PHARMACOLOGIC REGULATION OF CARDIAC ADRENERGIC RECEPTORS

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ABSTRACT

Cardiac adrenergic receptors were studied using radiolabeled ligands. The properties of the receptors were then characterized under various hormonal and pharmacological regulation.

Adrenergic receptor binding was measured in various membrane preparations using the potent beta-adrenergic antagonist [^{3}H]dihydroalprenolol ([^{3}H]DHA) to label the beta receptors and the potent alpha-adrenergic antagonist [^{3}H]dihydroergocryptine ([^{3}H]DHE) to label the alpha receptors. Binding of both radioactive ligands to their respective receptors exhibited stereoselectivity for the (-) and (+) isomers, high affinity and saturability, all of which are consistent with specific interaction of the ligands with adrenergic receptors. Beta-adrenergic receptor binding was also characterized in intact adult cardiac myocytes. This characterization consisted of labeling the beta receptors with a beta-adrenergic agonist, [^{3}H] hydroxybenzylisoproterenol ([^{3}H]HBI), as well as the antagonist [^{3}H]DHA. Strict stereospecificity and saturability were observed for both radiolabeled ligands in the isolated cells as it had been in the membrane preparations. The number of binding sites as assessed by Scatchard analysis using either ligand was similar, with [^{3}H]DHA yielding 88 fmol/mg protein and [^{3}H]HBI yielding 85 fmol/mg protein, which corresponded to approximately 200,000 beta receptors/heart cell.

To examine the subcellular localization of beta adrenergic

receptors, as well as other membrane constituents, a membrane fraction enriched in sarcolemma (S1) and another enriched in sarcoplasmic reticulum (SR) were isolated from a membrane vesicle preparation. These fractions were obtained by sucrose density gradient centrifugation, yielding various subfractions. The most dense subfraction (SR) contained the highest K^+,Ca^{++}-ATPase activity and virtually no Na^+,K^+-ATPase activity, adenylate cyclase activity, sialic acid or beta-adrenergic receptors. All of these latter parameters were concentrated 3-7 fold in the lightest subfraction (S1) providing evidence that these constituents are exclusively localized to the sarcolemma.

Of the numerous possible factors which may regulate adrenergic receptors, two particular ones, pharmacologic regulation by guanine nucleotides and hormonal regulation by thyroid hormone, were chosen to focus on in detail. The heart is dually innervated by sympathetic and cholinergic nerves. Cholinergic agonists attenuate cAMP generation in response to hormones which activate the beta-adrenergic receptor/adenylate cyclase system. This intriguing phenomenon led to the investigation of the effect of cholinergic agonists on beta-adrenergic receptor affinity for catecholamines. In cardiac homogenates, guanine nucleotides decreased the affinity of the beta-adrenergic receptors for isoproterenol without altering the affinity of the receptors for propranolol. The muscarinic cholinergic agonist methacholine antagonized this guanine nucleotide-induced change in affinity of the beta-adrenergic receptors for beta agonists. This antagonism by methacholine was reversed by atropine. Methacholine had no effect on beta-adrenergic receptor affinity for the agonist isoproterenol in the absence of guanine nucleotides. Methacholine was shown to attenuate adenylate

cyclase activity in the presence of GTP but not Gpp(NH)p or NaF suggesting that muscarinic cholinergic agonists can regulate both beta-adrenergic receptor affinity and adenylate cyclase activity by modulating the effects of the coupler GTP. Similar methacholine effects were seen on the beta-adrenergic receptor affinity for catecholamines using isolated cardiac myocytes. Thus one mechanism of cAMP regulation in the heart may be by reciprocal modulating effects of the two limbs of the autonomic nervous system, regulating both beta-adrenergic receptor affinity for catecholamines and also adenylate cyclase activity.

Receptor regulation by such hormones as thyroxine may have particular significance in the heart since abnormalities in the cardiovascular system are some of the most dramatic manifestations of altered thyroid state in patients and experimental animals. Therefore, membrane preparations from hearts of eu-, hyper- and hypothyroid rats were analyzed in an attempt to identify biochemical changes in the three thyroid states. Cardiac membranes from hyperthyroid rats showed a 40% decrease in total apparent number of alpha-adrenergic receptors and a 43% increase in total apparent number of beta-adrenergic receptors relative to euthyroid controls. Cardiac membranes from hypothyroid rats showed an approximate 25% decrease in apparent number of both alpha- and beta-adrenergic receptors. Total sialic acid content remained similar regardless of thyroid state. In addition Na^+,K^+-ATPase and K^+,Ca^{++}-ATPase activities remained unchanged in hearts from hyperthyroid rats but were decreased approximately 40% in hearts from hypothyroid rats. These results suggest that with hyperthyroidism, abnormalities in the cardiovascular system such as increased sensitivity to catecholamines may reflect an alteration in the approximate number of alpha- and beta-adrenergic receptors

of the S1 without alteration of the K^+,Ca^{++}-ATPase of the SR. The same biochemical parameters are more diffusely altered with hypothyroidism, however, with changes occurring at the level of the S1 as well as the SR.

These studies, using radiolabeled ligands, have demonstrated that cardiac adrenergic receptors may be hormonally and pharmacologically regulated.

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