Effect of pertussis toxin on hormonal responsiveness of rat hepatocytes

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The ureogenic action of epinephrine in hepatocytes from normal adult rats is mediated through activation of α_1-adrenoceptors. β-Adrenoceptors in addition to α_1-adrenoceptors, became involved in mediating this effect in cells from animals treated with pertussis toxin. The accumulation of cyclic-AMP in response to epinephrine or isoproterenol was markedly increased in hepatocytes from pertussis-treated rats as compared to that observed in control cells. The accumulation of cyclic-AMP due to glucagon was also increased. It is suggested that pertussis toxin may release a constraint on adenylate cyclase activity by blocking the inhibitory coupling mechanism (N_i) or some other entity involved in the regulation of the activity of this enzyme.

Pertussis toxin *α_1-Adrenoceptor* *β_2-Adrenoceptor* *Glucagon* *Ureogenesis* *Adenylate cyclase regulation*

1. INTRODUCTION

Three types of adrenoceptors are present in rat hepatocytes; i.e., α_1-, α_2- and β_2-adrenoceptors [1,2]. These adrenoceptor subtypes are coupled to different mechanisms of signal transduction: β-adrenergic activation results in the stimulation of adenylate cyclase whereas α_2-adrenergic activation results in inhibition [3,4]; the mechanism of signal transduction for α^1-adrenergic amines is not well known, but it seems to involve a calcium signalling system associated to phosphatidylinositol turnover [3,5] and other unknown factors [6].

α_1-Adrenoceptors are the main mediators of the metabolic actions of epinephrine in hepatocytes from normal adult rats [5,7–9]; the physiological significance of α_2-adrenoceptors in rat hepatocytes is unknown and β_2-adrenoceptors seem to play, if any, a minor role. However, there are certain physiological conditions in which β_2-adrenoceptors become involved in mediating the metabolic actions of epinephrine in liver cells. Thus, β_2-adrenoceptors are involved in the actions of epinephrine in hepatocytes from fetal [10], juvenile [11–13], hypothyroid [6,14,15] or adrenalectomized rats [16,17]. β_2-Adrenoceptors also play significant roles in the hepatic actions of catecholamines during cholestasis [18] and in hepatocytes formed during the initial stages of liver regeneration after partial hepatectomy [19].

Pertussis toxin, a protein isolated from the culture medium of *Bordetella pertussis,* markedly decreases receptor-mediated inhibitions of adenylate cyclase and magnifies stimulations of this enzyme in some cells [20–24]. Furthermore, we have observed that the administration of pertussis toxin to rats markedly diminishes in their adipocytes the α_1-adrenergic-mediated stimulation of phosphatidylinositol labeling [25].

This study describes the effect of pertussis toxin on the sensitivity of adult rat hepatocytes to adrenergic amines, glucagon, vasopressin and angiotensin II.

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2. MATERIALS AND METHODS

The sources of materials were as in [6,9,12,19]. Pertussis toxin was isolated from pertussis vaccine concentrates generously provided by the National Institute of Hygiene of México. The toxin was purified about 2000-fold by gel filtration, adsorption and ion-exchange chromatography [21]. Female Wistar rats were injected intraperitoneally with 15 μg toxin/100 g body wt in a single dose, 3 days before the experiment was performed. The effects of the toxin lasted for at least 1 week. Hepatocytes were isolated and incubated as in [9]. Urea, cyclic-AMP and phosphatidylinositol labeling were assayed as in [6,9,19].

3. RESULTS

Epinephrine stimulated ureogenesis in a dose-dependent fashion in hepatocytes from control rats (fig.1). In agreement with our previous report [9], the β-adrenergic agonist isoproterenol was without effect on ureogenesis (fig.1), and the action of epinephrine was blocked by the α_1-adrenergic-selective antagonist, prazosin (fig.2). Interestingly, in hepatocytes from pertussis toxin-treated rats, the dose–response to epinephrine was slightly shifted to the left as compared to the control (fig.1) and isoproterenol produced a clear dose-dependent stimulation of ureogenesis in these cells (fig.2) which was blocked by propranolol (not shown). Consistent with these findings, the stimulation of ureogenesis produced by epinephrine in cells from treated rats was not blocked by either propranolol or prazosin alone, but it was blocked in the presence of both antagonists (fig.2). The results indicate that both α_1- and β-adrenoceptors are involved in the actions of epinephrine in hepatocytes from pertussis toxin-treated rats.

The stimulation of ureogenesis produced through activation of α_1-adrenoceptors in hepatocytes from control and pertussis toxin-treated rats were very similar (epinephrine plus propranolol columns, fig.2). To further evaluate the α_1-adrenergic sensitivity of the cells, the action of epinephrine on phosphatidylinositol labeling was studied. It was observed that this α_1-adrenergic action of epinephrine was not affected by the administration of pertussis toxin (fig.3). The effects of the calcium-dependent hormones, vasopressin and angiotensin

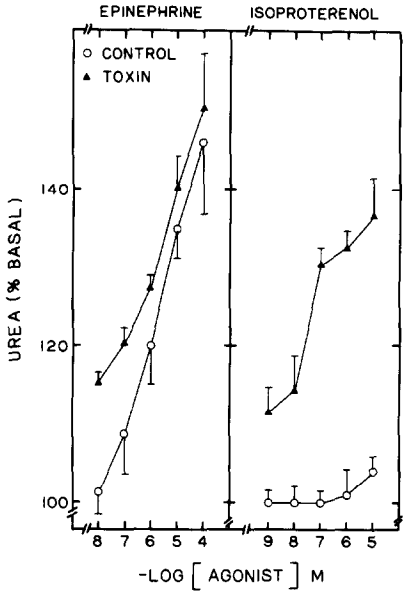

Fig.1. Dose–response curves to epinephrine and isoproterenol. Hepatocytes were incubated in 1 ml Krebs-Ringer–bicarbonate buffer containing 1% albumin, 10 mM glucose, 10 mM glutamine and 2 mM ornithine for 60 min at 37°C. Basal ureogenesis was 26 ± 3 and 24 ± 4 nmol/mg cell wet wt in cells from control and toxin-treated rats, respectively. Results are the means, and vertical bars represent SEM of 6–8 expt performed in duplicate.

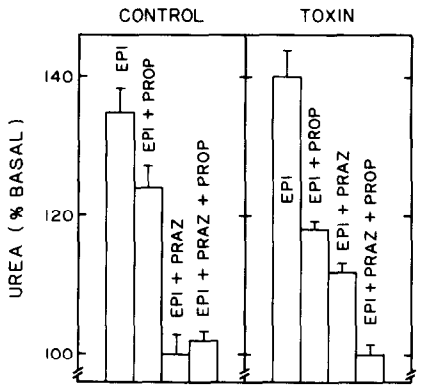

Fig.2. Effect of epinephrine and adrenergic antagonists on ureogenesis: 10^{-5} M epinephrine (EPI); 10^{-5} M prazosin (PRAZ); 10^{-5} M propranolol (PROP). Other indications as in fig.1.

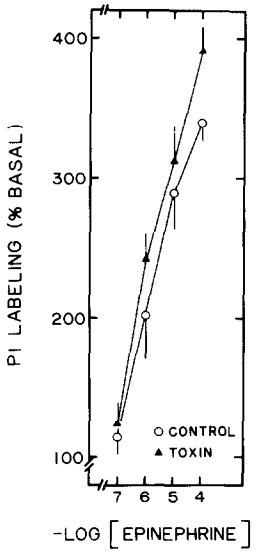

Fig.3. Effect of epinephrine on the labeling of phosphatidylinositol. Cells were incubated in the medium described in fig.1, supplemented with 10 μCi of $^{32}P_i$. Basal incorporation of label into phosphatidylinositol was 2160 ± 330 and 1920 ± 360 cpm/10 mg cell wet wt in cells from control and toxin-treated rats, respectively. Other indications are as in fig.1.

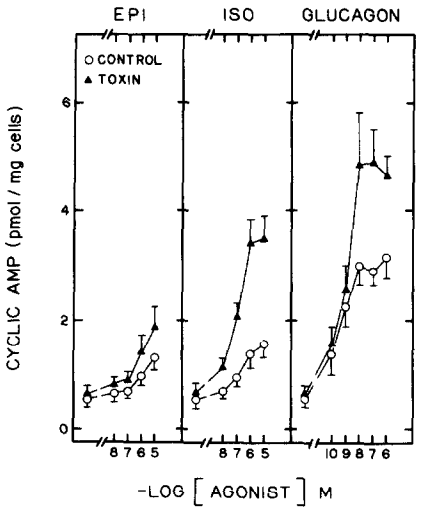

Fig.4. Effect of epinephrine, isoproterenol or glucagon on cyclic-AMP accumulation. Cells were incubated in the buffer described in fig.1, supplemented with 100 μM theophylline. Incubations were stopped 2 min after the addition of the agents. Other indications are as in fig.1.

II, on ureogenesis and phosphatidylinositol labeling [26] were not affected by the administration of pertussis toxin (not shown).

To further evaluate the β-adrenergic sensitivity of hepatocytes from persussis toxin-treated rats, the accumulation of cyclic-AMP in response to epinephrine or isoproterenol was studied. It was observed that the accumulation of cyclic-AMP due to β-adrenergic activation reached a higher maximum in cells from treated animals as compared to the controls; however, the EC_{50} was similar for both conditions. Interestingly, the accumulation of the cyclic nucleotide due to glucagon also reached a higher maximum in cells from pertussis toxin-treated rats as compared to that in the controls (fig.4).

Experiments were performed to determine the α_2-adrenergic sensitivity of hepatocytes from treated animals and compare such sensitivity with that in the control cells. However, no consistent effect on cyclic-AMP levels was produced by α_2-adrenergic activation in cells from either control or treated animals. Similar difficulties in demonstrating α_2-adrenergic effects on whole cells have been reported in [13].

4. DISCUSSION

Administration of pertussis toxin enhances the β-adrenergic responsiveness of rat liver cells, as evidenced by the increased accumulation of cyclic-AMP in response to epinephrine or isoproterenol. It is interesting to note that in cells from control rats both adrenergic agonists consistently produced 50–75% increases in cyclic-AMP levels (propranolol-sensitive). However, such increases in the level of the cyclic nucleotide do not seem to be metabolically significant, at least when ureogenesis is the metabolic parameter measured; i.e., isoproterenol increases cyclic-AMP levels but is not effective in stimulating ureogenesis and the effect of epinephrine on ureogenesis is totally blocked by prazosin, but not its action on cyclic-AMP.

The increased β-adrenergic responsiveness of liver cells from pertussis toxin-treated animals is reflected both in cyclic-AMP accumulation and in ureogenesis. The effect of pertussis toxin, however, does not seem to be exclusive for the actions of β-adrenergic amines; maximal accumulation of

cyclic-AMP due to glucagon is also enhanced in cells from treated animals. The main action of pertussis toxin seems to be to block the action of receptors coupled inhibitorily to adenylate cyclase [20–24]. However, we have observed that the action of agents that stimulate adenylate cyclase through receptor-mediated processes, such as isoproterenol or ACTH in hamster fat cells is enhanced in cells from pertussis toxin-treated animals [21]. Similar enhancements by pertussis toxin of receptor-mediated activations of adenylate cyclase have been observed in other cells [22–24]. Furthermore, we have observed that the accumulation of cyclic-AMP produced by forskolin is also magnified in cells from pertussis-toxin treated hamsters [21]. Our interpretation of the results is that either:

(i) The basal activity of the inhibitory coupling mechanism (N_i) maintains a constraint on adenylate cyclase, and that such constraint is released by the action of the toxin; or

(ii) That pertussis toxin may affect entities involved in the mechanism of activation of the enzyme.

Administration of pertussis toxin to rats markedly diminished the responsiveness of their adipocytes to α_1-adrenergic activation, as reflected by a decreased stimulation of phosphatidylinositol labeling by epinephrine [25]. In liver cells from pertussis toxin-treated rats, α_1-adrenergic responsiveness does not seem to be affected. Authors in [27] observed that the administration of pertussis toxin to rats diminishes the activation of liver phosphorylase by the subcutaneous administration of epinephrine. These authors suggested that the hyperinsulinism caused by pertussis vaccine can account for the attenuated activation of phosphorylase and hyperglycemia produced by epinephrine. Our results indicate that the adrenergic sensitivity of hepatocytes of pertussis toxin-treated rats is not diminished, but actually increased (β-adrenergic component), and support the suggestion that the hyperinsulinism might explain the results obtained in [27] in whole animals.

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