External Transport of Beta-Adrenergic Binding Sites in Ischemic Myocardium

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Abstract. The properties of beta-adrenergic receptors were studied in normal and in flow restricted regions of the dog heart. Purified cardiac membrane preparations and papillary muscle preparations were isolated from control and ischemic areas and tested a) following chronic beta-receptor blockade with metipranolol or exaprolol, and b) after acute regional myocardial ischemia. A significant reduction in the sensitivity of the heart muscle preparations from compromized heart for isoprenaline resulting in a reduced affinity of beta--adrenergic receptors to exaprolol was observed. Quantitative ligand binding data showed higher numbers of (^{3}H) dihydroalprenolol /(^{3}H) DHA/ binding sites in the membrane fraction obtained from compromized compared to control myocardium. The ratio of intra- to extracellular beta-adrenergic receptors decreased from 1.35 to 0.55 in the membrane fractions obtained from the compromized hearts. Pretreatment of experimental animals with metipranolol or propranolol attenuated the observed increase in the total number of beta-adrenergic receptor sites in myocardial membrane fractions from ischemic hearts. These data suggest preferential distribution of beta-adrenergic binding sites from intracellular to membrane fractions in flow restricted regions of the dog heart after coronary occlusion.

Key words: Beta-adrenoceptors — Myocardial membranes — Radioligand binding — Myocardial ischemia

Introduction

Both the number and affinity of beta-adrenergic receptors in the heart are capable to downregulate in the response to beta-adrenergic agonists (Minneman et al. 1979; Torda et al. 1981). Recent studies have shown that myocardial ischemia is also associated with a change in beta-adrenergic receptors (Mukherjee et al. 1979; Maisel et al. 1985). We report herein changes in the distribution

of the membrane binding sites and in the characteristics of beta-adrenergic receptors in the heart muscle after exposure to regional myocardial ischemia. The aim of our study was to identify the membrane binding sites in the heart muscle and to obtain evidence for specific membrane distribution of beta-adrenergic receptors in the dog heart under conditions of acute ischemic injury and chronic administration of beta-adrenergic receptor blocking drugs.

Materials and Methods

Experiments were carried out on papillary muscles isolated from young dogs anesthetized with pentobarbital (30 mg . kg^{-1} intravenously) and heparinized (300 i.u. Heparin Spofa).

Isolated heart muscle preparation

After rapid removal, the papillary muscles were placed in a 10 ml bath containing Krebs-bicarbonate solution (PSS) of the following composition (in mmol . l^{-1}): NaCl 113.0; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.18; $MgSO_4$ 1.17; $NaHCO_3$ 20.0; glucose 10.0. The solution was equilibrated with 95% O_2 and 5% carbon dioxide. The temperature of PSS was 27 °C in control and 37 °C in compromized preparations. The muscle length ranged from 8—11 mm and the calculated cross-sectional area was 0.9—1.2 mm^2. Stimulation was accomplished using punctate platinum electrodes placed parallel to the muscle. A Disa-Multistim Stimulator was used and pulses of 3 ms duration and voltage not exceeding 20 % above the threshold (usually from 1 to 1.5 V) were applied at the rate of 12/min. The method used to assess the effects of inotropic interventions has been described in detail elsewhere (Dřímal and Boška 1973). Briefly, the tendinous end of the muscle was attached to an RCA-force displacement transducer and isometric force was recorded on an oscillograph Elema. The tension was differentiated electronically to provide the rate of tension development (dF/dt). Chronic pretreatment of experimental animals with metipranolol or propranolol included two daily doses of 0.1 mg . kg^{-1} intravenously each for 7 consecutive days. Isoprenaline was added to the bathing solution in 9 cumulative concentrations in a range 1 μmol . l^{-1} — 0.1 mmol . l^{-1}. The antagonist was added in 3 concentrations in 30 min intervals. In 24 experiments with anesthetized dogs with open thoracic cavity myocardial ischemia was produced by the occlusion of the descending anterior branch or the circumphlexus branch of the left coronary artery (30 min) with subsequent reperfusion (Dřímal 1983). Specimens of myocardial muscle, mostly papillary muscles and trabeculae, were isolated from the marginal zone of the regional myocardial ischemia and from the posterior part of the left ventricle supplied by the nonoccluded coronary artery.

Myocardial membranes

After removal of fat, connective tissue and the great coronary vessels, the myocardial sample was minced and placed quickly in 50 mmol . l^{-1} buffer (Tris-HCl, pH 7.55, at 4 °C). The sample was homogenized in 35 ml buffer with Ultraturrax at setting 8 for 30 s, and then the procedure was repeated after one minute of cooling. The homogenate was centrifuged further at 1500 × g for 10 min. at 4 °C. The supernatant was further centrifuged at 105,000 × g for 40 min. The resultant pellet was resuspended in assay buffer (50 mmol . l^{-1} Tris-HCl, 5 mmol . l^{-1} $MgCl_2$; pH = 7.55) and

the membranes, approximately 0.20 mg of protein, were incubated for 20 min. at 25 °C with (^{3}H) dihydroalprenolol in a total volume of 400 μl. From the supernatant a light membrane fraction was prepared that sedimented in sucrose buffer at 130,000 × g (8—60% sucrose gradient, according to Kwan et al. (1983). On electron microscopy these light membranes were represented by vesicles, functionally these fractions were nonresponsive to isoproterenol-stimulated adenylate cyclase and quanylimidodiphosphate.

Nonspecific binding of (^{3}H) DHA was defined as the radioactivity bound to membrane protein which was not displaced by a high concentration of the beta-antagonist propranolol. Incubations were terminated by diluting the complete incubation mixture with ice-cold incubation solution followed by rapid filtration through Whatman GFC glass filters. Origin of drugs and chemicals: (^{3}H)4,6propyl-(^{3}H)Dihydroalprenolol (Specific activity 70 Ci/mmol) was obtained from Amersham (England), (—) exaprolol was from Institute for Drug Research, Modra, propranolol-hydrochlorid was a gift from ICI Macclesfield (England), N-isopropyl-DL-Noradrenaline- hydrochloride was from Fluka AG, Buchs (Switzerland). All other chemicals were of the highest chemical grade available.

Results

Physiological and Functional Studies

Baseline values before isoprenaline administration were similar in both groups (Table 1). In both groups (control and compromized papillary muscle preparations) isoproterenol increased the force and velocity of isometric contraction and reduced the duration of the active state. The response to isoproterenol in the presence of (—) exaprolol was markedly reduced in both groups. Parallel shifts to the right of isoproterenol concentration — response curves were observed in these experiments (Fig. 1). Analysis of the results (Schild 1949) showed that (—) exaprolol yielded a control ventricular muscle pA_2 value of 8.29 ± 0.02, and a slope not significantly different from 1. In papillary muscle preparations obtained from compromized hearts a pA_2 value of 8.29 ± 0.01 and a slope of 0.96 were observed after (—) exaprolol. The of the difference between control and compromized tissue was 4.06, indicating a slight change in the potency of the compound in the compromized myocardium.

Table 1. Functional parameters of isolated papillary muscle preparations

Parameter	Control	Compromized[1]
DAS[2] (ms)	630 ± 45	450 ± 34
F_{max} (mN)	10.96 ± 1.50	7.50 ± 1.08

[1] Coronary occlusion (30 min) and reperfusion (20 min) of the *r. interventricularis anterior* of the left coronary artery in situ, analysis in vitro.

[2] Duration of the active state of isolated heart muscle (papillary muscle preparations, 0.7 Hz, 3 ms, 1.2 V; stabilization for 60 min.

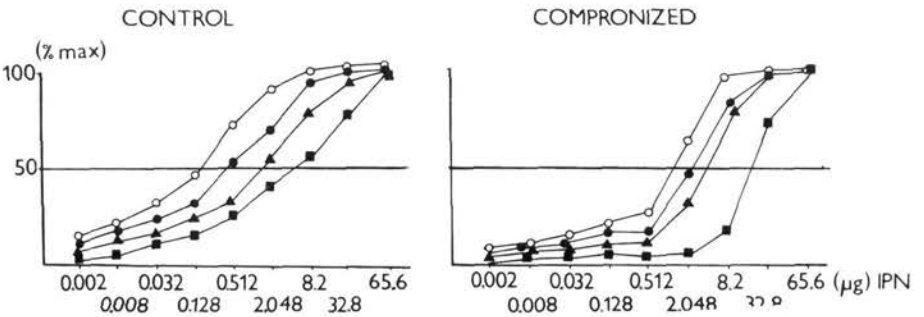

Fig. 1. Isoproterenol concentration-response curves in isolated dog papillary muscle preparations ($n = 40$) in the absence and in the presence of three increasing concentrations of the antagonist (—) exaprolol in control ($t = 27\,°C$) and in compromized ($t = 37\,°C$) tissue. Abscissa: concentration of isoproterenol (μg), ordinate: the percentage of maximal increase in force of isometric contraction. The increase in velocity of contraction measured as dF/dt (see Methods) produced by isoproterenol in isolated muscle preparations was similar to that in force. ○ — control, ● — 3×10^{-8} mol/l (—) exaprolol, ▲ — 3×10^{-7} mol/l (—) exaprolol, ■ — 3×10^{-6} (—) exaprolol, $N = 20$.

Binding Assays of Beta-Adrenoceptors

(^{3}H) DHA bound to the heart membranes in a saturable manner and with a high affinity to a single binding site (Fig. 2). Specific receptor binding of (^{3}H) DHA was defined as binding displaceable by 5.0 μmol.l^{-1} of propranolol. Quantitative ligand binding experiments in native myocardial membranes obtained from control (top) and from metipranolol pretreated hearts (bottom) showed saturability of specific binding and a high affinity. Scatchard analysis (Scatchard 1949) of saturation isotherms yielded a dissociation constant ($K_D = 12.5$ nmol.l^{-1} and a total number of binding sites (B_{max}) = 1035 ± 80 fmol.mg^{-1} protein in control, and $K_D = 11.2$ and $B_{max} = 1020 \pm 60$ fmol.mg^{-1} of protein in propranolol-pretreated dog heart.

Inhibition of (^{3}H) DHA Binding to Myocardial Beta-Adrenergic Receptors by Propranolol

Specific binding of (^{3}H) DHA to membranes of dog myocardium was inhibited by beta-adrenoceptor antagonists (Fig. 3). Propranolol displaced (^{3}H) DHA from the binding site with a K_i value of 2.69 nmol.l^{-1}, consistently with the above results of Scatchard analysis for this ligand. The apparent Hill slope ($_nH$) was 0.983 in the normal and 1.103 in the ischemic dog heart. These results

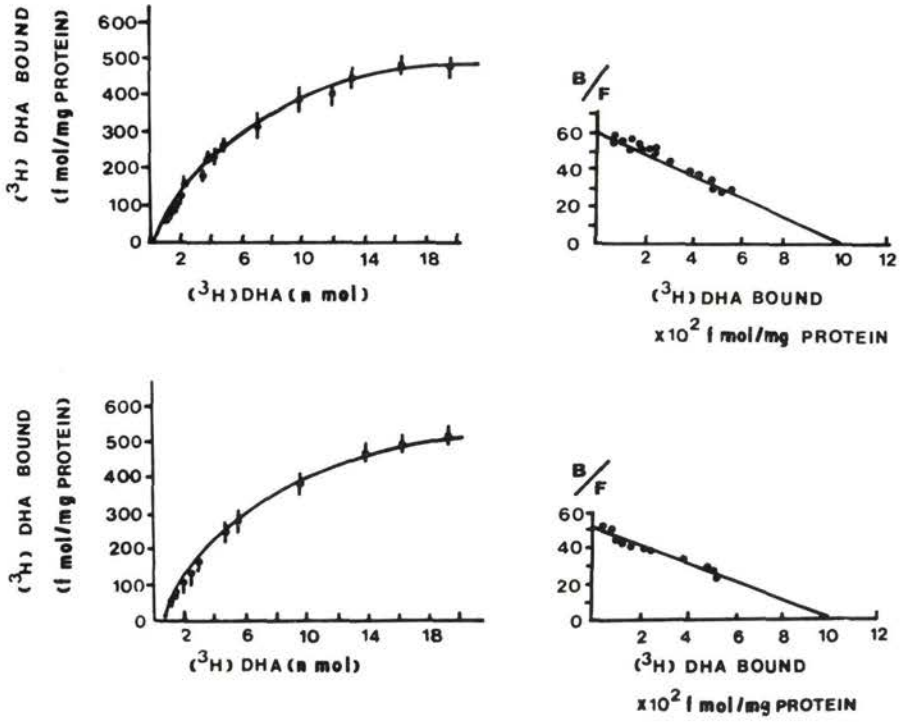

Fig. 2. Binding of (^{3}H) DHA to membrane preparations from control dog hearts (*top*) and from hearts obtained from chronically pretreated animals (*bottom*). Specific binding (fmol/mg of protein) plotted by the method of Scatchard (1949). Points represent Mean ± SEM by triplicates from five experiments in each group. Control — K_D = 12.5 n mol, B_{max} = 1035 ± 80 fmol/mg protein. Pretreated — K_D = 11.2 nmol, B_{max} = 1020 ± 60 fmol/mg protein.

suggest a common high affinity site of action for both the (^{3}H) ligand used and for propranolol. Propyl-4/(2-hydroxy-3-isopropylamino)-propoxy/carbanilate-hydrochloride (BL-343-A) also inhibited (^{3}H) DHA binding; however, the calculated Hill coefficients for this drug were below one ($0.58P < 0.05$) suggesting the presence of more than one class of binding sites and a possible partial agonist activity of this compound.

Additional experiments were also performed with (^{3}H) DHA isoprenaline as well as BL-343-A. In all cases the steepness factors of the competition curves were indistinguishable from those obtained in previous group of experiments. The heterogeneity of binding was apparent when BL-343-A was used as competitor. Nonlinear regression analysis of inhibition curves for this drug gave K_D values significantly different from those for propranolol. In order to explain the reduced sensitivity of the ischemic dog myocardium to isoprenaline, and also to

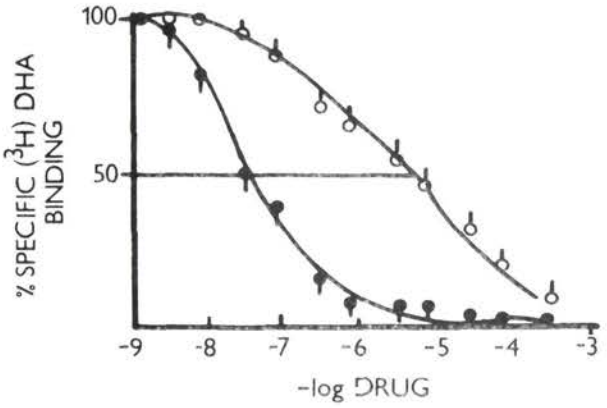

Fig. 3. Competition by propranolol a beta-adrenoceptor antagonist, and a carbanilate derivative BL-343-A with specific (^{3}H) binding, in dog myocardial membranes obtained from normal heart. (^{3}H) DHA was incubated in the absence and in the presence of twelve different concentrations of the antagonist propranolol (●) and BL-343-A (○). Nonspecific binding was determined as (^{3}H) DHA binding remaining in the presence of 5 μmol $.l^{-1}$ or propranolol. Each point represents the Mean ± SEM of at least five experiments performed in triplicate. Maximum specific binding (%) represents the binding of the antagonist.

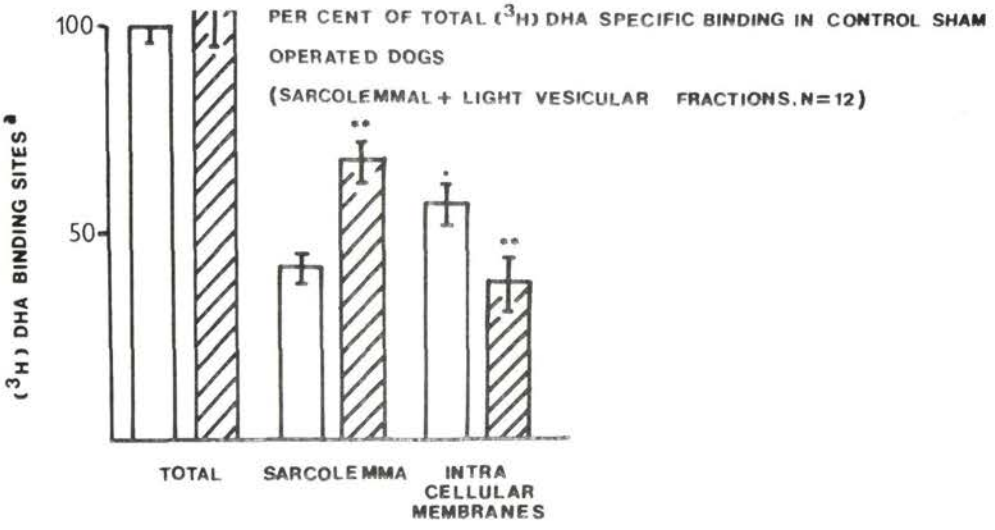

Fig. 4. Identification of (^{3}H) DHA binding sites in the membrane and the light vesicular fraction of dog myocardium. Total (^{3}H) DHA binding: B_{max} in the sarcolemmal and B_{max} in the corresponding light vesicular fraction. Control (□) assays in samples obtained from area of heart muscle of sham operated dogs; Compromised (▨) assays in samples obtained from area of heart muscle subjected to coronary occlusion and reperfusion. Note the preferential distribution of binding sites to sarcolemmal fractions in compromised myocardium ($n = 12$).

beta-adrenergic receptor blockade in physiological studies with (^{3}H) DHA, we identified the beta-adrenergic binding sites in two functionally distinct fractions of the dog myocardium obtained from both control and compromized hearts. In these experiments binding assays were routinely carried out with the purified membrane and with light vesicular, presumably intracellular, fractions. The total number of beta-adrenergic binding sites was proportionally distributed in the membrane and in the intracellular fraction in the control heart, but a preferential distribution to the membrane compared to the intracellular fraction was observed in the membranes obtained from the compromized myocardium (B_{max} = 1680 ± 216 and 928 ± 120 fmol . mg^{-1} protein for purified membrane and intracellular fraction respectively). There was no difference between B_{max} in the membrane and in the light vesicular fractions obtained from hearts of dogs chronically pretreated with the highly lipofilic beta-adrenergic receptor blocking compound metipranolol (Table 2).

Table 2. The total number of beta-adrenergic receptor sites (B_{max}) in the membrane and light vesicular fractions in chronically pretreated ischemic hearts.

	Membrane Fraction	Intracellular Vesicles
B_{max}	487 ± 95	530 ± 70

Values are Mean ± SEM of three experiments in triplicate determinations. Pretreatment with metipranolol 0.1 mg . kg^{-1} intravenously twice a day, for five days.

Discussion

The present results show that the properties of (^{3}H) DHA binding to both the membrane and the intracellular fractions of the dog myocardium was such as can be expected for an interaction with beta-adrenergic receptors. The results of functional studies on isolated papillary muscle preparations and also ligand binding data to two functionally different membrane fractions of the dog myocardium suggest a shift in the prevalence of distribution of beta-adrenergic binding sites and possibly also functional beta-adrenergic receptors from the intracellular to the membrane fraction in the ischemic heart. The values of K_D and B_{max} for (^{3}H) DHA (binding obtained in the present study for myocardial membranes from the dog heart are in agreement) with reported data obtained under similar experimental conditions but in other species (Schumann and Brodde 1979; Venter 1982). As compared to papers by Maisel et al. 1985 and Torda et al. 1981, the total number of beta-adrenergic receptors per mg of membrane protein in compromized dog heart appears to be higher than that in hearts of stressed guinea pigs or rats. It may be objected that the decreased

sensitivity of the compromized myocardium to isoprenaline observed in our functional studies is in contradiction to the total binding of (^{3}H) DHA to the membrane fraction after ischemia. This would indicate that the observed increase in the total number of binding sites in the membrane fraction may not be directly related to the increase in functional beta-adrenergic receptors recycling from the receptosomes shortly after ischemia and reperfusion. Our finding of a marked reduction of the distribution of (^{3}H) DHA binding to the membrane fraction after pretreatment with propranolol or metipranolol may suggest recycling of beta-adrenergic receptor sites and short term regulations in the myocardium.

Whether after recycling these receptors are coupled to adenylate-cyclase and are "functional" in the broadest sense remains to be established.

Finally, our saturation studies with the use of propranolol suggested the presence of a single component of low affinity binding, and are in agreement with the data of our competition studies and with Hill coefficients typical of beta-antagonists. This provides a rational basis for the identification of outward transport of beta-adrenergic binding sites in the ischemic dog myocardium representing one of the important mechanisms of functional adaptation of the heart.

Recent studies by Devos et al. (1985) have shown a defective enzymatic system and functional depletion of guanine nucleotide-binding regulatory protein (N_S or G protein), in the dog myocardium 5h after ligation of the coronary artery. At present the possible physiological significance of functional depletion in the G protein participating in the control of adenylate cyclase for the recycling of beta-adrenergic receptors is not clear. We can only hope that future research will furnish basis for explanation and interpretation of mechanisms involved in the participation of internal components of the membrane, or specific membrane proteins in the turnover of beta-adrenergic receptors in compromized and chronically pretreated heart.

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