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Uptake, disposition and metabolism of histamine in isolated heart preparations

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Summary

1. The uptake and metabolism of histamine by the guinea-pig heart and by the isolated electrically driven guinea-pig left atria was studied with both labelled and unlabelled histamine.

2. Histamine uptake took place against a concentration gradient and, at low concentrations, this was blocked by hypoxia plus glucose deprivation or by a toxic dose of ouabain.

3. The histamine taken up was retained by cardiac tissues and mainly metabolized into methyl-histamine and other metabolites. The uptake and metabolism of histamine was higher in atria than in ventricles thus reflecting the regional distribution of endogenous histamine and mast cell numbers.

4. The uptake and metabolism of histamine were greatly reduced in hearts which had been depleted of mast cells, by combining cardiac anaphylaxis and (+)-tubocurarine (1 mg/ml), but pretreatment of guinea-pigs with 6-hydroxydopamine failed to affect uptake of total radioactivity in the isolated left atria.

5. In the guinea-pig heart histamine appears to be taken up, stored and metabolized in at least two pools, one of which is linked to the mast cells.

Introduction

The origin of histamine in the mammalian heart, where it is available for release in such conditions as cardiac anaphylaxis (Giotti, Guidotti, Mannaioni & Zilletti, 1966), has not been conclusively demonstrated; either histamine is formed *in situ* or the heart takes up histamine from the blood. How much of the histamine formed *in situ* contributes to the steady state levels of histamine in the heart is not clear: formation of histamine has been unequivocally shown only in the rat heart, although to a very negligible extent (Schayer, 1966; Maudsley, Radwan & West, 1967). On the other hand, all organs which have been examined, including the heart, take up histamine (Mannaioni, 1972). The first evidence of cardiac histamine uptake was given by Halpern, Newen & Wilson (1959) showing that after intravenous injection into rats the largest concentrations of the amine were found in the heart, lung, liver and kidney, mostly reflecting tissue blood flow. Further insight into the uptake of histamine by hearts of different animal species was afforded by *in vivo* studies on the fate of labelled histamine after intravenous infusion into the mouse and rat (Snyder, Axelrod & Bauer, 1964; Reilly & Schayer, 1970; 1971), cat (Johnson, Beaven, Erjavec & Brodie, 1966) and dog (Ryan & Brody, 1970). In this respect the guinea-pig seems to be unique since no histamine was found in the tissues 4 h after administration of the labelled amine (Schayer, 1952). Moreover,

a known amount of histamine instilled into the guinea-pig isolated heart was quantitatively recovered in the perfusate (Zilletti, Guidotti & Mannaioni, 1965) suggesting that in this species the heart is incapable of taking up and metabolizing histamine.

In an attempt to obtain more detailed information on the uptake and metabolism of histamine in the heart of this animal species, in which cardiac anaphylaxis is particularly marked (Feigen, Vaughan Williams, Peterson & Nielsen, 1960; Giotti *et al.*, 1966), accumulation and metabolism of both unlabelled and [^{14}C]-histamine has been measured in isolated heart preparations.

Methods

The experimental preparations consisted of the electrically driven (0·5 ms duration; twice threshold voltage; 120 beats/min) isolated left atrium of the guinea-pig and the isolated perfused guinea-pig heart. In the majority of the experiments the left atrium was bisected longitudinally from tip to base (Cervoni, Kirpekar & Schwab, 1966), so that one half could serve as a control, thus avoiding the variability of cardiac histamine levels (Giotti *et al.*, 1966). Each half was placed under a tension of 1 g in separate 8 ml muscle chambers and perfused with Tyrode solution at 30° C, through which a mixture of oxygen (97%) and carbon dioxide (3%) was bubbled. The composition of the perfusion fluid (mM) was: NaCl 136·9; KCl 2·7; $CaCl_2$ 1·8 (titrated); $MgCl_2$ 1·0; NaH_2PO_4 0·4; $NaHCO_3$ 11·9; glucose 5·6. The pH of the solution was 7.45.

The isolated heart was perfused with Tyrode solution at 38° C in a modified Langendorff apparatus, at a constant pressure of 40 cm water. Oxygen was bubbled through the perfusion fluid; the pH of the perfusate leaving the heart was 7·5–7·6. The apex of the ventricle was connected with an isometric Grass Transducer and contractions were recorded with a Battaglia-Rangoni polygraph.

Histamine incubation

Isolated atria

The uptake and metabolism of histamine was studied by means of two different procedures. In a first group of experiments, the atria were incubated with different histamine concentrations for varying periods of time. At the end of the experiments, control and treated halves were removed and pressed firmly between filter paper to remove as much fluid as possible; the atria were then weighed and assayed. The extracellular space of guinea-pig left atrium was evaluated by Goodford & Lüllmann (1961) as 26·9 ml/100 g wet weight; the total water content was evaluated by us according to the method of Brodie, Axelrod, Soberman & Levy (1949). The appropriate corrections were therefore made in the calculations. In the experiments in which low histamine concentrations were used, the preparations were incubated with the labelled amine (2-ring ^{14}C, Radiochemical Centre, Amersham, England; specific activity 54 mCi/mmol). In the experiments with histamine concentrations higher than 1 μg/ml, the preparations were incubated with unlabelled histamine.

In a second group of experiments, the atria were incubated with different histamine concentrations for constant periods of time. Unlike the former experiments, control and treated halves were now thoroughly washed and then weighed and assayed.

According to the concentrations of histamine in the medium, the preparations were perfused with labelled or unlabelled histamine.

When studying the relation of energy metabolism to the uptake process, both halves of the atrium were exposed to glucose-free Tyrode aerated with 97% nitrogen and 3% CO_2 or to toxic doses of ouabain. One half was then incubated with different amounts of unlabelled histamine while the other half was not. Both atrial halves were washed as described above and analyzed for histamine content. Further details of the incubation procedure are reported under the appropriate sections in the **Results**.

Isolated hearts

Perfusion of the isolated heart with radioisotopic histamine (100 ng/ml) was continued for 30 min ; at the end of this period, the preparation was washed for 10 min with isotope-free solution.

In the experiments designed to destroy myocardial mast cells, the hearts of sensitized animals were challenged with the antigen as described by Giotti *et al.* (1966) and then perfused with (+)-tubocurarine (1 mg/ml) for 30 minutes. The preparation was then washed for 20 min and perfused with radioactive histamine as described above.

Extraction and analysis

Routinely, unlabelled histamine was extracted and estimated biologically on the guinea-pig ileum as described by Giotti *et al.* (1966). In a few experiments the samples were assayed using a 2+2 design as described by Schild (1942) to test whether the slopes of the log dose-effect curves of the standard and the unknown were parallel.

When analyzing the uptake and metabolism of [^{14}C]-histamine, in all the experiments [^{14}C]-methyl-histamine was assayed by the method of Snyder *et al.* (1964). Suitable aliquots of perchloric acid extracts of heart tissues were assayed for total ^{14}C. Other aliquots were made alkaline and methyl-histamine was extracted in chloroform. After washing the chloroform layer, methyl-histamine was transferred to acid and counted.

The [^{14}C]-histamine assay was carried out according to the isotope dilution (BSH) method of Schayer (1968).

The difference between the total radioactivity and that referring to [^{14}C]-histamine and [^{14}C]-methyl-histamine accounted for the sum of other metabolites. All the samples were corrected for recovery and quenching using an internal reference standard.

Drugs used

6-Hydroxydopamine (Axel Kistner AB, Göteborg) was given by three intravenous injections (100 mg/kg) with a 16 h interval. The animals were killed 16 h after the last injection. Other drugs used were: histamine dihydrochloride (Calbiochem) ; ouabain (Sandoz) ; (+)-tubocurarine hydrochloride (Wellcome) ; pure ovalbumin (Erba) ; atropine sulphate (Merck) ; methysergide bimaleate (Sandoz) diphen-

hydramine hydrochloride (Parke-Davis); nicotine bitartrate (Merck); tyramine hydrochloride (Merck). All concentrations (w/v) are expressed as salts. The values of histamine are expressed as the base.

Results

Uptake of unlabelled histamine by guinea-pig left atrium

Different parts of the guinea-pig heart contained different amounts of histamine: the histamine levels found in the left atrium during these experiments are consistent with those reported by Giotti *et al.* (1966) in the same species. More adequate controls were carried out by bisecting the left atrium longitudinally and determining the histamine content of each half. The mean histamine content of seven preparations was not significantly different from the corresponding halves (mean values $12 \pm 2{\cdot}5$ and $14 \pm 2{\cdot}4$ μg/g half atrium) so that one half of the atrium could serve as a control for the other.

The uptake of histamine by the atria was measured after incubation times ranging from 5 to 20 minutes. The histamine concentration-ratio between tissues and medium was found to be higher than unity even after a 5 min incubation (Table 1). The values increased progressively and reached a steady state between 10 and 20 minutes.

The mean histamine content of atria incubated with histamine, 10, 50, 100 μg/ml, significantly increased over the corresponding controls (Table 2). The net uptake

TABLE 1. *The uptake of histamine by guinea-pig isolated left atrium*

Incubation time (min)	Histamine, μg/ml intracellular water: Control half atrium	Half atrium* incubated with histamine	Histamine** net uptake	Concentration† -ratio
5	$14{\cdot}4 \pm 2{\cdot}6$ (5)	$17{\cdot}7 \pm 2{\cdot}6$ (5)	3·3	1·7
7·5	$12{\cdot}2 \pm 2$ (4)	$25{\cdot}0 \pm 2{\cdot}3$ (4)	12·8	2·5
10	$21{\cdot}4 \pm 5$ (6)	$33{\cdot}2 \pm 7$ (6)	11·8	3·3
20	$23{\cdot}0 \pm 2{\cdot}3$ (7)	$34{\cdot}6 \pm 4{\cdot}3$ (7)	11·6	3·4

* Experimental half atrium was incubated with histamine, 10 μg/ml for the given times, and then blotted, weighed and extracted. ** Incubated minus non-incubated with histamine. † Obtained by dividing cardiac histamine levels after incubation by the external histamine concentration. No. of experiments in parentheses.

TABLE 2. *Histamine uptake and retention by guinea-pig left atrium: effect of inhibiting oxidative metabolism and glycolysis and of high doses of ouabain*

Histamine concentration in the medium* μg/ml	Treatment	Control half atrium	Half atrium incubated with histamine** μg/g wet wt.	Histamine net uptake†	*P* values
10 (6)	—	$15{\cdot}4 \pm 2{\cdot}0$	$25{\cdot}0 \pm 3{\cdot}0$	9·6	0·05
50 (6)	—	$11{\cdot}8 \pm 1{\cdot}7$	$21{\cdot}8 \pm 4{\cdot}8$	10	0·05
100 (6)	—	$14{\cdot}1 \pm 3{\cdot}0$	$47{\cdot}5 \pm 7{\cdot}5$	27·4	0·05
10 (5)	Hypoxia†	$12{\cdot}3 \pm 1{\cdot}2$	$14{\cdot}4 \pm 2{\cdot}7$	2·1	N.S.
50 (5)	+glucose	$10{\cdot}2 \pm 2{\cdot}0$	$14{\cdot}3 \pm 2{\cdot}5$	4·1	N.S.
100 (5)	deprivation	$18{\cdot}6 \pm 5{\cdot}3$	$41{\cdot}6 \pm 4{\cdot}3$	23	0·05
10 (5)	Ouabain†	$12{\cdot}3 \pm 1{\cdot}0$	$15{\cdot}0 \pm 2{\cdot}5$	2·3	N.S.
100 (5)	10^{-6} g/ml	$17{\cdot}6 \pm 2{\cdot}3$	$50{\cdot}0 \pm 5{\cdot}5$	33	0·01

* Number of experiments in parentheses. ** Experimental half atrium was incubated for 1 h with histamine, then washed 4 times with 500 ml Tyrode solution for 30 minutes. Control half was treated similarly but not incubated with histamine. † Incubated minus non-incubated with histamine. † Both halves were kept under N_2 or ouabain for a 30 min preincubation period. One half was then incubated with histamine.

of histamine did not increase linearly with the concentrations in the medium, the figures obtained in tissues incubated with 10 and 50 μg/ml being equal. However, an unexpectedly large uptake of histamine was observed at 100 μg/ml.

The combination of anoxia plus glucose deprivation completely impaired the ability of the preparations to take up and retain exogenous histamine (Table 2) when histamine concentrations in the medium were 10 and 50 μg/ml. At an external amine concentration of 100 μg/ml, this treatment did not significantly change the net uptake of histamine from the control values. Similar results were obtained with ouabain (Table 2).

Both the combination of anoxia plus glucose deprivation and ouabain failed to reduce the atrial endogenous histamine levels (Table 2).

Anoxia plus glucose deprivation reduced contractile amplitude by about 80% in 10 min; the contraction was partially restored after 30 min washing. In the experiments with ouabain, contraction amplitude was completely blocked in about 20 min and no contractions were restored, even after repeated washing. It is of interest to point out that the uptake and retention of exogenous histamine is independent of contraction amplitude since it takes place in the same manner in quiescent, non-electrically driven, preparations.

Uptake, metabolism and compartmentation of labelled histamine in isolated heart preparations

The uptake of histamine at extracellular concentrations below 10 μg/ml was measured after perfusion with [^{14}C]-histamine, since the sensitivity of the isotope technique made it feasible to study the uptake process occurring at lower histamine concentrations in the medium.

In the isolated atrium, the concentration-ratio ranged from 1·25 after 5 min incubation to 3·6 after 20 min incubation with [^{14}C]-histamine, 100 ng/ml (Table 3). However, only 3–7% of the radioactivity present in the atrium was unchanged [^{14}C]-histamine, the higher proportion being due to methyl-histamine and acid metabolites.

TABLE 3. *Uptake and metabolism of [^{14}C]-histamine by guinea-pig isolated left atrium*

	[^{14}C]-histamine		[^{14}C]-methyl-histamine		Total ^{14}C	^{14}C (tissue) / ^{14}C (perfusate)
Time of incubation* (min)	(d/min)/mm^3 intracellular water	% Total ^{14}C	(d/min)/mm^3 intracellular water	% Total ^{14}C	(d/min)/mm^3 intracellular water	
5	<2	—	63±13	54	115±24	1·25
10	6·1±1·9	3·1	97±12	49	196±30	2·02
20	24·4±9	7	112±18	32	347±33	3·6

* Atria were incubated with [^{14}C]-histamine, 100 ng/ml for the given times and then blotted, weighed and extracted.

TABLE 4. *Uptake, retention and metabolism of [^{14}C]-histamine by guinea-pig isolated left atrium*

No. of experiments*	(d/min)/mg wet weight			% of total ^{14}C	
	[^{14}C]-histamine	[^{14}C]-methyl-histamine	Total ^{14}C	[^{14}C]-histamine	[^{14}C]-methyl-histamine
7	10·5±1·3	65±5	132·4±5·8	8	49

* Atria were incubated with [^{14}C]-histamine (100 ng/ml) for 30 minutes. Incubation was followed by a wash-out period of 10 minutes.

That a small but definite amount of [^{14}C]-histamine is taken up and retained by guinea-pig atria (although the bulk of histamine taken up is catabolized) is confirmed in Table 4, in which the uptake and metabolism of histamine are studied after a 10 min wash-out period. Under these conditions, the total radioactivity was accounted for by [^{14}C]-histamine (8%), [^{14}C] methyl-histamine (49%) and the combination of other metabolites (46·5%).

In the isolated perfused heart, histamine uptake and metabolism were found to be unevenly distributed. The right atrium had the highest amounts of both [^{14}C]-histamine and its metabolic products ; the left ventricle the least (Table 6).

Whether exogenous histamine mixes with the pools of endogenous histamine in the heart was analyzed in the following experiments. The participation of adrenergic terminals in the histamine uptake process can be ruled out since pretreatment with 6-hydroxydopamine fails to change significantly the uptake of total radioactivity by the isolated atrium (Table 5). The deterioration of the adrenergic nerve terminals was checked by the absence of the excitatory response to tyramine (300 μg) and to nicotine (100 μg), and by the disappearance of the adrenergic nerves as seen in the fluorescence microscope (Catini & Balboni, unpublished observations).

TABLE 5. *Effect of 6-hydroxydopamine on* ^{14}C *content of the guinea-pig isolated left atrium incubated with* [^{14}C]*-histamine* (100 *ng/ml*)*

	(d/min)/mg wet weight	
Controls	Treated†	*P*
(12)	(6)	value
203±17	250±32	N.S.

* Atria were incubated with [^{14}C]-histamine for 30 minutes. Incubation was followed by wash-out period of 10 minutes. † Animals were given 3 injections of 100 mg/kg 6-hydroxydopamine i.v. over a period of 48 h and killed 16 h after the last injection. No. of experiments in parentheses.

The capacity for affecting the uptake and metabolism of histamine is greatly reduced in hearts which have been subjected to cardiac anaphylaxis and (+)-tubocurarine. Histamine uptake was significantly less affected than metabolism. Both processes were impaired in a fashion which strongly recalls the distribution of endogenous mast cells (i.e., the highest inhibition in the right atrium and the lowest in the left ventricle, Table 6).

TABLE 6. ^{14}*C Content of guinea-pig isolated heart incubated with* [^{14}*C]-histamine* (100 *ng/ml*)† *after histamine release by antigen* (*ATG*) *and* (+)*-tubocurarine* (*TC mg/ml*)†

	(d/min)/mg wet weight					
	[^{14}C]-Histamine		[^{14}C]-methyl-histamine		Total ^{14}C	
	Control	ATG+TC	Control	ATG+TC	Control	ATG+TC
	(7)	(4)	(7)	(4)	(7)	(4)
Right atrium	29±4	17±3*	49±8	20±2*	199±13	74±6*
Left atrium	20±3	12±1*	58±8	20±2*	150±12	60±4*
Right ventricle	15±2	9±0·5*	34±3	12±1*	107±8	48±6*
Left ventricle	14±3	10±2	27±6	9·7±1*	85±13	42±5*

† Hearts were incubated with [^{14}C]-histamine for 30 minutes. Incubation was followed by a wash-out period of 10 minutes. † Anaphylactic reaction was elicited by injection through aortic cannula of 0·1 ml of a 1% solution of ovalbumin. No. of experiments in parentheses. * Denotes significance of difference from controls.

Discussion

Guinea-pig cardiac tissues, when incubated with various concentrations of histamine (0·1, 10, 50, 100 μg/ml) took up and retained the amine. The uptake led to a concentration of histamine in the left atrium which was always greater than that in the perfusion medium. It is evident that this uptake is not an artifact. The method, which involved thorough washing of the tissues after incubation with histamine, precludes the possibility that histamine is merely adsorbed to the cell surface or taken up and loosely bound to the extracellular water space. The simultaneous inhibition of the oxidative and glycolytic metabolism, as well as ouabain at toxic doses, blocked the uptake process which operates at 10 and 50 μg/ml clearly indicating that metabolic energy is somehow required for the uptake operating at these concentrations. However, these treatments did not modify the uptake process occurring when the isolated atrium was perfused with a solution containing a higher concentration of histamine (100 μg/ml). These data suggest two mechanisms of transport of exogenous histamine. The first process is inhibited by metabolic poisons and by ouabain, and occurs at concentrations in the medium ranging from 0·1 to 50 μg/ml The second process is detected when very high perfusion concentrations are used (>50 μg/ml) and does not require metabolic energy.

Our observations on the uptake of histamine by the guinea-pig heart are in keeping with similar evidence obtained by several investigators working on different animal species (rat: Halpern *et al.*, 1959; Snyder *et al.*, 1964; Johnson, 1969; Johnson, 1970; Reilly & Schayer, 1970; 1971; mouse: Snyder *et al.*, 1964; Reilly & Schayer, 1970; 1971; cat: Johnson *et al.*, 1966; dog: Ryan & Brody, 1970). Only the guinea-pig appears incapable of accumulating histamine in tissues after intravenous injection of the labelled amine (Schayer, 1952; Lewis & Nicholls, 1971). The lack of histamine uptake was confirmed in the experiments reported by Zilletti *et al.* (1965) in which the isolated guinea-pig heart failed to take up histamine, probably because the amine was injected at a very high rate (0·125 (μg/g)/min) and for too brief a period of time (6 minutes).

However, on comparing the uptake of the preformed amine with the uptake followed by metabolism, the present experiments demonstrate that exogenous histamine readily gains access to the intracellular pools in which the bulk of histamine taken up is quickly metabolized by methylation and oxidation. Our results are in agreement with those reported by Cotzias & Dole (1952) and Valette, Cohen & Burkard (1956), who found that the guinea-pig heart was provided both with histaminase and with histamine-*N*-methyltransferase activity.

It is therefore possible to conclude that the uptake of the preformed amine is a minor process in building up the endogenous histamine levels. However, uptake followed by metabolism may represent an important mechanism of inactivation of released amine especially when histamine is released locally in high concentration as in cardiac anaphylaxis. It is also conceivable that the uptake of the unchanged form may become more significant after inhibition of the metabolizing enzymes. Quantitatively, the guinea-pig heart is more like the mouse heart in which both methyl-histamine and acid metabolites are formed, than the rat heart in which only negligible amounts of [^{14}C]-methyl-histamine can be detected (Snyder *et al.*, 1964).

The question arises as to whether exogenous histamine mixes with the pools of endogenous histamine in the heart. Administered histamine can be taken up by cells which normally contain histamine (an example being mast cells, which are known to be unevenly distributed in the guinea-pig heart, Guidotti, Zilletti & Giotti, 1967) or by other structures which do not normally contain the amine. Quantitative data on the occurrence of histamine in autonomic nerves are available from a number of studies beginning with Kwiatowski (Kwiatowski, 1943 ; Von Euler, 1966). However, the participation of adrenergic nerves in the histamine uptake process can be excluded by experiments in which 6-hydroxydopamine fails to change significantly the uptake of this amine by the isolated atrium. Our results are in agreement with those reported by Harvey (1971) showing that cardiac histamine is not contained in adrenergic nerves since 6-hydroxydopamine does not change histamine content in the guinea-pig heart and since the subcellular distribution of histamine and noradrenaline is different.

There is good evidence that, at least in some parts of the heart, the exogenous histamine does mix with the endogenous histamine. The uptake and metabolism of exogenous histamine is higher in atria than in ventricles, thus reflecting both the regional distribution of endogenous histamine and the frequency of mast cells (Giotti *et al.*, 1966). Moreover, the capacity to take up and metabolize the amine is largely prevented in hearts which have been depleted of mast cell histamine by combining (+)-tubocurarine with antigen: the uptake and metabolism are reduced in such a way as to recall the endogenous distribution of mast cells (the highest inhibition in the right atrium, the lowest in the ventricles). It is of interest to point out that in the mast cell-depleted hearts the diminution in total radioactivity is accounted for by a more pronounced decrease in the metabolism rather than in the uptake of the unchanged amine. This might indicate that cardiac mast cells take up and promptly metabolize histamine, while the storage of the taken-up amine principally occurs in other compartments (namely, cardiac muscle cells or other non mast cell tissues).

In conclusion, these findings suggest that histamine is promptly taken up by guinea-pig heart, stored and metabolized in at least two pools, one of which is linked to mast cells, the other being a non mast cell pool to be delineated through further experiments. The hypothesis that histamine is inactivated at the cardiac histamine receptor level through two metabolic pathways and even through the recapture of the released substance, mediated by a cellular uptake process, is of special interest, since these mechanisms could be regarded as important factors in limiting the action of a potent agonist, such as histamine, on the heart (Mannaioni, 1960 ; Flacke, Atanackovic, Gillis & Alper, 1967 ; Dean, 1968 ; Mannaioni, 1972 ; Levi, 1972).

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