Effects of Known and Suspected Neurotransmitter Substances and of Some Nucleotides on Isolated Mast Cells

Mast cells occur widely in the tissues of vertebrates[1] and rarely in the central nervous system[2–4]. Although ultrastructural studies have not revealed synaptic endings on mast cells anywhere[5–9], it has recently been shown that in the skin of the rat the proportion of cells showing signs of degranulation can be increased by antidromic stimulation of sensory nerves[10]. Since this effect is presumably brought about by the release of a neurotransmitter substance, it was decided to test the effects on isolated mast cells of the various substances known or suspected to be neurotransmitters. In view of the results obtained, the effects of a number of other compounds, all nucleotides, were subsequently tested.

Table I. Effects of known and suspected neurotransmitter substances on isolated rat peritoneal mast cells

Compound tested and concentration	Effect on mast cells
Acetylcholine chloride (2.25×10^{-7} — $2.25\times10^{-2}M$)	—
Acetylcholine chloride, as above, in the presence of eserine sulphate ($2.4\times10^{-4}M$)	—
Noradrenaline (as L-noradrenaline HCl) (2.45×10^{-7} — $2.45\times10^{-2}M$)	—
Serotonin (5HT) (as 5-hydroxytryptamine creatinine sulphate complex) (1.3×10^{-7} — $1.3\times10^{-2}M$)	—
Dopamine (3-hydroxytyramine HCl) (2.65×10^{-7} — $2.65\times10^{-3}M$)	—
Histamine dihydrochloride (2.7×10^{-7} — $2.7\times10^{-2}M$)	—
Glycine (6.5×10^{-7} — $6.5\times10^{-2}M$)	—
Monosodium L-glutamate (2.95×10^{-7} — $2.95\times10^{-2}M$)	—
L-glutamine (3.4×10^{-7} — $3.4\times10^{-2}M$)	—
L-aspartic acid (3.75×10^{-7} — $3.75\times10^{-3}M$)	—
γ-aminobutyric acid (GABA) (4.85×10^{-7} — $4.85\times10^{-2}M$)	—
Adenosine-5′-triphosphate disodium salt. $3H_2O$ (ATP) (8.25×10^{-8} — $8.25\times10^{-7}M$)	—
(2.64×10^{-6} — $8.25\times10^{-3}M$)	+

+, degranulation; —, no degranulation

Technique. Mast cells from the peritoneal cavities of young rats were isolated by density gradient centrifugation in Ficoll[11]. The cells from each rat, when isolated and washed, were suspended in 1.0 ml of Hanks's balanced salt solution (BSS). Aliquots of 0.1 ml of this suspension were incubated with equal volumes of solutions in the same BSS of the compounds to be tested[12], for 3 min at 37 °C in different wells of polystyrene haemagglutination trays. All concentrations of test substances cited are those finally achieved in the mixtures. After incubation, the mixtures of test solutions and cell suspensions were smeared into glass slides which were then dried on a hotplate, fixed in Carnoy's fluid and stained with toluidine blue.

Control aliquots of mast cell suspension were incubated with BSS (which had no effect), distilled water (which disrupted the cells osmotically), compound 48/80 (which degranualed mast cells at concentrations of 5×10^{-6} mg/ml upwards) and polymixin B (which degranulated the cells at concentrations of 5×10^{-2} mg/ml upwards). These control incubations indicated that the isolated cells had retained their known pharmacological responsiveness[13].

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[12] All the compounds used were purchased from Sigma Chemicals Ltd, London S.W.6. The reagents were dissolved in BSS and the pH adjusted to 7.4 if necessary. The solutions were stored between preparation and use at —20°C for not more than 1 month.
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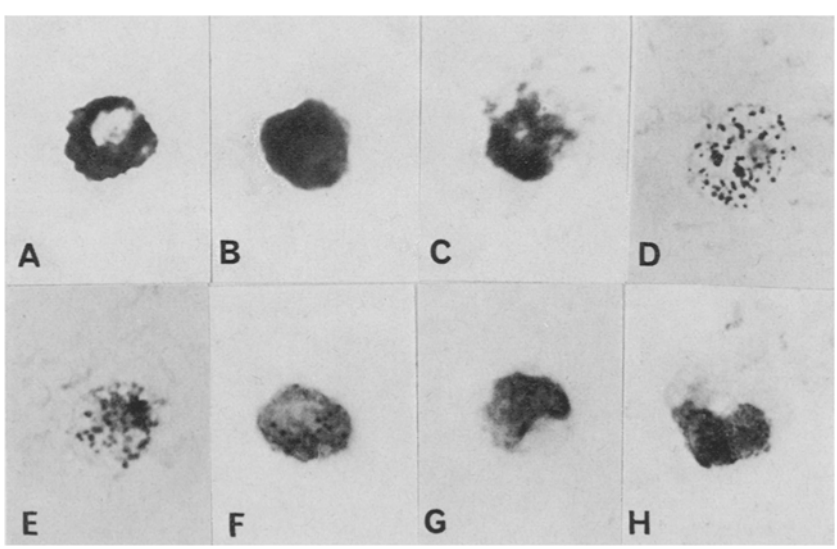

Fig. 1. Photomicrographs of mast cells stained with toluidine blue, to show the effects of treatment with adenosine triphosphate (ATP).
A) ATP, $8.25\times10^{-8}M$ (intact).
B) ATP, $8.25\times10^{-7}M$ (intact).
C) ATP, $8.25\times10^{-6}M$ (degranulated).
D) ATP, $8.25\times10^{-4}M$ (degranulated).
E) ATP, $8.25\times10^{-3}M$ (degranulated).
F) ATP, $1.15\times10^{-7}M$ in presence of 5.0 µg/ml oligomycin (intact).
G) ATP, $6.6\times10^{-7}M$ (intact).
H) ATP, $6.6\times10^{-7}M$ in presence of 5.0 µg/ml oligomycin (degranulated).

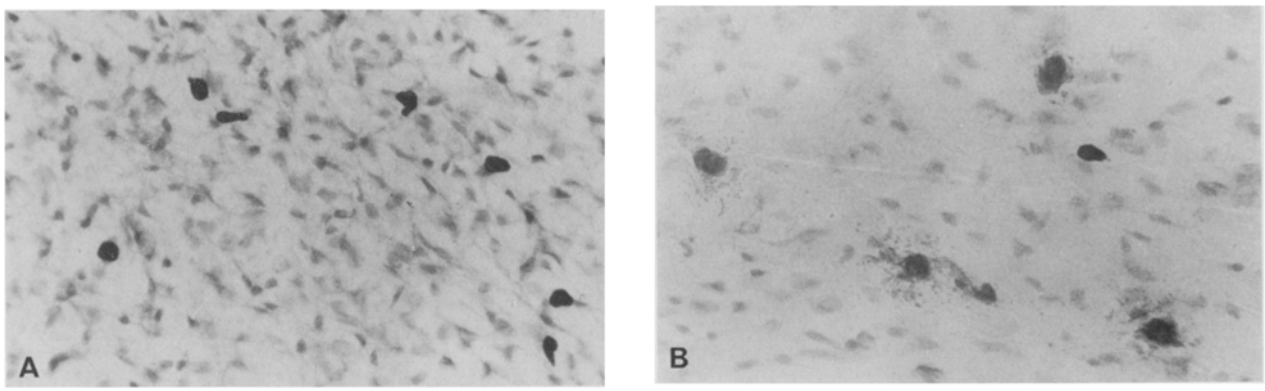

Fig. 2. Photomicrographs of rat mesentery incubated with ATP and subsequently fixed and stained as described in text. A) Incubated with ATP, $2.06 \times 10^{-6} M$. Intact mast cells. B) Incubated with ATP, $4.125 \times 10^{-6} M$. Most of the mast cells are degranulated.

In view of the results obtained with isolated mast cells, one of the tested substances, ATP, was incubated with rat mesentery as described later.

Neurotransmitter substances. The compounds listed in Table I, all of which have been suspected of being neurotransmitter substances[14], were tested in the concentrations stated, for 3 min at 37 °C.

The only substance which caused degranulation of peritoneal mast cells was ATP, in concentrations of $2.64 \times 10^{-6} M$ upwards. It was subsequently shown that ATP was active at $6.6 \times 10^{-7} M$ in the presence of oligomycin (5.0 µg/ml), which inhibits the hydrolysis of ATP by a membrane-associated enzyme[15].

ATP, in concentrations from 2.06×10^{-6} to $1.65 \times 10^{-5} M$, was also incubated for 10 min at 37 °C with pieces of mesentery taken from an adult male rat, as described by RILEY[16]. The mesenteric mast cells, as seen in Carnoy-fixed preparations stained with toluidine blue, were degranulated following incubation in $4.125 \times 10^{-6} M$ and higher concentrations of ATP, but were intact in mesentery incubated with $2.06 \times 10^{-6} M$ ATP or with BSS alone (Figure 2).

Table II. Effects of nucleotides other than ATP on isolated rat peritoneal mast cells

Compound tested and concentration	Effect on mast cells
Adenosine-5′-diphosphate disodium salt. $2.5H_2O$ (ADP)	
$(9.5 \times 10^{-8} - 9.5 \times 10^{-3} M)$	—
Adenosine-5′-monophosphate monosodium salt. $1.5H_2O$ (AMP)	
$(1.25 \times 10^{-7} - 1{,}25 \times 10^{-4} M)$	—
$(1.25 \times 10^{-3} - 1.25 \times 10^{-2} M)$	+
Adenosine-3′-5′-cyclic-monophosphoric acid (cyclic AMP) $(1.5 \times 10^{-7} - 1.5 \times 10^{-2} M)$	—
N^6, O^2-dibutyryladenosine-3:5′-cyclic monophosphoric acid $(1.0 \times 10^{-7} - 1.0 \times 10^{-3} M)$	—
Cytidine-5′-triphosphate sodium salt. $1.5H_2O$ (CTP)	
$(9.0 \times 10^{-8} - 9.0 \times 10^{-4} M)$	—
Guanosine-5′-triphosphate trisodium salt. $1.5H_2O$ (GTP) $(8.0 \times 10^{-8} - 8.0 \times 10^{-4} M)$	—
Thymidine-5′-triphosphate trisodium salt. $2H_2O$ (TTP) $(9.25 \times 10^{-8} - 9.25 \times 10^{-4} M)$	—
Uridine-5′-triphosphate trisodium salt. $4H_2O$ (UTP)	
$(8.0 \times 10^{-8} - 8.0 \times 10^{-4} M)$	—

+, degranulation; —, no degranulation

Nucleotides. In view of the mast cell-degranulating effect of ATP, other adenine nucleotides and other nucleoside triphosphates were tested against isolated mast cells, as shown in Table II. Of these, only the higher concentrations of AMP ($1.25 \times 10^{-3} M$ and above) brought about degranulation of the mast cells. The action of ATP is not, therefore, a property of nucleotides in general.

Photomicrographs of mast cells, indicating the responses to ATP, are shown in Figure 1.

Discussion. Of all the suspected neurotransmitters, only ATP degranulates isolated mast cells, and it does so at the low concentration of $2.64 \times 10^{-6} M$. This is of interest, since there is evidence that ATP is released by sensory nerve endings during antidromic stimulation of cutaneous nerves[17], a situation which is also associated with degranulation of mast cells in the skin[18].

DIAMANT and KRÜGER[19] have reported that ATP ($10^{-5} M$) causes release of histamine from isolated rat peritoneal mast cells, but state that this release is not associated with degranulation of the cells. This observation is at variance with the results of the present investigation. The discrepancy might be explained by the different techniques used, since granules liberated from the cells could escape detection when the cells were centrifuged after treatment with ATP[20]. However, DIAMANT and KRÜGER[21] observed distortion, but not degranulation of isolated mast cells treated with ATP (concentrations up to $4.0 \times 10^{-4} M$) while under observation by phase-contrast microscopy. Treatment with compound 48/80 under similar conditions[22] was followed by lysis of the plasmalemma and release of cytoplasmic granules.

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Résumé. Des suspensions de mastocytes péritoneaux isolées de rat furent incubées avec 10 substances différentes, connues ou presumées être des neurotransmitteurs. Seulement l'adenosine triphosphate (ATP) aux concentrations supérieures à $2.64 \times 10^{-6} M$ causèrent la dégranulation des mastocytes. L'ATP cause également la dégranulation des mastocytes dans le mésentère. L'action de l'ATP peut être responsible de la dégranulation des mastocytes cutanées observée après la stimulation antidromique des nerfs sensorieux, alors que de l'ATP est libéré dans la peau [23].

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Electrocardiographic Changes in Anaphylactic Shock of the Rabbit

Anaphylactic shock due to a challenge with heterologous proteins induces a predominantly histamine independent overall arterial vasoconstriction in intact animals, as well as in isolated organs which also includes the coronary arteries[1-9,11]. The anoxic effects of coronary constriction can be electrocardiographically demonstrated[5,7,8,11]. In man, most concern is directed towards the often dramatic decrease of arterial blood pressure, and clinical treatment mainly consists in an attempt to increase vascular resistance and to enhance cardiac activity by β-receptor stimulating substances such as epinephrine or isoprenaline, or even by the use of vascoconstricting agents such as norepinephrine or angiotensin. It therefore seemed of interest to reproduce the decrease of arterial blood pressure under experimental anaphylactic shock conditions and to establish the nature of its origin.

Materials and methods. Six white rabbits were sensitized twice in an interval of 10 days by 1.0 ml/kg horse plasma subcutaneously. The challenge was performed 3 weeks after the second injection by 2.0 ml/kg of plasma of the same horse given intravenously. The animals were anaesthetized by 22.5 mg/kg of Na-pentobarbital intravenously.

The arterial blood pressure was measured by direct puncture of the carotid artery with an Autocath-Teflon 3.6 F catheter which was connected to an electromanometric blood pressure unit with continuous rinsing of the catheter. Simultaneously with the blood pressure the electrocardiogram was recorded on a Cardiopan III T of F. Liechti AG, Ostermundigen (Switzerland).

Results. The results are summarized in the Table. Blood pressure values and electrocardiographic changes are reported together with the time elapsed after the challenge. The electrocardiographic changes mainly consisted in negativization of ST-T and/or ventricular arrhythmia. They were interpreted as signs of myocardial anoxia. In 3 cases, ventricular arrhythmia occurred at first while in 3 cases deformation of ST-T was first observed. In no case did the blood pressure decline more than 5 mm Hg systolically and/or diastolically at the onset of electrocardiographic changes. On the contrary, in 2 animals (No. 1 and 2) the blood pressure values had increased by 20/10 mm Hg and 30/25 mm Hg, respectively, at the onset of cardiac alterations. It may also be seen that a notable blood pressure decrease of at least 10 mm Hg occurred only after electrocardiographic changes had persisted for at least 20 sec.

Discussion. Our results obtained in intact rabbits in an early phase of anaphylactic shock indicate that heart reactions as evidenced by electrocardiographic changes are initially not due to a decrease of arterial blood pressure and coronary perfusion pressure, respectively. These ex-

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Carotid blood pressure in anaphylactic shock before and after challenge with reference to the time of onset of electrocardiographic changes

Animal No.	Blood pressure before challenge (in narcosis)	Blood pressure and time at the onset of				Blood pressure and time			
		Ventricular arrhythmia		Negativization of ST-T		at the earliest decline		at the lowest level	
	mm Hg	mm Hg	sec	mm Hg	sec	mm Hg	sec	mm Hg	sec
1	100/80	120/90	62	120/90	70	90/70	120	0/0	140
2	110/90	110/80	110	140/115	70	95/75	130	70/50	140
3	135/105	135/110	14	130/115	40	110/90	90	60/40	300
4	165/130	165/135	67	150/120	82	125/105	90	60/40	180
5	120/80	120/85	1080	115/80	65	—	—	(115/80)	(65)
6	130/80	120/70	45	130/75	35	100/50	55	50/25	110