Melittin stimulates liver glycogenolysis and the release of prostaglandin D₂ and thromboxane B₂

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Melittin stimulates glycogenolysis and induces vasoconstriction in perfused rat liver. The effect was rapid and associated with production and release of prostaglandin D₂ and thromboxane B₂. Indomethacin blocked the release of these eicosanoids and the stimulation of glycogenolysis induced by melittin. Ibuprofen blocked the release of prostaglandin D₂ induced by melittin and markedly attenuated that of thromboxane B₂. Interestingly, the initial burst of glucose output induced by melittin was not inhibited by ibuprofen, although the duration of the glycogenolytic action of the peptide was greatly diminished.

INTRODUCTION

It has been observed that some agents (including platelet-activating factor [1–5], phorbol myristate acetate [6–9], endotoxin [10] and Zymosan [11]) stimulate glycogenolysis in perfused liver, but not in isolated parenchymal liver cells. Vasoconstriction is associated with the glycogenolytic effect of these agents, and both responses are usually inhibited by indomethacin and bromophenacyl bromide [2,4,5,7,9], which are inhibitors of cyclo-oxygenase and phospholipase A₂ respectively. Release of cyclo-oxygenase products by non-parenchymal cells and their subsequent action on parenchymal and contractile cells seem to explain, at least partially, the observed effects [2–5,7,9–11]. This new concept of modulation of liver metabolism by non-parenchymal cells has gained considerable interest in recent years (see [12,13] for reviews).

We studied the effect of two active components of insect venoms, melittin (bee venom) and mastoparan (wasp venom), on the release of glucose, prostaglandin D₂ (PGD₂) and thromboxane B₂ (TxB₂) by perfused liver. These peptides are known activators of phospholipase A₂ in several models [14,15]. It was observed that melittin stimulated glycogenolysis and the production of PGD₂ and TxB₂; mastoparan has no effect.

MATERIALS AND METHODS

Melittin, mastoparan, indomethacin, ibuprofen, (–)-adrenaline, glucose oxidase and peroxidase were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The kits used for the determinations of PGD₂ and TxB₂ were obtained from Amersham International (Amersham, Bucks., U.K.). BSA (fraction V) was obtained from Armour (Kankakee, IL, U.S.A.).

Experiments were performed with female Wistar rats (approx. 200 g) fed *ad libitum* with Purina rat chow. Livers were perfused at constant pressure with a non-recirculating haemoglobin-free perfusion system [2,7]. The perfusion medium was glucose-free Krebs–Ringer bicarbonate buffer saturated with O₂/CO₂ (19:1), pH 7.4, maintained at 37 °C. The CaCl₂ concentration was 1.3 mM. The perfusions were carried out between 8:00 and 11:00 h. Livers were perfused 45 min before addition of the agents to be tested; the last 15 min of this equilibration was in the presence of 0.25% BSA. To test the effects of indomethacin (10 μM) or ibuprofen (50 μM), the liver was perfused with one of

these inhibitors 15 min before the addition of melittin, and it was present throughout the experiment. The effluent perfusate was collected at 1 min intervals. When hepatocytes were used, they were isolated and incubated as previously described [2]. Glucose was assayed by the glucose oxidase procedure [16], and PGD₂ and TxB₂ were determined by radioimmunoassay. Results were corrected for the flow rate per minute and the wet weight of the liver.

RESULTS

Neither melittin (0.1–30 μg/ml) nor mastoparan (1–30 μg/ml) stimulates glycogenolysis in isolated hepatocytes, whereas in the same cells adrenaline (10 μM) or glucagon (100 nM) induced clear stimulations of this metabolic process [basal glycogenolysis 57 ± 5 nmol/mg cell wet wt. (n = 10) in 60 min; stimulations of ~ 2-fold with the hormones, results not shown]. In contrast, melittin (1 μg/ml) stimulated glycogenolysis in perfused liver (Fig. 1). Melittin at concentrations of 1, 3 or 10 μg/ml induced similar stimulations of glycogenolysis, but lower concentrations (0.3–0.01 μg/ml) did not (results not shown). The effect of melittin was rapid and reached its maximum 3–5 min after addition of the peptide to the perfusion medium, then gradually decreased, remaining ~ 50% above basal for at least 20 min. Addition of adrenaline induced a further increase in glycogenolysis (Fig. 1). Mastoparan (1–10 μg/ml) neither stimulated glycogenolysis nor affected the effects of either melittin or adrenaline on this parameter (results not shown).

As expected, melittin (but not mastoparan; results not shown) induced vasoconstriction, as reflected by a 20–25% decrease in the flow rate (Fig. 2); adrenaline further decreased the perfusion flow rate.

The effect of the cyclo-oxygenase inhibitors indomethacin and ibuprofen was next examined. Neither of these agents altered basal glycogenolysis (results not shown). Indomethacin (10 μM) abolished the glycogenolytic effect of melittin, but did not decrease the action of adrenaline on this parameter (Fig. 1). Interestingly, this cyclo-oxygenase inhibitor did not abolish the effect of melittin on the perfusion flow rate (Fig. 2).

Interestingly, ibuprofen (50 μM) did not block the initial burst of glucose output in response to melittin, although it markedly decreased the duration of the peptide action on this parameter

(Fig. 1). Ibuprofen did not decrease the effect of melittin on the perfusion flow rate (Fig. 2).

We next examined the effects of melittin, indomethacin and ibuprofen on the production of PGD_2 and TxB_2 ; the results are presented in Table 1. Basal production of PGD_2 was ~ 10 times that of TxB_2 ; melittin increased the release of PGD_2 5–8-fold and that of TxB_2 10–15-fold. Maximal effects were observed 3–5 min after addition of melittin. Indomethacin ($10 \mu\text{M}$) decreased the basal release of TxB_2 by 60–70 % and that of PGD_2 by ~ 50 %, and completely abolished the effect of melittin on the production of eicosanoids (Table 1). Ibuprofen ($50 \mu\text{M}$) also decreased the basal production of eicosanoids and the effect of melittin (Table 1). A small but significant increase in the production of TxB_2 by melittin was observed at 3 min in the presence of ibuprofen (Table 1). A higher concentration of this cyclo-oxygenase in-

hibitor ($200 \mu\text{M}$) abolished this effect on TxB_2 production, but, interestingly, it did not block the initial burst of glycogenolysis induced by melittin (results not shown).

DISCUSSION

The suggestion that release of cyclo-oxygenase products from non-parenchymal cells in perfused liver may modulate hepatocyte metabolism [2] has led to the discovery of some putative mediators. On the one hand, it has been observed that eicosanoids are almost exclusively produced by Kupffer and endothelial cells [17]; the major eicosanoid is PGD_2 [17], and its production is stimulated by agents that activate glycogenolysis in perfused liver, putatively via intercellular communication inside the liver ([5,9,10], but see also [8]). It has been shown that PGD_2 binds to parenchymal cells [17] and stimulates glycogenolysis [9,10,18,19]. There is also evidence that prostaglandins influence protein phosphorylation in liver cells [20], including the phosphorylation of glycogen synthase [21]. On the other hand, prostaglandin $\text{F}_{2\alpha}$ and thromboxane A_2 analogues increase portal pressure and

Fig. 1. Effects of melittin, adrenaline, indomethacin and ibuprofen on glycogenolysis in perfused liver

Livers from fed rats were perfused as described in the Materials and methods section. Melittin ($1 \mu\text{g}/\text{ml}$) and adrenaline (ADR) ($10 \mu\text{M}$) were added to the perfusion media. Livers were perfused in the absence (●) or the presence of either $10 \mu\text{M}$ -indomethacin (□) or $50 \mu\text{M}$ -ibuprofen (○). Means of 5–6 experiments are plotted. The S.E.M. values were 5–10 % of the means, and were omitted for clarity. Results are presented as percentages of basal glucose output, which was $54 \pm 6 \mu\text{mol}/\text{h}$ per g ($n = 16$).

Fig. 2. Effects of melittin, adrenaline, indomethacin and ibuprofen on the perfusion flow rate

Livers were perfused in the absence (●) or presence of either $10 \mu\text{M}$ -indomethacin (□) or $50 \mu\text{M}$ -ibuprofen (○). Melittin ($1 \mu\text{g}/\text{ml}$) and adrenaline (ADR) ($10 \mu\text{M}$) were added to the perfusion media. Means of five experiments are plotted. The S.E.M. values were 5–10 % of the means, and were omitted for clarity. Results are expressed as percentages of basal flow rate, which was $2.50 \pm 0.01 \text{ ml}/\text{min}$ per g ($n = 15$).

Table 1. Effects of melittin, indomethacin and ibuprofen on the production of TxB_2 and PGD_2 by perfused liver

The numbers of determinations in independent experiments are given in parentheses. Data are means $\pm$ S.E.M.: ^a $P < 0.001$ versus zero time, control; ^b $P < 0.001$ versus melittin (same times); ^c $P < 0.025$ versus zero time, control; ^d $P < 0.05$ versus zero time, ibuprofen; ^e $P < 0.05$ versus zero time, control; ^f $P < 0.001$ versus melittin (same times).

Time after melittin ($1 \mu\text{g}/\text{ml}$) addition (min)	TxB_2 (pg/min per g wet wt.)			PGD_2 (pg/min per g wet wt.)		
	Control	Indomethacin ($10 \mu\text{M}$)	Ibuprofen ($50 \mu\text{M}$)	Control	Indomethacin ($10 \mu\text{M}$)	Ibuprofen ($50 \mu\text{M}$)
0	100 ± 20 (18)	27 ± 2^a (18)	48 ± 1^c (5)	1270 ± 265 (6)	580 ± 130^e (6)	612 ± 17^e (8)
3	1390 ± 280^a (6)	$19 \pm 2^{a,b}$ (6)	$214 \pm 80^{b,d}$ (5)	9405 ± 2560^a (6)	$337 \pm 135^{e,f}$ (6)	$486 \pm 25^{e,f}$ (8)
5	1540 ± 230^a (6)	$23 \pm 5^{a,b}$ (6)	41 ± 3^b (5)	5830 ± 1360^a (6)	$350 \pm 100^{e,f}$ (6)	$513 \pm 29^{e,f}$ (7)
10	1015 ± 245^a (6)	$27 \pm 2^{a,b}$ (6)	74 ± 15^b (5)	6365 ± 1310^a (6)	$415 \pm 155^{e,f}$ (6)	730 ± 154^f (7)

stimulate liver metabolism [22,23]. It has been shown that the glycogenolytic and respiratory effects of thromboxane analogues are mainly due to ischaemia induced by the vasoconstriction [22,23].

Our present results indicate that melittin stimulates glycogenolysis and the production of eicosanoids in perfused liver through an indomethacin-sensitive process. It is known that indomethacin can alter Ca^{2+} transport [24,25]. The fact that ibuprofen did not block the initial stimulation of glycogenolysis induced by melittin suggests that (a) the blockade by indomethacin may involve effects of this agent beyond cyclo-oxygenase inhibition, and (b) the initial effect of the peptide may involve an action via non-cyclo-oxygenase-derived metabolites. However, the data with ibuprofen also clearly indicate that a large part of melittin-induced glycogenolysis is mediated via cyclo-oxygenase products. In addition, the data with indomethacin indicate that the changes in perfusion flow rate (vasoconstriction) are not sufficient in themselves to explain the glycogenolytic effect of the peptide venom.

It was recently reported that ibuprofen caused a consistent but statistically non-significant decrease in platelet-activating-factor-stimulated glucose release [26], which suggested that the glycogenolytic effect of this agent could be mainly secondary to vasoconstriction [2,3].

Large increases in the release of PGD_2 and TxB_2 have been observed with other agents [5,9,10,27]. Our present data add melittin to this list, and suggest that this peptide can be a useful tool to elucidate further the processes involved.

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