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RELATION OF MAST CELL CHANGES TO HYPOTHERMIA IN THE RAT

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Abstract—Hypothermia caused by compound 48/80 in rats appears to result from histamine and 5HT liberated from ruptured mast cells and is antagonized by a specific antihistamine, mepyramine, and by LSD without preventing the rupture of mast cells. However, hypothermia caused by CPZ and related phenothiazine derivatives which also rupture mast cells is based on a different mechanism in that: (i) the hypothermia is not antagonized by mepyramine; (ii) the hypothermia can be dissociated from histamine release by prior treatment with compound 48/80 to deplete tissue histamine and tissue mast cells; (iii) LSD abolishes the hypothermic activity of CPZ but not its mast-cell-disrupting activity. Since peripheral 5HT antagonists (BOL and UML 491) do not reduce CPZ hypothermia, the latter appears to be associated with release of central nervous system 5HT. The observation[3] that cyanide causes a reduction of both CPZ hypothermia and mast cell disruption was not confirmed.

THE mast cells are considered to be an important source of tissue histamine[1] and also 5-hydroxytryptamine (5HT).[2] Both amines, particularly the latter, administered exogenously induce hypothermia in rats, mice, and other animals. However, certain antagonists of histamine and 5HT (e.g. promethazine and chlorpromazine), also cause pronounced hypothermia. The relationship which exists between endogenous amines, mast cells and the induction of hypothermia is by no means clear. Recently Le Blanc[3] showed that chlorpromazine (CPZ) caused disruption of mast cells *in vivo* and postulated an association between the hypothermic effects of CPZ and the liberation of histamine and 5HT from mast cells. Furthermore Junqueira and Beiguelman[4] reported that cyanide prevented the mast cell disruption (*in vitro*) caused by certain histamine liberators, and Le Blanc[5] reported that cyanide reduced both the hypothermic effect and mast cell disruption *in vivo* caused by CPZ. The present paper deals with studies on the hypothermic effects of CPZ and other substances in relation to mast cell disruption, with specific reference to the effect of CPZ in histamine and 5HT depleted animals.

MATERIALS AND METHODS

Adult rats of Wistar Hooded or Canberra Black stock, 150–200 g weight were used.

Drugs and administration

All doses are expressed in terms of the corresponding salt. The following drugs were used: 2-chloro-10 (3′-dimethylamino-*n*-propyl)-phenothiazine hydrochloride (chlorpromazine CPZ); 10-(2-dimethylamino-1-propyl) phenothiazine hydrochloride

(promethazine); *dl*-10-(3-dimethylamino-2-methylpropyl) phenothiazine neutral tartrate (trimeprazine); 10-(3-dimethylaminopropyl)-2-(trifluoromethyl) phenothiazine hydrochloride (triflupromazine); 2-trifluoromethyl-10-(3′ 1″-methyl piperazinyl-4″-propyl) phenothiazine dihydrochloride (trifluoperazine); N-*p*-methoxybenzyl-N′N′-dimethyl-N-α-pyridylethylenediamine maleate (mepyramine maleate); β-dimethylaminoethyl benzhydryl ether hydrochloride (diphenhydramine hydrochloride); D-lysergic acid diethylamide tartrate (LSD); D-2-bromolysergic acid diethylamide bitartrate (BOL); 1-methyl-D-lysergic acid-butanolamide (UML 491, UML); N-ethyl-3-piperidyl*cyclo*pentylphenyl glycolate hydrochloride (JB 329); 5-hydroxytryptamine creatinine sulphate (5HT); histamine acid phosphate; reserpine; 2 : 4 dinitrophenol (DNP); compound 48/80.

All compounds were injected intraperitoneally in either normal saline or buffered solution. The injected volume varied from 0·2–0·8 ml and control animals received an equivalent volume of saline. Where antagonism to drug action was examined, the antagonist was given 5 min prior to the injection of the agonist.

Mast cell preparations

At the end of most experiments the animals were killed by cervical fracture, and a segment of the mesentery of the terminal ileum removed without stretching by the application of two plastic rings to sandwich the sample of mesentery. The rings holding the specimen of mesentery were placed in 70 per cent ethanol and stained with 1 per cent toluidine blue. Following dehydration and clearing the samples were removed from the rings, mounted in DPX and examined microscopically for mast cell morphology. The changes in the mast cells were recorded. No attempt was made to score the morphological changes quantitatively, because as previously reported[6] such values are considered too inaccurate for critical assessment, owing to sampling difficulties. The classification of mast cells proposed by Riley[7] as Types I and II according to their vascular relationships, size and staining characteristics, has been followed in recording the changes observed after various treatments.

Temperature measurements

The rectal temperature of the rats was measured with a thermistor probe or a rectal thermometer inserted to 40 mm depth. Temperature readings were taken before, and at half hourly intervals after the administration of the test compounds. The ambient temperature was kept at 20 ± 1 °C for all experiments. In most cases groups of six rats were used for each drug treatment and the readings for each time interval were averaged and the standard error calculated. The temperature was usually recorded for 2 hr after injection of the test compound, except for reserpine where a longer time interval was used and temperature taken hourly.

Oxygen Consumption

The total oxygen consumption (QO_2) for a group of from three to six rats at a time was measured by means of a closed-circuit constant-pressure respirometer. The oxygen consumption was recorded for a 30-min run in the respirometer before and after a particular treatment. The rats were weighed before the experiment and the QO_2 expressed as the mean ml O_2 consumed/100 g rat weight per min. The reduction of QO_2 caused by treatment is expressed as a percentage reduction of control values.

Depletion of amines

For acute histamine depletion compound 48/80 was given intraperitoneally in a single dose of 200 μg. Subacute histamine depletion was carried out in accordance with the dosage schedule of Riley and West by giving 48/80 twice daily over 4 days in increasing dosage (2 $\times$ 50 μg doses first day, and increasing by 50 μg per dose per day). This schedule has been shown to reduce the histamine content of most tissues to less than 10 per cent[1] and also produces some tissue 5HT reduction.[2]

Reserpine (10 mg/kg) was given to deplete tissue 5HT. Sixteen hours after injection of reserpine rats were severely tranquillized and hypothermic and previous assays of rat tissue 5HT content have shown a reduction to 10 per cent and 30 per cent of control values for spleen and skin, respectively.[8]

Histamine content of rat mesentery

The histamine content of rat mesenteries was determined for control and treated animals. Compounds were injected intraperitoneally in doses given under results and controls were injected with equivalent volumes of normal saline solution. From 16 to 40 hr later the rats were killed by cervical fracture and the mesenteries removed, dried between filter papers and weighed. Histamine extractions and separation from impurities were carried out as described by Code and McIntire.[9] The extracts were neutralised with sodium hydroxide and diluted with Tyrode solution to the required volume. The histamine content was estimated on the atropinized guinea pig ileum suspended in Mg^{2+} free Tyrode and aerated with oxygen, using histamine acid phosphate as the standard. To ensure that the dose–response relationship was the same for both the standard and test substance several dilutions of each were tested after matched responses were obtained. Extracts were retested after inhibition of histamine by mepyramine (5×10^{-8} g/l.) and no contraction resulted.

RESULTS

Effects of compounds on body temperature, mast cells and oxygen consumption

Chlorpromazine and other phenothiazines not only cause hypothermia and reduction in oxygen consumption in animals but also rupture mast cells. Mast cells[10] are rich in histamine and 5HT,[2] and these compounds are liberated when rupture of mast cells is induced. However, in the rat 5HT and to a lesser extent histamine administered in large doses, caused hypothermia, and this finding must be taken into account in deciding the cause of hypothermia induced by phenothiazines and other compounds. In Table 1 and Fig. 1, the effects of histamine and 5HT on rectal temperature, oxygen consumption and mast cells are shown together with corresponding results for CPZ and other phenothiazines (promethazine, trimeprazine, pipamazine, trifluoperazine and triflupromazine) and the antihistamines mepyramine and diphenhydramine.

In the rat histamine and 5HT in large doses caused slight reduction in rectal temperature with comparable reductions in QO_2, but no observable changes in the mast cells. Rowley and Benditt[11] also found no action on mast cells after 5HT or histamine administration. The hypothermic effect was of short duration, being maximal at 30 min (Fig. 1). On the other hand, chlorpromazine in large doses (35 mg/kg) caused severe and prolonged hypothermia, a marked reduction in QO_2 and rupture of mature Type II mast cells, leaving the perivascular Type I cells apparently intact, confirming the results of Högberg and Uvnas[10] who described mast cell rupture by certain phenothiazine derivatives. Other phenothiazines tested (promethazine, trimeprazine,

pipamazine, trifluoperazine and triflupromazine) also caused moderate to severe hypothermia, reductions in $\dot{Q}O_2$ and mast cell rupture similar to CPZ. Two antihistamines which are not phenothiazine derivatives (viz. mepyramine and diphenhydramine) were also tested. Mepyramine in large doses (60 mg/kg) caused a slight hypothermia, but failed to rupture mast cells; in larger (near lethal) doses of

TABLE 1. EFFECT OF HISTAMINE, 5HT AND ANTIHISTAMINIC COMPOUNDS ON BODY TEMPERATURE, MAST CELL RUPTURE* AND OXYGEN CONSUMPTION IN ADULT RATS

Treatment (number of rats)			Rectal temperature (°C ± s.e.) Initial	Rectal temperature (°C ± s.e.) 2 hr after intraperitoneal injection	Mast cell rupture	Percentage reduction in $\dot{Q}O_2$ (%)†
Histamine	66 mg/kg	(6)	37·2 ± 0·3	37·1 ± 0·1	intact	20
5HT	15 mg/kg	(6)	37·5 ± 0·2	35·7 ± 0·3	intact	8
CPZ	35 mg/kg	(6)	37·6 ± 0·1	30·8 ± 0·1	ruptured	43
		(6)	37·6 ± 0·2	30·6 ± 0·4	ruptured	43
		(6)	37·6 ± 0·2	29·9 ± 0·4	ruptured	34
		(6)	38·1 ± 0·2	32·4 ± 0·4	ruptured	—
		(5)	38·7 ± 0·2	33·0 ± 0·2	ruptured	—
		(5)	37·6 ± 0·3	32·7 ± 0·6	ruptured	—
		(6)	37·9 ± 0·1	31·5 ± 0·4	ruptured	—
		(6)	38·4 ± 0·2	31·8 ± 0·4	ruptured	35
		(6)	37·5 ± 0·2	28·9 ± 0·3	ruptured	—
		(6)	38·1 ± 0·1	32·9 ± 0·7	ruptured	48
Promethazine	35 mg/kg	(6)	37·4 ± 0·2	33·6 ± 0·3	ruptured	20
		(6)	37·8 ± 0·2	31·8 ± 0·5	ruptured	33
Trimeprazine	25 mg/kg	(3)	37·5 ± 0·5	32·9 ± 0·1	ruptured	—
	15 mg/kg	(6)	37·8 ± 0·1	35·6 ± 0·3	ruptured	7
Pipamazine	25 mg/kg	(6)	38·5 ± 0·3	33·2 ± 0·4	ruptured	—
Trifluoperazine	8 mg/kg	(6)	37·2 ± 0·2	35·6 ± 0·2	ruptured	33
Triflupromazine	25 mg/kg	(6)	37·7 ± 0·2	35·0 ± 0·3	ruptured	9
Mepyramine	60 mg/kg	(6)	36·7 ± 0·2	37·0 ± 0·2	intact	—
	80 mg/kg	(6)	38·1 ± 0·2	36·3 ± 0·2	intact	—
Diphenhydramine	60 mg/kg	(6)	38·6 ± 0·2	34·4 ± 0·4	scattered ruptured	—

* Rupture of mast cells (Riley Type II) in mesentery determined at 2 hr after injection.
† $\dot{Q}O_2$ for control rats was 0·208 ± 0·022 (s.d.) ml O_2/100 g rat per min.
$\dot{Q}O_2$ measured for 30 min before and after injection of drugs.

80 mg/kg, a moderate hypothermia resulted (Fig. 1), but still no rupture of mast cells was observed. Diphenhydramine caused a moderate lowering of rectal temperature and scattered rupture of mast cells in agreement with *in vitro* findings of Arunlakshana.[12] Therefore it appears that the weak to moderate hypothermic action of histamine and 5HT were not associated with mast cell rupture and apparently not dependant on the latter. On the other hand the profound hypothermic effect of CPZ and other phenothiazines tested, and that of diphenhydramine, were accompanied by rupture of mast cells.

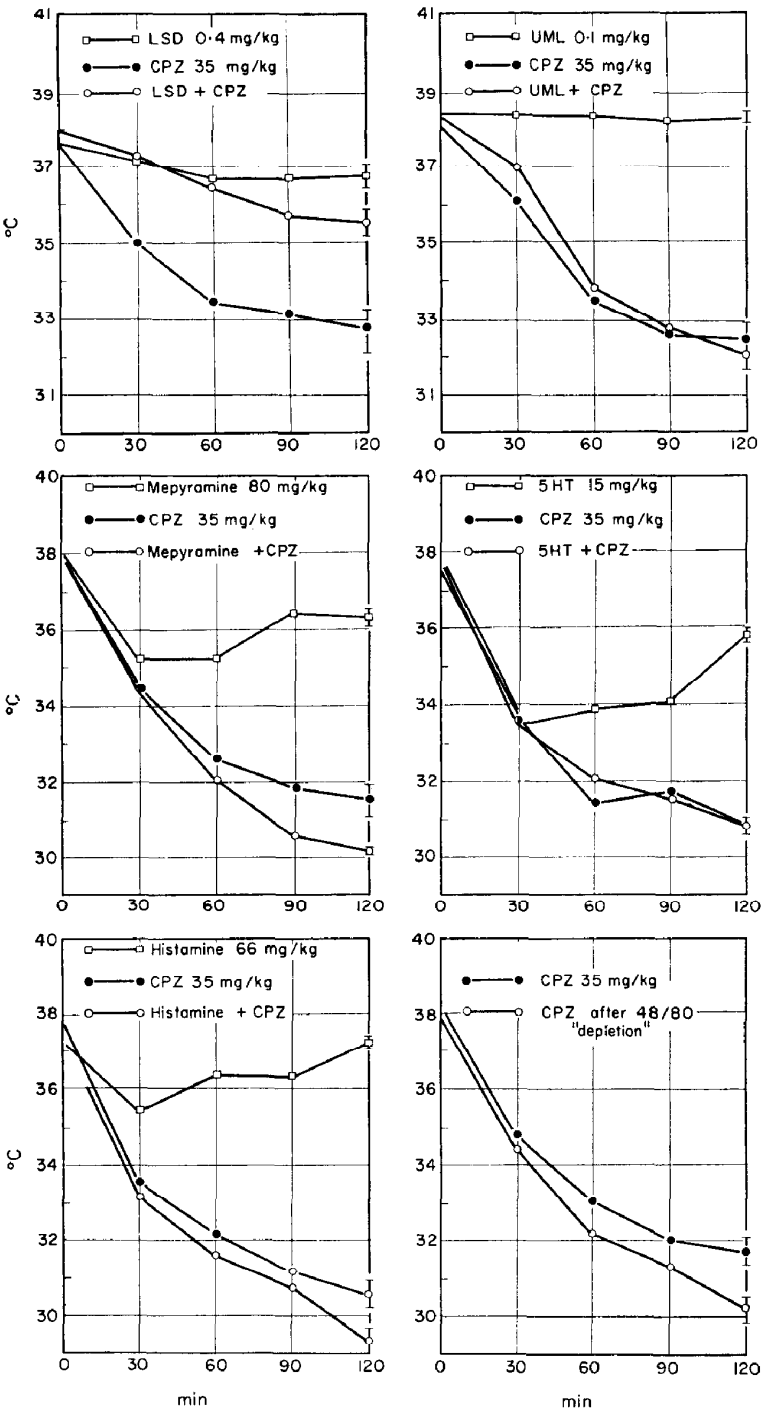

FIG. 1. Effect of CPZ and other compounds on rectal temperature of rats, both singly and in combination. Vertical lines at 120 min denote standard errors.

Since reserpine is particularly associated with the release and depletion of tissue 5HT, and compound 48/80 with corresponding effects on tissue histamine, these compounds were also examined for their effect on rectal temperature, QO_2 and mast cells in rats (Figs. 2 and 3). Reserpine caused a progressive fall in rectal temperature for 18–20 hr after injection (Fig. 2) with a marked reduction in QO_2 but did not cause

TABLE 2. HISTAMINE CONTENT OF RAT MESENTERIES AFTER THE ADMINISTRATION OF SOME PHENOTHIAZINE DERIVATIVES, COMPOUND 48/80, MEPYRAMINE AND RESERPINE

Treatment (number of rats)			Time of extraction after injection (hr)	Mean histamine content in μg/g tissue ± s.e.
Controls (saline)		(7)	24	10·9 ± 1·5
Compound 48/80	1 mg/kg	(6)	24	2·9 ± 0·8
Mepyramine	35 mg/kg	(6)	24	10·4 ± 0·8
CPZ	35 mg/kg	(6)	24	5·7 ± 1·0
Promethazine	35 mg/kg	(6)	24	5·6 ± 0·3
Trimeprazine	25 mg/kg	(6)	24	4·3 ± 0·4
Reserpine	10 mg/kg	(6)	16	8·8 ± 1·0
Reserpine	10 mg/kg	(6)	40	7·4 ± 0·9
Reserpine and CPZ		(6)	16 + 24	6·5 ± 0·7

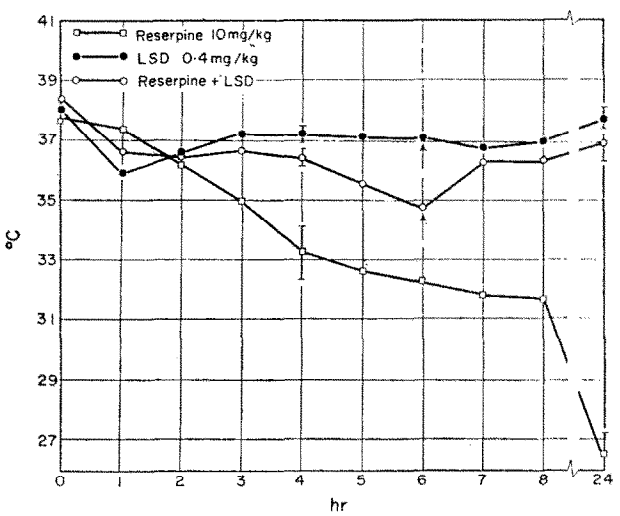

FIG. 2. Rectal temperature of rats treated with reserpine and LSD, singly or in combination. Arrows mark second intraperitoneal injection of a dose of 0·2 mg/kg LSD.

observable rupture of mast cells, although the latter appeared swollen and irregular 16 hr after injection. Compound 48/80 in single doses of 200 μg had a marked hypothermic action (Fig. 3) with a 30 per cent reduction in QO_2, and ruptured all Type II mast cells and most Type I mast cells. At the conclusion of a subacute course of administration of 48/80, the rectal temperature and QO_2 of treated animals did not differ from those of control animals, but only isolated mast cells were found in the mesenteries and subcutaneous tissues (Tables 4 and 5). It follows that a positive association of mast cell rupture and hypothermia holds for compound 48/80 but not for reserpine.

Two hours after the administration of the 5HT antagonists, BOL, UML and LSD there was no significant hypothermia and there were no observable effects on mast cells (Table 3, Fig. 1).

A hallucinogenic agent JB 329, which is an acetylcholine antagonist but has no anti-5HT or antihistamine action,[13] had no action on mast cells and produced only very slight hypothermia (Table 3).

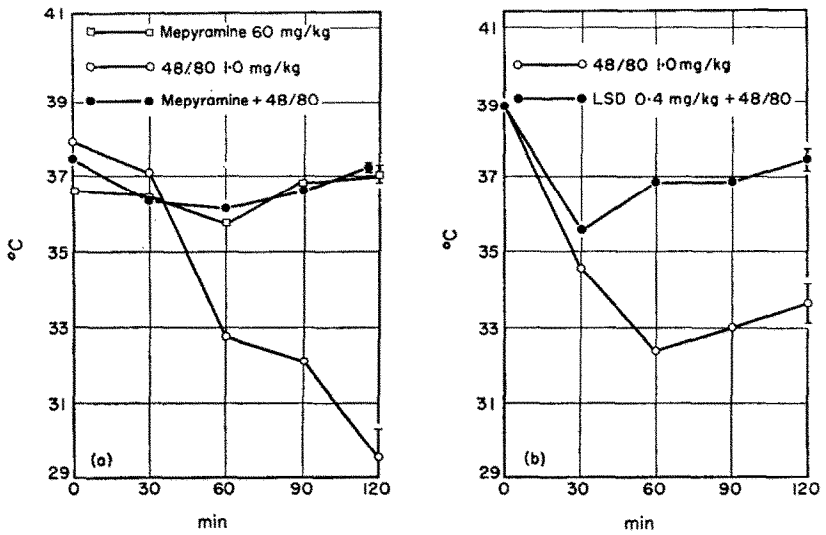

FIG. 3. Effect of (a) mepyramine and (b) LSD on rectal temperature of rats and on the hypothermic action of compound 48/80.

Histamine content of mesentery

Twenty-four hours after the administration of the phenothiazine derivatives, CPZ, promethazine and trimeprazine, assays of rat mesentery showed significant reductions in histamine content to approximately 50 per cent of control values (Table 2). The antihistamine mepyramine did not, however, significantly reduce tissue histamine. Twenty-four hours after administration of a single dose of compound 48/80 (200 μg) there was a great reduction in tissue histamine. Reserpine caused a slight but not significant reduction at 16 hr after injection but at 40 hr the reduction (30 per cent) was significant ($0{\cdot}05 > P > 0{\cdot}01$). It is concluded that whilst hypothermia may be associated with histamine release, drugs which cause the greatest fall in body temperature (and QO_2), e.g. reserpine, CPZ, may be associated with moderate to slight reductions in tissue histamine, whilst agents causing less severe hypothermia (e.g. 48/80) may cause profound reductions in tissue histamine.

Effect of amine antagonists on the actions of CPZ

Since CPZ causes mast cell rupture, release of tissue histamine and also 5HT, the effects of combining the administration of CPZ with antihistamines and anti-5HT drugs were examined, to test further the hypothesis that hypothermia may result secondarily from release either of endogenous histamine or 5HT from mast cells. Conversely the combination of CPZ and either histamine or 5HT was also examined to determine if the effects were additive.

BOL and UML 491 are peripheral 5HT antagonists but are not hallucinogenic. Pretreatment of animals with BOL in a dose which has been reported to prevent the action of endogenous 5HT[14] failed to modify CPZ induced hypothermia and mast cell rupture (Table 3, Fig. 1). UML 491 gave a similar result. LSD, which is a peripheral 5HT antagonist, but also a hallucinogen, markedly reduced the hypothermic action

TABLE 3. EFFECT OF PRETREATMENT* WITH HISTAMINE, 5HT AND AMINE ANTAGONISTS ON THE HYPOTHERMIA, MAST CELL RUPTURE AND QO_2 REDUCTION DUE TO CPZ (35 mg/kg) IN ADULT RATS

Treatment (number of rats)		Rectal temperature (°C ± s.e.) Initial	2 hr after CPZ	Mast cell rupture	% reduction in QO_2
CPZ	(6)	37·6 ± 0·2	30·6 ± 0·4	ruptured	43
Histamine 66 mg/kg	(6)	37·2 ± 0·3	37·1 ± 0·1	intact	20
Histamine and CPZ	(6)	37·7 ± 0·2	29·2 ± 0·4	ruptured	44
CPZ	(6)	37·6 ± 0·1	30·8 ± 0·1	ruptured	43
5HT 15 mg/kg	(6)	37·5 ± 0·2	35·7 ± 0·3	intact	8
5HT and CPZ	(6)	37·5 ± 0·2	30·6 ± 0·5	many intact	41
CPZ	(6)	38·1 ± 0·1	32·9 ± 0·7	ruptured	—
5HT 15 mg/kg	(6)	38·3 ± 0·2	35·1 ± 0·5	intact	—
5HT and CPZ	(6)	38·2 ± 0·2	31·4 ± 0·6	many intact	—
CPZ	(6)	37·9 ± 0·1	31·6 ± 0·4	ruptured	—
Mepyramine 80 mg/kg	(6)	38·1 ± 0·2	36·3 ± 0·2	intact	—
Mepyramine and CPZ	(6)	37·8 ± 0·1	30·0 ± 0·2	ruptured	—
CPZ	(6)	37·6 ± 0·2	29·9 ± 0·4	ruptured	34
BOL 3 mg/kg	(6)	37·4 ± 0·2	36·6 ± 0·3	intact	20
BOL and CPZ	(6)	36·9 ± 0·3	29·6 ± 0·6	ruptured	40
CPZ	(6)	38·1 ± 0·2	32·4 ± 0·4	ruptured	—
UML 491 0·1 mg/kg	(6)	38·4 ± 0·2	38·3 ± 0·1	intact	—
UML 491 and CPZ	(6)	38·3 ± 0·2	32·0 ± 0·5	ruptured	—
CPZ	(6)	37·6 ± 0·3	32·7 ± 0·6	ruptured	—
LSD 0·4 mg/kg	(6)	37·6 ± 0·3	36·7 ± 0·4	intact	—
LSD and CPZ	(6)	38·0 ± 0·3	35·5 ± 0·4	ruptured	—
CPZ	(5)	37·9 ± 0·2	33·1 ± 0·5	ruptured	—
JB 329 10 mg/kg	(5)	37·9 ± 0·2	36·4 ± 0·2	intact	—
JB 329 and CPZ	(5)	37·9 ± 0·2	32·1 ± 0·6	ruptured	—

* Compounds injected 5 min before the injection of CPZ.

of CPZ in rats (Fig. 1), as previously reported for mice,[15] but did not modify the mast cell rupture due to CPZ. Similarly LSD reduced the hypothermic action of reserpine in rats (Fig. 2), a finding also reported by Parkes[15] for mice. Another hallucinogen JB 329, which produced very slight hypothermia in rats had no effect on CPZ hypothermia or mast cell rupture. The antihistamine mepyramine, which had little hypothermic action *per se*, and which has been reported to be a most specific antihistamine with little anti-5HT activity[16] failed to modify CPZ induced hypothermia and mast cell rupture. Neither histamine nor 5HT in large doses, modified the effects of CPZ on body temperature and QO_2, but whilst histamine also failed to reduce the mast cell response to CPZ, 5HT caused a considerable reduction in the degree of mast cell rupture observed 2 hr after CPZ administration.

Effect of mepyramine and LSD on the action of compound 48/80

Since the specific antihistamine mepyramine failed to reduce either CPZ induced hypothermia or mast cell rupture, its effect against compound 48/80 was determined. Whilst mepyramine markedly reduced the hypothermia resulting from 48/80 (Fig. 3a) it did not affect the mast cell rupture caused by this substance. LSD also reduced the

TABLE 4. EFFECT OF AMINE DEPLETION BY SUBACUTE TREATMENT WITH 48/80* ON RECTAL TEMPERATURES, MAST CELL RUPTURE AND OXYGEN CONSUMPTION DUE TO THE PHENOTHIAZINES, CPZ (35 MG/KG), PROMETHAZINE (35 MG/KG) AND TRIMEPRAZINE (15 MG/KG)

Treatment (number of rats)	Rectal Temperature (°C ± s.e.)		Mast cell rupture	% reduction in QO_2
	Initial	2 hr after injection of phenothiazine derivative		
CPZ (6)	38·4 ± 0·2	31·8 ± 0·4	ruptured	35
48/80 (subacute depletion) and CPZ (6)	37·8 ± 0·2	30·2 ± 0·4	most mast cells disappeared	43
Promethazine (6)	37·4 ± 0·2	33·6 ± 0·3	ruptured	45
48/80 (subacute depletion) and Promethazine (6)	37·2 ± 0·1	33·2 ± 0·6	most mast cells disappeared	42
Trimeprazine (6)	38·8 ± 0·1	35·6 ± 0·3	ruptured	7
48/80 (subacute depletion) and Trimeprazine (6)	37·7 ± 0·1	35·2 ± 0·3	most mast cells disappeared	10

* Subacute depletion by 48/80 over 4 days injected twice daily, commencing with 50μg doses on first day and increasing total daily dose by an additional 100 μg per day. Rectal temperatures and oxygen consumptions were unaltered at the end of the depletion period.

hypothermic effect of 48/80, without preventing mast cell disruption (Fig. 3b). It would appear that the hypothermic effect of compound 48/80 is due to the release of both histamine and 5HT.

Effect of amine depletion on the action of CPZ and other compounds

(i) *Compound* 48/80. At the termination of subacute depletion of tissue histamine in rats by 48/80, as described under Methods, the rectal temperature and oxygen consumption were normal, but most mast cells (including Type I cells) had disappeared. CPZ administered to such depleted rats. caused as great a fall in rectal temperature and oxygen consumption as in control animals. Similarly the effect of promethazine and trimeprazine was not reduced by pretreatment with 48/80 (Table 4, Fig. 1).

(ii) *Reserpine.* Sixteen hours after administration of reserpine the rectal temperatures and oxygen consumptions of rats were greatly reduced and treatment with CPZ at this stage did not cause any further reduction in these values. The mast cells in reserpinized rats were swollen but intact and suffered less rupture as a result of administration of CPZ than in control animals (Table 5).

(iii) 48/80 *and reserpine.* In one experiment rats pretreated with 48/80 for 4 days were given reserpine. Sixteen hours later, the falls in rectal temperature and oxygen

TABLE 5. EFFECT OF CPZ (35 MG/KG) ON RECTAL TEMPERATURE, MAST CELLS AND OXYGEN CONSUMPTION IN RATS PRETREATED WITH RESERPINE (10 MG/KG INTRAPERITONEAL INJECTION 16 HR BEFORE CPZ) AND IN RATS PRETREATED WITH BOTH 48/80 (SUBACUTE DEPLETION) AND RESERPINE

Treatment (number of rats)		Rectal temperature C° ± s.e.			Mast cell rupture	% reduction QO_2
		Before reserpine	16 hr after reserpine	2 hr after CPZ (or 18 hr after reserpine)		
Reserpine only	(9)	—	32·0 ± 1·6	(31·0 ± 1·1)	swollen but intact	34*
CPZ only	(6)	—	38·1 ± 0·3	32·9 ± 0·7	ruptured	48
Reserpine and CPZ		—	32·3 ± 1·1	33·4 ± 1·0	many intact	30
CPZ only	(6)		37·5 ± 0·2	28·9 ± 0·3	ruptured	—
Reserpine and CPZ	(6)	37·9 ± 0 1	30·9 ± 1·2	29·9 ± 1·0	many intact	—
Reserpine only	(6)	37·9 ± 0·2	32·9 ± 0·5	(32·7 ± 1·0)	—	27*
48/80 and reserpine	(6)	38·0 ± 0·2	32·4 ± 0·4	(33·4 ± 0·4)	—	35*
48/80, reserpine and CPZ	(6)	38·0 ± 0·2	33·4 ± 0·4	34·4 ± 0·4	—	—

* QO_2 measured 18 hr after reserpine injections.

consumption were similar to those in controls treated with reserpine alone. CPZ given to rats already treated with both 48/80 and reserpine, did not cause a further fall in body temperature. In fact the temperature which was gradually returning to normal at the time of CPZ injection, maintained its upward trend (Table 5).

Effect of cyanide and 2:4-*DNP on the action of CPZ*

In an initial experiment NaCN in doses of 1 mg/kg failed to reduce the hypothermia and mast cell response elicited by CPZ (35 mg/kg) (Table 6). Since this result did

TABLE 6. EFFECT OF METABOLIC INHIBITORS ON THE FALL IN RECTAL TEMPERATURE AND MAST CELL RUPTURE DUE TO CPZ IN ADULT RATS*

Treatment			Rectal temperature (°C ± s.e.)		Mast cell rupture
			Initial	2 hr after injection(s)	
CPZ	35 mg/kg	(1)	38·0	32·5	ruptured
DNP	15 mg/kg		38·7 ± 0·2	39·9 ± 0·4†	intact
DNP and CPZ		(2)	37·5 ± 0·5	38·0 ± 1·0	ruptured
CPZ	35 mg/kg	(5)	38·7 ± 0·2	33·0 ± 0·2	ruptured
NaCN	1 mg/kg	(5)	39·0 ± 0·2	37·7 ± 0·1†	intact
NaCN and CPZ		(5)	38·2 ± 0·1	32·2 ± 0·4	ruptured
CPZ	10 mg/kg	(5)	36·8 ± 0·1	35·5 ± 0·5	ruptured
KCN	0·66 mg/kg	(4)	37·5 ± 0·2	38·0 ± 0·1	intact
KCN and CPZ			37·1 ± 0·2	34·7 ± 0·4	ruptured

* Albino rats were used in this experiment to conform with that of Le Blanc.[5]

† The doses of DNP and NaCN used caused an elevation of 50 per cent and a reduction of 35 per cent in QO_2, respectively.

not agree with the findings of Le Blanc[5] the experiment was repeated using the conditions described by the latter. However, in our experiments KCN (0·66 mg/kg) failed to reduce either the hypothermic effect or mast cell response due to CPZ (10 mg/kg).

2 : 4-Dinitrophenol (DNP) raised rectal temperatures in rats and increased oxygen consumption, but was without observable effect on mast cells. The administration of DNP prior to CPZ prevented the hypothermic action of CPZ but not the mast cell rupture.

Effect of refrigeration on rat mast cells in vivo

The mast cells in anaesthetized rats subjected to refrigeration for from 2 to 3 hr at − 10 °C, were intact, although rectal temperatures had fallen to 18–20 °C.

DISCUSSION

From the results obtained in this series of experiments it appears that the property of rupturing mast cells in the rat, with the resultant release of amines, is not related to the hypothermic action of CPZ and related compounds studied. Antagonists of both histamine and 5HT even in massive doses failed to reduce the hypothermia produced by CPZ in agreement with the results of Le Blanc and Rosenberg.[17] However, the lowering of rectal temperature due to compound 48/80 was reduced by large doses of a specific antihistamine (mepyramine) without altering the mast cell rupture. Thus it appears that only compound 48/80 produces hypothermia mediated through its amine releasing action. This is supported by the finding that a large dose of 48/80 administered to rats previously treated with this substance to cause disappearance of most tissue mast cells, has a much less effect on body temperature. Prior depletion of mast cell amines by compound 48/80 did not modify the hypothermia caused by CPZ, promethazine or trimeprazine—this suggests a dissociation of hypothermia from histamine release for these latter substances.

In these experiments, contrary to the findings of Le Blanc,[5] cyanide was ineffective against the temperature and mast cell effects of CPZ. However, DNP antagonized CPZ induced hypothermia whilst not preventing mast cell rupture. This latter result showing a lack of correlation between amine release and temperature effects is perhaps better shown with LSD, since DNP itself raises rat rectal temperature and thus could inhibit induced hypothermia by direct non-specific antagonism. In rats LSD itself produced a slight short lasting reduction in rectal temperature yet was effective in reducing the hypothermia caused by CPZ. Although LSD was apparently without effect on mast cells, and did not modify the mast cell response to CPZ, its antagonism of hypothermia produced by CPZ suggests that this effect of LSD may be due to: (1) its anti-5HT action against 5HT liberated peripherally; (2) its anti-5HT action against centrally liberated 5HT or (3) "hallucinogenic activity" possibly unrelated to 5HT. Since 5HT antagonists with much less central action, viz. BOL and UML 491, failed to reduce the effects of CPZ on body temperature, QO_2 and mast cells, the first proposition is not supported. On the other hand, the hallucinogen JB 329, which has marked peripheral anticholinergic action[13] but weak antihistamine and anti-5HT activity did not reduce either CPZ hypothermia or mast cell rupture. This makes the second proposition most likely, viz. that the hypothermic effect of CPZ is mediated through the release of central 5HT. Further, after administration of reserpine which depletes catechol amines and 5HT, CPZ exerted no further hypothermic action,

although it is difficult to judge quantitative effects in such reserpinized animals which are already hypothermic. However, it does appear that brain catechol amines and/or 5HT are involved in the hypothermic action although the mechanism is obscure. As Lessin and Parkes[18] suggested there does appear to be an association between c.n.s. depressant action and hypothermia, at least amongst the antihistamines studied here and for reserpine. However, it is more difficult to suggest which action precedes the other, although a logical sequence may be primarily a c.n.s. depressant action, consequent loss of motor activity and decreased heat production resulting in hypothermia.[19] In all cases reductions in temperature were accompanied by similar degrees of reduction in oxygen consumption. The mast cell rupture caused by some antihistamines would then be merely another of the many "side effects" of this group of compounds seen in varying degrees from mepyramine with no action on mast cells to the phenothiazine derivatives which disperse the granules in almost all Type II mast cells.

Certainly hypothermia itself does not alter the mast cells, as seen in rats which were subjected to refrigeration and whose rectal temperatures were as low as 18 °C. There seems to be a relationship between hypothermia, mast cell rupture and histamine release caused by compound 48/80 since injected histamine caused hypothermia, and this hypothermia as well as that due to compound 48/80 was antagonized by the specific antihistamine mepyramine, without preventing mast cell rupture caused by 48/80. This latter finding is at variance with the statements of Riley[20] that mepyramine antagonizes mast cell rupture due to both chemical histamine liberators and anaphylotoxins.

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