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EFFECT OF PROPRANOLOL ON β-ADRENOCEPTORS IN RAT HEARTS

STEPHEN P. BAKER & LINCOLN T. POTTER

Department of Pharmacology, University of Miami School of Medicine, P.O. Box 016189, Miami, Florida 33101, U.S.A.

The question of whether the chronic administration of propranolol modifies the numbers or properties of cardiac β-adrenoceptors was examined because of many reports suggestive of cardiac hypersensitivity following the withdrawal of β-antagonists. In three studies, rats were given (±)-propranolol, 30 mg/day, orally or intraperitoneally, for 1 to 7 weeks. The numbers and affinities of specific binding sites for radioactive dihydroalprenolol in whole hearts from 60 control and 75 test animals were found to be the same. Thus catecholamine deprivation does not exert an important regulatory effect on most β-receptors in the heart, as it does in the brain.

Introduction Most humans who stop taking a β-adrenoceptor blocking drug are temporarily aware of heightened sympathetic nervous activity (Miller, Olson, Amsterdam & Mason, 1975). Tachycardia and tremulousness are common about two days after withdrawal, when blocking activity becomes insignificant; thereafter signs and symptoms usually diminish gradually. Patients with angina soon learn to readjust their activities to avoid pain. However, a few (perhaps 5%; review: Shand & Wood, 1978) develop serious arrhythmias and/or coronary insufficiency which appear out of proportion to the inciting stress. It has therefore been suggested that therapy with β-antagonists may lead to a compensatory or rebound state in which the heart is hypersensitive to catecholamines. In favour of such a state are reports of increased cardiac responsiveness (both chronotropic and inotropic) to isoprenaline in normal volunteers (Boudoulas, Lewis, Kates & Dalamangas, 1977) and patients (Nattel, Rangno & Van Loon, 1978) 1 to 7 days after the withdrawal of propranolol, and a preliminary report that cardiac β-adrenoceptors double in numbers in rats given propranolol for 2 weeks (10 mg/kg every 8 h, intraperitoneally) (Glaubiger & Lefkowitz, 1977). Other studies, however, do not support the idea of a hypersensitive state. Faulkner, Hopkins, Boerth, Young, Jellet, Nies, Bender & Shand (1973) found no abnormality in the inotropic responsiveness of human atria to noradrenaline 2 days after withdrawal of propranolol, nor any changes in atria from rats which had received the drug for 2 months (30 mg/kg twice a day, intraperitoneally). No change was found in systolic time intervals in man during or after propranolol (Patano & Lee, 1976), nor in cardiac responses to infusions of isoprenaline in man (Faulkner *et al.*, 1973), or dogs (Myers & Horwitz, 1978) 2 to 7 days following withdrawal of this antagonist. Given such controversial findings, we have re-examined the question of whether the administration of propranolol modifies the numbers and/or ligand-binding properties of β-adrenoceptors in rat hearts.

Methods Male Sprague-Dawley rats weighing 120 to 300 g (in groups varying <50 g) received propranolol in their drinking water (1 mM) or were given the drug in 0.9% w/v NaCl solution (saline) intraperitoneally. Ingestion of 1 mM propranolol corresponds to a daily dosage of about 30 mg/kg. Control animals received water or saline without the antagonist.

Cardiac β-receptors were assessed with a well-established binding assay as described in detail elsewhere (Baker & Potter, 1980a, b). In brief, whole hearts were homogenized in hypotonic buffer, 1 M KCl was added to dissolve contractile and other proteins, and membranes having >95% of the receptors in homogenates were sedimented by centrifugation. Membranes were resuspended in buffer and repelleted once to complete the removal of propranolol. Unless otherwise noted, binding assays were carried out in triplicate with 6 nM (−)-[2,3-^{3}H-propyl]dihydroalprenolol (DHA) in the presence and absence of 10 μM alprenolol, and the difference in mean values was taken as specific binding. This concentration of DHA saturates about 75% of the specific binding sites (see Figure 1). Assay values are not corrected to full saturation.

(±)-Propranolol hydrochloride was provided by Ayerst Labs Inc., and (±)-alprenolol hydrochloride by Hässle. (−)-[2,3-^{3}H-propyl]dihydroalprenolol (DHA, 48 Ci/mmol) was purchased from New England Nuclear Corp.

Results Three studies were performed. During the first we anticipated changes in receptors with time, and therefore studied groups of 5 test and 3 control animals at seven 1-week intervals during the oral administration of propranolol, and one week after withdrawal. To our surprise, there was no significant effect of propranolol on the binding of DHA at any time: 40 test and 24 control animals yielded mean (±s.e. mean) values of 1.5 ± 0.3 and 1.4 ± 0.2 pmol/g tissue, respectively. We then repeated the dosage schedule

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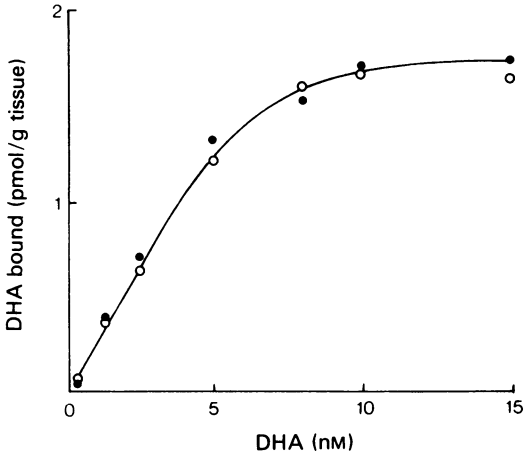

Figure 1 Effect of propranolol on the affinity of cardiac β-adrenoceptors for dihydroalprenolol (DHA). The samples were pooled membranes from 20 control rats (○) and from 20 animals that had received propranolol (30 mg/kg, i.p., per day) for 2 weeks (●).

used by Glaubiger & Lefkowitz (1977) precisely, with 20 test and 20 control animals, with the idea that intraperitoneal drug administration, or drug withdrawal for 8 h, might alter the results. Assay values were 1.3 ± 0.2 and 1.4 ± 0.3, respectively. Pooled test and control membranes from these animals were also used to assess specific binding with 0.6 to 15 nM DHA. The results shown in Figure 1 graphically demonstrate that propranolol does not alter either the overall number or the affinity of β-adrenoceptors. Analysis of these data by the method of Scatchard (not shown) yielded a dissociation constant for DHA of ~3 nM, in keeping with its known pharmacological activity and previous results (Baker & Potter, 1980a, b). One further study was carried out in which 15 test and 16 control rats were kept for 1 to 2 weeks in constant dark, to promote their activity, the ingestion of propranolol throughout each 24 h period, and doubled turnover of cardiac catecholamines (Lemmer & Saller, 1974). Again there was no significant change in β-receptors. In summary, 75 test and 60 control animals yielded the same mean assay values: 1.4 ± 0.3 pmol/g tissue.

Discussion The administration of high doses of propranolol to rats for weeks clearly does not cause major changes in the overall numbers of cardiac β-receptors or their affinity for DHA. These results are of interest in two contexts. First, they do not support the idea that changes in most cardiac β-receptors contribute to propranolol withdrawal phenomena. The 'propranolol withdrawal syndrome' in man (Shand & Wood, 1978) may be due simply to the way patients re-set their activities during the administration of β-antagonists. Alternatively a true 'hyper-adrenergic' cardiac state could result from other factors, including increased release of catecholamines, decreased amine uptake or metabolism, and/or modification in the production, levels and effects of cyclic adenosine 5'-monophosphate. Post-propranolol tests of these factors are still needed. Propranolol-induced changes in a small population of cardiac receptors, e.g. those in the sinus node or coronary arteries, also remain to be excluded. The difference between the present results and those of Glaubiger & Lefkowitz (1977) may be due to the fact that cardiac microsomes were assayed in the earlier study. In our hands such membrane preparations have only 5 to 10% of the β-receptors present in homogenates or our pellets. Conceivably a propranolol-sensitive fraction of the total membrane population was selected previously.

Second, and of considerable general interest, the present results show that prolonged catecholamine deprivation does not exert an important regulatory effect on most cardiac β-receptors. This result was unexpected, since it is well established that the number of nicotinic receptors in skeletal muscles varies inversely with the level of acetylcholine at synapses, and that β-receptors in the brain increase after adrenergic denervation, and decrease with imipramine (Molinoff, Sporn, Wolfe & Harden, 1978). We suspect that the lack of change of most cardiac β-receptors in response to catecholamine deprivation is a consequence of their diffuse distribution over myocardial cells, away from adrenergic nerves (Baker & Potter, 1980a). At such sites, cellular growth and surface area appear to be more important determinants of receptor numbers (Baker & Potter, 1980b) than catecholamine levels.

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