ELEVATED BETA-ADRENERGIC RECEPTOR NUMBER AFTER CHRONIC PROPRANOLOL TREATMENT

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SUMMARY: Treatment of rats with the beta-adrenergic antagonist propranolol for two weeks leads to a 100% increase in the number of beta-adrenergic receptors ((-)[^{3}H]dihydroalprenolol binding sites) in cardiac particulate fractions. No change in receptor affinity was observed. These findings may 1) represent the converse of agonist-induced desensitization which is associated with decreases in beta-receptor number, 2) provide a potential explanation for the clinically observed "propranolol withdrawal syndrome".

A variety of pharmacologically active molecules capable of altering cell function have been shown to have their primary interaction with the cell at specific receptor sites on the outer membrane surface(1). Combination of agonist molecules with these receptor sites sets in motion an amplification system which often begins with adenyl cyclase activation and results in significant changes in physiologic activity(1).

It has recently been demonstrated in a number of systems that changes in the occupancy of receptor sites leads to alterations in receptor number (2) and in some cases alterations in the responsiveness of the receptor-coupled amplification system as well. For example, Mukherjee et al., (2) have shown that chronic occupancy of frog erythrocyte beta-adrenergic receptors by agonists such as isoproterenol led to a decreased number of receptors in the erythrocyte cell membranes as assayed by (-)[^{3}H]dihydroalprenolol binding. This phenomenon was accompanied by desensitization of beta-receptor mediated adenyl cyclase activation by agonists. In the same system , occupancy of the receptor sites by the antagonist propranolol failed to alter receptor number (2).

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In rat brain however, it has been demonstrated that chronic blockade of dopaminergic receptors by the antagonist haloperidol led to an increased number of dopamine receptors measured by [^{3}H]haloperidol binding (3). Sporn et al., have shown (4) that chronic depletion of tissue norepinephrine with consequent diminished beta-receptor occupancy by agonist (produced by 6-hydroxydopamine treatment) led to an increase in beta-receptor number in rat brain. These observations suggest that there may be a dynamic relationship between receptor occupancy and receptor number.

Propranolol is a beta-adrenergic antagonist which is clinically useful in several disease states, most notably angina (5). Recently several reports have appeared documenting a number of untoward cardiac events occurring after acute discontinuation of chronic, high-dose propranolol therapy (6,7,8). It has been suggested that these "rebound phenomena" which include unstable angina, myocardial infarction and sudden death, may result from an increased sensitivity of cardiac tissue to beta-agonists (9). In view of this information and the data supporting a relationship between receptor occupancy and receptor number, we studied the effects of chronic, high-dose propranolol on beta-receptor number in rat hearts.

Male C-D strain rats (Charles River) were injected intraperitoneally every eight hours with a solution of (±)propranolol, 4 mg/ml in normal saline, at a dose of 10 mg/kg. Control animals were injected with similar volumes of normal saline. Eight hours after the last dose the animals were sacrificed and beta-receptor number was assayed in ventricular tissue according to previously published methods (10). Dissociation constants (K_D) and maximum binding for (-)[^{3}H]dihydroalprenolol were determined from Scatchard plots (11).

Data from a representative experiment are presented in the form of saturation and Scatchard plots in Figs. 1A and B. These

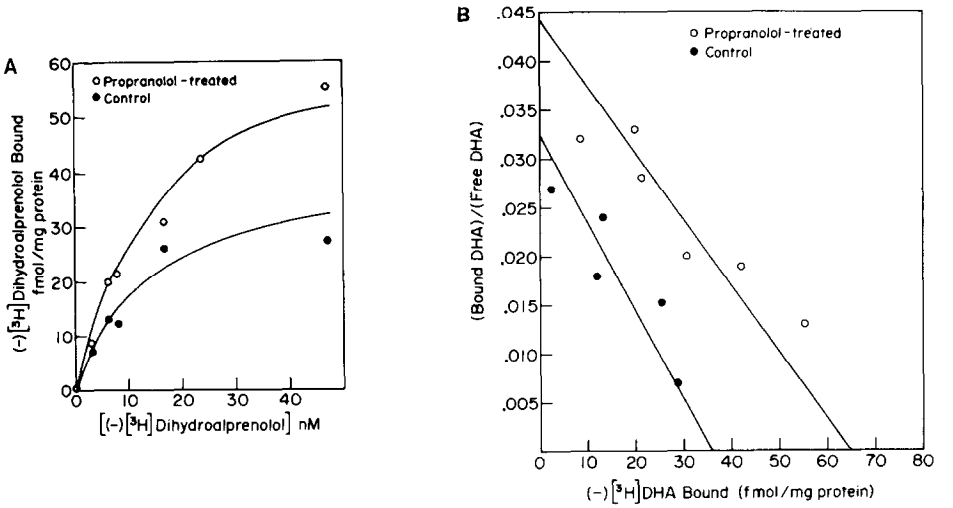

Fig.1A Specific (-)[^{3}H]dihydroalprenolol binding to ventricular membranes from control and propranolol treated rats as a function of (-)[^{3}H]dihydroalprenolol concentration. Groups of four control and treated rats were sacrificed by cervical dislocation and the ventricles rapidly extirpated and minced with a scissors to a particle size of 1-3 mm in 20 ml of ice-cold sucrose 0.25 M, Tris-HCl 5 mM, pH 7.4, $MgCl_2$ 1 mM. The ventricular pieces were allowed to settle and the supernatant decanted and replaced with 30 ml of fresh ice-cold buffer. The suspension was homogenized at 0^{o}C using 20 strokes of a motor-driven grooved Teflon-glass homogenizer . After cheese cloth filtration, the material was centrifuged at 4^{o}C for 10 min at 480 xg and the resulting supernatant centrifuged at 4^{o}C for 10 minutes at 30,000 xg. The pellet was resuspended in 40 ml of Tris HCl 50 mM, pH 7.5, $MgCl_2$ 10 mM, and centrifuged at 4^{o}C for 10 min at 30,000 xg. This step was repeated and the final pellet was suspended in 4-6 ml of Tris HCl 75 mM , pH 7.65, $MgCl_2$ 25 mM. The binding assay was initiated by adding 100 µl of the membrane preparation to 50 µl of (-)[^{3}H]dihydroalprenolol with and without (±)propranolol 3 X 10^{-5}M. The mixtures were incubated for 10 min at 37^{o}C with shaking. One hundred µl aliquots were then withdrawn and immediately dispersed in 5 ml of ice-cold Tris HCl 75 mM, pH 7.65, $MgCl_2$ 25 mM and filtered by suction through a 25mM Schleicher and Schuell No. 25 filter disc. The filters were washed four times with 5 ml of buffer, dried and counted. Specific binding was defined as the difference between binding in the absence and presence of (±)propranolol 10^{-5}M expressed as fmol bound/mg protein. Specific binding was usually 50-70% of total binding. A representative saturation curve with a membrane preparation derived from control and treated groups of animals is shown. Data points are means of closely agreeing duplicate observations.

Fig. 1B Scatchard plot of (-)[^{3}H]dihydroalprenolol binding to ventricular membranes from control and propranolol treated rats. Data of Figure 1A have been replotted. Experimental details are as described in legend to Figure 1A.

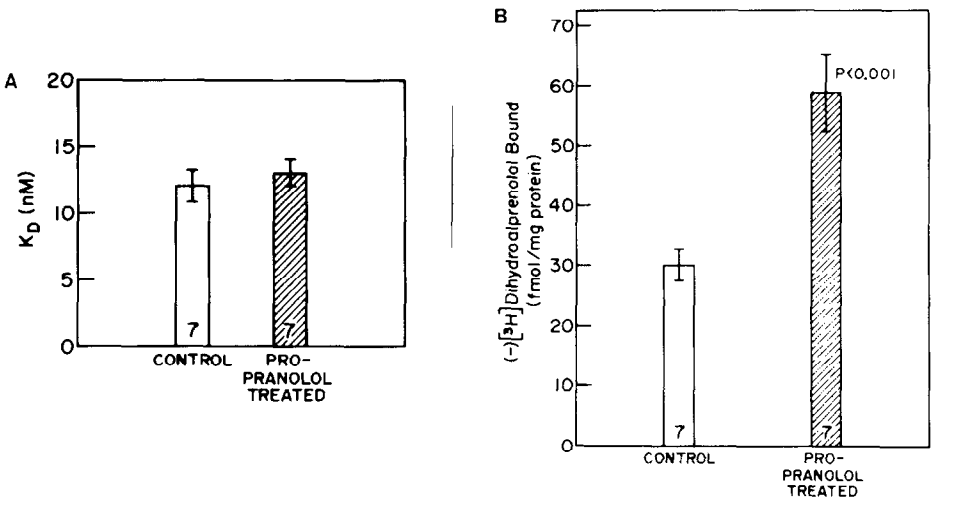

Fig. 2 A) Dissociation constants (K_d) and B) Maximum specific binding of (-)[^{3}H]dihydroalprenolol in ventricular membranes from control and propranolol treated rats. Membranes were prepared and (-)[^{3}H]dihydroalprenolol binding assays performed as described in Figure 1 legend. Dissociation constants and maximum (-)[^{3}H]dihydroalprenolol binding were determined from least square fits to Scatchard plots of the data. The results represent the mean ± S.E. of seven experiments. Statistical analysis was performed by Student's t test (unpaired).

indicate an increase in the number of beta-adrenergic receptor binding sites in the hearts from propranolol-treated rats without major alteration in the affinity of binding. Data from 7 such experiments are presented in Figs. 2A and B. Although the affinity for (-)[^{3}H]dihydroalprenolol was not significantly changed by chronic propranolol treatment (13.0 vs 11.9 nM: $p > 0.5$) the number of beta-receptors per milligram of membrane protein underwent an almost 100% increase (58.7 vs 29.9 fmol/mg; $p < 0.001$).

The mechanism responsible for the increase in receptor number observed in the present experiments is not known. Possibilities include: 1) de novo synthesis of additional receptors in an attempt to compensate for a chronic decrease in agonist occupancy associated with persistent, high-level antagonist blockade; 2) a decreased

rate of degradation of receptors occupied by the antagonist; 3) reversal of a chronic "desensitizing" or receptor lowering effect of endogenous catecholamine agonist.

Whether the increased number of cardiac beta-adrenergic receptors demonstrated in the present experiments is reflected in altered physiologic function has not been determined. It is possible that chronic, high-level, antagonist blockade of agonist occupancy results in an increased sensitivity to agonist and the increased number of beta-receptors represents part of this change in sensitivity. Such a finding would provide a possible explanation for the "rebound phenomena" observed after acute discontinuation of chronic propranolol therapy in man.

In favor of this view is the observation that chronic haloperidol administration to rats caused an increase in dopamine receptor number (3) and a leftward shift in the dose response curve of apomorphine - stimulated motor activity (12). In brains of rats treated with intraventricular 6-hydroxydopamine Sporn et al., have observed (4) an increase in beta-receptor number and an increase in basal and isoproterenol stimulated cAMP accumulation. Further, Palmer et al., (13) found an increased sensitivity of ventricular adenyl cyclase to norepinephrine after chronic surgical cardiac denervation in dogs. Thus, several systems exist where chronic decreased agonist occupancy of receptors results in increased agonist sensitivity as measured by biochemical and pharmacological responses.

By contrast it has been reported that there is not an increased sensitivity to agonist as measured by changes in inotropy and chronotropy after acute discontinuation of chronic propranolol administration both in rats and man (14). Interpretation of these experiments is open to criticism however since it is difficult

to exclude the presence of residual propranolol in the physiological preparations used for these studies.

In conclusion it has been demonstrated that chronic administration of a beta-adrenergic antagonist, propranolol, to rats is associated with significant "up-regulation" of cardiac beta-adrenergic receptor number. This regulatory effect, which is opposite to that exerted by agonist catecholamines in other systems may have important physiological and clinical implications.

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