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INVOLVEMENT OF MICROTUBULES IN THE ISOPROTERENOL-INDUCED 'DOWN'-REGULATION OF MYOCARDIAL β-ADRENERGIC RECEPTORS

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The number of cardiac β-adrenergic receptors identified by [^{3}H]dihydroalprenolol binding decreases in a concentration-dependent manner during prolonged administration of isoproterenol. Loss of membrane β-receptors is paralleled by the appearance of [^{3}H]dihydroalprenolol binding activity in the cytosol. This redistribution of receptors is prevented by colchicine and vinblastine but not lumicolchicine. Cardiac receptor desensitization is, therefore, dependent on microtubules and may be influenced by agents interfering with tubulin polymerization.

Prolonged exposure of β-adrenergic receptors to their agonists is known to decrease the magnitude of agonist-mediated physiological responses [1]. This subsensitivity is associated with loss of membrane-bound β-adrenergic receptors and/or adenylate cyclase activity through pathways which may involve internalization of the recognition site of the receptors [2–4]. Attenuation of physiological responses to β-adrenergic stimulation through 'down-regulation' of the receptors has important functional implications for the contractile behavior of the myocardium which is strongly influenced by catecholamines. Although adrenergic mechanisms are probably not indispensable for the beat-to-beat regulation of pump function in the normal heart [5], they play an important role in regulating contractility during cardiac hypertrophy and failure. Occurrence of receptor densensitization, therefore, severely limits the extent to which adrenergic mechanisms can be utilized for support of the failling myocardium and study of the pathways involved in this desensitization phenomenon is of more than just theoretical interest. In this report, we present evidence that the microtubular system is involved in the process of internalization during in vivo exposure of cardiac β-receptors to isoproterenol.

Experiments were carried out on male Sprague-Dawley rats weighing 250–350 g. Hearts wre homogenized in 0.25 M sucrose/50 mM Tris-HCl (pH 7.4)/10 mM $MgCl_2$ with a Polytron PT-20 at a setting of 3 for 15 s, twice. The homogenates were then spun at 700 × *g* for 10 min and the supernatant was either used for [^{3}H]dihydroalprenolol binding or further centrifuged at 30000 × *g* for 20 min and then assayed for β-adrenergic receptors (cytosolic fraction). The 30'000 × *g* pellet was washed by homogenization in 50 mM Tris-HCl (pH 7.4)/10 mM $MgCl_2$ followed by centrifugation at 30000 × *g* for 20 min. The pellet (membrane fraction) was resuspended in buffer and used for [^{3}H]dihydroalprenolol binding assay.

Identification of β-adrenergic receptors was carried out as previously described [6]. The assay medium contained 50 mM Tris-HCl/10 mM $MgCl_2$ (pH 7.4), 0.4–0.6 mg membrane or 1.0–1.5

mg cytosolic protein, 5 nM [^{3}H]dihydroalprenolol (England Nuclear Comp., spec. act. 58.5 Ci/mmol) in a total volume of 0.2 ml. Controls contained 10^{-5} M (±)-propranolol to determine nonspecific binding. Incubation was carried out at 25°C for 15 min and [^{3}H]dihydroalprenolol binding was determined as previously described [6].

The effect of colchicine on the isoproterenol-induced 'down' regulation of cardiac β-adrenergic receptors was studied in vivo. Rats were injected either with colchicine (0.2 mg/100 g body weight intraperitoneally) followed, 2 h later, by isoproterenol (2.0 mg/kg subcutaneously) or with isoproterenol (2.0 mg/kg s.c.) alone. Pretreatment with colchicine was designed to allow the formation of the colchicine-tubulin complex prior to isoproterenol administration. Animals were sacrificed 1–6 h later, the 30000 × *g* supernatant as well as membranes were isolated and [^{3}H]dihydroalprenolol binding was determined. As shown in Fig. 1, [^{3}H]dihydroalprenolol binding to both fractions was relatively stable in control and colchicine-treated animals. In isoproterenol-treated animals, however, a progressive decrease in [^{3}H]dihydroalprenolol binding by membranes was noted with increasing length of exposure to isoproterenol. This was paralleled by a significant increase in assayable β-receptors in the 30000 × *g* supernatants. The extent of [^{3}H]dihydroalprenolol binding by the cytosolic fraction was, nevertheless, considerably less than by the membrane fraction even in the isoproterenol-treated rats. In sharp

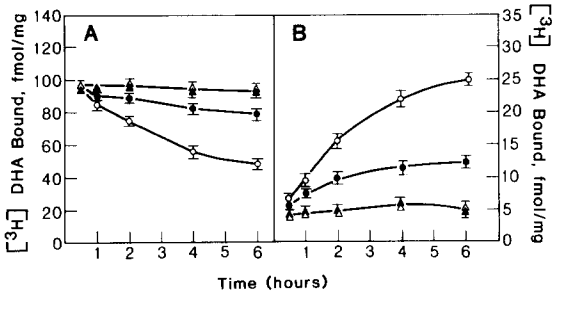

Fig. 1. The effect of isoproterenol and colchicine on [^{3}H]dihydroalprenolol ([^{3}H]DHA) binding to cardiac membranes (A) and cytosolic fraction (B). Animals were injected with either colchicine (△), colchicine + isoproterenol (●) or isoproterenol (○) and were killed 0.5–6 h later. Animals injected with 0.9% NaCl alone (▲) served as controls. Results represent mean ± S.E. for six experiments.

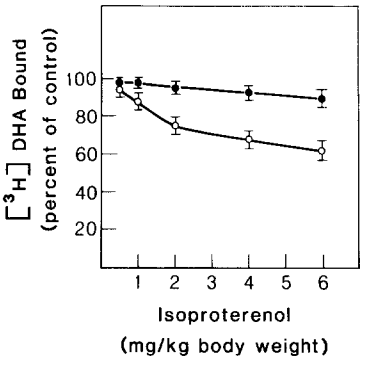

Fig. 2. Dependence of [^{3}H]dihydroalprenolol ([^{3}H]DHA) binding to cardiac membranes from rats treated with isoproterenol (○) and colchicine + isoproterenol (●). Results are expressed as percent of binding to membranes of control animals (injected with saline alone) and represent mean ± S.E. for six experiments.

contrast to these results, the loss of β-receptors in myocardial cell membranes of animals treated with colchicine plus isoproterenol was considerably attenuated as was the appearance of [^{3}H]dihydroalprenolol binding activity in the cytosol. Colchicine was effective in preventing loss of cardiac β-receptors over a wide range of isoproterenol doses (Fig. 2).

A similar redistribution of β-adrenergic receptors between the membrane and cytosolic fractions

TABLE I

EFFECT OF ISOPROTERENOL ON CARDIAC β-ADRENERGIC RECEPTORS AND ITS MODIFICATION BY COLCHICINE, VINBLASTINE AND LUMICOLCHICINE

Rats were injected with the indicated substances and [^{3}H]DHA binding to the membrane and soluble fractions was determined. Results represent mean ± S.E. for six animals in each group.

Experimental group	[^{3}H]Dihydroalprenolol binding (fmol/mg)	
	30000 × *g* pellet	30000 × *g* supernatant
Control (0.9% NaCl)	89 ± 6	6 ± 2
Isoproterenol	47 ± 5 *	18 ± 4 *
Colchicine + isoproterenol	72 ± 6	10 ± 2
Alprenolol + isoproterenol	85 ± 7	5 ± 2
Vinblastine + isoproterenol	68 ± 7 *	12 ± 2
Lumicolchicine + isoproterenol	85 ± 9	6 ± 3

* $P < 0.01$ compared to controls.

was noted following short-term in vivo administration of isoproterenol (Table I). Animals were injected (a) with isoproterenol (2.0 mg/kg s.c.) daily for 4 days, (b) colchicine (0.5 mg/kg i.p.) followed, 2 h later, by isoproterenol (2.0 mg/kg s.c.) daily for 4 days, (c) alprenolol (20 mg/kg i.p.) followed, 30 min later, by isoproterenol (2.0 mg/kg s.c.) daily for 4 days, (d) vinblastine (0.6 mg/kg i.p.) followed, 2 h later, by isoproterenol (2 mg/kg s.c.), (e) lumicolchicine (0.5 mg/kg i.p.) followed, 2 h later by isoproterenol (2 mg/kg s.c.), or (f) 0.9% NaCl (controls). Following death, the membranes and $30\,000 \times g$ supernatant were separated and tested for [^{3}H]dihydroalprenolol binding. As shown in Table I, stimulation of [^{3}H]dihydroalprenolol binding in the $30\,000 \times g$ supernatant and loss of activity in the corresponding pellet was prevented both by colchicine and alprenolo.

We interpret these results as evidence that 'down regulation' of cardiac β-receptors by isoproterenol is partly due to internalization of the receptors as a consequence of agonist-receptor interaction. In support of this contention, the characteristic redistribution of [^{3}H]dihydroalprenolol binding activity between membrane and cytosolic fractions was not seen in rats injected with both isoproterenol and alprenolol (Table I). Secondly, the [^{3}H]dihydroalprenolol binding activity in the cytosol had the same K_d as in the membranes (membranes: 2.1 ± 0.1 nM, cytosol: 2.4 ± 0.2 nM). Thirdly, the competition of various agonists with [^{3}H]dihydroalprenolol for binding to the receptor was similar in the two fractions and unaltered by colchicine (results not shown). Previous studies with the frog erythrocytes had shown that shuttling of the β-receptors between the plasma membrane and cytosol is one pathway for 'down regulation' of the receptors in the presence of agonists [7]. This is reminiscent of the internalization of receptors for some polypeptide hormones [2–4] and contrasts with the 'uncoupling' of receptors to adenylate cyclase reported for other models [8].

Our results implicate the microtubular system in the desensitization myocardial β-adrenergic receptors. The protection against isoproterenol-induced desensitization was shared by vinblastine but not lumicolchicine (Table I), a finding which argues against a non-specific inhibitory effect of colchicine. Furthermore, at concentrations of 1–50 μM, colchicine has no direct effect on [^{3}H]dihydroalprenolol binding by either homogenates or cardiac membranes while higher concentrations are inhibitory. These data agree with recent reports [9] that colchicine has no direct effect on the number of β-adrenergic receptors of synaptic membranes. During in vivo administration of isoproterenol, interference with myocardial microtubular assembly appears to attenuate the loss of membrane-bound β-receptors probably by inhibiting their translocation to the cytoplasm. The in vitro effects of colchicine are also compatible with inhibition of an agonist-induced mobility change of the receptors even though internalization cannot be identified in broken cell preparations.

It has been shown [10] that unoccupied β-adrenergic receptors are aggregated and are slowly dispersed by agonists; furthermore, increased mobility of the β-receptors is not involved in adenylate cyclase activation but may be needed for 'desensitization' to occur. It is likely that microtubules are essential for the agonist-mediated receptor mobility and that interference with this receptor dispersion is an early step in colchicine action.

Since catecholamines have important influences on the contractile behavior of normal and diseased myocardiums, their interactions with the receptor-adenylate cyclase system have drawn particular attention. With few exceptions, the number of β-receptors is decreased in the hypertrophied and failing myocardium [11–13]. Since the levels of circulating catecholamines are elevated, sometimes strikingly, in cardiac failure [14], 'loss' of β-receptors may reflect desensitization. This has functional implications since it deprives the myocardium of an important support of contractile capacity. It is not known whether changes in microtubule assembly in the course of hypertrophy and failure contribute to the apparent loss of beta receptors. It is suspected, however, that desensitization may partly explain the limited success of β-adrenergic stimulants in sustaining a beneficial hemodynamic effect in patients with heart failure [13]. Manipulation of the cardiac microtubular system may afford a novel mechanism for amplifying the inotropic influence of such agents.

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