Influence in vitro of High-Energy Adenosine Phosphates and Related Compounds on Anaphylaxis of Isolated Smooth Muscle

On the significance of adenosine-5'-triphosphate (ATP) in the shock state, apparently contrary evidence has been presented. It has been suggested that ATP is one of the chemical mediators in allergy, playing a role in the production of anaphylactic shock[1,2]. Further, evidence has been obtained for the release of ATP into the plasma from the blood cells and tissue under various pathological conditions[3-5]. It is an established fact that ATP and other adenine nucleotides can evoke marked vasodilatation and reduction of blood pressure when administered intravascularly[6,7].

In the clinical field, studies have been carried out on ATP as a means in the prevention or management of shock, and its beneficial effects have been indicated[8,9].

To add a definite finding on one aspect of this issue, we adopted the anaphylaxis system of smooth muscle in vitro, and studied the effect on it of ATP along with other phosphorylated compounds of bioenergetical significance: adenosine-5'-diphosphate (ADP), creatine-phosphate (Cr-P), and D-glucose-6-phosphate (G-6-P).

Materials and methods. Smooth muscle pieces measuring 2 cm in length when relaxed were obtained from the uterine horns of guinea-pigs of Hartley strain: For the present experiment, animals weighing between 300 and 600 g were sacrificed by a blow at the base of the skull. All of the smooth muscle pieces from one uterus were passively sensitized in vitro by immersion in 2.0 ml of anti-bovine serum albumin (BSA) rabbit antiserum for 1 h at 4 °C with pure oxygen bubbled through the serum every 5 min. The antiserum was prepared in the following procedure: 3 ml of 1% BSA was administered by i.v. route to albino rabbits weighing between 2.5 to 3.5 kg. A series of 7 injections every other day was made, and the rising titer of the anti-BSA antibody was measured by Ouchterlony's method[10]. Antisera showing positive precipitation against 1:128 dilution of 1% BSA were pooled and used for the passive sensitization as mentioned above.

As a rule, except for a temperature of 4 °C for the storage of muscle pieces, standard procedure for the anaphylaxis of isolated smooth muscle organ[11] was followed, and the reaction was released with 6.25 mg of BSA added to the 20 ml tissue bath in the presence of 4.65 μM of the test compound which had been added 1 min prior to the antigenic challenge. Iso-osmotical amount of sodium chloride was added to the tissue bath containing the control piece of smooth muscle obtained from the same uterus as the test piece. ATP and Cr-P were prepared by Wako Pure Chemical Industries Ltd., Osaka, Japan; ADP and G-6-P were purchased from Sigma Chemical Co., St. Louis, USA.

The effect of a particular compound on anaphylaxis was discussed on the basis of the magnitude of isotonic muscular contraction: The reaction was expressed in terms of histamine equivalent according to the dose-response curve obtained with histamine dihydrochloride on the same muscle piece. Every muscular response was observed in the complete absence of contraction which might be spontaneous or caused by any factors other than antigenic challenge or histamine dosage.

Results and discussions. The results are summarized in the Table. The present experiment shows that ATP, at a level lower than 1% of that normally found in the blood of humans[12], is capable of marked intensification of anaphylactic reaction in vitro of smooth muscle from virgin guinea-pig uterus. ADP also showed similar effect. Cr-P, however, showed insignificant influence on the anaphylactic reaction, and G-6-P had statistically significant inhibitory action on the anaphylaxis system.

Induction by ATP of histamine release from mast cells of rat has been observed, and it is explained on the basis of energy supply by ATP to the energy dependent system of histamine release[13]. Moreover, the addition of high-energy phosphate bond to adenosine is known to be associated with the potentiation of its pharmacological activity such as the increase of the tonus of smooth muscle[4]. One or the combination of these actions of ATP might explain the intensification of the anaphylactic reaction shown in the present experiment. The inhibitory effect of G-6-P on the anaphylactic reaction shows a contrast to the enhancement by D-glucose of histamine release from mast

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Effects of high-energy phosphates on anaphylaxis in vitro of smooth muscle from guinea-pig uterus

Compounds tested	No. animals	Histamine equivalent[a] of anaphylactic response in the presence of the compound tested	Histamine equivalent of control response	P	Influence (%)
ATP	7[b]	9.31 ± 4.90[d]	2.63 ± 1.36[d]	<0.01	+254
ADP	7[b]	10.2 ± 9.37	4.48 ± 5.92	<0.02	+129
G-6-P	7[c]	1.72 ± 0.78	2.32 ± 1.14	<0.05	—25.9
Cr-P	7[c]	2.01 ± 1.76	2.34 ± 1.26	>0.05	

[a] μg of histamine dihydrochloride added in 20 ml tissue bath. [b] Mean values on 2 pieces, for control and test, respectively, were used as representing the individual animal. [c] One pair of muscle pieces was tested for each animal. [d] Mean ± standard deviation.

cells of rat by ATP[14]. On this point, as well as the mechanisms by which adenosine phosphates influence anaphylaxis as observed, further investigation is being conducted in our laboratory[15].

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Zusammenfassung. Im Schultz-Dale'schen Versuch wird festgestellt, dass Adenosin-Triphosphat und Adenosin-Diphosphat die anaphylaktische Reaktion verstärken. D-Glukose-6-Phosphat wirkt abschwächend, während Kreatin-Phosphat keinen Einfluss auf die Reaktion hat.

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Effect of Adrenaline on the in vitro Enzyme Secretion in the Guinea-Pig Pancreas

It is known in several animal species that injection of adrenaline brings about a marked inhibition of the exocrine secretion of the pancreas[1–4]. The mechanism of this effect is still debated. Hence, the inhibition has been interpreted either as the result of a direct action of the drug on the secretory cells[4] or as the consequence of vasoconstriction in the splancnic area[3].

Two types of exocrine cells are known to be present in the pancreas: the acinoductular cells, which secrete water and electrolytes, and the acinar cells which are responsible for the secretion of digestive (pro)enzymes. Recently HUBEL[5] has provided convincing evidence, obtained by in vitro experiments, indicating that in the rabbit pancreas secretion of water and electrolytes is inhibited by adrenaline by a direct mechanism acting at the cell level. In a system of pancreatic tissue slices we have investigated the in vitro effect of adrenaline on the other type of pancreas exocrine cells, the acinar cells.

In tissue slice systems the rate of in vitro enzyme secretion is usually determined by assaying the activity of one or more secretory enzymes released in the incubation medium. Such a method is rather insensitive because it does not permit discrimination between the enzyme molecules which are actually secreted over the time of the experiment and those which at the beginning of the experiment were already secreted and stored within the duct system. Recently JAMIESON and PALADE[6] have developed a more sensitive radiochemical procedure based on the determination of radioactive proteins released in the medium during a pulse-chase experiment. In our work we have used both the traditional and the new radiochemical procedures and in neither case we were able to demonstrate any effect of adrenaline on in vitro pancreatic enzyme secretion.

Methods. Male albino guinea-pigs (gift of Sigurtà Drug Co., Milan, Italy) weighing ~600 g were starved for 18–20 h.

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Effect of adrenaline on the rate of secretion of pancreas tissue slices incubated in vitro

		A		B	
	Treatment	TCA-insoluble dpm in medium (%)	% of controls	α-amylase (mg maltose/5 min at 30°/mg protein)	% of controls
I	None	4.6 (3.6–5.9)	100	8.8 (7.3–11.0)	100
	Adrenaline 3 μM	5.8 (4.2–7.5)	125	7.2 (6.7–7.7)	72
	Adrenaline 30 μM	4.6 (2.7–6.5)	100	8.5 (7.0–10.2)	98
	Adrenaline 300 μM	4.4 (2.4–6.4)	94	9.0 (6.4–11.6)	102
II	Caerulein	13.1 (9.2–18.2)	285	14.3 (9.8–17.1)	162
	Caer. + Adr. 3 μM	10.8 (10.7–11.0)	237	13.1 (9.0–17.3)	149
	Caer. + Adr. 30 μM	9.4 (8.4–10.5)	210	14.1 (10.0–18.2)	160
	Caer. + Adr. 300 μM	10.8 (9.9–11.7)	237	11.4 (8.5–14.4)	130
III	Carbamoylcholine	11.1 (8.2–16.5)	241	10.8 (8.1–13.6)	123
	Carb. + Adr. 3 μM	11.1 (8.7–14.5)	241	13.7 (9.5–16.5)	155
	Carb. + Adr. 30 μM	13.3 (9.6–18.9)	289	10.6 (8.6–13.7)	121
	Carb. + Adr. 300 μM	12.9 (9.6–18.9)	280	10.6 (8.6–13.7)	121

Slices were first labeled for 5 min with ^{14}C-L-leucine, then chased for 80 min in a non-radioactive medium and finally reincubated for 20 min in the latter containing various concentrations of adrenaline, alone (I) or in the presence of either caerulein (II) or carbamoylcholine (III). In A) the rate of secretion is estimated by the amount of radioactive protein recovered in the medium, expressed as percentage of radioactive proteins present in slices + medium; in B) by the activity of the secretory enzyme α-amylase recovered in the medium, expressed as mg of maltose formed in 5 min of incubation at 30 °C per mg of slice protein. Values given are averages of 2 to 6 experiments. Ranges are in parentheses.