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Alterations in Cardiac Autonomic Receptors following 6-Hydroxydopamine Treatment in Rats

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SUMMARY

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To characterize the regional changes in β-adrenergic and muscarinic cholinergic receptors in rat hearts following 6-hydroxydopamine (6-OHDA) treatment, receptor binding assays using the specific ligands [^{3}H]dihydroalprenolol (DHA) (β-adrenergic receptor) and [^{3}H]quinuclidinyl benzilate (QNB) (muscarinic cholinergic receptor) were performed in five regions of control and treated hearts. In addition, norepinephrine (NE) concentration and choline acetyltransferase activity in hearts were assayed to characterize the model. Rats were treated with 2 × 50 mg/kg i.v. injections of 6-OHDA · HBr at 24-h intervals and the hearts were removed 1, 2, and 3 weeks later. The specific [^{3}H]DHA binding was significantly higher in the ventricles and intraventricular septum than in the right atria of control rat hearts, suggesting a regional variation for β-adrenergic receptors. Following 6-OHDA treatment, the specific [^{3}H]DHA binding to right atria, left ventricles, and ventricular septae significantly increased compared to similar regions of control rat hearts. The B_{max} value in left ventricles was significantly increased, by 32, 38, and 17%, respectively, at 1, 2, and 3 weeks without a change in the K_d, suggesting an increase in the density of β-adrenergic receptors. There was a marked (60–80%) reduction of NE levels in all regions of 6-OHDA-treated rat hearts. These results suggest a relationship between the reduction of endogenous NE and the development of the increased density of β-adrenergic receptors in the rat hearts following 6-OHDA treatment. Specific [^{3}H]QNB binding was significantly higher in atria than in ventricles of control rat hearts. The binding to each region was unchanged at 1 and 2 weeks following 6-OHDA treatment, while it was significantly increased in most regions at 3 weeks. There was a 30% increase in the B_{max} in right and left atria without a change in the K_d, suggesting a change in the receptor density. The choline acetyltransferase activity was not significantly altered in any region of 6-OHDA-treated rat hearts. The present study has demonstrated that destruction of noradrenergic nerve terminals in rats with 6-OHDA leads to an increase in the density of β-adrenergic receptors and a decrease in the NE concentration and that most of the cardiac muscarinic cholinergic receptors are not located on adrenergic neurons.

INTRODUCTION

The autonomic nervous system regulates blood pressure, heart rate, and myocardial contractility. The regulation of neurotransmitter release is important in the sequence of control of these parameters. One of the major regulatory sites may be the presynaptic receptor which is presumed to inhibit the release of neurotransmitter. The concept of presynaptic regulation of neurotransmitter release has been well reviewed (1).

Chronic alterations in the amount of a neurotransmitter at the synapses produce changes in responsiveness of postsynaptic cells to the transmitter. Denervation of adrenergically innervated tissues results in a supersensitivity of pharmacological responsiveness to exogenously applied catecholamines (2, 3). The catecholaminergic

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neurons have been selectively destroyed by the administration of the neurotoxin, 6-OHDA[3] (4–6). In rat hearts, a significant increase in the basal activity and also in the catecholamine stimulation of myocardial adenylate cyclase following 6-OHDA or reserpine treatment has been reported (7). Although several studies of the effects of catecholamine depletion such as reserpine and guanethidine on cardiac tissues have been reported (7, 8), to our knowledge, none has shown changes in cardiac β-adrenergic receptors following 6-OHDA treatment.

There is some pharmacological evidence compatible with the concept that the release of NE from the sympathetic nerves in isolated rabbit heart may be regulated by muscarinic cholinergic presynaptic receptors on the noradrenergic neurons (9). Recently, Sharma and Banerjee (10) found a significant (50%) decrease in the number of cardiac muscarinic cholinergic receptors following treatment of rats with 6-OHDA, suggesting the existence of muscarinic cholinergic receptors on the sympathetic nerve endings. On the other hand, Story *et al.* (11) have shown a significant increase in the number of muscarinic cholinergic receptors in rat hearts after 6-OHDA treatment. Previous receptor studies in the heart have not been done for each major region. Since different regions of the heart have varying densities of receptors which may be related to different physiological functions (12), a regional study appears important for the analysis of receptor alteration after drug treatment. To study the control of neurotransmitter release in the hearts, we have characterized in detail the regional distribution of β-adrenergic and muscarinic cholinergic receptors and also the time course of the development of receptor changes in the heart following 6-OHDA treatment. In addition, we determined the endogenous NE concentration and CAT activity in these tissues to characterize the model. In the present study the changes in β-adrenergic and muscarinic cholinergic receptors have been determined by using the potent β-adrenergic antagonist, [^{3}H]DHA, and the muscarinic receptor antagonist, [^{3}H]QNB. β-Adrenergic and muscarinic cholinergic receptors have been labeled in the peripheral and central nervous systems using [^{3}H]DHA (13, 14) and [^{3}H]QNB (12, 15, 16), respectively. Our results demonstrate that in the 6-OHDA-treated rats, there is a decrease in cardiac NE concentration and an increase in cardiac β-adrenergic receptors. In addition, we found an increase in cardiac muscarinic cholinergic receptors with no change in CAT activity.

MATERIALS AND METHODS

6-OHDA treatment. Male Sprague–Dawley rats (150–200 g) were divided into two groups of 9 or 10 rats each. Rats of the first group were injected i.v. with 6-hydroxydopamine hydrobromide, in two doses of 50 mg/kg at 24-h intervals (corresponding to 2 × 34 mg/kg of 6-OHDA, free base). Treated rats had a similar increase in body weight and heart weight as control animals. The control rats received an equivalent volume (0.1 ml/100 g) of 0.001 N HCl. Three or four rats from each group were concurrently sacrificed 1, 2, or 3 weeks after the first dose of 6-OHDA or of 0.001 N HCl. Three separate sets of experiments were performed and the results were averaged. In a preliminary experiment, we have examined specific binding of [^{3}H]QNB and [^{3}H]DHA to five regions of rat hearts at 2 days following treatment with this dose schedule of 6-OHDA. We did not detect any significant change in the specific binding of either ligand in any region. Since degenerating adrenergic neuronal structures are present for several days following 6-OHDA treatment (6), no immediate change was expected. Therefore, the present experiments were done after 1 week. 6-OHDA hydrobromide was dissolved in 0.001 N HCl saturated with nitrogen gas (4).

Tissue preparation. Intact hearts were carefully removed from control and 6-OHDA-treated rats and chilled in 0.9% NaCl. Hearts were perfused through the aorta with 0.9% NaCl (4°C) until the coronary arteries were cleaned of blood. Additional blood clots, connective tissue, large vessels, and fat were trimmed and removed. Dissection of the rat heart into its regions was performed at 4°C. The free wall of the right ventricle was incised and removed. The right atrium, including its floor, and the intraatrial septum were dissected, followed by the free wall of the left atrium and its floor. Finally, the intraventricular septum was dissected from the free wall of the left ventricle. Tissues were minced with scissors, mixed with 40 vol of buffer, and homogenized using a Polytron (setting No. 6, Brinkman Instruments) for 30 s. The homogenates were filtered through four layers of cheesecloth and the filtrate was then centrifuged at 50,000*g* for 20 min. The pellet was resuspended with the homogenizer in the original volume of buffer and utilized in the [^{3}H]DHA and [^{3}H]QNB binding assays.

Radioreceptor assays. [^{3}H]DHA binding was performed as described by Chen *et al.* (14). The incubation medium used for the binding assay was 50 mM (Na/K)-phosphate buffer, pH 7.4. Specific [^{3}H]DHA binding was experimentally determined from the difference between counts bound in the absence and presence of 0.1 μM (−)-propranolol. In the routine assay, 5 mg of tissue (or 0.5 mg of protein) and [^{3}H]DHA (final concentration, 0.29 nM), in parallel sets with the presence and absence of 0.1 μM (−)-propranolol, were incubated in a total volume of 2 ml of 50 mM (Na/K)-phosphate buffer at 21°C. Since the concentration of [^{3}H]DHA was not saturating, the binding obtained is proportional to the maximum value (about 50%), unless the K_d changes. In the experiments presented, the K_d did not change significantly. The concentration was chosen to improve the ratio of specific binding to nonspecific binding and improve the reliability of single observations (14). After 30 min, the reaction mixture was filtered under reduced pressure through Whatman glass-fiber filters (GF/B). The filters were rinsed four times with 4 ml of (Na/K)-phosphate buffer (21°C). The filters were added to plastic vials and 8 ml of scintillation fluid (2 liters of toluene, 1 liter of Triton X-100, and 16 g of Omnifluor) was utilized

[3] Abbreviations used: 6-OHDA, 6-hydroxydopamine; NE, norepinephrine; [^{3}H]DHA, (−)-[^{3}H]dihydroalprenolol; [^{3}H]QNB, [^{3}H]quinuclidinyl benzilate; CAT, choline acetyltransferase; cyclic AMP, adenosine cyclic 3′,5′-monophosphate; RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; SEP, intraventricular septum; ANOVA, a hierarchical single-factor analysis of variance.

to extract the radioactivity from the filters for at least 12 h at room temperature. The radioactivity was determined in a liquid scintillation counter (Searle, Model 6893) with 45% efficiency. All assays were conducted in duplicate. In a typical study specific binding was more than 50% of the total binding.

Analogous experiments were performed using [^{3}H]-QNB to measure the cholinergic muscarinic receptor using methods previously described (12). Specific [^{3}H]-QNB binding was experimentally determined from the difference between counts bound in the absence and presence of 1 μM atropine sulfate. In the routine assay, 1.25 mg of tissue and [^{3}H]QNB (final concentration, 0.1 nM), in the presence or absence of 1 μM atropine, were incubated in 2 ml of Na/K phosphate buffer, pH 7.4, at 37°C for 60 min. Since the concentration of [^{3}H]QNB was not saturating, the binding obtained is proportional to the maximum value (about 70%), unless the K_d changes. In the present experiments, the K_d did not change significantly. All assays were conducted in duplicate. In a typical experiment more than 90% of the binding was specific.

Endogenous norepinephrine determinations. The level of norepinephrine (NE) in the heart was estimated by a sensitive radioenzymatic assay procedure (17). This assay involves the *in vitro* methylation of NE by the enzyme phenylethanolamine-*N*-methyltransferase (PNMT) in the presence of the radiolabeled methyl donor [^{3}H]*S*-adenosylmethionine.

Fresh or frozen hearts dissected into five regions were homogenized in 10 vol (ventricles, septum) or 20 vol (atria) of cold 0.1 N perchloric acid in a polytron homogenizer. After standing for 10 min in ice, the homogenates were centrifuged (4°C, 10,000*g*, 10 min). An aliquot of the supernatant fluid was used to assay NE. The sample (50 μl) was incubated for 30 min at 37°C in a reaction mixture which contained 35 μl of 1 M Tris–HCl buffer (pH 8.6, in 5 g/100 ml EDTA, 0.1 mM dithiothreitol), 5 μl of [^{3}H]*S*-adenosylmethionine (2.5 μCi), and 10 μl of PNMT (0.22 unit) in a final volume of 150 μl. An internal standard consisting of 1.0 ng authentic NE was run with each assay. The reaction was stopped by the addition of 2 ml of 0.5 M sodium phosphate buffer, pH 10.0, containing 5 g/ml EDTA, 0.1 mM dithiothreitol, and 150 mg alumina. The alumina was washed and the [^{3}H]epinephrine was eluted in 1 ml of 0.1 N perchloric acid, 50 μl of 0.2 N acetic acid containing 25 μg epinephrine and 100 μg *S*-adenosylmethionine chloride. Then 100 μl of a saturated solution of phosphotungstic acid was added. The tubes were centrifuged for 5 min at a low speed and 1.0-ml aliquots of the supernatant were transferred to a second tube containing 1 ml of 1 M potassium phosphate, pH 7.15, and 10 ml of 1% (v:v) diethylhexylphosphoric acid–toluene. After mixing vigorously and centrifuging at low speed, 9 ml of the clear organic phase was transferred to a vial containing 400 μl of Liquiflor and counted. Standard curves were linear in the range of 0.1–10 ng NE in the left ventricle.

Choline acetyltransferase assay. The activity of CAT in the heart was determined by using an assay modified from previously described methods (18). Frozen hearts were homogenized in 20 vol of distilled H_2O (0.5% Triton X-100) with a Polytron (setting No. 6) for 30 s. The homogenate (5 μl) was incubated for 20 min at 37°C in a reaction mixture which contained 1 mM EDTA, 80 mM sodium phosphate buffer, 0.15 mM eserine, 5 mM $MgCl_2$, 2 mM choline, 300 mM NaCl, and 100 μM acetyl CoA (23.3 μM [^{14}C]acetyl CoA). The reaction was stopped by placing the tubes into an ice bath. After the addition of tetraphenyl boron (50 mg/ml dissolved in 3-heptanone), the tubes were shaken vigorously and centrifuged to break the emulsion. A 50 μl aliquot of the top layer was counted.

Statistical analysis. Statistical analysis of the data was performed using either a double-tailed Student's *t* test or a hierarchical single-factor analysis of variance (ANOVA) (19). Receptor binding data from the saturation studies were analyzed by a rearranged Scatchard equation corresponding to the linear equation, $B = -K_d(B/F) + B_{max}$, where B is the specifically bound [^{3}H]ligand, F is the free concentration of [^{3}H]ligand, K_d is the apparent dissociation equilibrium constant, and B_{max} is the total receptor density. When the binding data are plotted in this manner, the negative of the slope of the regression line gives the dissociation constant, K_d, and the Y intercept is B_{max}.

Materials. (−)-[^{3}H]Dihydroalprenolol (48.6 Ci/mmol), [^{3}H]quinuclidinyl benzilate (29.4 Ci/mmol), [^{3}H]*S*-adenosylmethionine (10.9 Ci/mmol), [^{14}C]acetyl coenzyme A (52.0 mCi/mmol), and Liquiflor were obtained from New England Nuclear Corp., Boston, Mass. (−)-Propranolol hydrochloride, atropine, norepinephrine bitartrate, epinephrine bitartrate, dithiothreitol, *S*-adenosylmethionine chloride, 6-hydroxydopamine hydrobromide, phosphotungstic acid, diethylhexylphosphoric acid, phenylethanolamine-*N*-methyltransferase (100 units/mg protein), and alumina were obtained from Sigma Chemical Company. Other drugs and material were obtained from the pharmaceutical company of origin or commercial sources.

RESULTS

Effect of 6-OHDA treatment on β-adrenergic receptors in the rat heart. Previous experiments with mouse heart homogenates have shown that the specific binding of [^{3}H]DHA was saturable and stereospecific with the IC_{50} for (−)propranolol of 0.38 nM and for (+)-propranolol of 113 nM (14). Similar results were obtained with rat heart homogenates.

Using equilibrium experiments to determine the dissociation constant (K_d) and the maximum number (B_{max}) of receptor sites by Scatchard analysis (20), a K_d = 0.27 ± 0.02 nM and a B_{max} = 2.96 ± 0.11 fmol/mg tissue were obtained in 12 experiments using rat left ventricles (Table 2). The β-adrenergic receptors were measured in five regions of hearts from control and 6-OHDA-treated rats. There was a definite regional variation for the β-adrenergic receptors in the control rat hearts. As shown in Table 1, the specific binding of [^{3}H]DHA was significantly higher in the ventricles and intraventricular septum than in the right atrium. Since there was no developmental (1–3 weeks) change in the specific [^{3}H]DHA binding to each region of control rat hearts, the control data for each region were pooled (Tables 1 and 2).

At 1, 2, and 3 weeks after the injection of 6-OHDA, the

TABLE 1

Effect of 6-OHDA treatment on specific binding of [^{3}H]DHA to five regions in rat hearts

Saline control and 6-OHDA-treated rats were sacrificed 1, 2, and 3 weeks after injection. The assays for [^{3}H]DHA binding in the five regions of rat hearts were performed according to standard procedures described in Methods. Tissue homogenates (5 mg) were incubated with 0.29 nM [^{3}H]DHA at 21°C for 30 min. Specific [^{3}H]DHA binding was experimentally determined as the difference between total binding and nonspecific binding in parallel assays in the absence and presence of 0.1 μM (−)-propranolol. The values are given as fmol of DHA bound per mg tissue and are the means ± standard errors. The number of determinations was: control, N = 22; 6-OHDA treatment, N = 8–11.

Region	Specific [^{3}H]DHA bound (fmol/mg tissue)			
	Control[a]	6-OHDA treatment[b]		
		1 week	2 weeks	3 weeks
Right atrium	1.19 ± 0.04	1.45 ± 0.08	1.59 ± 0.04	1.55 ± 0.07
Left atrium	1.28 ± 0.05	1.33 ± 0.09	1.43 ± 0.07	1.55 ± 0.10
Right ventricle	1.35 ± 0.06	1.39 ± 0.10	1.37 ± 0.09	1.45 ± 0.08
Left ventricle	1.42 ± 0.05	1.61 ± 0.06	1.84 ± 0.07	1.71 ± 0.07
Intraventricular septum	1.49 ± 0.06	1.57 ± 0.08	1.63 ± 0.05	1.76 ± 0.10

[a] The control data for each week were analyzed by a hierarchical single-factor analysis of variance (ANOVA). The analysis revealed that the means for each region did not differ with time ($P > 0.05$). Therefore, the pooled data are presented. However, the means for the different regions were not all equal ($P < 0.05$). The Newman-Keuls multiple range test showed that the means for the RV, LV, and SEP were significantly different from the mean for RA ($P < 0.05$).

[b] For the 6-OHDA-treated tissue, the data for each region were analyzed by an ANOVA. It was found that for each region there was no significant effect with time ($P > 0.05$). Thus, for a given region, the data for all the weeks were pooled and compared to the corresponding control region using Student's t test. The following treated regions were found to be significantly different from their controls: RA ($P < 0.001$), LV ($P < 0.001$), and SEP ($P < 0.05$).

TABLE 2

Effect of 6-OHDA treatment on the dissociation constant (K_d) and receptor density (B_{max}) of [^{3}H]DHA binding in homogenates of rat left ventricles

Saturation isotherms and a Scatchard analysis of [^{3}H]DHA binding in the left ventricles from control and 6-OHDA-treated rats were performed as described in Fig. 1 and the dissociation constants (K_d) and the number of binding sites (B_{max}) were estimated. The values are the means ± standard errors. The number of determinations was: control, N = 12; 6-OHDA treatment, N = 4–8.

	Control[a]	6-OHDA treatment[b]		
		1 week	2 weeks	3 weeks
K_d (nM)	0.27 ± 0.02	0.32 ± 0.04	0.36 ± 0.04	0.26 ± 0.03
B_{max} (fmol/mg tissue)	2.96 ± 0.11	3.90 ± 0.45	4.07 ± 0.15	3.46 ± 0.12

[a] The control data for each week were analyzed by an ANOVA. The analysis indicated that the means for the K_d and the B_{max} of control groups were not significantly different between weeks ($P > 0.05$). Therefore, the pooled data are presented.

[b] The ANOVAs were run on the K_d and B_{max} values obtained from the 6-OHDA-treated tissue. It was found that the K_d value did not differ between weeks ($P > 0.05$); however, there was a significant difference between weeks for the B_{max} data ($P < 0.05$). The Newman-Keuls multiple range test indicated that the means for B_{max} were significantly different between 2 and 3 weeks ($P < 0.05$) but not between 1 and 2 weeks ($P > 0.05$). Since there were no differences with time for the K_d data from the treated tissue, all the values were pooled and compared to controls using Student's t test. It was found that there was no significant difference ($P > 0.05$). Since the ANOVA showed that there were differences with time for the B_{max} data, each week was compared separately to controls using Student's t test. The B_{max} for the treated LV was found to be significantly different from the control: 1 week ($P < 0.05$), 2 weeks ($P < 0.001$), and 3 weeks ($P < 0.01$).

specific binding of [^{3}H]DHA to the homogenates of RA, LV, and SEP significantly increased in 6-OHDA-treated rats compared to control rats (Table 1). The maximum enhancement of the specific [^{3}H]DHA binding to these regions was seen at 2 weeks after the 6-OHDA injection. Saturation isotherms for [^{3}H]DHA binding to LV homogenates from control and 6-OHDA-treated (2 weeks) rats and a subsequent Scatchard plot were performed (Fig. 1). Table 2 shows the K_d and B_{max} for specific [^{3}H]DHA binding in the LV. The number (B_{max}) of [^{3}H]DHA binding sites was 3.90 ± 0.45 (N = 3) at 1 week, 4.07 ± 0.15 (N = 8) at 2 weeks, and 3.46 ± 0.12 (N = 8) at 3 weeks after 6-OHDA (Table 2). Thus, the number of binding sites significantly increased, by 32% (1 week), 38% (2 weeks), and 17% (3 weeks), in 6-OHDA-treated rat LV as compared with control preparations. The B_{max} of [^{3}H]DHA binding in the LV at 3 weeks was significantly ($P < 0.05$) lower than that at 2 weeks. The injection of 6-OHDA did not change the K_d significantly. Therefore, it seems likely that the increase in the specific binding of [^{3}H]DHA after 6-OHDA treatment is primarily due to a change in receptor density rather than a change in affinity. 6-OHDA treatment of rats had no significant effect on the specific binding of [^{3}H]DHA to the LA and RV homogenates at 1 and 2 weeks. Thus, there is a regional variation in the development of increased receptor density in cardiac β-adrenergic receptors following the chemical denervation by 6-OHDA treatment.

Effect of 6-OHDA treatment on muscarinic cholinergic receptors. The binding of [^{3}H]QNB in rat heart homogenates was stereospecific, with the IC_{50} of dexetimide being 1 nM and the IC_{50} of levetimide being 1450 nM, in agreement with Fields *et al.* (12). Scatchard analysis in control rat left ventricle homogenates demonstrated a K_d of 60.5 ± 4.3 pM and a B_{max} of 15.2 ± 0.8 fmol/mg tissue (N = 13) (Table 4). Table 3 shows the specific [^{3}H]QNB binding in five regions of hearts from control and 6-OHDA-treated rats. The control data for each region in Tables 3 and 4 were pooled since there was no developmental change in the specific [^{3}H]QNB binding to five regions of control rat hearts. There is a significant regional variation for the muscarinic cholinergic receptor between the atria and ventricles. The specific binding of [^{3}H]QNB in control rat hearts was much higher in atria than in ventricles, in agreement with previous reports (12). The specific binding of [^{3}H]QNB was not significantly changed in each region of rat heart after 1 week following the injection of 6-OHDA as compared with control preparations. At 2 weeks, there was an increase in [^{3}H]QNB binding in the RA and LV by 13 and 19%, respectively. Interestingly, at 3 weeks [^{3}H]QNB binding significantly increased in the RA, LA, and SEP. Since there was an approximately 35% increase in the [^{3}H]QNB binding in the RA and LA, saturation isotherms and a Scatchard analysis were carried out for these regions. Figure 2 shows the Scatchard plot in right atria from control and 6-OHDA-treated (3 weeks) rats. The B_{max} in

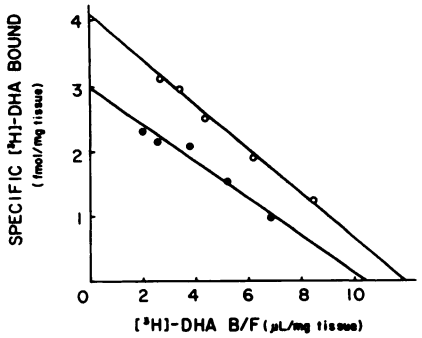

FIG. 1. *Scatchard plot of [³H]DHA binding to left ventricular homogenates from control (●) and 6-OHDA-treated (○) rats*

Animals received two doses of 50 mg/kg (i.v.) of 6-OHDA·HBr at 24-h intervals and were sacrificed 2 weeks later. The assays for [³H]DHA binding in left ventricles were performed according to standard procedures described in Methods. The abscissa is B/F = specific [³H]DHA bound (fmol/mg tissue)/free concentration of [³H]DHA of the medium (pM). The ordinate is specific [³H]DHA bound (fmol/mg tissue). The apparent K_d and receptor density B_{max} are: control—0.27 ± 0.02 nM and 2.96 ± 0.11 fmol/mg tissue, correlation coefficient r = 0.94; 6-OHDA treatment—0.36 ± 0.04 nM and 4.07 ± 0.15 fmol/mg tissue, r = 0.98. Each point represents the average of duplicate determinations from 12 (control) and 8 (treated) animals.

RA and LA was 25.6 ± 1.3 and 22.6 ± 1.3 fmol/mg tissue, respectively, in control rats (N = 4) and 33.9 ± 1.4 and 28.7 ± 1.8 fmol/mg tissue, respectively, in treated rats (N = 4) (Table 4). Thus, there was a 32% (RA) and 29% (LA)

TABLE 3

Effect of 6-OHDA treatment on specific binding of [³H]QNB to five regions of rat hearts

Saline control and 6-OHDA-treated rats were sacrificed 1, 2, and 3 weeks after injection. The assays for [³H]QNB binding in the five regions of rat hearts were performed according to standard procedures described in Methods. Tissue homogenates (1.25 mg) were incubated with 0.1 nM [³H]QNB at 37°C for 1 h. Specific [³H]QNB binding was experimentally determined as the difference between total binding and nonspecific binding in parallel assays in the absence and presence of 1 μM atropine. The values are given as fmol of QNB bound per mg tissue and are the means ± standard errors. The number of determinations was: control, N = 22; 6-OHDA treatment, N = 8–11.

Region	Specific [³H]QNB bound (fmol/mg tissue)			
	Control[a]	6-OHDA treatment[b]		
		1 week	2 weeks	3 weeks
Right atrium	13.6 ± 0.4	13.5 ± 0.7	15.4 ± 0.5	18.3 ± 0.5
Left atrium	14.7 ± 0.6	12.8 ± 0.7	13.9 ± 0.8	18.9 ± 0.4
Right ventricle	8.17 ± 0.28	8.95 ± 0.51	9.18 ± 0.8	10.0 ± 1.0
Left ventricle	9.25 ± 0.42	9.83 ± 0.45	11.0 ± 0.7	10.7 ± 0.9
Intraventricular septum	8.78 ± 0.37	8.65 ± 0.28	8.85 ± 0.42	11.4 ± 0.7

[a] The control data for each week were analyzed by an ANOVA. The analysis revealed that the means for each region did not differ with time ($P > 0.05$). Therefore, the pooled data are presented; however, the means for the different regions were not all equal ($P < 0.05$). The Newman-Keuls multiple range test showed that the means for the RA and LA were significantly different from the means for RV, LV, and SEP ($P < 0.001$).

[b] For the 6-OHDA-treated tissue, the data for each region were analyzed by an ANOVA. It was found that there was a significant difference between weeks for RA, LA, and SEP ($P < 0.05$) but not for RV and LV ($P > 0.05$). The Newman-Keuls multiple range test indicated that the means for RA, LA, and SEP were significantly different between 1 or 2 and 3 weeks ($P < 0.05$). According to Student's t test, the means for the treated RA, LA, and SEP at 3 weeks were found to be significantly different from their controls ($P < 0.001$). All the values for RV and LV were pooled and it was found that the mean for LV was significantly different from the control ($P < 0.05$) but that for RV was not.

TABLE 4

Effect of 6-OHDA treatment on the dissociation constant (K_d) and receptor density (B_{max}) of [³H]QNB binding in rat atria and left ventricles

Saturation isotherms and a Scatchard analysis of [³H]QNB binding in the right and left atria from control and 6-OHDA-treated rats were performed as described in Fig. 2 and the dissociation constant (K_d) and the number of binding sites (B_{max}) were estimated. The values are the means ± standard errors. The number of determinations was: RA and LA, control and 6-OHDA treatment, N = 4; LV, control, N = 3, 6-OHDA treatment, N = 3–7.

Region	Control	6-OHDA treatment		
		1 week	2 weeks	3 weeks
Right atrium[a]				
K_d (pM)	59.4 ± 4.4			68.2 ± 3.8
B_{max} (fmol/mg tissue)	25.6 ± 1.3	—	—	33.9 ± 1.4
Left atrium[a]				
K_d (pM)	58.2 ± 7.4			64.8 ± 5.5
B_{max} (fmol/mg tissue)	22.6 ± 1.3	—	—	28.7 ± 1.8
Left ventricle[b]				
K_d (pM)	60.5 ± 4.3	60.8 ± 7.5	66.2 ± 9.7	49.4 ± 3.0
B_{max} (fmol/mg tissue)	15.2 ± 0.8	19.2 ± 1.2	18.0 ± 1.5	14.6 ± 1.2

[a] The K_d of 6-OHDA-treated RA and LA was not significantly different from each control according to Student's t test, but the B_{max} was different ($P < 0.05$).

[b] The data were analyzed by an ANOVA. The analysis indicated that the means for K_d and B_{max} of control and 6-OHDA-treated LV were not significantly different between weeks ($P > 0.05$). Thus, all the values of K_d and B_{max} in the treated LV were pooled and compared to the pooled controls using Student's t test. It was found that both the K_d and the B_{max} of 6-OHDA-treated LV did not significantly differ from controls.

increase in the B_{max}, whereas the K_d in both regions was not significantly changed by 6-OHDA treatment. These results indicate that the increase in the [³H]QNB binding to RA and LA at 3 weeks after 6-OHDA treatment results from a change in receptor density.

Effect of 6-OHDA treatment on endogenous norepinephrine levels. The regional concentration of endogenous NE in hearts from control and 6-OHDA-treated (2 weeks) rats is shown in Fig. 3. In control rat hearts, the NE concentration was much higher in the RA than in the LA and higher in the RV than in the LV. The regional distribution of NE in rat heart, determined by the radioenzymatic assay procedure, is consistent with the previous reports determined by bioassay (21). There was a marked (60–80%) reduction of the NE concentration in each region of rat heart following 6-OHDA treatment. At 2 weeks after 6-OHDA treatment, the NE concentration was most significantly reduced in the RA and LV among the five regions (to about 20% of control values). Interestingly, these regions showed significant increases in specific [³H]DHA binding at 2 weeks after 6-OHDA treatment.

The time course of change in NE concentration of LV following 6-OHDA treatment was examined. Since the analysis (ANOVA) indicated that the NE concentration in control LV was not significantly different with time ($P > 0.05$), all the control values were pooled. The NE

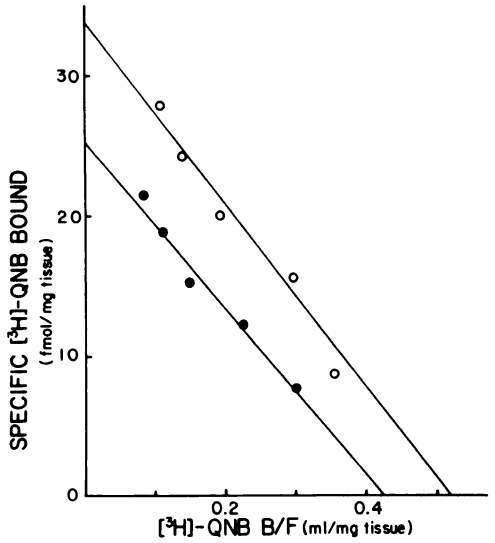

FIG. 2. *Scatchard plot of [^{3}H]QNB binding to right atrial homogenates from control (●) and 6-OHDA-treated (○) rats*

The assays for [^{3}H]QNB binding in right atria were performed according to standard procedures described in Methods. The abscissa is B/F = specific [^{3}H]QNB bound (fmol/mg tissue)/free concentration of [^{3}H]QNB of the medium (pM). The ordinate is specific [^{3}H]QNB bound (fmol/mg tissue). The apparent K_d and receptor density B_{max} are: control—59.4 ± 4.4 pM and 25.6 ± 1.3 fmol/mg tissue, correlation coefficient r = 0.95; 6-OHDA treatment—68.2 ± 3.8 pM and 33.9 ± 1.4 fmol/mg tissue, r = 0.96. Each point represents the average of duplicate determinations from four animals.

concentration in control LV was 0.47 ± 0.03 (N = 14) μg/g fresh weight, and that in 6-OHDA-treated LV was 0.10 ± 0.01 (1 week, N = 6), 0.10 ± 0.01 (2 weeks, N = 8), and 0.14 ± 0.01 (3 weeks, N = 8) μg/g fresh weight, respectively. Thus, at 1 and 2 weeks after the treatment, the NE concentration was significantly ($P < 0.001$) reduced, to 20% of control values and to 30% at 3 weeks. There was a significant ($P < 0.05$) increase (40%) in the NE concentrations in the LV at 3 weeks as compared with that at 1 or 2 weeks. The recovery of endogenous NE concentrations following 6-OHDA treatment has also been reported in rat hearts (4).

In another set of experiments, rats were treated with two i.v. doses of 6-OHDA·HBr, 50 mg/kg, on the first day and two doses of 68 mg/kg 1 week later, according to Sharma and Banerjee (10). The animals were sacrificed 2 and 3 weeks after the first dose of 6-OHDA for endogenous NE determinations in hearts. NE concentrations in the LV from these 6-OHDA-treated rats were 0.059 ± 0.005 μg/g fresh weight (N = 4) and 0.058 ± 0.005 μg/g fresh weight (N = 4), respectively, at 2 and 3 weeks. Thus, NE levels in the LV were reduced to 13% of control values, more markedly than the values obtained previously, and there was no recovery of NE levels in these rats at 3 weeks. In contrast to the animals treated with two doses of 50 mg/kg i.v., which showed no change in body weight compared with the control group, the latter group of animals showed an altered growth pattern (35–45% decrease in weight compared to controls at the time of sacrifice).

Effect of 6-OHDA treatment on choline acetyltransferase activity. CAT is usually considered as a marker for cholinergic neurons. Ekstrom (22) found that chemical sympathectomy by 6-OHDA caused an increase in the total CAT activity in the rat submaxillary gland. To determine if the activity of cholinergic nerves in the hearts may be affected by 6-OHDA treatment, the CAT activity in five regions from control and 6-OHDA-treated rats was assayed. The control data for each week were analyzed by an ANOVA. The analysis revealed that the CAT activity for each region did not differ with time ($P > 0.05$). Therefore, the pooled data for CAT activity are presented (nmol ACh formed/g fresh weight of tissue/min): RA = 23.1 ± 1.5 (N = 4), LA = 11.4 ± 0.7 (N = 4), RV = 12.2 ± 0.5 (N = 20), LV = 10.3 ± 0.4 (N = 20), and SEP = 11.1 ± 0.4 (N = 20). The Newman–Keuls multiple range test showed that the enzyme activity of the RA was significantly ($P < 0.001$) greater than that of the other regions, in agreement with previous reports (23). Since the CAT activity for each region of 6-OHDA-treated rats did not differ with time ($P > 0.05$), all the values in the treated tissues were pooled and compared to controls. It was found that the CAT activity was not significantly altered in any region of the 6-OHDA-treated rats as compared with the control rats.

DISCUSSION

The major finding of our study is the development of increased receptor density of both cardiac β-adrenergic and muscarinic cholinergic receptors in rat hearts following 6-OHDA treatment. There is a regional variation in the development and extent of the increase in receptor density for both receptors after 6-OHDA treatment. In addition, the treatment resulted in a marked reduction of endogenous NE concentrations with no change in CAT activity.

Little information is available regarding the regional

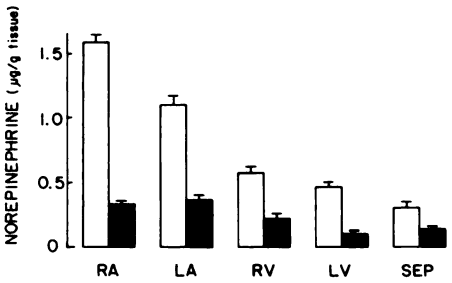

FIG. 3. *Regional distribution of NE in rat hearts and its reduction by 6-OHDA treatment*

Animals received two doses of 50 mg/kg (i.v.) of 6-OHDA·HBr and were sacrificed 2 weeks later for assay of endogenous NE as described in Methods. Each region (RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; SEP, ventricular septum) from control and 6-OHDA-treated rats was homogenized in 10 or 20 vol of cold 0.1 N perchloric acid and centrifuged for 10 min at 10,000g; 50 μl of supernatant aliquots was used for radioenzymatic NE assay. The white column shows the concentration of NE in five regions from control rat hearts, and the black column from 6-OHDA-treated rat hearts. Each column (μg of NE per g fresh weight of tissue) represents the mean (±standard error) of determinations from 6 control and 3 treated rats for RA, LA, RV, and SEP and from 20 control and 6 treated rats for LV. The values from 6-OHDA-treated preparations are significantly different from control values (RA, LA, and LV, $P < 0.001$; SEP, $P < 0.02$; RV, $P < 0.01$).

distribution of cardiac [^{3}H]DHA binding sites. We found a significant regional distribution which was different from either the distribution of endogenous NE or the distribution of [^{3}H]QNB binding sites. There is nearly an inverse relationship between the NE content and the [^{3}H]DHA binding sites when both are expressed per unit of fresh tissue weight. Treatment of rats with 6-OHDA resulted in a significant increase in specific [^{3}H]DHA binding in RA and LV at all times studied. Scatchard analysis of saturation isotherms indicated that the enhancement of specific [^{3}H]DHA binding in LV is due to an increase in the receptor density which was maximal at 2 weeks. The affinity was unchanged. Chronic treatment of rats with guanethidine using a dosage schedule that produces permanent neuronal destruction has resulted in an increase in the number of β-adrenergic receptors in the ventricles (8). As compared to guanethidine, 6-OHDA rapidly destroys noradrenergic neurons with a high degree of selectivity (6). Also, treatment of rats with 6-OHDA or reserpine causes a significant increase in basal myocardial ventricular adenylate cyclase activity and in the enzyme stimulated by NE, isoproterenol, NaF, or guanylimidodiphosphate (7).

Using the cat's nictitating membrane preparation, Trendelenburg and Weiner (2) proposed two mechanisms for the development of supersensitivity after various procedures and agents, that is, the presynaptic supersensitivity seen after cocaine treatment or 24 h after surgical denervation and a postsynaptic supersensitivity occurring about 2 weeks after surgical denervation. In the isolated perfused hearts from 6-OHDA-treated rats (24) and cats (25) (12–16 days), an increase (3- to 10-fold) in the sensitivity (measured by the positive chronotropic response) to NE was observed. This denervation supersensitivity in pharmacological response seems to be greater than the increase in the β-adrenergic receptor density following 6-OHDA treatment obtained in the present study. NE uptake could be expected to be virtually abolished in hearts after 6-OHDA treatment (4, 6). Since the denervation supersensitivity after 6-OHDA treatment was of a similar magnitude as the maximal supersensitivity obtained with cocaine or desipramine, Haeusler *et al.* (25) suggested that the observed denervation supersensitivity in physiological experiments using 6-OHDA treated hearts was primarily related to the altered NE uptake and not postsynaptic supersensitivity. Early appearance of presynaptic supersensitivity was also mentioned in isolated rat atria and ventricles after a single intravenous injection of 6-OHDA since there occurred a supersensitivity to NE without any change in the response to isoproterenol (26). In contrast, our results are compatible with the development of postsynaptic supersensitivity of β-adrenergic receptors in rat atria and ventricles following 6-OHDA treatment. In view of the previous literature, this mechanism probably accounts for a relatively small component of the observed supersensitivity of the sympathectomized heart and may be very difficult to demonstrate in physiological experiments.

In our study, treatment with 6-OHDA produced a marked (60–80%) reduction of endogenous NE in each region of heart, which is consistent with previous reports (4, 5, 26). At 2 weeks, when the increase in receptor density of β-adrenergic receptors in the RA and the LV was maximal, the reduction of NE was also most significant (80%) in these regions. This finding is consistent with previous pharmacological observations that supersensitivity of pharmacological responsiveness to catecholamines seen in rat hearts (24) and in cat hearts and/or nictitating membranes (2, 25, 27) was maximal 2 weeks after treatment with 6-OHDA or reserpine and surgical denervation. The increased receptor density of the β-adrenergic receptors in LV (Table 2) had significantly declined at 3 weeks after 6-OHDA treatment, in parallel with a small but significant recovery of NE in LV (compared to 1 or 2 weeks). The recovery of endogenous NE concentration suggests the regeneration of sympathetic nerve terminals in the LV of 6-OHDA treated rats as previously described (4, 5).

The treatment of rats with 6-OHDA had complex effects on the cardiac muscarinic cholinergic receptors. [^{3}H]QNB binding in the RA, LA, and SEP increased significantly at 3 weeks. The enhancement in RA and LA was shown to be a change in receptor density and not in the affinity. Although Story *et al.* have found a 50% increase (11) and also no change (28) in the maximal binding of [^{3}H]QNB to membrane fragments from 6-OHDA-treated rat heart ventricles (2 weeks), our data indicated no significant change in [^{3}H]QNB binding in the ventricles at 2 weeks (Tables 3 and 4). The CAT activity in each region was not significantly altered at any time after 6-OHDA treatment, suggesting that nonspecific cholinergic neuronal destruction did not occur. Acetylcholine levels (29) and CAT activity (30) in rat brain are reportedly unaltered 15 days after the intraventricular treatment with 6-OHDA. It is possible that the increase in cardiac β-adrenergic receptors by 6-OHDA treatment resulted in the alteration in the muscarinic cholinergic receptors since the ratios at 3 weeks of [^{3}H]-QNB binding to [^{3}H]DHA binding are not different in the controls or treated animals. Recently, Sharma and Banerjee (10) concluded that the 50% decrease in the specific binding of [^{3}H]QNB following 6-OHDA treatment was due to a loss of presynaptic muscarinic receptors located on the noradrenergic neurons. In the present study, we did not find a significant decrease in specific [^{3}H]QNB binding in any region of rat hearts at any time after 6-OHDA treatment. We therefore suggest that most of the cardiac muscarinic cholinergic receptors are localized postsynaptically and are not related to adrenergic neurons.

In conclusion, we have shown that the destruction of noradrenergic nerve terminals in rats with 6-OHDA leads to an increase in the density of both β-adrenergic receptors and muscarinic cholinergic receptors in the heart. There is a regional variation in the development and extent of the increased receptor density for these receptors. The majority of muscarinic cholinergic receptors does not appear to be localized on adrenergic nerve terminals.

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