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Adrenaline-induced desensitization of liver adenylate cyclase*

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Recently several authors have observed that the exposure of intact cells to adrenaline or prostaglandin E_1 resulted in desensitization of the adenylate cyclase systems to the respective hormones. This phenomenon has been documented on the level of the adenosine-3′,5′-monophosphate (cAMP) response in intact cells as well as on the level of adenylate cyclase isolated from hormone-treated cells and it may represent a mechanism of physiological or pharmacological tolerance or resistance by which cells protect themselves against prolonged exposure to hormonal stimuli and elevated levels of cAMP. To date, hormone-induced desensitization of adenylate cyclase has been observed mainly in isolated and cultured cell systems [1–6] and it remains to be shown to what extent this effect can be seen also in intact tissues and organs. Accordingly, we have attempted to demonstrate the desensitization of liver adenylate cyclase by adrenaline treatment of liver slices.

Livers were obtained from adult and newborn (3–7 days of age) Wistar rats. The tissue was cut mechanically with a tissue chopper into cubes ("slices") of about 1-mm length. The slices were collected in ice-cold saline and rinsed several times with a buffer containing 110 mM NaCl, 4.9 mM KCl, 1.2 mM $MgSO_4$, 25 mM Na_2HPO_4, 5.5 mM D-glucose and 0.1% purified bovine serum albumin, adjusted to pH 7.4 and aerated with pure oxygen. About 300 mg of slices were incubated in 5 ml of this medium at a temperature of 37° with gentle shaking with and without varying doses of adrenaline (L-ephinephrine bitartrate, Sigma Chemical Co.; stock solutions prepared in 0.9% NaCl). After incubation the flasks were chilled in ice water and the slices were then rinsed twice with ice-cold buffer and briefly centrifuged. Following decantation of the supernatant, about 5 vol of Tris HCl (pH 7.5) containing 1 mM $MgCl_2$ were added and the mixture was homogenized and adenylate cyclase prepared in form of a low speed, washed membrane fraction as previously described [7]. Enzyme activity was measured using ATP-alpha-^{32}P as the substrate and separating the cAMP produced by ion exchange thin-layer chromatography on PEI-cellulose [8]. Assays were carried out in triplicate and contained in a volume of 0.05 ml: 40 mM Tris HCl (pH 8), 5 mM $MgCl_2$, 0.1% bovine serum albumin, 0.1 mg/ml creatine phosphokinase, 10 mM sodium creatine phosphate, 10 mM aminophylline, 0.5 mM cAMP and 0.1 mM ATP-alpha-^{32}P (International Chemical Nuclear Co.; ca. 600,000 cpm). After addition of enzyme protein (about 0.3 mg/ml), incubations were carried out for 10 min at 37°. Basal and hormone-stimulated (0.1 mM adrenaline) activities were measured and hormone sensitivity of the enzyme was expressed as Δ% stimulation.

In several experiments we observed and confirmed that partial desensitization of adenylate cyclase to adrenaline could be achieved by preincubation of liver slices with 10^{-5} M adrenaline provided albumin was included in the buffer. No effect was seen in the absence of albumin. The basis for this requirement is not understood: it may be related to the binding of adrenaline, or of a factor involved in desensitization, to the albumin. The glucagon sensitivity of the enzyme remained unaffected; Table 1 demonstrates a typical result. Although slices from both adult and newborn animals showed this effect, the more pronounced adrenaline sensitivity of adenylate cyclase from livers of newborns [7] caused us to use these for most experiments.

Table 1. Effect of adrenaline treatment of liver slices on hormone sensitivity of adenylate cyclase

Enzyme*	% Stimulation by 10^{-4} M Adrenaline	% Stimulation by 0.02 mg/ml Glucagon
Control	79 ± 13	131 ± 25
Adrenaline-pretreated	23 ± 6†	107 ± 13‡

* Three batches of liver slices from adult rats each were incubated for 1 hr with and without 10^{-5} M adrenaline prior to preparing the enzymes as described in the text. The means ± S.E.M. of enzyme activities obtained from each batch of slices are listed.

† Significantly different from control ($P < 0.05$).

‡ Not significantly different from control.

When "desensitized" slices, treated for 60 min with 10^{-5} M adrenaline, were washed several times to remove the hormone and reincubated for periods up to 90 min, no significant recovery of the adrenaline sensitivity of adenylate cyclase was observed. In the case of adenylate cyclase from Ehrlich ascites cells, recovery of adrenaline sensitive adenylate cyclase took place within 30–60 min of reincubation [6] and in the case of human fibroblast cultures periods of up to 24 hr were required [3]. The present experiment is thus not conclusive since extended time periods were not investigated. It has also been observed that trace amounts of catecholamine prevent recovery [3]; the efficiency of removal of adrenaline from liver slices by washing steps has not been evaluated by us.

The dose dependence of desensitization is shown in Fig. 1. At 10^{-6} M adrenaline desensitization was notable and at 10^{-5}–10^{-4} M it became maximal, representing a 50–75% loss of hormone sensitivity compared to controls. This result was confirmed in further experiments. A study of the time course of adrenaline action revealed that within 30 min an almost maximal effect was obtained (Fig. 2). We have not yet attempted to achieve more extensive or complete desensitization of the cyclase by combining high doses of adrenaline with longer incubation periods. Incubations of more than 90 min led to notable physical deterioration of the slices. We also noticed in some experiments a drop in basal activity or yield and of hormone sensitivity of the enzyme after 60–90 min of incubating slices in the absence of adrenaline; however, these changes were below 10 per cent of the control values at zero time and the effect of adrenaline treatment on the hormone sensitivity of the enzyme was always significant compared to control slice incubations of the same duration.

The sensitivity of the adenylate cyclase to glucagon was not changed in any of the experiments above. Adrenaline thus induces a hormone-specific desensitization as has been established by other authors for cyclases sensitive to both adrenaline and prostaglandin E_1 [1, 2]. The mechanism underlying hormone-induced desensitization of adenylate cyclase is not known.

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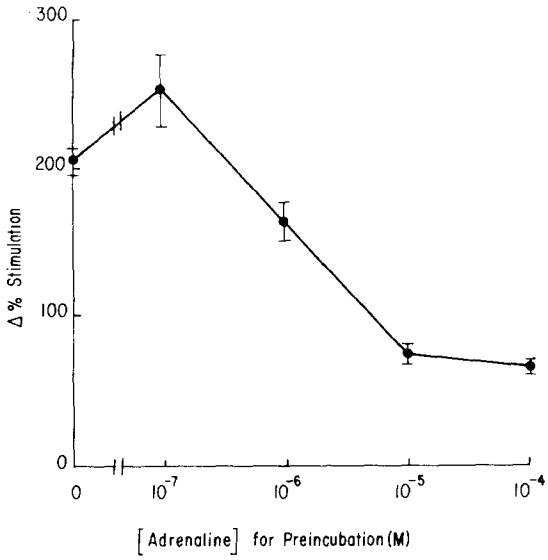

Fig. 1. Dose dependence of desensitization. Slices were preincubated for 1 hr at various adrenaline concentrations prior to preparation of adenylate cyclase. Each point represents the mean ± S.E.M. of three batches of slices (new-born rats).

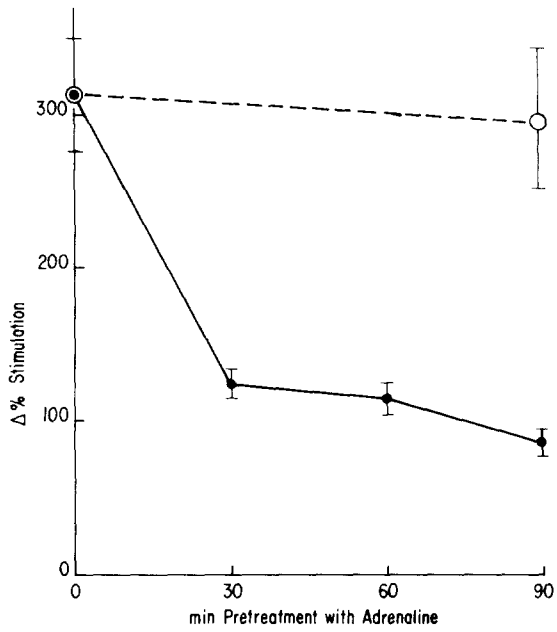

Fig. 2. Time dependence of desensitization. For each point three batches of slices (new-born rats) were incubated with 10^{-5} M adrenaline for various times prior to preparation of adenylate cyclase. One point (○) represents a control incubated without adrenaline for 90 min. Means ± S.E.M.

A loss of the β-adrenergic receptor binding function has been noted in catecholamine-treated frog erythrocytes [5]. It is possible that a cAMP-dependent phosphorylation reaction on the level of adenylate cyclase or the hormone receptor is involved.

The significance of the present desensitization phenomenon remains to be investigated. The chronic treatment of rats with adrenaline was found to lead to a suppression of the adrenaline-induced glycolytic response [9]. It is possible that this pharmacological effect is based on the desensitization of liver adenylate cyclase. It should be noted that the concentrations of adrenaline used in the present study were considerably higher than those found *in vivo*, and this fact should be taken into account when considering a possible physiological relevance of our data.

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Non-specificity of sulphydryl inhibition of the alpha adrenergic response

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Several reactive chemical groups have been suggested as possible binding sites for agonists and antagonists on the alpha adrenergic receptor [1–5]. Prominent amongst these suggestions is that of the involvement of the sulphydryl group [1, 6, 7].

Protein has been suggested as the foundation material for the structure of the alpha adrenergic receptor [8, 9], and irreversible alpha receptor blocking agents have been shown to interact with protein and its constituents [9–11]. In view of the relationship between free sulphydryl groups