine*	10^{-6}	13	90	87
	10^{-5}	94	—	—
Dichloroisoprenaline*	10^{-6}	55	49	25
	10^{-5}	95	75	73
Pronethalol	10^{-6}	66	65	64
	10^{-5}	95	—	85
Chlorpromazine*	10^{-6}	79	31	—
	10^{-5}	99	82	—
Guanethidine*	$2{\cdot}5 \times 10^{-6}$	0	24	50
Imipramine	5×10^{-6}	98	12	35

TABLE 7

EFFECT OF ANTIHISTAMINES ON THE UPTAKE AT 37° C OF [^{14}C]-HISTAMINE AND [^{14}C]-5-HYDROXYTRYPTAMINE BY MAST CELLS

Figures give percentage inhibitions of control uptake

Antihistamine		Uptake of								
Drug	Concentration (g/ml.)	5-Hydroxytryptamine at μg/ml.			Histamine at μg/ml.					
		0·03	0·3	3·0	0·1	1·0	10·0	0·03	0·3	3·0
Mepyramine	10^{-6}	89	70	21	91	36	0	—	—	—
Promethazine	10^{-6}	0	0	0	—	—	—	77	21	0
	10^{-5}	79	73	56	—	—	—	—	—	—

of histamine and was without effect on the uptake of 5-hydroxytryptamine at any concentration.

Cocaine. Cocaine reduces the uptake and possibly the storage of noradrenaline and of 5-hydroxytryptamine by various tissues (Trendelenburg, 1959; Whitby, Hertting & Axelrod, 1960; Burn & Burn, 1961; Hertting, Axelrod, Whitby & Patrick, 1961; Muscholl, 1961; Stacey, 1961; Iversen, 1963). Tables 6 and 8 show the inhibition of uptake of 5-hydroxytryptamine by mast cells produced by cocaine (10^{-5} and 10^{-6} g/ml.). When the cells were incubated with increasing concentrations of 5-hydroxytryptamine the inhibition was decreased. Cocaine inhibited the uptake of 5-hydroxytryptamine (2 μg/ml.) less at 25 than at 37° C (Table 8). In no experiment did cocaine affect the uptake of histamine.

TABLE 8

EFFECT OF COCAINE ON UPTAKE OF [^{14}C]-5-HYDROXYTRYPTAMINE BY MAST CELLS

Figures show percentage inhibitions of control uptake

Concentration of cocaine (g/ml.)	Uptake of 5-hydroxytryptamine at μg/ml.				
	0·03	0·3	3·0	2·0	
	37° C	37° C	37° C	25° C	37° C
10^{-5}	96	78	52	20	51
10^{-6}	79	54	32	0	21

Since cocaine reduces the uptake of noradrenaline by other tissues it was of interest to see if it had the same effect with mast cells, where the mechanism of noradrenaline uptake appeared to differ from that for 5-hydroxytryptamine and to resemble that for histamine uptake (Fig. 2). The concentration of noradrenaline was 2 μg/ml. as this gave a rate of uptake similar to that found with 5-hydroxytryptamine (0.03 μg/ml.) and with histamine (2 μg/ml.). Cocaine (10^{-5} and 10^{-6} g/ml.) was without effect on the uptake of noradrenaline (Table 6).

Phenoxybenzamine. Phenoxybenzamine depresses the actions both of 5-hydroxytryptamine and of histamine on smooth muscle (Gaddum & Picarelli, 1957) and

reduces the uptake of noradrenaline by various tissues (Hertting *et al.*, 1961). The effect of phenoxybenzamine on the uptake of amines by mast cells is shown in Tables 6 and 9. At 37° C, with concentrations of amines necessary to give similar rates of uptake, phenoxybenzamine (10^{-6} g/ml.) had little effect on the uptake of 5-hydroxytryptamine but decreased the uptake of histamine by 90% and that of noradrenaline by 87%. Table 9 also shows that phenoxybenzamine was more effective in reducing

TABLE 9

EFFECT OF PHENOXYBENZAMINE (10^{-6} G/ML.) ON THE UPTAKE OF [^{14}C]-5-HYDROXYTRYPTAMINE AND [^{14}C]-HISTAMINE BY MAST CELLS AT 25 AND 37° C

Figures show percentage inhibitions of control uptake

5-Hydroxytryptamine			Histamine		
	Uptake at			Uptake at	
Concentration (μg/ml.)	25° C	37° C	Concentration (μg/ml.)	25° C	37° C
0·03	—	13	2·0	74	91
2·0	38	43			

uptake when the concentration of 5-hydroxytryptamine was increased to 2 μg/ml, both at 37 and at 25° C. It had a slightly smaller effect on histamine uptake at 25 than at 37° C.

Substances which block adrenaline β-receptors. Dichloroisoprenaline and pronethalol, which block the β-receptors for catechol amines, were equally effective in reducing the uptake of 5-hydroxytryptamine, of histamine and of noradrenaline (Table 6).

Chlorpromazine. Chlorpromazine is an antihistamine and antagonizes the action of 5-hydroxytryptamine on smooth muscle; it also depresses the uptake of noradrenaline and of 5-hydroxytryptamine in various tissues (Dengler, Spiegel & Titus, 1961 ; Stacey, 1961). Chlorpromazine (10^{-6} g/ml.) was more than twice as effective in reducing the uptake of 5-hydroxytryptamine as in reducing the uptake of histamine (Table 6) ; at 10^{-5} g/ml. it was equally effective against both amines.

Guanethidine. Guanethidine blocks the uptake of noradrenaline by the heart (Hertting, Axelrod & Patrick, 1962; Bhagat & Shideman, 1963). With mast cells, guanethidine (2.5×10^{-6} g/ml.) inhibited the uptake of noradrenaline and of histamine but was without effect on the uptake of 5-hydroxytryptamine (Table 6).

Imipramine. A reduction in the uptake of 5-hydroxytryptamine by platelets in the presence of imipramine was described by Marshall, Stirling, Tait & Todrick (1960) and by Stacey (1961). Day & Green (1962b) showed that imipramine inhibited uptake both of 5-hydroxytryptamine and of histamine by mast cells in culture. In their experiments histamine was present at 0.4 μg/ml. and 5-hydroxytryptamine at 0.46 μg/ml. and inhibition of uptake was measured after 20 hr ; in the experiments described here the amines were added to give equal rates of uptake in the control cells in the first hour. Imipramine (5×10^{-6} g/ml.) inhibited the uptake of 5-hydroxytryptamine by 98%, and that of histamine by only 12%, compared with 69% and 32% in the experiments of Day & Green (1962b). The uptake of noradrenaline was inhibited by 35% (Table 6).

Competition between amines for uptake

To discover whether one amine affected the uptake of another, an unlabelled amine at a high concentration (20 μg/ml.) was added to the medium 30 min before the addition of either [^{14}C]-5-hydroxytryptamine or [^{14}C]-histamine. The cells were incubated for 1 hr with the labelled amine. 5-Hydroxytryptamine, (−)-noradrenaline and tryptamine all inhibited histamine uptake but only tryptamine decreased the uptake of 5-hydroxytryptamine to a significant extent (Table 10).

TABLE 10

EFFECT OF A HIGH CONCENTRATION OF ANOTHER AMINE IN THE SOLUTION ON UPTAKE AT 37° C OF [^{14}C]-5-HYDROXYTRYPTAMINE AND [^{14}C]-HISTAMINE BY MAST CELLS

Figures show percentage inhibitions of control uptake

Amine at 20 μg/ml.	Uptake of [^{14}C]-5-Hydroxytryptamine at 0·03 μg/ml.	Uptake of [^{14}C]-Histamine at 2 μg/ml.
5-Hydroxytryptamine	—	74
Histamine	0	—
(−)-Noradrenaline	16	69
Tryptamine	97	82

TABLE 11

EFFECT OF EQUIMOLAR CONCENTRATIONS OF ANOTHER AMINE ON UPTAKE AT 37° C OF [^{14}C]-5-HYDROXYTRYPTAMINE AND [^{14}C]-HISTAMINE BY MAST CELLS

Figures give percentage inhibitions of control uptake. Concentrations : 5-hydroxytryptamine 0·03 μg/ml. ; histamine, 2 μg/ml.

Unlabelled amine	Uptake of [^{14}C]-5-Hydroxytryptamine	Uptake of [^{14}C]-Histamine
5-Hydroxytryptamine	–	36
Tryptamine	0	12
(−)-Noradrenaline	–	37

The uptake of [^{14}C]-histamine was also inhibited by an *equimolar* concentration of 5-hydroxytryptamine, tryptamine or (−)-noradrenaline, although tryptamine only had a small effect (Table 11). Tryptamine did not affect the uptake of an equimolar concentration of [^{14}C]-5-hydroxytryptamine.

DISCUSSION

Processes of amine uptake

The uptake of 5-hydroxytryptamine and of histamine by neoplastic mast cells, when taken from the mouse and incubated *in vitro*, was similar to that which occured with the same cells in culture (Day & Green, 1962b). One of the most interesting results was that the uptake of 5-hydroxytryptamine, although resembling that of histamine under some conditions, also showed the following remarkable differences: (1) With low concentrations in the medium (up to 1 μg/ml.), the initial rate of uptake of 5-hydroxytryptamine was much faster than that of histamine. When the initial rate of uptake was plotted against concentration the results fitted the Michaelis-

Menten equation. (2) This fast rate of uptake depended much more on temperature than did uptake of histamine; it was reduced by 80% by lowering the temperature to 25° C. (3) A high *p*H inhibited uptake of 5-hydroxytryptamine at low concentrations to a much greater extent than uptake of histamine. Stacey (1961) stated that a change of *p*H in the range used in these experiments would have very little effect on the degree of ionization of 5-hydroxytryptamine; therefore this need not be considered in accounting for the effect of high *p*H. (4) The fast component of 5-hydroxytryptamine uptake was reduced by cocaine, chlorpromazine and imipramine. The uptake of 5-hydroxytryptamine by platelets (Born & Gillson, 1959) is inhibited by cocaine (Stacey, 1961) and so is the uptake of noradrenaline by the heart (Iversen, 1963). Chlorpromazine and imipramine diminish the uptakes of 5-hydroxytryptamine, noradrenaline and tryptamine in various tissues (Dengler *et al.*, 1961; Stacey, 1961; Pletscher, Kunz, Staebler & Gey, 1963). In all these examples, uptake of the amines is thought to be by an active process.

One interpretation of the results is that there is an active component in the uptake of 5-hydroxytryptamine by mast cells, that is a component which requires energy from cellular metabolism to function. On the other hand, it is known from earlier work that these cells contain heparin to which 5-hydroxytryptamine is bound (Green & Day, 1963). Since uptake of any substance by active transport implies that the substance is at a higher chemical potential inside the cell than outside, the results do not show conclusively that 5-hydroxytryptamine is taken up by active transport. The problem is rather similar to that of the mechanism of uptake of 5-hydroxytryptamine by platelets (Born & Gillson, 1959; Stacey, 1961).

A second process contributed to the uptake of 5-hydroxytryptamine at high concentrations (1 to 3 μg/ml.) or when the fast component was abolished, for example by lowering the temperature. This process resembled that for the uptake of histamine, which had the following characteristics: (1) The initial rate of uptake was proportional to the concentration of the amine up to 2 μg/ml. (2) When the temperature was lowered to 25° C the uptake was reduced to a small extent. (3) A high *p*H caused only a small reduction in uptake. Thus *p*H 8.6 reduced uptake of histamine and of 5-hydroxytryptamine (2 μg/ml.) by only 28 and 23% respectively. (4) The uptake of histamine was not affected by cocaine, chlorpromazine (at least at low concentrations), or imipramine; in contrast, histamine uptake was reduced by phenoxybenzamine which inhibited uptake of 5-hydroxytryptamine only at high concentrations.

The uptake of tryptamine and of noradrenaline resembled that of histamine. These results support the conclusion that histamine, tryptamine and noradrenaline are taken up by diffusion only, but that diffusion also plays a part in the uptake of 5-hydroxytryptamine. The concentration ratios for histamine were 11 to 20 and the ratios for tryptamine and noradrenaline were similar. It is known that histamine, like 5-hydroxytryptamine, is bound to heparin in the cells (Green & Day, 1963). Since the sulphomucopolysaccharides of mast cells have a high affinity for these amines (Green, Roberts & Day, 1963), it is possible that the affinity extends to tryptamine and noradrenaline which can be bound in the cells, although they do not occur there naturally (Day & Green, 1962a).

Competition between amines for uptake

The inhibition of the uptake of histamine by a high concentration of 5-hydroxytryptamine, but not *vice versa,* may indicate that there are some sites in the cells which bind both 5-hydroxytryptamine and histamine, whilst others bind only 5-hydroxytryptamine. However, it might also mean that both amines are bound at the same sites but that 5-hydroxytryptamine is taken up in "preference" to histamine, a possibility which is supported by the fact that, in equimolar concentrations, 5-hydroxytryptamine inhibited the uptake of histamine. It should be pointed out that rats and mice are the only species in which mast cells contain 5-hydroxytryptamine as well as histamine. Heparin obtained from mast cells of rats and mice has an unusual component which is not found in bovine heparin; it has been suggested that it is this unusual heparin which binds 5-hydroxytryptamine and explains its storage in these mast cells (Green & Day, 1963).

Tryptamine at high concentrations inhibited uptake both of 5-hydroxytryptamine and of histamine. Possibly tryptamine is bound to all types of storage sites in the cells and, at high concentrations, it takes up most of the available sites. Noradrenaline inhibited only the uptake of histamine, which may indicate that it becomes attached to sites which bind histamine or 5-hydroxytryptamine but not to " specific " sites for 5-hydroxytryptamine. This interpretation is supported by the results with guanethidine and phenoxybenzamine, which inhibited uptake of histamine and of noradrenaline but not that of 5-hydroxytryptamine. If guanethidine and phenoxybenzamine produce their effects by blocking binding sites, then the results show that histamine and noradrenaline are bound at the same sites but that there are separate sites for 5-hydroxytryptamine.

Effects of antagonists

The ability of a substance to antagonize the action of 5-hydroxytryptamine or histamine on smooth muscle was no indication of its effect on the uptake of these amines by mast cells. In general, any antagonist (except dichloroisoprenaline and pronethalol) which inhibited the uptake of 5-hydroxytryptamine did not affect the uptake of histamine or reduced it only slightly, and *vice versa.* There are two ways in which these antagonists might produce their effects: either they affect the penetration of the amine into the cell or they prevent it from being bound once it has entered the cell. If the antagonists prevent binding, the results again point to the existence of separate receptor sites for 5-hydroxytryptamine and histamine, since substances which blocked the uptake of one amine did not usually affect the other. Another possibility, that some cells take up 5-hydroxytryptamine only and others histamine only, is unlikely because the cells are all descendants of a single cell (Green & Day, 1960).

The antagonists which prevented the uptake of 5-hydroxytryptamine but not that of histamine were cocaine, chlorpromazine and imipramine. These drugs block the active uptake of amines by other tissues (Dengler *et al.,* 1961; Stacey, 1961; Iversen, 1963; Pletscher *et al.,* 1963) and they probably have a similar action on mast cells. If the uptake of 5-hydroxytryptamine is both by an active process and by diffusion, it is interesting to work out what the action of cocaine on this uptake should be theoretically and to compare this with the experimental results. When

5-hydroxytryptamine was present in the solution at a concentration of 2 μg/ml., about one-third of its uptake could be attributed to diffusion. Assuming that cocaine did not interfere with diffusion but inhibited the active process completely, then uptake should be reduced by 60 to 70%. In fact, cocaine caused an inhibition of 51%, so that the theoretical and experimental results agree quite well. The effect of cocaine on the uptake of 5-hydroxytryptamine was much smaller at 25° C, as would be expected if most of the uptake at this temperature were by diffusion.

Of the other antagonists used, methysergide, lysergic acid diethylamide, bromolysergic acid diethylamide, phenoxybenzamine and guanethidine all inhibited the uptake of histamine but not that of 5-hydroxytryptamine at low concentrations. In contrast, other workers suggest that these antagonists inhibit the active uptake of amines (Dengler *et al.*, 1961; Hertting *et al.*, 1961; Stacey, 1961; Hertting *et al.*, 1962). Some of the differences in the results may be due to differences in the concentration of the antagonists used. Thus, in our experiments, phenoxybenzamine at low concentrations (10^{-6} g/ml.) caused a *preferential* inhibition of the uptake of histamine and of noradrenaline; the uptake of 5-hydroxytryptamine was also depressed if the concentration of phenoxybenzamine was increased tenfold. The simplest interpretation of our results with these antagonists is that they all reduce diffusion into the cells; the fact that phenoxybenzamine and bromolysergic acid diethylamide reduced the uptake of 5-hydroxytryptamine at high concentrations and at 25° C supports this interpretation. An alternative interpretation is that these antagonists block binding sites in the cells. If this is so, they must block those sites which bind both histamine and 5-hydroxytryptamine but not sites which are specific for 5-hydroxytryptamine. This does not explain why bromolysergic acid diethylamide and phenoxybenzamine are effective in reducing the uptake of high concentrations of 5-hydroxytryptamine at 37 and at 25° C, unless the 5-hydroxytryptamine taken up by diffusion is bound at different sites from the 5-hydroxytryptamine which is taken up actively.

Dichloroisoprenaline and pronethalol probably block all binding sites to some extent, since they depressed the uptake of 5-hydroxytryptamine, of histamine and of noradrenaline.

It has been suggested that antihistamines compete with histamine not only at its site of action, the histamine receptor, but also at its storage site in cells (Mota & Dias da Silva, 1960). If antihistamines also compete for storage sites of 5-hydroxytryptamine in neoplastic mast cells, this may explain the observation that antihistamines inhibited the uptake both of histamine and of 5-hydroxytryptamine competitively. These results are not easily interpreted as effects on uptake since it seems improbable that the different processes for uptake of 5-hydroxytryptamine and of histamine should be affected by the same antagonist. It has been shown that some antihistamines are able to release histamine from tissues (Arunlakshana, 1953) and from mast cells (Mota & Dias da Silva, 1960); but it is unlikely that release plays any part in our experiments since the concentrations of antihistamines which inhibited uptake were lower than those required for histamine release. Why promethazine was so much more effective in inhibiting the uptake of histamine than that of 5-hydroxytryptamine is difficult to explain unless, again, promethazine has a

greater affinity for sites binding histamine and 5-hydroxytryptamine than for specific 5-hydroxytryptamine sites.

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