Canine Myocardial Beta-Adrenergic, Muscarinic Receptor Densities After Denervation: A PET Study

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In an effort to better understand cardiac neurotransmission, PET was serially used in dogs to assess changes in ventricular muscarinic (MR) and beta-adrenergic receptor (β-AR) densities following chemical or surgical denervation. **Methods:** Beta-adrenergic and MR receptor concentrations were studied in beagle dogs nine days after chemical sympathectomy (using the neurotoxin 6-hydroxydopamine) or 3–7 wk and 23–28 wk after surgical intrapericardial denervation. **Results:** In control dogs (n = 13), global β-AR and MR concentrations were 32 ± 4 and 62.2 ± 10.4 pmole/ml tissue, respectively. Nine days after 6-hydroxytk; 1opamine (n = 8), hemodynamic tests and MIBG scintigraphy demonstrated the destruction of cardiac sympathetic innervation. Beta-adrenergic density increased by 190% (p < 0.001) while MR density remained unchanged. Three to 7 wk after surgery (n = 5), hemodynamic tests and MIBG scintigraphy demonstrated both parasympathetic and sympathetic denervations. Beta-adrenergic density was increased by 219% while MR concentration remained unchanged. Twenty-three to 28 wk after surgery, atrial innervation was restored (hemodynamic tests) while ventricular sympathetic innervation was not (MIBG scintigraphy). Beta-adrenergic density remained high. **Conclusion:** The present study demonstrates the ability of PET to serially assess myocardial receptor concentrations. The absence of change in MR density and the prolonged up-regulation of β-AR following heart denervation are the main findings of the present study.

Key Words: CGP 12177; methyl quinuclidinyl benzylate; PET; beta-adrenergic receptors; muscarinic receptors; heart; denervation

J Nucl Med 1995; 36:140–146

Cardiac transplantation severs sympathetic and parasympathetic nerves fibres and causes extrinsic cardiac denervation. Acute changes in beta-adrenergic receptor (β-AR) density following denervation have been studied early after surgical (1) or chemical sympathetic denervation (2,3) using in vitro binding techniques. In animals, partial reinnervation by the sympathetic nervous system usually occurs within 6 mo to 1 yr following either homotopic or autotransplantation (4–9). Long-term studies of cardiac reinnervation were based on the assessment of chronotropic responses to sympathetic or electrical stimulations (5,9) or on histopathological criteria (6). Recently late cardiac reinnervation has been proven in heart transplanted patients using ^{11}C-hydroxyephedrine (10). This metaraminol derivative is actively taken up by the sympathetic nerve terminals and therefore explores the sympathetic presynaptic function. One component of the postsynaptic function, the β-AR or muscarinic receptor (MR) density, has not been determined at a time period well after denervation. Myocardial norepinephrine content is nearly absent immediately after transplantation or chemical denervation. Later, it remains low (4) or increases to subnormal levels (7,8,11). In contrast, myocardial acetylcholine levels remain subnormal (12).

The present investigation was undertaken to determine noninvasively the changes in β-AR and MR densities following cardiac denervation in dogs. The myocardial receptor concentrations were assessed using PET, which has the advantage of allowing serial measurements in the same animal. In addition, PET also provides information concerning the regional distribution of myocardial receptors. This method has already been validated in humans using the hydrophilic β-blocking agent ^{11}C-CGP 12177 as a ligand for β-AR (13). The feasibility of the measurement of myocardial MR with PET with the membrane impermeant antagonist ^{11}C-methyl quinuclidinyl benzylate (MQNB) was demonstrated in dogs (14–16). These methods were applied to dogs studied at baseline, 9 days after chemical sympathectomy or 3–7 and 23–28 wk after surgical denervation. Atrial innervation was serially assessed with hemodynamic tests while an eventual sympathetic ventricular reinnervation was assessed through the sympathetic presynaptic uptake of ^{123}I-metaiodobenzylguanidine (MIBG).

MATERIAL AND METHODS

Animals used in the study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory

Animals of the Institute of Laboratory Animal Resources, National Research Council.

Thirteen dogs (mean weight 14 kg) were used to establish baseline values of β-AR and MR density. Five dogs were used to establish control values for ^{123}I-MIBG myocardial uptake.

Surgical Denervation Procedure

Among the 13 dogs, 5 were surgically denervated using an intrapericardial technique (17). Briefly, surface ECG was attached and right femoral artery and vein were catheterized for arterial and central venous pressure monitoring and fluid infusion. Left thoracotomy was performed through the fourth intercostal space and the pericardium was cradled. The denervation procedure consisted of: (a) sectioning of the ventrolateral cardiac nerve at the border of the left superior pulmonary vein; (b) transection of pericardial reflections and epicardium on left atrium towards the right atrium through the transverse sinus towards the inferior vena cava over the left inferior pulmonary veins; (c) sharp dissection of connective tissue around main, left and right pulmonary arteries, then around ascending aorta; (d) dissection of all connective tissue on the right atrium, around the superior vena cava dividing the azygos vein and (e) completion of dissection in the interatrial groove, towards inferior vena cava around right pulmonary vein. Finally, the pericardium was loosely closed and the chest closed on two aspirating tubes which were removed 4 hr postoperatively. To allow dogs to recover, PET experiments (1 wk apart), MIBG scintigraphy and hemodynamic tests were performed 3–7 wk after surgery. The same dogs also underwent PET, scintigraphic and hemodynamic studies 23–28 wk after surgical denervation, using the same procedures.

Chemical Sympathectomy Procedure

To study early changes (9th day) in receptor density, eight dogs (four dogs for each ligand) underwent a chemical sympathectomy using 6-hydroxydopamine (6-OHDA, 50 mg/kg i.v.). This compound causes a selective destruction of sympathetic nerve endings while vagal fibers are unaffected. The acute adverse effects of 6-OHDA administration were prevented using propranolol and phentolamine (18). PET experiments, MIBG scintigraphy and hemodynamic tests were performed 7–9 days later.

Hemodynamic Assessment of Atrial Denervation

The completeness of atrial denervation was confirmed by 2 procedures within 3–4 days of the PET scanning. Phenylephrine (10 μg/kg) and nitroglycerine (15 μg/kg) were injected intravenously and changes in heart rate and arterial blood pressure were continuously monitored during drug infusions (1).

Scintigraphic Assessment of Sympathetic Ventricular Denervation

To scintigraphically evaluate ventricular sympathetic innervation, all dogs (either chemically or surgically denervated) underwent ^{123}I-MIBG scintigraphy. This norepinephrine analog is taken up by the intact sympathetic nerve endings (19). MIBG scintigraphy was performed 7–9 days after chemical denervation, 3–7 wk and 23–28 wk after surgical denervation. In order to minimize extraneuronal uptake (uptake-2), important in dogs (20), the animals were pretreated with intravenous dexametasone (10 mg) 1 hr before the injection of ^{123}I-MIBG (1.5 mCi). Four hours after MIBG administration, the dogs were imaged with a gamma camera for 15 min (anterior view of the chest). The heart to lung activity ratio was used to compare myocardial MIBG uptake in control and denervated dogs.

PET Determination of β-AR and MR Density

Beta-adrenergic and MR concentrations were determined 9 days after chemical denervation, 3–7 wk and 23–28 wk after surgical denervation.

Radiosynthesis of ^{11}C-CGP 12177

The pharmacological active enantiomer (2S) CGP 12177, ((2S)-4-(3-t-butylamino-2 hydroxypropoxy)-benzimidazol-2-one), was synthesized and labeled with ^{11}C, the synthesis being accomplished from (2S)-3-tosyloxy-1,2-propanediol acetonide. The enantiomeric excess was greater than 98%. Carbon 11-CGP 12177, in the S form was obtained with a specific radioactivity of 350 to 1200 mCi/μmole (21).

Radiosynthesis of ^{11}C-MQNB

MQNB was labeled with high specific radioactivity using ^{11}C by methylation of QNB with ^{11}C-methyliodide (22). Labeled compound with a specific radioactivity ranging from 600 to 2000 mCi/μM at the moment the injection was purified using HPLC.

PET Data Acquisition

For the dogs which underwent measurement of both β-AR and MR densities, the two PET studies were performed 1 wk apart. Female beagle dogs were anaesthetized with pentobarbital, intubated and artificially respired. For study of MR, blood samples were obtained from the femoral artery. Dogs were positioned in the TTV01 time-of-flight PET scanner. Each slice was 13 mm thick and spatial transverse resolution was 12 mm. Transmission scans were obtained with a rotating ^{68}G source and used for attenuation correction of the emission scans.

PET Experimental Protocol

One Degree β-AR Study. The protocol included two injections (23). A trace dose of ^{11}C-CGP (3–5 nmole) at the beginning of the experiment and 30 min later and a mixture of labeled (6–8 nmole) and unlabeled (35 nmole) CGP was administered as a slow bolus over 1 min. The PET examination lasted 70 min. The scanning protocol consisted of 66 images (18 × 10 sec, 7 × 1 min, 5 × 2 min, 2 × 5 min, 18 × 10 sec, 7 × 1 min, 5 × 2 min, 4 × 5 min images).

Two degree MR Study. The protocol included 3 injections (14). A first dose of ^{11}C-MQNB (2–4 nmole) was injected, and 30 min later an excess of unlabeled MQNB (0.5 μmole) was injected, and forty minutes later, a mixture of labeled (4–8 nmole) and unlabeled (1.25 μmole) MQNB was injected. The PET examination lasted 120 min. The scanning protocol consisted of 82 images (12 × 10 s, 8 × 1 min, 10 × 2 min, 8 × 1 min, 16 × 2 min, 12 × 10 s, 8 × 1 min, 8 × 5 min images). Since the identification model parameters required the knowledge of the plasma time-activity curve as input function, 64 arterial blood samples (0.5 ml) were collected. Blood ^{11}C radioactivity was measured in a gamma counting system and the blood time-activity curves were corrected for the decay of ^{11}C from the time of the first injection.

PET Data Processing

Myocardial time-concentration curves were measured from regions of interest (ROIs) encompassing the left ventricular myocardium, the septum or the lateral wall (the apex was excluded from the regional study). Carbon-11-CGP and ^{11}C-MQNB concentrations were obtained after correction for ^{11}C decay and expressed as pmole/g after normalization using the specific radioactivity measured at the beginning of the PET experiment. Data were corrected for partial volume effect using postmortem measurements of left ventricular wall thickness (4 dogs) and a recovery factor measured on a heart phantom. In dogs of this size, the

	Control n = 13	6OHD (9 days, n = 4)	Postsurgery (3–7 wk, n = 5)	Postsurgery (23–28 wk, n = 5)
MR: B'_{max} (pmole/ml)	62.2 ± 10.4	57.8 ± 10.2	64 ± 9.6	55.5 ± 5.7
Septal wall	57.4 ± 8.7	53.7 ± 8.5	58.9 ± 17.8	51.5 ± 15
Lateral wall	54.3 ± 9	50.5 ± 8.7	55.8 ± 18	48.2 ± 15
K_dV_r (pmole/ml)	0.07 ± 0.017	0.057 ± 0.013	0.064 ± 0.007	0.067 ± 0.017
β-AR: B'_{max} (pmole/ml)	32.7 ± 4	62.2 ± 10	68.9 ± 15.7	61.3 ± 7.9
Septal wall	30.8 ± 6	60 ± 6.5	64 ± 17	52.4 ± 20
Lateral wall	27.3 ± 6	51.6 ± 6	58.6 ± 16	59.5 ± 7.7

MR = muscarinic receptors; B'_{max} = maximal density of available receptors; V_r = PET volume of reaction in which the free ligand can react with the receptor sites; and $K_d = k_{-1}/k_{+1}$.

thickness of the septum is equal to that of the lateral wall (12 mm). The ratio of true-to-measured concentration was equal to 0.45 for a 12 mm thickness in the phantom calibration experiment performed on the TTV01 PET system. Therefore, true concentrations were obtained by dividing the measured concentration values by this 0.45 recovery coefficient.

Calculation of β-AR Density

The graphical method (23) is based on a specific experimental protocol and justified by the properties of the CGP kinetics. The receptor concentration is estimated by using two experimental myocardial concentration values calculated from the PET time-concentration curve. This approach relies on a difference in the kinetics of the radiotracer when injected alone or with an excess of unlabeled ligand. It is based on the following differential equation:

$$dB(t) = k_{+1}/V_R(B'max - B(t))F(t) - k_{-1}B(t), \quad \text{Eq. 1}$$

where B(t) and F(t) are the molar concentration of bound and free ligand, respectively; V_R is the volume of reaction for free ligand in tissue; k_{+1} is the association rate constant and k_{-1} is the dissociation rate constant. The method is based on an uptake measurement where association of the tracer to the receptor dominates the kinetics and the small effect of dissociation ($k_{-1}B(t)$ in the equation) is accounted for in the analysis by exponential extrapolation.

Calculation of MR Density

The compartmental model used is a nonequilibrium, nonlinear one, justified by the properties of the MQNB kinetics (14). It included two steps: first, a transport of the ligand from blood to a free ligand compartment and second, a classical ligand-receptor interaction. The rate constant p characterized the transfer of ligand from blood to tissue; k characterized the transfer from tissue to blood and V_R is defined as the fraction of the ROI delineated by PET, in which the ligand can react with receptors. The product $p \cdot V_R$ is the clearance of the ligand. The model parameters introduced in the ligand-receptor interactions were similar to those used in in vitro studies: the concentration of available receptors (B'max) and the association and dissociation rate constants (k_{+1} and k_{-1}, respectively). By fitting the mathematical model to time-concentration curves, it was possible to obtain estimates of parameters $p \cdot V_R$, k, B'max, k_{+1}/V_R, k_{-1}. The volume of reaction V_R was deduced by assuming that the transport between blood and tissue was passive and the two parameters p and k had the same value. Thus, V_R could be estimated from the $p \cdot V_R/k$ ratio.

Plasma Catecholamine Determination

At the beginning of each PET study, blood samples (5 ml) were drawn. Plasma norepinephrine and epinephrine concentrations were determined by using high-pressure liquid chromatography (24).

Statistical Analysis

Numerical data are expressed as mean ± s.d. ANOVA for repeated measures was used to compare receptor densities and MIBG myocardial uptake in the four experimental conditions (control, chemical denervation, early and late surgical denervation). Bonferroni t-test was used to compare mean values between each situation.

RESULTS

Control Dogs

Beta-adrenergic concentrations were 32 ± 4, 30.8 ± 6 and 27.3 ± 6 pmole/ml tissue in the left ventricular ROI, in the septum and in the lateral wall, respectively (Table 1). For MR, the corresponding values were 62.2 ± 10.4, 57.4 ± 8.7 and 54.3 ± 9 pmole/ml tissue, respectively (Table 1). The density of β-AR and MR was slightly higher in the septum than in the lateral wall but this difference was not significant. Hemodynamic response to vaso-active drugs are presented in Table 2. MIBG heart-to-lung ratio was 3.14 ± 0.12 (Fig. 1). Plasma norepinephrine and epinephrine concentrations are presented in Figure 2.

6-OHDA Denervated Dogs

Nine days after 6-OHDA administration, there was no increase in heart rate following nitroglycerine infusion indicating the completeness of the sympathetic chemical denervation (Table 2). The response to phenylephrine infusion was similar to that observed in control dogs. MIBG myocardial uptake was similar to that of the lung (Fig. 1) confirming the destruction of sympathetic nerve endings. Plasma norepinephrine and epinephrine concentrations were not different from those of control dogs (Fig. 2).

TABLE 2
Hemodynamic Assessment of Denervation

		Control (n = 5)	6-OH DA (9 days, n = 8)	Postsurgery (3–7 wk, n = 5)	Postsurgery (23–28 wk, n = 5)
Phenylephrine (10 μg/kg)	ΔHR (b/min)	−23	−24	0	−24
	ΔMAP (mmHg)	+41	+38	+33	+29
Nitroglycerine (15 μg/kg)	ΔHR (b/min)	+26	+1	0	+33
	ΔMAP (mmHg)	−22	−23	−21	−23

HR = heart rate; MAP = mean arterial pressure; Δ = changes control minus trial; 6-OH DA-6-hydroxydopamine.

Beta-adrenergic density increased by 190% (p < 0.001; Table 1) while MR density and affinity constant remained unchanged (Table 1). Regional distributions of β-AR and MR are shown in Table 1.

Surgically Denervated Dogs

Early Study: 3 to 7 Weeks. There was no significant change in heart rate following nitroglycerine or phenylephrine infusion (Table 2). Plasma norepinephrine and epinephrine values were not different from those of control dogs (Fig. 2). Changes in MIBG heart-to-lung ratio (Fig. 1), β-AR and MR densities (Table 1) were similar to those observed 9 days after chemical sympathectomy. Global as well as regional concentrations of β-AR were increased (p < 0.001) while MR density and affinity constant remained unchanged (Table 1).

Late Study: 23 to 28 Weeks. Response to infusion of vasoactive drugs was similar to that observed in control dogs (Table 2) suggesting that both atrial sympathetic and parasympathetic innervations had recovered. Plasma norepinephrine and epinephrine values were not different from those of control dogs (Fig. 2). MR density as well as the

affinity constant remained unchanged (Table 1). MIBG scintigraphy still demonstrated a lack of significant ventricular sympathetic innervation (Fig. 1). Global myocardial β-AR density remained increased (210% versus control, p < 0.001) to a similar extent to that observed 3–7 wk after surgery. Septal density of β-AR was slightly lower (p = 0.1) than that measured 3–7 wk after surgery while the lateral wall β-AR density remained unchanged (Table 1). Individual data showed that in four dogs septal β-AR density decreased by an average of 23% in the septum while it remained unchanged in one dog.

DISCUSSION

Neuroimaging techniques with PET have recently begun to be applied to the study of cardiac physiology and disease. They represent a potent means to investigate noninvasively cardiac neurotransmission under different conditions. The present study demonstrates the ability of PET to serially measure myocardial receptor concentrations for the follow-up of animals in experimental conditions. The
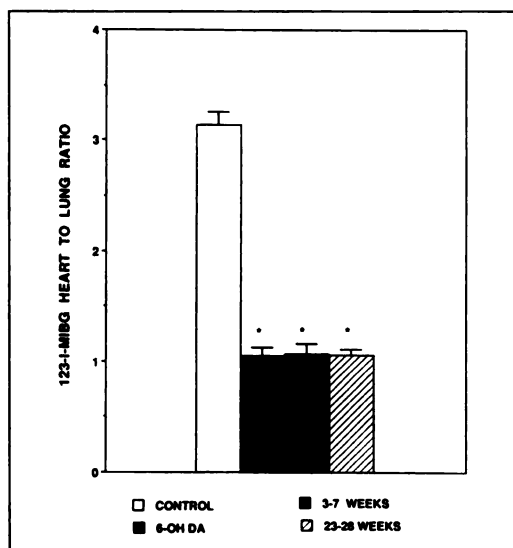

FIGURE 1. Myocardial 123I-MIBG uptake (calculated as the heart to lung ratio) in control and denervated dogs. *p < 0.05 versus control dogs.
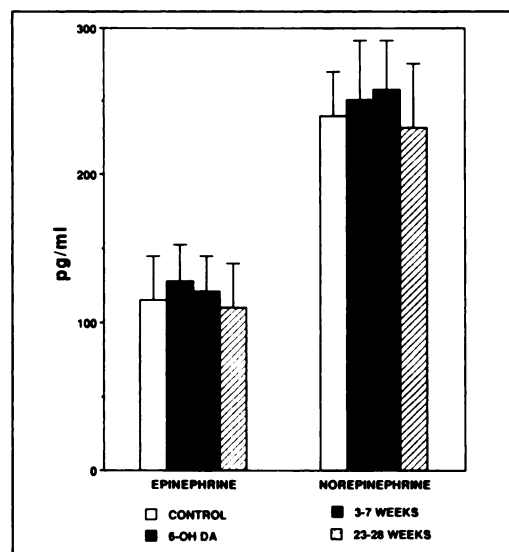

FIGURE 2. Plasma norepinephrine and epinephrine concentrations in control and denervated dogs. No change in plasma catecholamine concentrations was observed after chemical or surgical denervation.

main findings of this study is the lack of changes in MR concentration and the prolonged up-regulation of β-AR following cardiac denervation. Study of regional distribution of β-AR did not show a clear decrease in β-AR septal concentration long after denervation, a fact which could have suggested a local increase in norepinephrine content due to a partial reinnervation as found in heart transplant patients (10).

Methodological Considerations

Ligands Used in the Study.

MQNB: MQNB is a hydrophilic ligand which binds only to externalized MR (25) contrary to the lipophilic QNB which binds to both externalized and internalized MR. Both ligands bind to three muscarinic receptor subtypes M1, M2 and M3. M1 receptors may be present on sympathetic nerves and parasympathetic ganglia, M2 receptors on cholinergic nerves and M3 receptors on smooth muscles. As AF-DX-116, which selectively antagonizes M2 muscarinic receptor subtype, was not available as ^{11}C-labeled ligand, the measurements performed in the present study concern all three subtypes of receptors. This may be a limitation of our study, but in Lewis rats after lung transplantation, there was no change in subgroups of muscarinic receptors (26).

CGP 12177: This high affinity beta-blocking agent binds to both β_1–β_2 AR (27). During in vivo experiment in beagle dogs, the specific binding of S-CGP 12177 could be estimated >90% (personal data). It is hydrophilic and binds only to externalized β-receptors (28). These properties are different from those of ligands commonly used for in vitro studies (^{3}H-dihydroalprenolol, ^{125}I-pindolol or ^{125}I-iodocyanopindolol).

PET Methodology.

A limitation of the present study is inherent to PET methodology. The atria which receives dense cholinergic innervation cannot be studied with PET because of its thin wall. The absolute quantification of tissular labeled ligand concentration can be altered by changes in ventricular wall thickness or impaired wall motion. To account for left ventricular thickness, a recovery coefficient based on postmortem measurements was used. It is likely that the use of this coefficient had no significant effect on the relative changes in receptor concentrations since the same dogs were studied before and after denervation. Furthermore, there is no change in myocardial thickness or significant alteration in resting left ventricular function after denervation (29).

An attempt was made to study the regional distribution of β-AR and MR. In most of the dogs, the septal receptor density was slightly higher than that in the lateral wall. Since the septal and lateral myocardial thickness is similar in beagle dogs, this factor cannot account for the observed difference. Spillover from cavity to myocardium can explain this difference since it affects differently the septum and the lateral wall. For the septum, spillover from right and left ventricular blood affects the quantification. For the lateral wall, spillover comes mainly from the left ventricu-

lar cavity since the uptake of CGP 12177 or MQNB by the lungs is low. Furthermore, wall motion (excursion from diastole to systole of the lateral wall) can account also for this difference since septum motion is reduced to thickening. This problem does not affect the meaning of our results since the same dogs were studied before and after denervation.

MIBG Scintigraphy. Iodine-123-MIBG scintigraphy was used to assess ventricular sympathetic nerve ending integrity. Planar imaging and calculation of the relatively insensitive index were used to assess presynaptic function. SPECT imaging could not be used because of the very low myocardial uptake of the tracer following denervation and the inability to obtain a clear delineation of the myocardium from adjacent structures (lung and liver). Therefore, an eventual regional recovery of presynaptic sympathetic function could not be assessed. The absence of significant global ventricular MIBG uptake, observed 23–28 wk after denervation, cannot exclude the presence of some ventricular sympathetic reinnervation. To detect such a partial regional reinnervation, a PET norepinephrine analog would have been necessary (30), but a tracer such as ^{76}Br-metabromobenzylguanidine was not available in our laboratory at the time of the study.

Muscarinic Receptor Density

No alteration in total population of MR was observed following chemical denervation. Previous results of in vitro studies concerning changes in MR density after 6-OHDA remain conflicting. A loss (31,32), no change (33,34) and an increase (35) in MR density were found. These discrepancies could be due to interspecies and/or to methodological differences (ligands and preparation of membranes).

In the present study, no change in MR density was observed either early or late after surgical denervation. This result is discrepant with that of Vatner (1) who found a slight (30%) but statistically significant decrease of MR density. It is likely that such a small change cannot be detected with the PET-MQNB protocol we have used. In fact, the standard errors in the measured MR density are about 10% (14). Furthermore, the s.d. in the control population is rather large.

Following vagotomy in rats and cats, or heart transplantation in rats, ventricular choline acetyltransferase activity is reduced by 80% while acetylcholine content is not significantly reduced (12,36). Because the cell bodies of post ganglionic parasympathetic nerves reside inside the myocardial wall, these neurons are not removed at surgery and therefore the ventricular density of MR could remain unchanged.

The atrial vagal reinnervation has been shown to be present 4 wk after surgery (7). In our study, no reflex bradycardia was observed following a pressor infusion of phenylephrine, suggesting that the atrial vagal innervation was not yet restored 3–7 wk after surgery. This lack of bradycardia could be due to the use of sodium pentobar-

bital as anaesthetic agent since it has been shown that vagal inhibition of heart rate is depressed by barbiturates (7).

Beta-Adrenergic Density

Following denervation (either chemical or surgical), early and late up-regulations of β-AR were observed. There was no change in plasma norepinephrine and epinephrine levels, findings in accordance with those of Vatner et al. (1). The absence of significant ventricular MIBG uptake found in the present study confirms the absence of significant sympathetic innervation following chemical denervation or surgical denervation. The increase in β-AR found in dogs by PET is in accordance with the up-regulation of myocardial β-AR observed in rabbits 2 wk after 6-OHDA administration (3). This is also in accordance with previous data (1) obtained in the same surgical model but using an in vitro binding technique with a lipophilic ligand. Similar findings were also observed in baboons after autotransplantation (37). The fact that the present results are in accordance with those obtained with lipophilic ligands suggests that both the total number and the number of externalized binding sites are increased after sympathetic denervation.

A similar decrease in norepinephrine myocardial content was found 7 days after 6-OHDA administration (20) or after the intrapericardial surgical procedure (29). Therefore, both methods of denervation strongly decrease norepinephrine heart content and induce a similar up-regulation of β-AR. Furthermore, both procedures of denervation induce a similar supersensitivity to norepinephrine (1,38). Left-ventricular NE content remains very low for at least 1 to 2 yr (4).

The atrium is the first to reinnervate 3 to 4 mo after surgery (4). Nine to 14 mo later, a progressive ventricular sympathetic reinnervation occurs from the base to the apex (4). In the present study, 23–28 wk after surgery, vagosympathetic atrial innervation was restored (hemodynamic tests). This finding is in accordance with previous studies (5,7,9). It is likely that late partial sympathetic reinnervation (1–2 yr) occurs, but the density of ventricular neurons remained too low to restore normal ventricular norepinephrine levels (4).

Comparison with Human Findings

The prolonged myocardial up-regulation of β-AR following denervation observed in dogs is in contrast with the unchanged β-AR density found in endomyocardial biopsies from heart transplanted patients (39,40). Similarly, no change in β-AR density in transplanted patients was observed using ^{11}C-CGP 12177 and the same PET methodology (41). To our knowledge, early determination (before 15 days) of β-AR density have not been published and a transient up-regulation cannot be excluded in humans.

Four to 5 mo after heart transplantation in humans, there is no evidence of significant sympathetic reinnervation assessed by tyramine infusion (42) or by the myocardial uptake of ^{11}C-hydroxyephedrine (10). Post-transplant human myocardial norepinephrine content was found to be

reduced by 98% (40); a value similar to that observed in dogs following denervation. Many facts can account for the difference in regulation of β-AR density after denervation or transplantation; interspecies difference; the frequency of at least minimal rejection episodes and the use of steroids. Furthermore, in heart transplanted patients, plasma norepinephrine concentration shows a trend towards elevated values at rest (43); while in denervated dogs it remains normal. Cyclosporine may also contribute to increased plasma norepinephrine (44). Rejection and immunosuppressive therapy are likely causes of this lack of up-regulation since there is an up-regulation of β-AR in nonhuman primates after cardiac autotransplantation (37).

Late after heart transplantation in humans (3.5 ± 1.3 yr), retention of ^{11}C-hydroxyephedrine was found to be higher in the proximal anterior wall and septum (10). This is in accordance with electrophysiological (stimulation of stellate ganglia), hemodynamic tests (infusion of norepinephrine and cryptenamine; local measurement of left ventricular function using strain-gauge arches), and biochemical (myocardial norepinephrine content) studies which demonstrated that reinnervation begins in the atria (3–4 mo) and then progresses from the base (6 mo) to the apex (9–14 mo) of the left ventricle (4) in dogs. Our results did not clearly suggest that sympathetic reinnervation began in the septal wall. It is likely that the time elapsed between surgery and the second PET study (23–28 wk) was too short.

Data concerning MR density following human heart transplantation are scarce in spite of the greater clinical utilization of cardiac transplantation. After heart-lung transplants in humans, there was no change in bronchoconstrictor response to acetylcholine and lung MR density (45). An unchanged myocardial MR density after heart transplantation, using the same PET methodology was also reported (46). Similarly, the absence of supersensitivity to intracoronary infusion of acetylcholine suggested the functional integrity of ventricular MR after transplantation in humans (47).

CONCLUSION

The development of PET techniques allows in vivo serial assessment of the role of β-AR receptors in the hypersensitivity response to denervation. The comparison of the present results concerning the changes in β-AR concentration with those obtained with lipophilic ligands suggests that both total and externalized β-AR were increased after denervation. In contrary to what is observed in humans following heart transplantation, denervation in dogs induced a prolonged up-regulation of β-AR. PET can contribute to a better understanding of the regulation of cardiac function by the autonomic nervous system.

ACKNOWLEDGMENTS

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