release histamine from mast cells pre-treated with 2–4 dinitrophenol (DNP) in the absence of glucose, places this factor in a group of histamine releasing agents like antigen[5,6], compound 48/80[6,7], chymotrypsin[8,9] and dextran[10], whose action on rat mast cells is also inhibited by DNP in the absence of glucose.

The lack of an in vitro, direct histamine releasing action of rattlesnake venom phospholipase A agrees with reports showing the absence of a mast cell degranulating or histamine releasing effect of the phospholipase A present in bee venom[11–15].

Zusammenfassung. Die chromatographische Auftrennung von Klapperschlangen- (*Crotalus durissus terrificus*) Gift ermöglicht, den Unterschied zwischen Phospholipase A und einer Histamin freisetzenden Komponente dieses Giftes zu bestimmen. Letztere ist ein hitzelabiles, nicht dialysierbares Protein (enzymatisch), leicht von Crotamin unterscheidbar. Phospholipase A ist nicht fähig, Histamin aus isolierten Ratten-Mastzellen freizusetzen. Der hier isolierte Histaminliberator wirkt nicht auf mit 2, 4-Dinitrophenol vorbehandelte Mastzellen.

A. M. ROTHSCHILD

Department of Pharmacology, Faculty of Medicine of Ribeirão, Preto (Brazil), 20th March 1967.

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Effect of Some Substances on the Mitochondrial Swelling Induced by Diphtheria Toxin in Chicken Embryo Heart Cell Cultures

Recently it has been observed that diphtheria toxin induces a remarkable mitochondrial swelling in chicken embryo heart cell cultures and in other primary cell cultures, whereas any swelling effect is detectable in the mitochondria of established cell lines as HeLa cells and RC 37 cells. Crude, highly purified and crystalline diphtheria toxin exhibit the same effect[1,2].

In this paper we have studied the effect of some well-known inhibitors of the mitochondrial swelling, as the blocking agents for the respiratory chain on the swelling induced by diphtheria toxin and the effect of ATP and seroalbumin which are related to mitochondrial contraction.

Materials and methods. Cell cultures. Chicken embryo heart cell cultures were obtained from 6-day-old chick embryos as reported elsewhere[1,2]. The cells were cultured in the ROSE's chambers[3]. The culture medium did not contain antibiotics.

Diphtheria toxin and other reagents. Highly purified diphtheria toxin (Lilly, containing 1120 Lf/ml, Lot. L 00087) and crystalline diphtheria toxin (Wellcome, Lot. RX 7238, containing after dilution with 5 ml of Hanks' BSS, 4.300 Lf/ml) were used. Diphtheria toxin was diluted in Hanks' BSS to the following concentrations: 0.5, 1.0, 5.0, 10 and 20 Lf/ml. The following reagents of the highest purity were employed: Amytal (5-ethyl-5-isoamyl-barbituric acid) (Lilly), KCN (B.D.H.), sodium azide (Merck), Rotenone (B.D.H.), ATP (adenosine triphosphate) (Sigma), seroalbumin (Sigma). All these substances were dissolved, immediately before use, in Hanks' BSS to obtain the final concentrations indicated in the Tables I and II.

Experimental. About 20 h after cell establishment, the nutrient medium was eliminated and substituted with diphtheria toxin-test substance mixture. The ROSE's chambers were then placed under a Leitz Ortholux phase-contrast microscope equipped with a thermoregulated box (37°C) and continuously observed during a period of 30 min. For testing the action of ATP and Seroalbumin the

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Table I. Mitochondrial swelling in diphtheria toxin treated cells (chicken embryo heart cells) in presence of some electron transport blocking agents. +, thickened mitochondria; ++, swollen mitochondria; +++, swollen mitochondria with differences in optical density.

	KCN			Sodium Azide			Amytal			Rotenone		
Diphtheria toxin	1 μM	10 μM	1 mM	1 μM	10 μM	1 mM	1 μM	10 μM	1 mM	1 μM	10 μM	1 mM
0.5 Lf/ml	—	—	—	—	—	—	—	—	—	++	++	+–
1.0 Lf/ml	—	—	—	—	—	—	—	—	—	+++	+++	+
5.0 Lf/ml	—	—	—	—	—	—	—	—	—	+++	+++	+++
10 Lf/ml	—	—	—	++	++	+–	—	—	—	+++	+++	+++
20 Lf/ml	—	—	—	+++	+++	++	++	+	+	+++	+++	+++

cell cultures were inoculated with diphtheria toxin dilutions. After 15 min the diphtheria toxin was eliminated using a syringe with a hypodermic needle and substituted with ATP or seroalbumin dilutions. Some cultures were treated with diphtheria toxin at the same concentrations but without any test substance, or with plain Hanks' BSS as a control.

Results. The effect of test substances is summarized in Table I. KCN prevents the swelling at all concentrations and for all toxin concentrations. Amytal and sodium azide are also very active preventing agents but they are ineffective for higher toxin concentrations. Rotenone gives an inconstant effect with the lower toxin concentration but it is completely ineffective using toxin concentrations higher than 1.0 Lf/ml (see Table I). ATP and seroalbumin promote actively the contraction of diphtheria toxin swollen mitochondria, but with higher toxin concentrations the mitochondria do no return to their filamentous shape and only a reduction in mitochondrial size can be observed (Table II).

Discussion. The findings reported in this paper show that the mitochondrial swelling induced by diphtheria toxin is an electron transport-dependent swelling and that this swelling is reversible, within certain limits, by ATP and seroalbumin. Anaerobiosis induced by KCN, Amytal, sodium azide and by other electron transport blocking agents inhibits the swelling induced by several substances in isolated liver mitochondria and the active electron transfer can be considered as a requisite for the swelling[4].

These findings support also the assumption that the toxin induced swelling is an active phenomenon. This concept is supported also by the action of ATP and seroalbumin which are related to mitochondrial contraction and promote the reversal of swelling induced by several substances in isolated mitochondria[5-7]. It is interesting to observe that ATP and seroalbumin are active on the diphtheria toxin induced swelling for toxin concentrations ranging between 0.5 Lf/ml and 5.0 Lf/ml. It is conceivable that, with higher toxin concentrations, an irreversible damage of the mitochondrial membranes occurs[8].

Riassunto. E stato studiato l'effetto di alcune sostanze sul rigonfiamento mitocondriale indotto dalla tossina difterica. Il KCN, l'Amytal e l'azide sodica prevengono il rigonfiamento. ATP e sieroalbumina ripristinano, entro certi limiti, la forma filamentosa in mitocondri rigonfiati dalla tossina difterica.

F. PARADISI

Clinica delle Malattie Infettive, Università di Napoli, Ospedale Gesù e Maria, Napoli (Italy), 13th February 1967.

Table II. Reversal of swelling induced by ATP and seroalbumin in the mitochondria of diphteria toxin-treated cells (chicken embryo heart cells cultured in vitro). —, ineffective; + and ++, partial reversal of swelling; +++, complete reversal of swelling

Diphtheria toxin	ATP			Seroalbumin		
	10 μM	1 mM	10 mM	0.5 mg/ml	1.0 mg/ml	2.0 mg/ml
0.5 Lf/ml	—	+++	+++	—	++	+++
1.0 Lf/ml	—	+++	+++	—	++	+++
5.0 Lf/ml	—	++	+++	—	+	++
10 Lf/ml	—	—	++	—	—	+
20 Lf/ml	—	—	++	–	–	+

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Electronmicroscopic Localization of 5-hydroxydopamine (3,4,5-trihydroxy-phenyl-ethylamine), a New 'False' Sympathetic Transmitter

In previous studies we have shown that pretreatment of cats with 5-hydroxydopa leads to a marked norepinephrine depletion and to an accumulation of 5-hydroxydopamine (5-HDA) in sympathetically innervated organs[1]. Concomitantly, the contractile response of the nictitating membrane and of the isolated perfused spleen to sympathetic nerve stimulation is greatly diminished, and 5-HDA is liberated as a transmitter into the splenic perfusion fluid.

It seemed to be of interest whether the replacement of the physiological transmitter norepinephrine (NE) by 5-HDA and its possible metabolites is accompanied by ultramorphological changes. Precipitation of 5-HDA with glutaraldehyde and the instaneous reduction of osmium tetroxide in the test tube seemed to provide favourable prerequisites for the electronmicroscopic localization of this new sympathetic transmitter substances.

Cats were given 4 × 20 mg/kg 5-HDA i.p. over a period of 48 h. Four h after the last dose, small pieces of iris, vas deferens, heart and spleen were removed, fixed in 3% glutaraldehyde (buffered at pH 7.4 with 0.1 M phosphate buffer), overfixed in 2% osmium tetroxide and embedded in epon for electronmicroscopic studies. The residual parts

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