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# Homologous β-adrenergic desensitization in isolated rat hepatocytes

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Hepatocytes from hypothyroid rats have a marked β-adrenergic responsiveness. Preincubation of these hepatocytes with isoprenaline induced a time-dependent and concentration-dependent desensitization of the β-adrenergic responsiveness without altering that to glucagon (homologous desensitization). The desensitization was evidenced both in the cyclic AMP accumulation and in the stimulation of ureagenesis induced by the β-adrenergic agonists. Under the same conditions, preincubation with glucagon induced no desensitization. Propranolol was also unable to induce desensitization, but blocked that induced by isoprenaline. Pertussis-toxin treatment did not alter the homologous β-adrenergic desensitization induced by isoprenaline.

## INTRODUCTION

Desensitization is a widespread regulatory phenomenon in which cell exposure to a hormone, neurotransmitter, autacoid, or in general to external stimuli, results in a decreased cell responsiveness. Two major types of desensitization have been described: (1) homologous desensitization, when the cell sensitivity to the desensitizing agent is decreased, whereas that to other agents remains unchanged; and (2) heterologous desensitization, when the stimulation by one agonist results in a more general effect decreasing cell responsiveness to several structurally unrelated agents, working through different receptors.

The β-adrenergic-receptor-linked adenylate cyclase has been one of the most widely studied systems, and both homologous and heterologous types of desensitization have been described [reviewed by Harden (1983) and Sibley & Lefkowitz (1985)].

Rat hepatocytes from normal adult rats scarcely respond to β-adrenergic agonists. However, there are a number of normal and pathological conditions in which β-adrenoceptors play an important role in the actions of catecholamines in hepatocytes; they include fetal-rat liver (Sherline *et al.*, 1974), juvenile rats (Blair *et al.*, 1979), hepatic hyperplasia after surgical or chemical partial hepatectomy (Aggerbeck *et al.*, 1983; Huerta-Bahena *et al.*, 1983), hypothyroidism (Malbon *et al.*, 1978; Corvera *et al.*, 1984), adrenalectomy (Bitensky *et al.*, 1970) and cholestasis (Aggerbeck *et al.*, 1983). Furthermore, acquisition of a β-adrenergic response by adult rat hepatocytes during primary culture has also been observed (Nakamura *et al.*, 1983).

Using primary cultures of rat liver parenchymal cells, Gurr & Ruh (1980) and Noda *et al.* (1984) observed that isoprenaline induces β-adrenergic desensitization. Similarly, Reilly & Blecher (1982) observed that cultured differentiated RL-PR-C hepatocytes are desensitized to isoprenaline after exposure to this β-adrenergic agonist. On the contrary, using perfused rat liver, Morgan *et al.* (1982) observed no β-adrenergic desensitization even after successive β-adrenergic stimulations. We further examined the phenomenon of β-adrenergic desensitization, using fresh hepatocytes from hypothyroid rats. Our results indicate that short-term exposure to isoprenaline leads to an homologous desensitization of the β-adrenoceptor.

## MATERIALS AND METHODS

6-*N*-Propyl-2-thiouracil, methylisobutylxanthine, (−)-isoprenaline, (±)-propranolol, urease, L-glutamine and L-ornithine were obtained from Sigma Chemical Co. Cyclic [$^3$H]AMP (32 Ci/mmol) was obtained from New England Nuclear. Pertussis toxin was purified from vaccine concentrates, generously provided by the National Institute of Hygiene (México), by the method of Sekura *et al.* (1983). Glucagon was generously given by Eli Lilly.

Female Wistar rats (approx. 250 g) fed *ad libitum* were used. Hypothyroidism was induced by giving the rats water containing 0.015% propylthiouracil for 40–50 days; hypothyroidism was assessed by decreased weight gain, dryness of the fur and decreased blood concentrations of tri-iodothyronine (Corvera *et al.*, 1984). Pertussis toxin (25 μg/100 g, intraperitoneally) was administered 3 days before the experiment was performed. Angiotensin II produces a 30–45% decrease in the accumulation of cyclic AMP induced by glucagon in hepatocytes; administration of this dose of toxin completely blocked the ability of angiotensin II to decrease glucagon-induced cyclic AMP accumulation (see Pobiner *et al.*, 1985; Lynch *et al.*, 1986).

Hepatocytes were isolated by the method of Berry & Friend (1969). Isolation, washings and incubation of the cells were done in Krebs–Ringer bicarbonate buffer (120 mM-NaCl, 5 mM-KCl, 1.3 mM-$CaCl_2$, 1.2 mM-$KH_2PO_4$, 1.2 mM-$MgSO_4$, 10 mM-$NaHCO_3$) saturated with $O_2/CO_2$ (19:1), pH 7.4 at 37 °C.

To induce desensitization, the cells (40 mg/ml) were preincubated in the absence or presence of agonists; after

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this preincubation, the cells were washed by centrifugation and resuspension three times with Krebs–Ringer bicarbonate buffer at 37 °C, and the effects of the agents were tested. The washing procedure took 3–4 min. In all the experiments, the cells obtained from a liver were divided into two groups (control and experimental), and they were processed in parallel. To determine the rate of ureagenesis, the cells were incubated in buffer supplemented with 10 mM-glutamine and 2 mM-ornithine for 60 min; urea was quantified enzymically (Gutman & Bergmeyer, 1974) in the cell supernatants. Cyclic AMP accumulation 2 min after the addition of the agents was quantified in cells plus medium by the method of Gilman (1970) as modified by Brown *et al.* (1971). In the cyclic AMP experiments 100 $\mu$M-methylisobutylxanthine was present to inhibit phosphodiesterase activity.

## RESULTS

To examine whether $\beta$-adrenergic activation in fresh hepatocytes leads to desensitization, liver cells were incubated for different times in the absence or presence of 100 $\mu$M-isoprenaline; after this preincubation, the cells were washed three times and incubated for 2 min in the presence of 1 $\mu$M-isoprenaline and 100 $\mu$M-methylisobutylxanthine, the reaction was stopped and cyclic AMP accumulation determined. Fig. 1(*a*) shows that during prolonged incubation there was some decrease (approx. 20–25%) in the accumulation of cyclic AMP induced by isoprenaline in cells preincubated in the absence of any agent; this was at least partially due to a decrease in viability (~ 10%), as evidenced by Trypan Blue exclusion and lactate dehydrogenase release; such decrease in cell viability was identical in the control and desensitized group. However, much faster and more impressive was the diminution observed in the cells preincubated with the $\beta$-adrenergic agonist. The magnitude of the desensitization and the time course of the process can be more clearly observed if the data are normalized as the percentage of the response of cells preincubated without agonist (Fig. 1*b*). Preincubation with the agonist produced a decrease in the response of approx. 50%; it took place rather rapidly, with a $t_{\frac{1}{2}}$ of approx. 3 min, and reached a near-maximal desensitization at 15 min. This time of preincubation was used in all the following studies.

The effect of the concentration of agonist during the preincubation was next studied, and the results are presented in Fig. 2. The desensitization induced by isoprenaline was concentration-dependent, with a maximal desensitization observed in the range of 10–100 $\mu$M-isoprenaline and an $EC_{50}$ (concn. giving 50% of maximum effect) of approx. 50 nM. To obtain the maximal desensitization, 100 $\mu$M-isoprenaline was used during the preincubation in all the following studies.

Further experiments were performed in order to define (A) the type of desensitization, (B) its metabolic repercussion, (C) if cyclic AMP accumulation, i.e. cyclase activation, was required or if the desensitization was due to receptor occupation or activation, and (D) if the guanine-nucleotide-binding regulatory protein ('Ni') was involved in the desensitization process.

The accumulation of cyclic AMP induced by different concentrations of isoprenaline or glucagon in cells

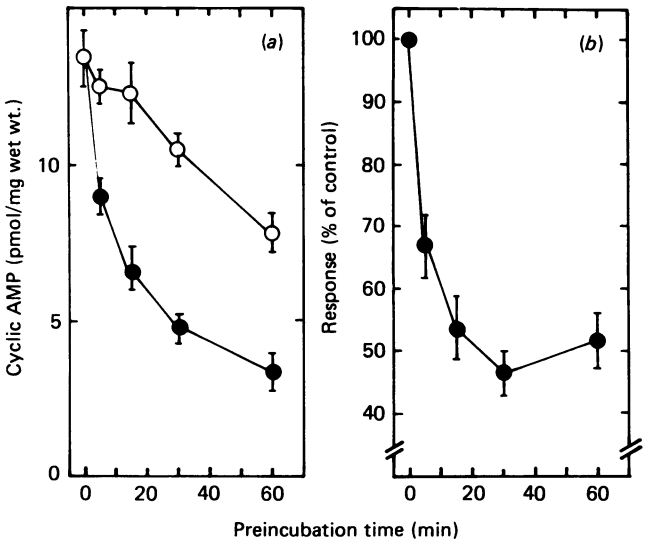


**Fig. 1. Time course of the isoprenaline-induced desensitization in hepatocytes**

Hepatocytes were preincubated without (○) or with (●) 100 $\mu$M-isoprenaline; the cells were washed and rechallenged with 1 $\mu$M-isoprenaline in the presence of 100 $\mu$M-methylisobutylxanthine at the times indicated. (*b*) presents the normalization of the data expressed as percentages of control (preincubated without agonist) response. Mean values are plotted, and vertical lines represent the S.E.M. for four experiments using different cell preparations.

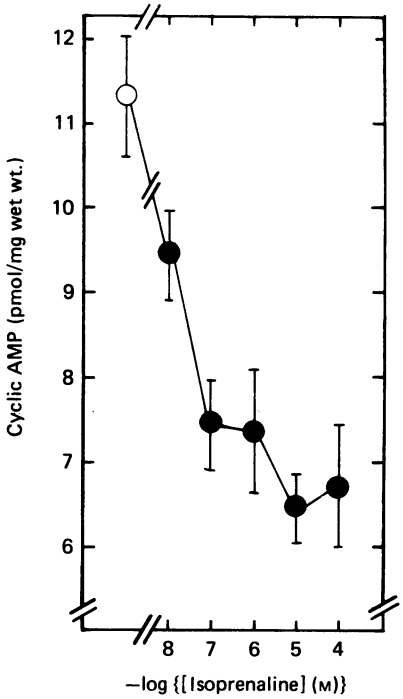


**Fig. 2. $\beta$-Adrenergic desensitization as a function of isoprenaline concentration during preincubation**

Hepatocytes were incubated for 15 min with the concentrations of isoprenaline indicated (○, control), washed, and challenged with 1 $\mu$M-isoprenaline in the presence of 100 $\mu$M-methylisobutylxanthine. Mean values are plotted, and vertical lines represent the S.E.M. for four determinations using different cell preparations.

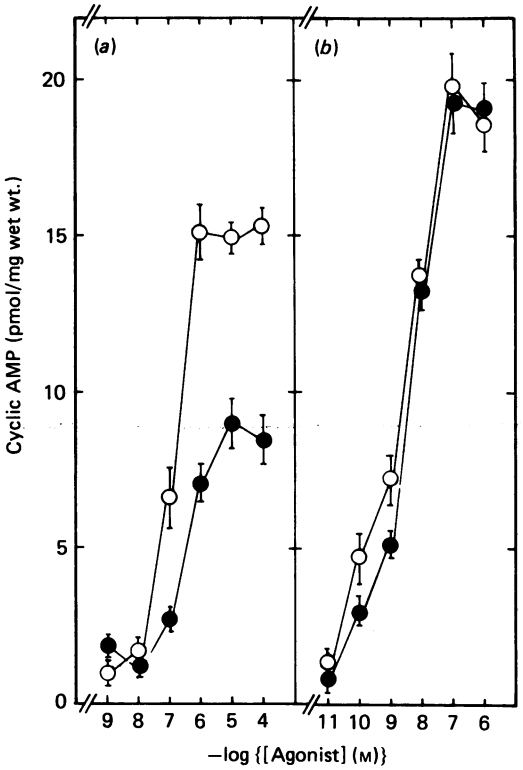


**Fig. 3. Effect of preincubation with isoprenaline on the accumulation of cyclic AMP induced by isoprenaline or glucagon**

Hepatocytes were incubated in the absence (○) or presence (●) of 100 μM-isoprenaline, washed and incubated with different concentrations of isoprenaline (*a*) or glucagon (*b*). Mean values are plotted, and vertical lines represent the S.E.M. for four experiments using different cell preparations.

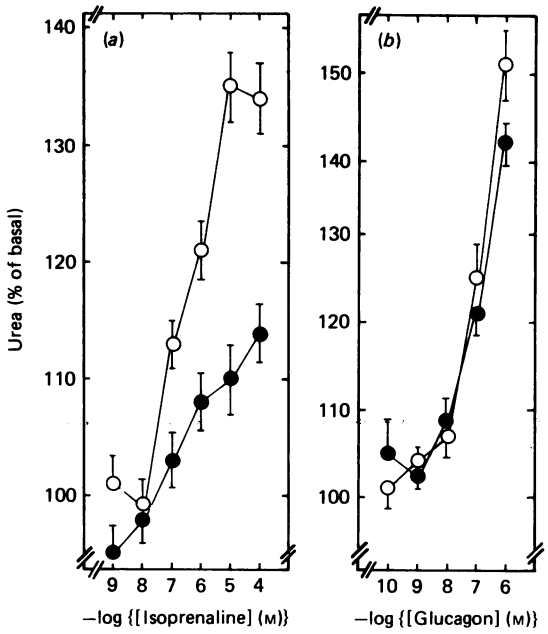


**Fig. 4. Effect of preincubation with isoprenaline on the effects of isoprenaline (*a*) and glucagon (*b*) on ureagenesis**

Results are expressed as the percentage of basal urea synthesis, which was 35±2 and 40±3 nmol/mg wet wt. of cells in cells preincubated in the absence (○) and presence (●) of isoprenaline respectively. For other information see Fig. 3.

preincubated in the absence or presence of 100 μM-isoprenaline was studied. The data presented in Fig. 3 show that preincubation with the β-adrenergic agonist markedly decreased (~ 50%) the maximal cyclic AMP accumulation induced by isoprenaline, but that there was no change in the $EC_{50}$ (~ 300 nM). Interestingly, the accumulation of cyclic AMP induced by glucagon was almost identical in cells preincubated in the absence or presence of isoprenaline (Fig. 3*b*). The data clearly indicate that the desensitization induced is homologous and not due to a general deleterious effect on the cells or the adenylate cyclase complex.

To determine if the preincubation with the β-adrenergic agonist has metabolic consequences, the ability of isoprenaline and glucagon to stimulate ureagenesis was studied (Fig. 4): both did so in a concentration-dependent fashion. The effect of isoprenaline was markedly diminished in cells preincubated with the β-adrenergic agonist, as reflected by both a diminished effect in the range of concentrations 100 nM–100 μM and a flat concentration–response curve as compared with the control (Fig. 4*a*). In contrast, preincubation with isoprenaline affected neither the magnitude nor the $EC_{50}$ of the concentration–response curve for glucagon (Fig. 4*b*).

When the cells were preincubated with 10 μM-propranolol in the absence or presence of isoprenaline, no desensitization of the β-adrenergic actions on cyclic AMP accumulation or on ureagenesis was detected (results not shown). The data indicate that receptor occupation (by the antagonist) was not sufficient to elicit desensitization and that activation was required, as reflected by the ability of the agonist to desensitize and that of the antagonist to block the desensitization.

Glucagon can markedly activate adenylate cyclase in these cells (Fig. 3). Therefore, the effect of preincubation with a maximally effective concentration of glucagon (100 nM) for 15 min was studied. It was observed that preincubation with glucagon did not induce β-adrenergic desensitization, as reflected by cyclic AMP accumulation (Fig. 5) or ureagenesis (Fig. 6). Interestingly, the effects of glucagon on both parameters were not affected by preincubation with the peptide.

Results from several laboratories have indicated that pertussis toxin can block some desensitization processes, suggesting a role of 'Ni' (Heyworth *et al.*, 1984; Wilson *et al.*, 1986). Therefore, we studied the effect of pertussis-toxin treatment on the β-adrenergic desensitization. Hepatocytes from pertussis-toxin-treated hypothyroid rats were incubated in the absence or presence of 100 μM-isoprenaline, washed, and re-challenged with different concentrations of the β-adrenergic agonist for the determination of cyclic AMP accumulation (Fig. 7). In spite of the treatment with the toxin, exposure to the β-adrenergic agonist blunted the second response (Fig. 7). Another observation was that in these cells the basal cyclic AMP concentrations were consistently higher in cells preincubated with the β-adrenergic agonist than those of cells preincubated without any agent (see legend to Fig. 7). Therefore, on a fold-over-basal basis, the response was markedly desensitized. To study the reason for tnese high basal concentrations of cyclic AMP, the

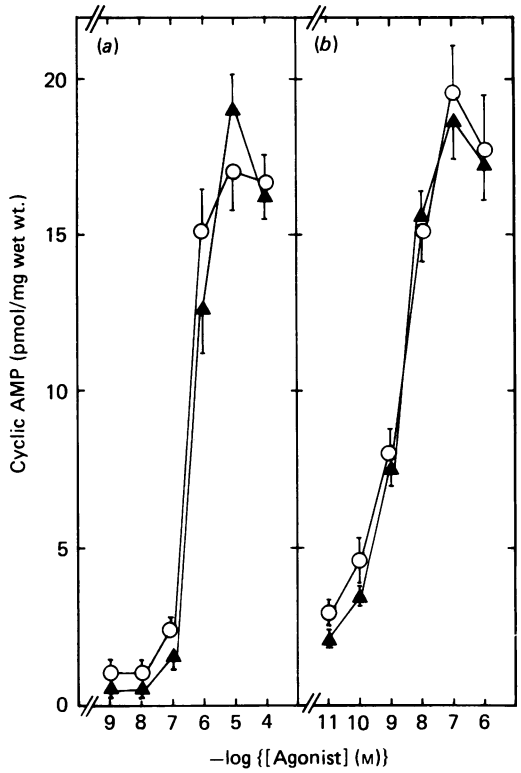


**Fig. 5. Effect of preincubation with glucagon on the accumulation of cyclic AMP induced by isoprenaline or glucagon**

Hepatocytes were preincubated in the absence (○) or presence (▲) of 100 nM-glucagon for 15 min, washed, and incubated with different concentrations of isoprenaline (*a*) or glucagon (*b*). Mean values are plotted, and vertical lines represent the S.E.M. for eight or nine experiments using different cell preparations.

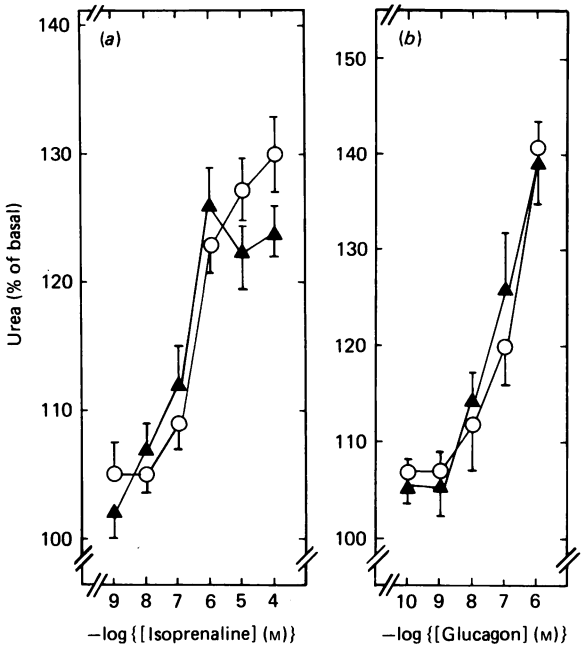


**Fig. 6. Effect of preincubation with glucagon on the effects of isoprenaline (*a*) and glucagon (*b*) on ureagenesis**

Results are expressed as percentage of basal urea synthesis, which was 40 ± 2 and 38 ± 3 nmol/mg wet wt. in cells preincubated in the absence (○) and presence (▲) of glucagon respectively. For other details see Fig. 5.

time course of cyclic AMP accumulation (in the absence of methylisobutylxanthine) induced by β-adrenergic stimulation was studied comparatively in cells from pertussis-toxin-treated rats and the control hypothyroid rats. The ascending part of the curves was very similar; in both conditions the maximal cyclic AMP accumulation was reached at 2 min (Fig. 8). However, some differences were observed in the descending part of the curves. The decrease in cyclic AMP accumulation was fast in the controls and returned to basal values at 60 min. In contrast, with cells from toxin-treated animals the decrease was slower, and a tendency to reach a plateau at concentrations 2–3-fold higher than the initial basal value (zero time) was observed (Fig. 8).

The desensitization induced by short-term β-adrenergic action was long-lived. We incubate the cells for as long as 150 min without observing that the desensitized cells recover the β-adrenergic responsiveness to values comparable with those of control cells incubated in parallel (results not shown). Longer incubations were not attempted, since the viability after this long incubation decreased to 75% or less.

## DISCUSSION

The present results indicate that short-term β-adrenergic activation leads to desensitization in hepatocytes. Our results are in agreement with those of Lam & Bar (1976), who observed that incubation of rat liver slices with adrenaline for as little as 30 min markedly desensitizes β-adrenergic stimulation of membrane adenylate cyclase activity, but does not affect glucagon-stimulated activity. Similarly, Gurr & Ruh (1980) and Noda *et al.* (1984) observed that in primary cultures of rat hepatocytes β-adrenergic activation induces homologous desensitization. In these studies using cultured cells, the agonist induced a rapid initial desensitization (in less than 1 h) (Gurr & Ruh, 1980), followed by a slower progress of the process (several hours) (Gurr & Ruh, 1980; Noda *et al.*, 1984). This desensitization after a long exposure to agonist is associated with a marked decrease in the number of membrane β-adrenoceptors (Noda *et al.*, 1984). Also in agreement with our data is the finding of Gurr & Ruh (1980) that the desensitization induced by adrenaline was poorly reversible. Reilly & Blecher (1982), using a cloned cell line (RL-PR-C hepatocytes), also observed that 1 h exposure to isoprenaline induces marked desensitization of β-adrenergic-stimulation adenylate cyclase activity.

In contrast with all these data are the findings of Morgan *et al.* (1982), who observed that the glycogenolytic response of perfused liver does not become desensitized even after successive short-term β-adrenergic stimulation. It is difficult to compare our results with those of Morgan *et al.* (1982); it is possible that in their model (perfused liver) a large receptor reserve may exist for the final response (glycogenolysis) or that other factors or non-parenchymal cells could play a role in the effects. In our study the desensitization was reflected both in cyclic AMP accumulation and in a metabolic parameter (ureagenesis).

It has been observed that cyclic AMP can induce in

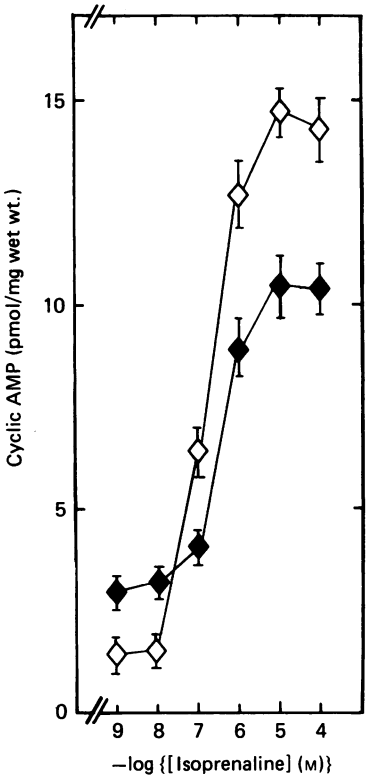


**Fig. 7. Effect of pertussis-toxin treatment on the β-adrenergic desensitization induced by isoprenaline**

Hepatocytes were preincubated in the absence (◇) or presence (◆) of 100 μM-isoprenaline, washed and re-challenged with different concentrations of isoprenaline. Basal cyclic AMP accumulations were $1.14 \pm 0.16$ and $3.68 \pm 0.21$ pmol/mg wet wt. of cells ($P < 0.001$) in cells preincubated in the absence or presence of isoprenaline respectively. Mean values are plotted, and vertical lines represent the S.E.M. for six experiments using different cell preparations.

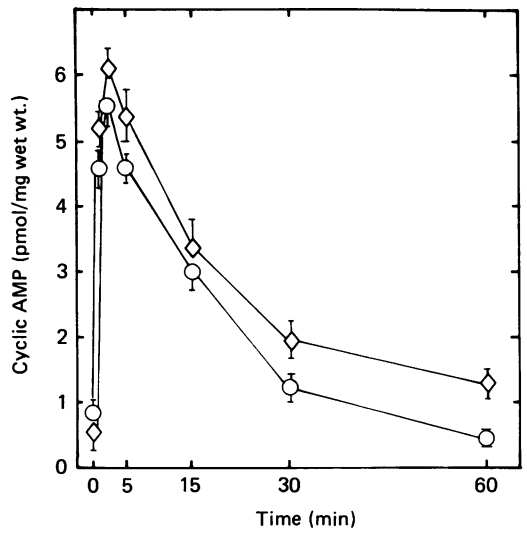


**Fig. 8. Effect of pertussis-toxin treatment on isoprenaline-induced cyclic AMP accumulation**

Hepatocytes from control (○) or pertussis-toxin-treated (◇) hypothyroid rats were incubated for the times indicated with 1 μM-isoprenaline (in the absence of methylisobutylxanthine). Mean values are plotted, and vertical lines represent the S.E.M. for five experiments using different cell preparations.

some cells β-adrenergic desensitization (Sibley & Lefkowitz, 1985), and the possibility that the cyclic AMP-dependent protein kinase (protein kinase A) could be involved has also been suggested (Benovic *et al.*, 1985). However, in our system glucagon stimulates adenylate cyclase through a process similar to that of the β-adrenoceptor (i.e. through 'Ns', the guanine-nucleotide-binding regulatory protein involved in adenylate cyclase activation) and to a similar extent as does isoprenaline in these cells. The inability of glucagon to induce β-adrenergic desensitization indicates that none of these factors (protein kinase A, cyclic AMP, the catalytic subunit of adenylate cyclase or 'Ns') is involved in the process of desensitization observed in this study. Propranolol does not induce β-adrenergic desensitization, which indicates that simple occupation of the receptor is not enough to trigger the process and that receptor activation is required. Benovic *et al.* (1986) reported the identification of a novel β-adrenergic receptor kinase that phosphorylates the agonist-occupied form of the receptor and which could probably be involved in the homologous types of desensitization. The possibility that this kinase could be involved in the effect here described is considered as a very attractive possibility. However, receptor phosphorylation and the role of this or any other protein kinase remains to be specifically demonstrated.

Heyworth & Houslay (1983) have observed that glucagon triggers a rapid decrease of adenylate cyclase activity in hepatocyte membranes; they suggested that the desensitization occurs at the level of 'Ns'. This desensitization is blocked by pertussis toxin (Heyworth *et al.*, 1984). We observed no desensitization induced by glucagon. The desensitization observed by Heyworth & Houslay (1983) is very rapid (5 min) and gradually disappears. In our experiments a preincubation of 15 min with glucagon, followed by washing (3–4 min), was used; therefore it is highly probable that our failure to observe any desensitization induced by glucagon could be due to the conditions employed. The fact that pertussis toxin does not block the homologous β-adrenergic desensitization here presented further indicates that the process involved differs from that reported by Heyworth *et al.* (1984). Clark *et al.* (1986) reported that the homologous desensitization of the β-adrenoceptor in lymphoma cells is not altered by pertussis-toxin treatment, which is consistent with our findings in hepatocytes. Other authors have reported desensitization by glucagon in whole cells, but a much longer exposure (several hours) is required (Plas & Nunez, 1975; Noda *et al.*, 1984).

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