Autoradiographic Characterization of Beta Adrenergic Receptors in Coronary Blood Vessels and Myocytes in Normal and Ischemic Myocardium of the Canine Heart

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Abstract. This light microscopic autoradiographic study was performed to test the hypotheses that (*a*) the density of beta adrenergic receptors (BAR) may differ in various components of the heart and (*b*) BAR in certain components of the heart may exhibit a selective response to pharmacologic and pathological stimuli. Blocks of canine left ventricle were frozen and tissue sections cut and incubated in $(-)[^3H]$dihydroalprenolol (DHA) to label the BAR. For total and nonspecific binding, serial sections were incubated with and without 10^{-5} M ($\pm$)propranolol. Scintillation spectrometry of sections demonstrated rapid binding, saturability, stereospecificity, a dissociation constant (K_D) of 3.2 ± 0.5 nM (SD) (*n* = 3), and a maximal binding of 31.3 ± 3.1 fmol/mg of tissue protein. Isoproterenol was 12.5 times more effective than norepinephrine in displacing DHA. Sections incubated with 10^{-5}–10^{-8} M metoprolol, a beta one selective antagonist, demonstrated a K_D of 0.7×10^{-6} M. For autoradiography, emulsion-coated coverslips were attached to the slides. After exposure, the slides were developed and stained, and grain density quantified. Specific BAR binding (*n* = 4 dogs) was $1,047\pm131$ (SEM) grains/10^{-2} mm^2 for myocardial arterioles, 219 ± 30 for myocardial arteries, 31 ± 12 for the proximal left anterior descending coronary artery (LAD), and 231 ± 34 for cardiac myocytes. Specific binding in the presence of 10^{-5} M metoprolol was reduced $\sim75\%$ for both arterioles and myocytes. However, at 10^{-6} M metoprolol, the percent reduction in specific DHA binding was greater for myocytes (50%) than for arterioles (0%), and at 10^{-7} M metoprolol, the percent reduction in specific DHA binding was 17% for myocytes with no reduction over arterioles. After 1 h of LAD occlusion, a selective increase (18%) in BAR density occurred over cardiac myocytes, but not over blood vessels in the ischemic myocardium. Thus, (*a*) specific BAR binding was five times greater in arterioles than in small arteries and myocardium and 34 times greater than in the proximal LAD; (*b*) BAR of myocytes were more sensitive than those of arterioles to displacement by the beta one selective antagonist, metoprolol; and (*c*) a selective increase in BAR occurs in cardiac myocytes but not in blood vessels after 1 h of ischemia in this experimental model.

Introduction

Catecholamines exert their physiological and pathophysiological effects by binding to specific catecholamine receptors. Physiological and radioligand binding studies have indicated that the heart has populations of beta one and beta two receptors (1, 2), and alpha one receptors (3). With regard to the localization of these receptors, studies as early as 1948 suggested that changes in myocardial contractility appear to be mediated primarily by the beta receptor, whereas changes in coronary blood vessels can involve alpha and beta receptors (4).

Physiological and radioligand binding studies have advanced the understanding of the beta adrenergic system in various pathophysiological states. These studies have documented changes in myocardial beta adrenergic receptor number with chronic beta adrenergic blockade, thyroid disorders, congestive heart failure, and acute ischemia (5, 6). Furthermore, the changes in receptor number have been correlated with changes in tissue responsiveness to catecholamines (5, 6).

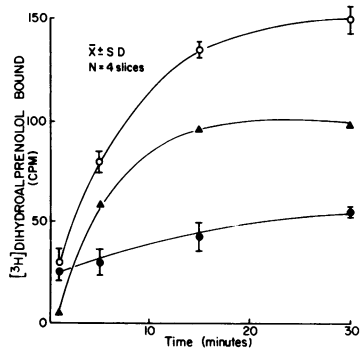*Figure 1.* Total (○), specific (▲), and nonspecific (●) binding of 2 nM (−)[³H]DHA as a function of incubation time. Specific binding reached equilibrium at ~15 min. Nonspecific binding was determined by incubation of sections in [³H]DHA in the presence of 10^{-5} M (±)propranolol. Each point is derived from four tissue slices.

The standard radioligand binding assays are performed on tissue homogenates which are prepared from relatively large amounts of tissue and contain membranes from several cell types. These limitations have stimulated the search for autoradiographic and histochemical techniques to localize the beta-adrenergic receptor (7–9). Recently, the autoradiographic localization of tritiated isoproterenol was reported in rat heart (10). However, no attempt was made to prevent diffusion of the ligand during histological processing, and because the ligand was injected, no correlation with biochemical data could be obtained. We used light microscopic autoradiography with levo-

tritiated dihydroalprenolol (DHA),[1] (−)[³H]DHA, and a highly specific beta adrenergic antagonist (5) to study the distribution of beta adrenergic receptors over cardiac myocytes and blood vessels in the canine heart, using a technique that minimizes diffusion of the ligand and can be validated biochemically (7, 8). We also wanted to determine whether pharmacological and pathological interventions would produce selective alterations in beta receptors over various tissue components of the heart which could be detected by this method. We chose the beta one selective antagonist metoprolol as a pharmacological intervention and 1 h of myocardial ischemia as a pathological intervention. These studies were based on the hypotheses that (*a*) the density of beta adrenergic receptors may differ in various components of the heart and (*b*) beta adrenergic receptors in certain components of the heart may exhibit a selective response to pharmacological and pathological stimuli.

Methods

Tissue preparation. Mongrel dogs were anesthetized with sodium pentobarbital and placed on a respirator. The chest was opened and the

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1. *Abbreviations used in this paper:* BAR, beta adrenergic receptor; CMC, cardiac muscle cell; DHA, dihydroalprenolol; K_D, dissociation constant; LAD, left anterior descending coronary artery; MA, myocardial artery.
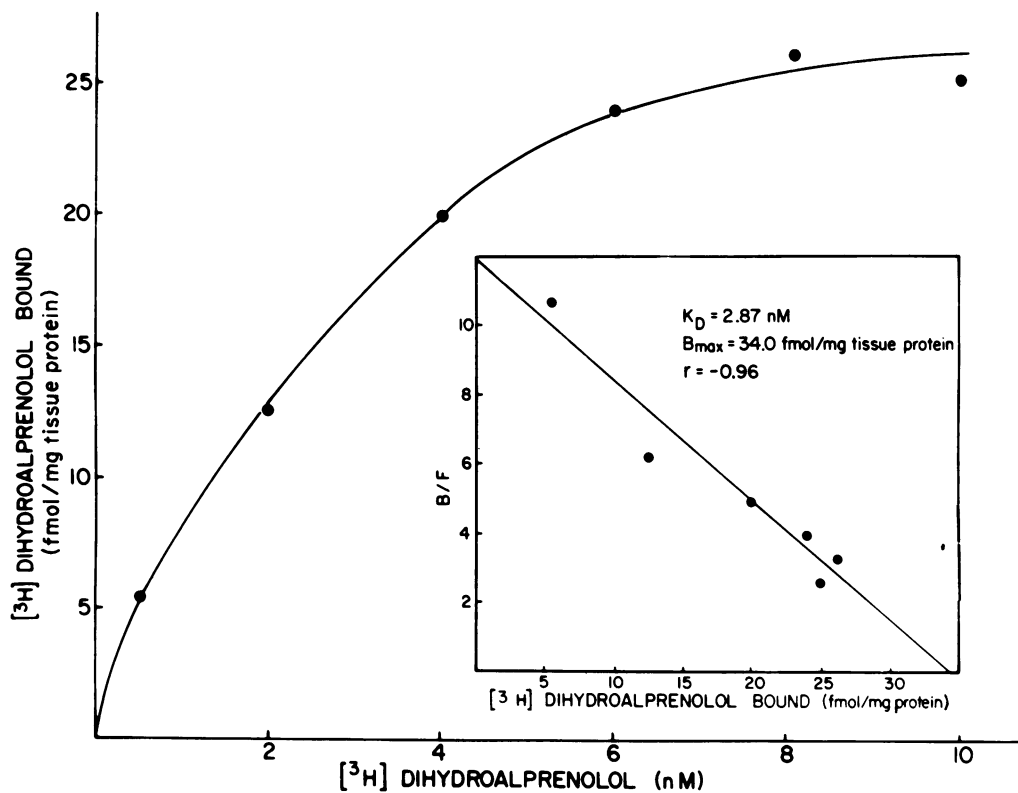

Figure 2. This is a representative curve which plots specific binding of [³H]DHA to tissue sections of dog myocardium as a function of increasing concentration of [³H]DHA. Specific binding is defined as (−)[³H]DHA binding displaceable by 10^{-5} M (±)propranolol. Specific binding ranged from 77% of total binding at 0.5 nM [³H]DHA, to 32% of total binding at 10 nM [³H]DHA, decreasing as the concentration of [³H]DHA increased. *Inset:* Scatchard plot of [³H]DHA binding. The ratio (B/F) of bound [³H]DHA (femtomole per milligram tissue protein) to free [³H]DHA (nM) is plotted as a function of the [³H]DHA bound/milligram tissue protein.
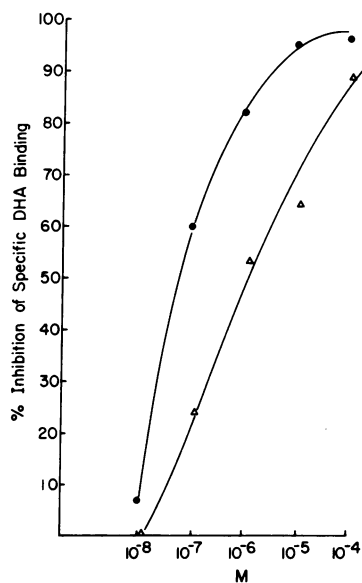
Figure 3. The percent inhibition of specific [³H]DHA binding (2 nM) as a function of increasing concentrations of (−)isoproterenol and (−)norepinephrine. Isoproterenol is 12.5 times more effective at displacing [³H]DHA than norepinephrine. ●, Isoproterenol $IC_{50} = 8 \times 10^{-8}$; △, norepinephrine $IC_{50} = 1 \times 10^{-6}$.

heart removed and placed on ice. In one series of experiments, the proximal left anterior descending coronary artery (LAD) and several branch vessels were ligated to create an area of intense cyanosis in the anterior left ventricular wall. After 1 h of ischemia the heart was removed.

Blocks of left ventricular myocardium, 3 mm thick, were quickly obtained from the hearts. A block also was taken from the LAD, 2 cm distal to the ostium of the left coronary artery. Routinely, the blocks were fixed in 0.1% paraformaldehyde in phosphate-buffered saline for 30 min at 0–2°C, and then were snap frozen in a slurry of isopentane cooled in liquid nitrogen. In some experiments, blocks of tissue were frozen without prior fixation. The blocks were stored for one or more days in liquid nitrogen before processing. Tissue sections were cut 10 μm thick in a cryostat microtome and thaw-mounted on acid-washed, subbed slides.

Receptor binding. The slides with tissue sections were incubated at room temperature for 30 min in 0.17 M Tris-HCl buffer, pH 7.4, with 10 mM $MgCl_2$. The sections were incubated with nanomolar concentrations of tritiated (−)DHA, (−)[³H]DHA (34.1–49.4 Ci/mM, New England Nuclear, Boston, MA), in the presence or absence of various other ligands. In different experiments these ligands were: 10^{-5} M (±)propranolol, 10^{-10}–10^{-5} M (−)propranolol, 10^{-10}–10^{-5} M (+)propranolol, 10^{-5} M phentolamine, 10^{-8}–10^{-4} M (−)isoproterenol, 10^{-8}–10^{-5} M (−)norepinephrine, and 10^{-8}–10^{-4} M metoprolol. After incubation with ligands, the slides were washed twice in ice-cold buffer for two 10-min periods, then quickly dipped in distilled water and dried.

In biochemical experiments, the sections were dried, scraped from the slides with a razor blade, placed in scintillation vials, and 5 ml of scintillation fluid added. Four tissue slices were used for each determination. Radioactivity was measured in a liquid scintillation counter. Binding curves and Scatchard plots were generated by standard techniques (5). Tissue protein was measured according to the method of Lowry et al. (11). In displacement experiments, the IC_{50} was derived from the curve, and the dissociation constant (K_D) was calculated according to the method of Cheng and Prusoff (12).

Autoradiography. Acid-washed coverslips were coated by dipping into NTB 2 emulsion (Eastman Kodak Co., Rochester, NY) (diluted 1:1 with water) at 43°C, air dried for 3 h, and stored over desiccant. The emulsion-coated coverslips were attached to the slides with tissue sections in the dark with glue (Super Glue No. 3., Loctite Corp., Cleveland, OH), which was placed on one end of the slide. After the glue set, squares of Teflon (⅛ in. thick) were put on top of the coverslips and the assemblies were held together with No. 20 binder clips. The assemblies were stored with desiccant at 2–4°C.

After several weeks exposure, the autoradiographs were developed. The binder clips and Teflon were removed in the dark, the coverslips were gently bent away from the tissue sections, and a rubber spacer was placed between the slide and coverslip. The emulsion was developed in D19 (1:1 with water) for 4 min at 18°C, rinsed with deionized water for 15 s, fixed in Kodak fixer (Eastman Kodak Co.) for 5 min, and rinsed in distilled water for 20 min. The tissues were fixed in Carnoy's solution and stained with Meyer's hematoxylin and eosin. The spacers were removed after the tissues had dried and the coverslips were mounted with Permount (Fisher Scientific Co., Pittsburgh, PA).

Autoradiographic grain distribution was analyzed with a Zeiss microscope (Carl Zeiss, Inc., New York) at a magnification of 1,250 using a square grid subdivided into 400 smaller squares. Five large grid areas were analyzed over the subendocardial myocytes, the subepicardial myocytes, and the media of the proximal LAD. The number of squares of the grid into which the wall of a small artery (>55 μm o.d.) or an arteriole (<55 μm o.d.) fit was recorded, and the grains in that area were counted. All arterioles with a definite smooth muscle layer and all small arteries in a tissue section were counted. This was an average of nine arterioles, and three small arteries per tissue section. Nonspecific grain density over serial sections from the same block incubated in (−)[³H]DHA plus 10^{-5} M (±)propranolol was subtracted from total grain density over sections incubated in (−)[³H]DHA to determine the grain density specific for binding to beta adrenergic receptors.

Statistics. The t test, analysis of variance, and the Kruskall-Wallis test were used as appropriate (13).

Results

Biochemical characteristics of receptor binding. The slide incubation, tissue scraping, and scintillation counting procedures were used to obtain biochemical data regarding receptor binding with the technique used for autoradiography. Binding of (−)[³H]DHA to the tissue sections reached equilibrium after 15 min of incubation at room temperature (Fig. 1). The binding was not affected by brief fixation of tissue in 0.1% paraformaldehyde. Binding curves indicated a saturable binding process (Fig. 2). Data were obtained by incubating sections in 0.5–10 nM (−)[³H]DHA in the presence or absence of 10^{-5} M (±)propranolol. With Scatchard analysis, the K_D for (−)[³H]DHA binding was 3.2±0.5 nM (SD) ($n = 3$ experiments), and the maximal binding (B max) was 31.3±3.1 fmol/mg tissue protein (Fig. 2, inset). Specific binding ranged from 77% of total binding at 0.5 nM [³H]DHA to 32% of total binding at 10 nM [³H]DHA. Specific binding decreased as the concentration of [³H]DHA increased. For most experiments, a concentration of 2 nM [³H]DHA was chosen, since it provided a relatively high concentration and relatively high specific binding (∼70%).

Slide-mounted tissue sections were incubated with varying concentrations (10^{-4}–10^{-10} M) of (+) and (−)propranolol in the
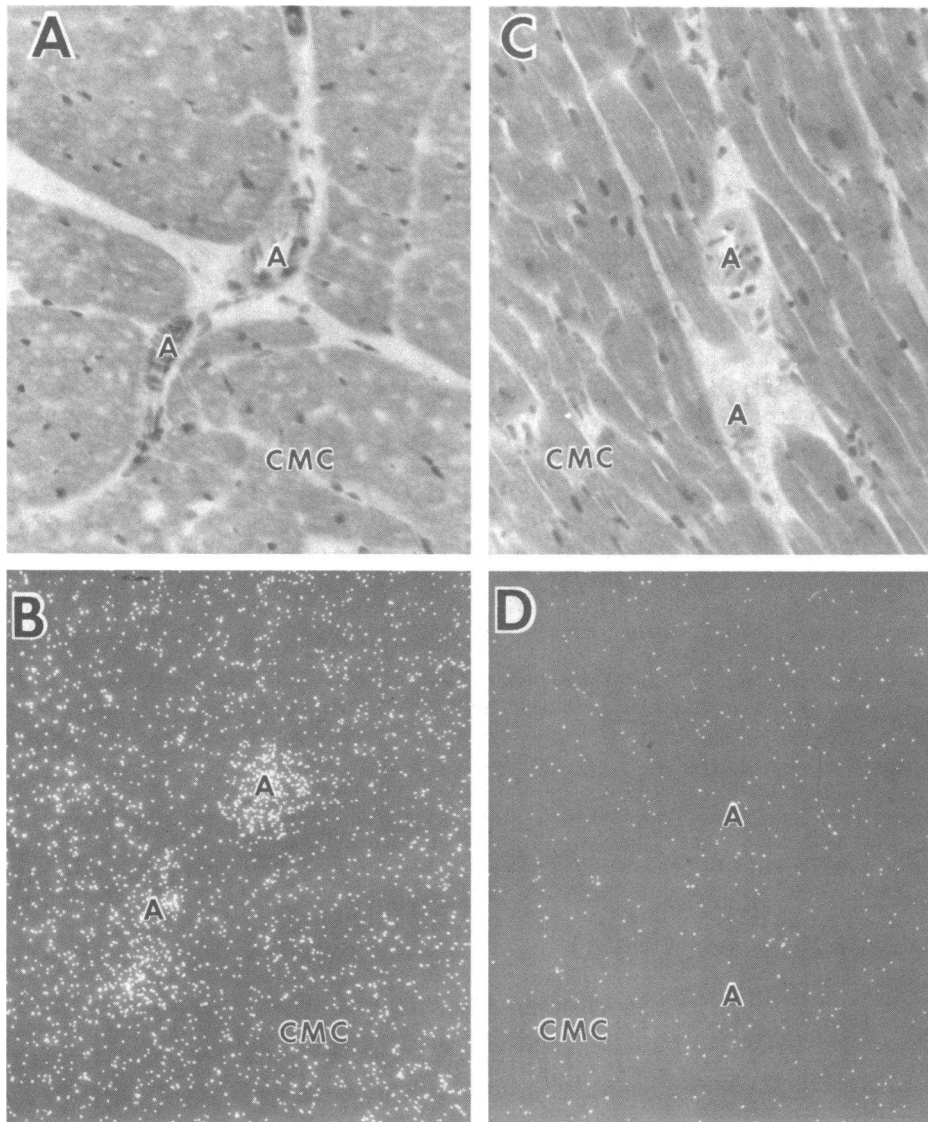
Figure 4. Photomicrographs of [^{3}H]DHA autoradiographs taken at 200 $\times$ magnification. Pictures on the top are taken with a bright-field condensor and pictures on the bottom are the same fields taken with a dark-field condensor. (*A* and *B*) A tissue section incubated with 2 nM [^{3}H]DHA (i.e., total binding). Note the increased grains over the arterioles (*A*) when compared with cardiac muscle cells (CMC). (*C* and *D*) A serial section incubated with [^{3}H]DHA in the presence of 10^{-5} M ($\pm$)propranolol (i.e., nonspecific binding). Note the reduction in grain density and the equal distribution of grains over myocytes and arterioles.

presence of 2 nM ($-$)[^{3}H]DHA. The ($-$)propranolol was over 100 times more effective (IC$_{50}$ = 9 $\times$ 10^{-10}) than the (+)propranolol (IC$_{50}$ = 1 $\times$ 10^{-7} M) in displacing ($-$)[^{3}H]DHA, indicating that the method for labeling beta-adrenergic receptors shows stereospecificity. In another experiment, no displacement of 2 nM ($-$)DHA was measured in the presence of 10^{-5} M phentolamine, an alpha adrenergic antagonist.

Tissue sections were incubated with varying concentrations (10^{-4}–10^{-8} M) of ($-$)isoproterenol and ($-$)norepinephrine in the presence of 2 nM ($-$)[^{3}H]DHA (Fig. 3). Isoproterenol was 12.5 times more effective at displacing ($-$)[^{3}H]DHA (IC$_{50}$ = 8 $\times$ 10^{-8}) than norepinephrine (IC$_{50}$ = 1 $\times$ 10^{-6}), in accordance with binding characteristics of the beta adrenergic receptor (4).

Sections also were incubated with 10^{-5}–10^{-8} M metoprolol, a beta one selective antagonist, in the presence of 2 nM ($-$)[^{3}H]DHA. Metoprolol exhibited a K_D of 0.7 $\times$ 10^{-6} M.

Autoradiographic localization of ($-$)[^{3}H]DHA binding sites. Autoradiographic grains appeared to be distributed uniformly over cardiac myocytes in normal canine myocardium (Figs. 4 and 5). Arterioles were heavily labeled when compared with the myocytes. Serial sections incubated with [^{3}H]DHA in the presence of propranolol had fewer grains, and grains were equally distributed over myocytes and arterioles (Fig. 4). Tritiated DHA binding could not be localized to the sarcolemma or any other subcellular organelle. However, there were obvious demarcations between grain density over myocytes, connective tissue, and areas of the slides without tissue (background). Specific binding was similar over cardiac myocytes in the subepicardial (208$\pm$29
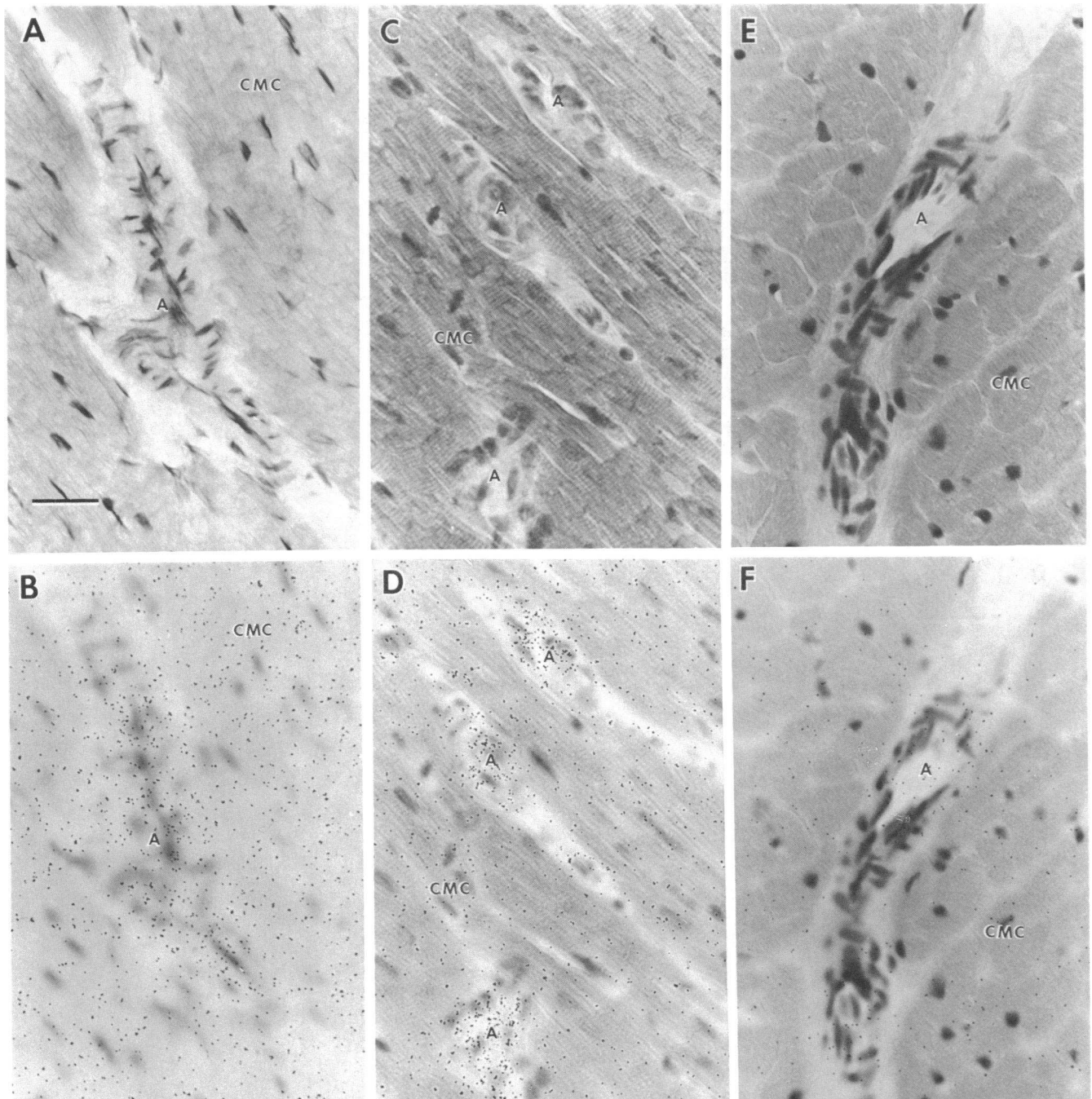
Figure 5. Photomicrographs of [³H]DHA autoradiographs taken at 750 × magnification. Pictures on the top row were taken with tissue in focus and pictures on the bottom row were the same field taken with grains in focus. (*A* and *B*) A tissue section incubated with 2 nM [³H]DHA (i.e., total binding). Note that grain density indicative of beta adrenergic receptor binding is moderate over the CMC and more intense over the myocardial arteriole (*A*). (*C* and *D*) A tissue section incubated with 2 nM [³H]DHA in the presence of 10^{-6} M metoprolol, a beta one selective antagonist. Note that binding is decreased over the CMC but is not decreased over myocardial arterioles (*A*). (*E* and *F*). A tissue section incubated with 2 nM [³H]DHA in the presence of 10^{-5} metoprolol. Note that binding is decreased over both the CMC and the myocardial arterioles (*A*). Scale bar, 20 μm.
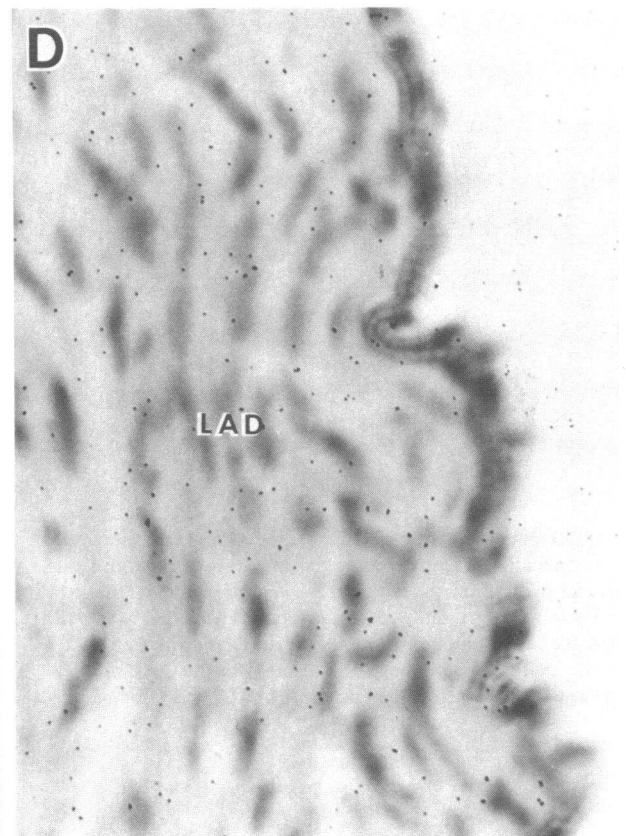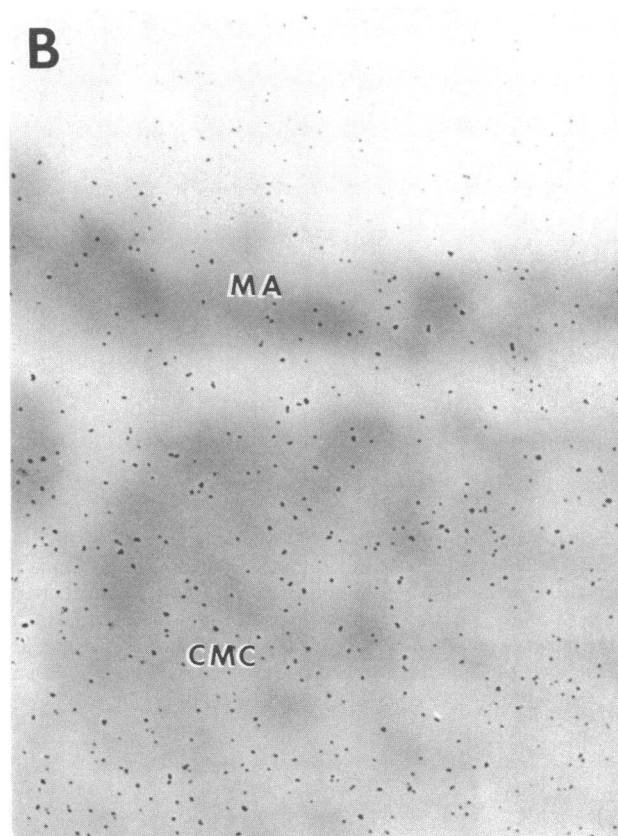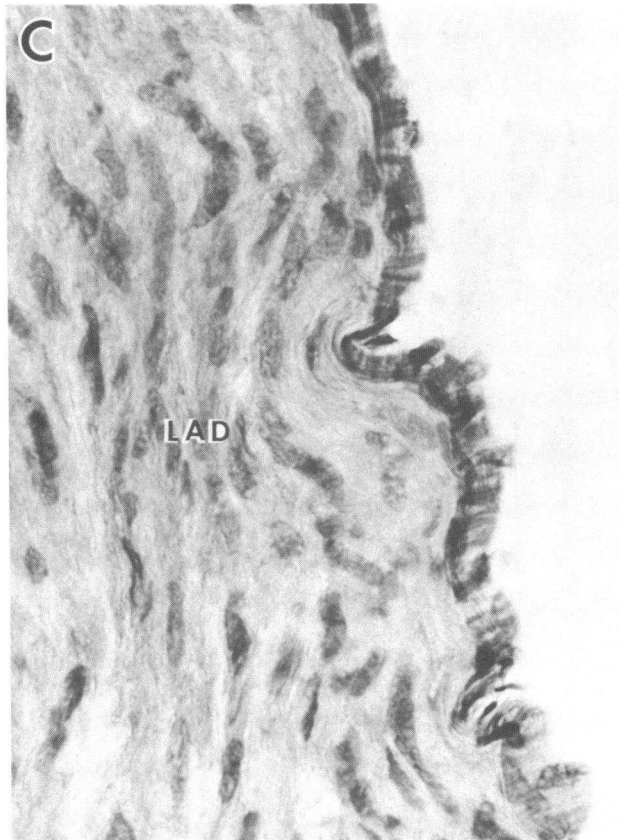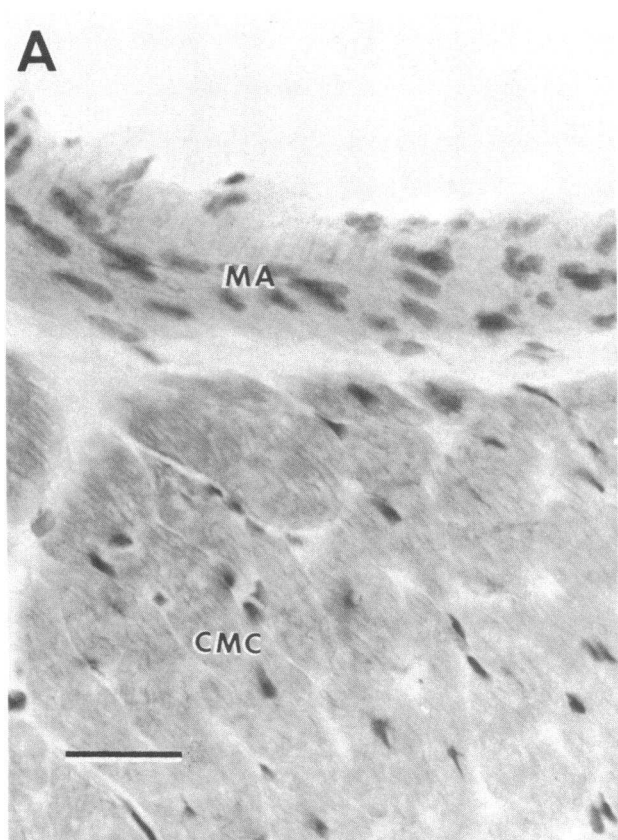
Table I. Autoradiographic Distribution of Specific (−) [³H]DHA Binding Sites in the Normal Canine Heart (n = 4 dogs)

Site	Grains/10^{-2} mm²
CMC	231±34 (SEM)[a]
Myocardial arterioles (<55 μm o.d.)	1,047±131[b]
MA	219±30[a]
Proximal LAD	31±12[c]

a ≠ b ≠ c with the Kruskall-Wallis test ($P < 0.001$).

(SEM) grains/10^{-2} mm²) and subendocardial (182±25) regions of the anterior left ventricle and the subepicardial (212±12) and subendocardial (196±26) regions of the posterior left ventricle ($n = 3$ dogs). The average specific binding over cardiac myocytes was 67% of total binding. The grain density over the walls of small to medium-sized arteries was slightly less than over surrounding cardiac myocytes (Table I) (Fig. 6, *A* and *B*). The grain density over the wall of the proximal LAD was low (Table I) (Fig. 6, *C* and *D*).

Many arterioles had a much greater specific grain density than both surrounding cardiac myocytes and myocardial arteries (Table I). We analyzed vessels with a definite smooth muscle layer and with outer diameter of <55 μm. Specific binding over arterioles was five times greater than over surrounding cardiac myocytes and arteries. Nevertheless, after determining the percent area of myocardium occupied by arterioles, it was calculated that specific binding by arterioles represented 1.38±1.29% (SD) of total myocardial specific binding activity. The grain density over arterioles was less homogeneous than over arteries, with areas of increased binding within segments of the same vessel. There was also greater variability among the counts over arterioles than the counts over the cardiac myocytes in the same tissue. The grain density over veins, venules, and capillaries was low.

Autoradiographic characterization of metoprolol effects. The effects of the beta one selective adrenergic antagonist, metoprolol, on [³H]DHA binding was dose dependent (Table II, Fig. 5). Metoprolol at 10^{-5} M resulted in a 75% reduction in [³H]DHA binding over all structures studied, i.e., cardiac myocytes and arterioles (Table II, Fig. 5). However, the absolute numbers of grains over arterioles was five times higher than over cardiac myocytes. The proximal LAD was not studied because specific DHA binding was very low. At 10^{-6} M metoprolol, [³H]DHA binding to cardiac myocytes was reduced by 50%, whereas binding to arterioles was not significantly reduced (Table II, Fig. 5). At 10^{-7} M metoprolol, 17% inhibition of [³H]DHA binding

occurred over cardiac myocytes, but there was no inhibition over arterioles (Table II).

In the biochemical studies with metoprolol, specific [³H]DHA binding to myocardial sections was reduced by 82% with 10^{-5} M metoprolol, 49% in the presence of 10^{-6} M metoprolol, and 17% in the presence of 10^{-7} M metoprolol. Thus, the percent displacement in the biochemical studies was similar to the displacement of [³H]DHA by metoprolol found over cardiac myocytes in the autoradiographic binding studies (Table II).

Autoradiography of ischemic vs. control myocardium. Specific binding of (−)[³H]DHA was increased significantly (average of ~18%) over cardiac myocytes from anterior myocardium subjected to 1 h of ischemia (Table III). The same result was obtained when the data were analyzed with a repeated-measures analysis of variance ($P < 0.003$). There was no significant difference between binding over cardiac myocytes in the subendocardial and subepicardial regions of the ischemic area. We were unable to demonstrate any significant differences between arterioles from ischemic and nonischemic myocardium. However, the number of blood vessels available for analysis was relatively small, and there was greater variability of binding over blood vessels.

Discussion

The system of in vitro labeling of tissue sections, developed by Young and Kuhar (7) and Palacios and Kuhar (8) for brain tissue, offers several advantages for studying receptors in heart and other organs. Specifically, this technique allows quantification of nonspecific binding and total binding in serial tissue sections; it offers the potential of simultaneously studying several receptor systems in the same tissue, and it is not dependent on vascular delivery or diffusion of ligand. The latter point is particularly important in analyzing relative differences in binding to different tissue components, such as blood vessels and cardiac myocytes. In addition, the technique allows pharmacological verification of the receptor system being studied. We performed biochemical studies to characterize the properties of receptor binding with the technique used for autoradiography. The results of these experiments indicated that the method demonstrates authentic beta adrenergic receptor activity. With the autoradiographic method, we could easily separate the grain density over cardiac myocytes, blood vessels, connective tissue, and background. However, the resolution of this system was not adequate to localize (−)[³H]DHA binding to compartments of individual cells.

A most striking result of our study was the distribution of beta adrenergic receptors over blood vessels. We found a fivefold

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Figure 6. Photomicrographs of [³H]DHA autoradiographs taken at 750 × magnification with tissue in focus (*top row*) and with grains in focus (*bottom row*). (*A* and *B*) Myocardial artery (MA). (*C* and *D*) Proximal LAD. The photomicrographs show silver grains over sec- tions incubated with 2 nM DHA (i.e., total binding). Note that binding over the MA is slightly less than the adjacent CMC and that binding is very low over the LAD. Scale bar = 20 μm.

Table II. Specific (−)[³H]DHA Binding in the Presence and Absence of Metoprolol

Incubation conditions	CMC		Myocardial arterioles	
	Grains/10^{-2} mm^2	% Inhibition	Grains/10^{-2} mm^2	% Inhibition
2 nM DHA (n = 3 dogs)	200±23 (SEM)		1,000±174	
2 nM DHA + 10^{-5} M metoprolol	46±8	76.8±3.4[a]	250±92	70.1±14.0[a']
2 nM DHA (n = 3 dogs)	338±24		1,165±155	
2 nM DHA + 10^{-6} M metoprolol	168±8	50.0±1.2[b]	1,225±168	−4.5±3.9[b']
2 nM DHA (n = 3 dogs)	338±24		1,165±155	
2 nM DHA + 10^{-7} M metoprolol	275±12	17.4±8.5[c]	1,201±141	−2.8±7.4[b']

a ≠ b ≠ c and a ≠ b′ with one-way analysis of variance ($P < 0.001$).

greater density of autoradiographic grains over arterioles as compared with small and medium-sized arteries and a 34-fold greater density compared with the proximal LAD. These results may be related to the high concentration of adrenergic nerve terminals associated with coronary arterioles (14). They are also consistent with physiologic studies demonstrating variation in the response pattern to various adrenergic interventions according to the size of the coronary vessel (15). These results may have important implications regarding the regional control of coronary blood flow by the adrenergic nervous system. Although the specific binding over arterioles was very high, its contribution to total myocardial binding was small, averaging only 1.4%.

Pharmacological studies done in rat demonstrate both beta one and beta two receptors in the heart (2), with a predominance of beta one receptors (85%). Both beta one and beta two receptors have been identified in cerebral microvessels. However, the majority of beta receptors in this preparation were of the beta two type (85%) (16). In our studies, we found that 10^{-6} and 10^{-7} M metoprolol displaced [³H]DHA over cardiac myocytes but not over arterioles, suggesting that the cardiac myocytes are a major site of localization of beta one receptors in the canine heart. The dose-related binding over the cardiac myocytes was

Table III. Autoradiographic Distribution of Specific (−)[³H]DHA Binding Sites in Control vs. Ischemic Myocardium after 1 H of Occlusion of the Proximal LAD

Tissue	Cardiac myocyctes subepicardium (n = 6)	Cardiac myocytes subendocardium (n = 6)	Arterioles (n = 5)
Control myocardium*	234±31	240±31	1,173±147
Ischemic myocardium*	275±29‡	283±33‡	1,335±138

* Data are expressed as grains/10^{-2} mm^2 ($\bar{x}$±SEM).
‡ $P < 0.01$; ischemic vs. control myocardium, paired t test.

similar to myocardial binding of metoprolol determined by scintillation spectrometry; however, the binding to arterioles was not. This probably reflects the low contribution of arterioles to the beta receptor population in the heart. Definitive data on the numbers of beta one and beta two receptors over different tissue compartments could only be obtained with additional autoradiographic studies using varying concentrations of several beta one and beta two selective antagonists. It is also interesting to note that the selective response of cardiac muscle cells and blood vessels to metoprolol is dose dependent. This finding is in agreement with clinical findings in which the beta one selectivity of metoprolol is lost when plasma levels of the drug are high (17).

Using membrane binding assays with (−)[³H]DHA, Mukherjee et al. (18–20) have previously reported no regional differences in beta adrenergic receptor number in the left ventricle of sham-operated dogs. However, in myocardium subjected to 1 h of ischemia, there was a significant increase in beta adrenergic receptor numbers, although binding affinity was not different between ischemic and nonischemic areas. The topographical distribution of the increased population of receptors was not determined. Our autoradiographic data extend the previous findings by demonstrating that ischemia produces a change in the number of beta adrenergic receptors predominantly in cardiac muscle cells rather than in other structures of the heart. The increase in beta adrenergic receptor number in ischemic myocardium was greater in the previous studies (18–20), which used membrane homogenates (60% increase) than in the present autoradiographic study (18% increase). This difference could be due to interanimal variation in the degree of ischemia or to detection of different subgroups of the total population of beta adrenergic receptors by the two methods. In addition to the receptors on the cell membrane, the autoradiographic method could have visualized receptors in the cytosol, which were physiologically inactive (21, 22). The mechanism responsible for the increased beta adrenergic receptor number in ischemic cardiac myocytes is unclear. The phenomenon may be related to unmasking of sarcolemmal receptors, possibly due to changes in membrane fluidity (23), or to inhibition of membrane receptor internalization, and ATP-dependent process (21).

In conclusion, using an autoradiographic technique developed by Young and Kuhar (7) and Palacios and Kuhar (8), we have determined the distribution of beta adrenergic receptor binding over canine cardiac myocytes and myocardial blood vessels. We also have characterized the response of the beta adrenergic receptors of these structures to the selective beta one adrenergic receptor antagonist, metoprolol. In addition, we have verified an increase of specific $(-)[^3H]DHA$ binding over cardiac myocytes in both subendocardial and subepicardial regions of ischemic myocardium. Data obtained in this study have demonstrated directly the distribution of beta adrenergic receptors in striated myocytes and blood vessels of the canine heart, and have shown that changes in beta adrenergic receptor density occur selectively in certain components of the heart in response to pharmacological and pathological perturbations.

Acknowledgments

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