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Catecholamine action on thyroid adenylate cyclase: evidence for inhibitory α-adrenoreceptors

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Cyclic AMP is known to mediate some of the control mechanisms involved in thyroid functions [1, 2]. The existence of adrenergic nerve endings has been shown in the gland [3, 4], and catecholamines were demonstrated to stimulate cyclic AMP [5–7] and iodine release [8], probably through β-adrenergic receptors.

More recently, however, it was shown that α-adrenoreceptors might exist in the thyroid, but their roles and modes of action remained conflicting. It was first suggested that at least some of the adenylate cyclase complexes were stimulated through α-adrenergic binding sites while others were sensitive to β-adrenergic agents [9]. Maayan *et al.* [10] later showed that α-adrenergic agonists could reversibly inhibit TSH and dibutyryl cyclic AMP-stimulated T3 and T4 release without altering cyclic AMP response to TSH activation.* They were thus led to postulate that α-adrenergic agonists were active on a step beyond cyclic AMP synthesis. However, Yamashita *et al.* [11] found that norepinephrine could inhibit TSH-elicited cyclic AMP increase in dog thyroid slices. This effect was abolished by the antagonist phentolamine.

In an attempt to resolve some of these conflicting data, the action of catecholamines and α-adrenergic antagonists was analysed on a broken cell preparation of horse thyroid. The results presented here suggest that adenylate cyclase may be stimulated by β agonists and inhibited by α agents in the same tissue, according to a mechanism not unlike the one exisiting in platelets [12, 13].

Materials and methods. Epinephrine, norepinephrine, isoproterenol, phenylephrine, propranolol, Tris–HCl, dithiothreitol and ascorbic acid were bought from Sigma; ATP, cAMP, phosphocreatine and creatine kinase from Boehringer Mannheim; phentolamine came from Ciba–Geigy and dihydroergocryptine from Sandoz. [8-^{3}H]-cyclic AMP (20–30 Ci/mmole) and [α-^{32}P]-ATP (0.5–10 Ci/mmole) were obtained from the Radiochemical Centre (Amersham, U.K.). Neutral aluminium oxide was from Macherey Nagel.

Horse thyroids were obtained from the local slaughterhouse. The fibrous capsula was removed and the glands were chopped with scissors. The tissue was homogenized in 10 vol. ice-cold Tris–HCl (25 mM, pH 7.4), sucrose (0.3 M), dithiothreitol (1 mM) with four strokes of a loose-fitting teflon–glass homogenizer. The homogenate was filtered through two layers of cheesecloth and was centrifuged for 15 min at 1000 *g*. The pellets were resuspended in 2 vol. Tris–HCl (50 mM, pH 7.4) and used immmediately. The whole procedure took place at 4° or lower and lasted for less than three hours.

As previously described [14], incubations took place for 15 min at 30° in 200 μl of a medium containing 50 mM Tris–HCl, pH 7.4, 3 mM [α-^{32}P]-ATP (0.8–1.5 × 10^6 c.p.m.), 5 mM $MgCl_2$, 2 mM[8-^{3}H]-cyclic AMP (20–40 × 10^3 c.p.m.), 0.1% bovine serum albumin, 10 mM creatine phosphate, 0.3 mg/ml phosphocreatine kinase and approximately 10 mg/ml protein of the thyroid pellet. Catecholamines were dissolved in 6 mM thiourea, 6 mM ascorbic acid just prior to use and were added in a 5 μl volume. Controls contained the same concentration of ascorbic acid and thiourea (e.g. 0.15 mM of each). The reaction was started by the addition of the thyroid preparation and stopped by adding 100 μl ice-cold carrier solution (cyclic AMP 20 mM, ATP 50 mM). Labeled cyclic AMP was separated from ATP by elution on dry alumina columns using Tris–HCl, 50 mM, pH 7.4. Losses of cyclic AMP either through degradation by phosphodiesterases during the incubation or by any other mechanism were monitored by [8-^{3}H]-cyclic AMP recovery. They accounted for less than 40 per cent. Contamination of the eluate by ^{32}P (enzyme blank) was less than 10 per cent of the lowest measured radioactivity, and was subtracted from the results. The results are expressed as picomoles cyclic AMP formed per minute and per milligram protein (determined by the method of Lowry); each point is the mean of 4 measurements. Preliminary experiments had shown the reaction to be a linear function of time up to 20 minutes.

Results. Figure 1 illustrates the action of various adrenergic agents on thyroid adenylate cyclase, and their interaction with dihydroergocryptine, an α-adrenergic antagonist. The β-adrenergic agonist isoproterenol, at 10^{-5} M—a concentration known to elicit submaximal stimulation in our system [7]—stimulated the enzyme, while phenylephrine, epinephrine and norepinephrine, all possessing α-adrenergic agonist properties, were ineffective at the same concentration. Addition of dihydroergocryptine (10^{-5} M) did not modify significantly base- or isoproterenol-stimulated levels; however, in the presence of epinephrine or norepinephrine (thus inhibiting the α-adrenergic component of their actions) a highly significant activation was evoked ($P < 0.001$, using Student's *t*-test). No consistent effect was seen with phenylephrine. In another experiment epinephrine and dihydroergocryptine, used together at the concentration of 10^{-5} M each, stimulated adenylate cyclase from 11.5 ± 0.6 pmoles cyclic AMP/min/mg protein to 20.3 ± 0.9 pmoles cyclic AMP/min/mg proteins; however,

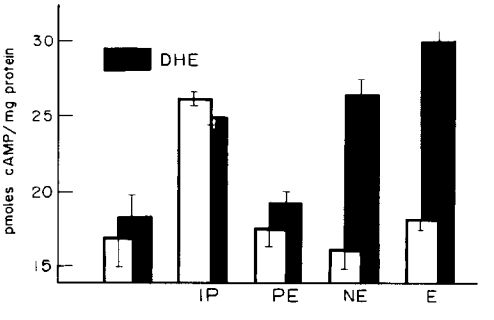

Fig. 1. Action of catecholamines on horse thyroid adenylate cyclase, in the absence or presence of dihydroergocryptine (DHE) 10^{-5} M. All agents were used at a concentration of 10^{-5} M. IP = Isoproterenol, PE = phenylephrine, NE = norepinephrine, E = epinephrine. The actions of isoproterenol, NE + DHE and E + DHE were all highly significant ($P < 0.001$, Student's *t*-test), while the effect of phenylephrine was not. Duration of the incubation was 15 min. Results are expressed as picomoles cyclic AMP formed per minute and per mg of protein.

* Abbreviations used: TSH, thyroid stimulating hormone; T3, triiodothyronine, T4, thyroxine.

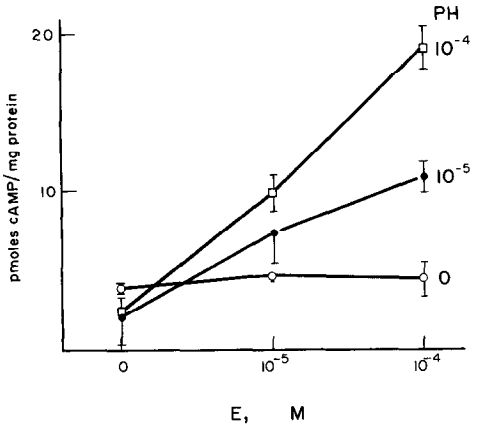

Fig. 2. Action of epinephrine (E) and phentolamine (PH) at 10^{-5} and 10^{-4} M on horse thyroid adenylate cyclase. Each curve represents the effect of epinephrine at increasing phentolamine concentrations. Epinephrine or phentolamine alone had no significant action.

the addition of the β-adrenergic blocker propranolol at 10^{-5} M to epinephrine and dihydroergocryptine reduced the activity to 12.2 ± 0.8 pmoles cyclic AMP/min/mg protein. Propranolol had no significant action on base level. This suggests that the stimulation of adenylate cyclase by epinephrine in the presence of an α-adrenergic antagonist is due to β-adrenoreceptors.

In order to test the specificity and the concentration dependence of the action of α-adrenergic blockers, various concentrations of epinephrine were studied in conjunction with phentolamine, as shown in Fig. 2. Epinephrine or phentolamine alone had no effects at 10^{-5} and 10^{-4} M, although when combined they stimulated adenylate cyclase in a concentration-dependent way.

To strengthen further the hypothesis that α-agonists could inhibit thyroid adenylate cyclase, the action of norepinephrine was investigated on isoproterenol-stimulated adenylate cyclase (Fig. 3). Norepinephrine (10^{-5} M)

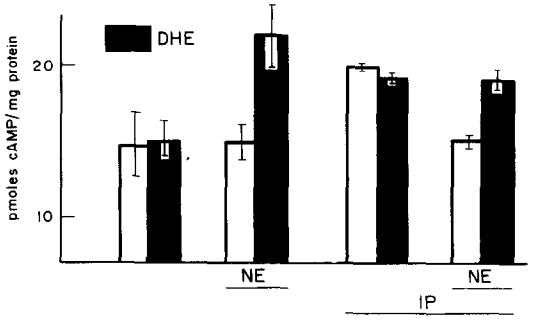

Fig. 3. Inhibition of the stimulation by isoproterenol in the presence of norepinephrine. Isoproterenol (IP), norepinephrine (NE) and dihydroergocrytpine (DHE) were all used at 10^{-5} M. DHE had no action on base (first two bars) or isoproterenol stimulated (third group of columns) levels. It revealed a stimulation by norepinephrine (second group). Norepinephrine inhibited the action of isoproterenol in the absence, but not in the presence of DHE (fourth pair of bars). This effect was highly significant ($P < 0.001$, Student's *t*-test).

was found to inhibit the activation by isoproterenol significantly ($P < 0.001$). The addition of dihydroergocryptine (10^{-5} M) brought back the activity to the same level as with isoproterenol alone.

Discussion. Catecholamines which possess α- and β-adrenergic properties are able to stimulate thyroid adenylate cyclase but only when the α component of their activity is inhibited by phentolamine or dihydroergocryptine. Furthermore, norepinephrine inhibits the stimulation by isoproterenol. These findings raise the question of the existence of inhibitory α-adrenoreceptors directly linked to adenylate cyclase in the thyroid.

Inhibitory α-adrenoreceptors have been demonstrated in various tissues, including melanocytes [15], adipocytes [16], platelets [12, 13], endocrine pancreas [17], heart [18], ileal mucosa [19] and neuroblastoma × neuroglioma hybrid cultured cells [20].

Maayan *et al.* [10] showed that α-adrenergic agonists could inhibit TSH-induced T3 and T4 release, without modifying cyclic AMP levels, suggesting an action on a regulatory step located beyond cyclic AMP synthesis. Our results suggest that agonist may also act directly on adenylate cyclase: in our broken cell preparation the cyclic AMP formation was measured directly from [α-^{32}P]-ATP, monitoring any possible cyclic AMP losses by the recovery of [8-^{3}H]-cyclic AMP present in the medium since the beginning of the measurement. Such a direct inhibition by α-adrenoreceptors is in line with data on adenylate cyclase of platelets [12, 13] and neuroblastoma × neuroglioma cells [20]. It substantiates the hypothesis of Yamashita *et al.* [11] using thyroid slices. The divergence from the work of Maayan *et al.* [10] could be related to the different animal species used, as the catecholamine actions are known not to be identical in the thyroid of different animals [4].

We were able to show that norepinephrine, which possesses the α-adrenergic properties in addition to certain β-adrenergic characteristics, could inhibit the action of an almost pure agonist, i.e. isoproterenol. This suggests that the action of the adrenergic receptor is not restricted to antagonizing TSH stimulation as already demonstrated [10, 11] but also inhibits activation through the β-adrenoreceptors in the thyroid. It would explain why mixed α–β-adrenergic agonists are ineffective when used alone and require blocking of the α component to stimulate adenylate cyclase. A similar potentiation of catecholamine action has been found in adipocytes [16] and platelets [12].

The properties of the thyroid α-adrenergic response was thus similar to the one in platelets. The same concentration range of agonists and antagonists was found to be effective in both systems. However, in the horse thyroid we did not find any significant modification of basal activity by epinephrine or norepinephrine alone, as opposed to what was shown in thrombocytes. An inhibitory component could have been masked by the stimulatory action and thus pass unnoticed due to the difficulty of measuring small decreases of an already low basal level. Alternatively, in the thyroid, α-adrenergic receptors may act by inhibiting the activation and coupling of the catalytic subunit of adenylate cyclase with the activatory receptors. Yamashita *et al.* [11] similarly failed to demonstrate inhibition of basal cyclic AMP levels by norepinephrine in conditions antagonizing TSH activation.

In conclusion, isoproterenol, a β-adrenergic agonist, was found to stimulate adenylate cyclase in a 1000 *g* pellet of horse thyroids, while phenylephrine, epinephrine and norepinephrine were ineffective at the same concentration of 10^{-5} M. However, when the α-adrenergic component of epinephrine or norepinephrine was antagonized by dihydroergocryptine (10^{-5} M), both agents were found to be stimulatory. Similarly, phentolamine could induce stimulation of adenylate cyclase by epinephrine in a concentration-dependent way. Norepinephrine was able to inhibit the stimulation by isoproterenol; this inhibition was absent

when dihydroergocryptine was added. These data suggest the existence of α-adrenergic receptors inhibiting the stimulation of thyroid adenylate cyclase by isoproterenol.

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Effect of acetaminophen (paracetamol) and its antagonists on glutathione (GSH) content in rat liver

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Acetaminophen (paracetamol) overdose may be fatal due to hepatic centrilobular necrosis [1, 2]. Mitchell and his colleages showed that liver necrosis was due to covalent binding to macromolecules of a reactive metabolite [3] and proposed a protective role for reduced glutathione (GSH)* in mice [4] and man [5].

It has been shown that acetaminophen toxicity can be decreased by oral administration of charcoal which slows the absorption of the drug from the intestinal tract [6]. Other treatments are based on the administration of a variety of substances, including methionine [7] and *N*-acetyl cysteine [8, 9], to counteract the toxic effects of acetaminophen. According to the GSH protective hypothesis of Mitchell *et al.* [4, 5], these compounds may act by preventing GSH depletion. Other authors have proposed a direct binding of the antagonists of acetaminophen to a reactive metabolite of the drug [10].

In this communication we report the effect of methionine and of *N*-acetyl cysteine on GSH depletion due to acetaminophen overdose and also that high doses of *N*-acetyl cysteine alone decrease hepatic GSH content in rats.

Animals. Wistar rats were fed on a standard diet for rats and mice. (Sandersa Industrial S.A. Pinto, Madrid, Spain). They always had free access to food and water. Although some previous studies on acetaminophen toxicity in rats have been carried out in fasted animals [10] we used well fed rats because fasting significantly decreases hepatic GSH content in rats [11].

Acetaminophen and aminoacids were dissolved in a small volume of physiological saline and injected intraperitoneally (i.p.). *N*-acetyl cysteine was injected i.p. as a 10% aqueous solution. It was tested that injection of similar amounts of both solvents did not affect hepatic GSH content. The dose of acetaminophen injected was 0.5 g/kg body wt (3.3 mmoles/kg). A similar dose had been previously used by other authors [10].

Chemicals. Acetaminophen was a gift of the Department of Pharmacy of the Faculty of Medicine (Valencia, Spain). Amino acids and other chemicals were of the highest purity available.

Determination of GSH and GSSG. Reduced glutathione (GSH) was determined by the glyoxalase method of Racker [12].

Rats were killed by cervical dislocation and livers removed, weighed and homogenized in 2% perchloric acid in physiological saline. Analysis of GSH in rapidly frozen livers showed that the above procedure did not affect the hepatic GSH content. Normal GSH concentration was 5.40 ± 0.37 μmoles/g (four observations). Oxidized glutathione (GSSG) was determined as previously described [13].

* Abbreviations used: GSH, reduced glutathione; GSSG, oxidized glutathione.