Histamine Release and Mast Cell Degranulation Induced by 2-4 Dinitrophenol in Rat Tissues

Although the dependence on energy metabolism of the histamine releasing process has been demonstrated[1-4], the biochemical nature of this event, as well as of the related extrusion of granules from mast cells remains unknown. The present note reports on an unexpected histamine releasing and mast cell degranulating effect of 2–4 dinitrophenol (DNP), which could yield further insight into the problem of mast cell amine release.

Fragments of rat mesentery or diaphragm kept at low temperature from the moment of their removal from the animal, were incubated with DNP at 37 °C, in 0.01 M phosphate buffer, pH 7.4 containing (in meq/l): Na, 143; K, 5.6; Ca, 3.0; Mg, 2.8; Cl, 154. After incubation, the tissues were removed and prepared for microscopic examination of mast cells or bioassay of released histamine as previously described[2]. Oxygen consumption was measured in a Warburg apparatus[2].

Mast cell alterations induced by DNP were of a localized character quite similar to those observed in rat mesentery treated with catecholamines[5]. Figure 1 shows the effect of concentration, time, temperature of preincubation and glucose on the percentage of rat mesentery mast cells showing evidence of degranulation after contact with DNP. $3 \times 10^{-5} M$ DNP was ineffective after 20 min incubation; a ten-fold higher concentration brought about morphological changes in a statistically significant number of mast cells within 1 min ($p \leq 0.05$, Student's t-test on paired samples). This effect was not significantly increased in tissue incubated for 20 min with DNP. Preincubation of the tissue for 20 min at 37 °C prevented the action of DNP; this inhibition was not observed in mesentery kept at 0 °C for the same time. 0.1% glucose, added together with the phenol, inhibited mast cell degranulation.

Figure 2 shows that $3 \times 10^{-4} M$ DNP caused a statistically significant release of histamine from the isolated diaphragm of the rat. This effect was also inhibited in tissue preincubated for 20 min at 37 °C or treated with 0.1% glucose.

The characteristic stimulation of oxygen consumption in tissue exposed to DNP, is a reflection of the uncoupling of mitochondrial oxydative phosphorylation. The histamine-releasing and mast cell degranulating action of DNP does not seem to be directly associated with the stimulation of cell respiration. As shown in Figure 3, maximal enhancement of the oxygen consumption of the rat isolated diaphragm could be produced by $3 \times 10^{-5} M$ DNP, a concentration which fails to produce mast cell degranulation or histamine release.

It is generally admitted that DNP does not inhibit ATP synthesis by cellular processes like glycolysis, which are not directly linked to the mitochondrial electron transport chain. When added to the isolated rat diaphragm, DNP actually causes intense glycogen depletion[6], suggestive of enhanced glycolysis leading to transiently increased levels of non-mitochondrially originated ATP. Since a histamine releasing action of ATP has been demonstrated[7], it is conceivable that a sudden stimulation of glycolytic synthesis of ATP could lead to the mast cell degranulation and histamine release

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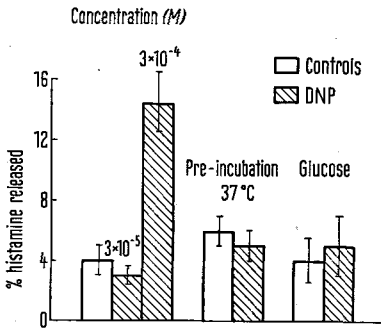

Fig. 2. Effect of 2–4 dinitrophenol (DNP) on histamine release from rat isolated diaphragm. Experimental details were as indicated for Figure 1.

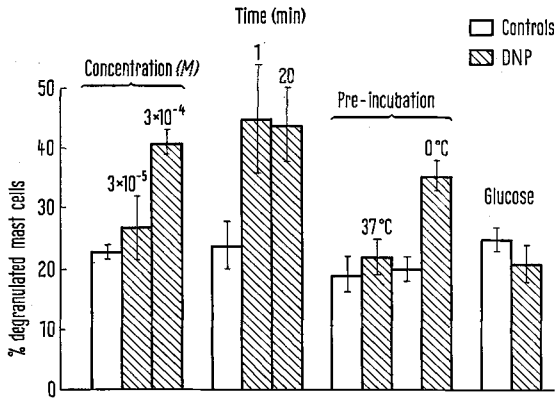

Fig. 1. Effect of 2–4 dinitrophenol (DNP) on rat isolated mesentery mast cell degranulation. Incubations were run for 20 min except where otherwise indicated. Preincubation time, 20 min; glucose, 0.1%. All values are averages of 4–8 experiments.

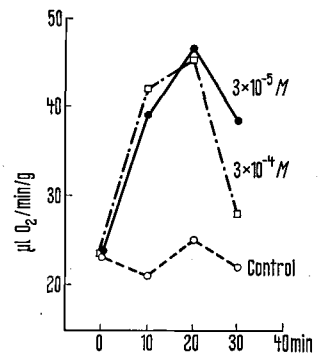

Fig. 3. Effect of 2–4 dinitrophenol on the oxygen consumption of the rat isolated diaphragm. Values are averages of 3 experiments.

observed in DNP-treated rat tissues. These processes were inhibited by glucose. Rat mast cells seem to be capable of considerable aerobic glycolytic activity[8]: by placing a heavy demand on ATP required for its transport into the cell, exogenous glucose could prevent ATP, resulting from DNP-stimulated processes, from reaching effective concentration levels at selected mast cell sites important for granule extrusion and histamine release.

Resumen. El 2-4-dinitrofenol es capaz de producir degranulación rápida aunque parcial de mastocitos y liberación de histamina de los tejidos de la rata in vitro. Estos efectos solamente pueden ser demostrados en tejidos mantenidos a baja temperatura antes del tratamiento; son inhibidos por glucosa. La estimulación del consumo de oxígeno no parece ser la causa de los efectos del dinitrofenol.

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Pyruvate Kinase Activity in Brain, Liver and Brown Adipose Tissue and the Effect of Cortisone in Suckling Rats

Pyruvate kinase (E.C. 2.7.1.40) is one of the rate limiting enzymes of glycolysis and hence it is probable that changes in its activity reflect changes in the formation of acetyl CoA from different precursors. In the rat liver activity decreases at birth and increases again when the high fat milk diet is replaced by the solid high carbohydrate laboratory diet[1,2]. In brown adipose tissue postnatal changes are just the opposite to those found in the liver[1] and it is hence difficult to correlate changes found in this tissue with changes in the composition of the diet. It was therefore of interest to examine development of PK activity in other tissues and to ascertain the effect of cortisone administration, which in general speeds up development of the adult enzyme pattern[3] and also inhibits PK activity in the liver of adult rats.

Rats aged 7 days were injected i.m. with 5 mg/100 g body weight cortisone (SPOFA) for 3 days and were sacrificed on the tenth day of life. Pyruvate kinase activity was determined as described previously[1].

It is apparent from the Table that the activity of this enzyme in both brain and muscle changes in a way similar to that found in liver. Activity falls at birth, is low during the suckling period and increases again after weaning. If a high fat diet is fed from the time of weaning (day 14) activity in the liver remains decreased while it is elevated in brown fat, but that in the brain is not affected. For liver this has been reported[2].

Administration of cortisone has an equivocal effect on brain pyruvate kinase, decreases activity in the liver and increases it in brown adipose tissue (Table). Adrenalectomy on day 14 postnatally has no effect on enzyme activity 4 days later in any of the tissues examined.

These results indicate that either cortisone does not directly affect the metabolic patterns of all the tissues examined or else that pyruvate kinase activity in the different organs is represented by different enzymes (isozymes have been demonstrated[4]). We incline towards the first alternative for the following reasons: Gluconeogenesis is accentuated in the suckling period[5] and after cortisone administration[6]. This is the case in the liver and apparently cortisone given to suckling rats further accentuates this process in this organ. Obviously this results in increased glucose formation which in turn could stimulate glycolysis in brown adipose tissue. It can, of course, be argued that a similar change should occur in the brain. Why this is not so is not clear, particularly since cortisone is known to decrease the rate

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Pyruvate kinase activity in muscle (gastrocnemius), brain (cortex), brown adipose tissue and liver of developing rats (μmoles/mg protein per min ± S.E.)

Organ	Fetus	1 day	10 days		30 days	
			0	cortisone	ND[a]	HF[b]
Muscle	15 ± 0.5	9 ± 0.5	15 ± 0.8	15 ± 0.7	30 ± 1.5	
Brain	12 ± 1.1	4 ± 0.3	4.5 ± 0.4	4 ± 0.7	11 ± 0.8	10.5 ± 1.1
Brown fat			11.5 ± 0.6	16.4 ± 0.7	3.4 ± 0.03	5.7 ± 0.4
Liver			3.6 ± 0.2	2.3 ± 0.1	4.2 ± 0.12	3.0 ± 0.1

[a] ND, normal pellet diet. [b] HF, high fat diet (60% margarine, 30% casein, no carbohydrate) fed from day 14.