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(+)-[^{3}H]Isradipine and [^{3}H]Glyburide Bindings to Heart and Lung Membranes From Rats With Monocrotaline-Induced Pulmonary Hypertension

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ABSTRACT—We examined the binding of a 1,4-dihydropyridine-sensitive Ca^{2+} channel ligand, (+)-[^{3}H]isradipine (PN200-110), and that of an ATP-sensitive K^+ (K_{ATP}) channel ligand, [^{3}H]glyburide, to heart, lung and brain membranes isolated from Sprague-Dawley rats made pulmonary hypertensive by monocrotaline, a pyrrolizidine alkaloid. A single subcutaneous injection of monocrotaline increased right ventricular systolic pressure, a measure of pulmonary arterial pressure, and the thickness of the right ventricular free wall in 3 to 4 weeks. The (+)-[^{3}H]PN200-110 and [^{3}H]glyburide binding site densities (B_{max}) were reduced in hypertrophied right ventricles when normalized per unit protein in comparison with those of age-matched control (sham) rats, whereas the values of the dissociation constant (K_d) of both ligands bound to the hypertrophied right ventricle were not significantly changed. The [^{3}H]PN200-110 binding to the lung membranes of the monocrotaline-induced pulmonary hypertensive rats was increased. The results indicate that the change in the binding of 1,4-dihydropyridine Ca^{2+} and K_{ATP} channel ligands to heart membranes may contribute to the pathological alteration of cardiopulmonary structure and functions in rats with pulmonary hypertension induced by monocrotaline.

Keywords: Ca^{2+} channel, ATP-sensitive K^+ channel, Monocrotaline, Pulmonary hypertension, Binding assay

A single injection of monocrotaline (MCT), a pyrrolizidine alkaloid from *Crotalaria spectabilis*, leads to progressive pulmonary hypertension resulting in right ventricular hypertrophy and cardiac failure in rats (1, 2). Various etiologic factors pertaining to the genesis of MCT-induced pulmonary hypertension, including an increase in endogenous endothelin-1 production (3) and a decrease in NO production (4), have been reported; and the curative effects of an overwhelming number of drugs on the experimental disease have been assessed; e.g., voltage-dependent L-type Ca^{2+} channel blockers such as 1,4-dihydropyridines (DHP) (nifedipine, 5; amlodipine, 6) and non-DHP (verapamil, 7; semotiadil, 8).

It becomes evident that ion channels and pharmacological receptors are susceptible to diseases and that their functional properties can be changed, as well as their expression at the messenger RNA and protein levels. Despite much information on L-type Ca^{2+} channels and ATP-sensitive K^+ (K_{ATP}) channels in the circulatory system, the data obtained in experimental models of cardiac diseases, including ventricular hypertrophy, heart failure, and cardiomyopathy, are still controversial. As there is an argument as to whether the MCT-induced pulmonary hypertension mirrors the functional changes in human pulmonary hypertension (9), it is important to pharmacologically characterize the experimental model of the disease.

The purpose of the present study was to investigate the status of L-type Ca^{2+} channels and K_{ATP} channels in the rat with MCT-induced pulmonary hypertension. The K_{ATP} channel is composed of at least two subunits, a K^+-channel subunit and a sulfonylurea receptor, and glyburide, a sulfonylurea receptor antagonist, has been used to identify K_{ATP} channels. Although both high and low radioligand binding sites for [^{3}H]glyburide have been identified, the high affinity component referred to as a

sulfonylurea receptor (10) has been considered to play a more important role in the cardiovascular system (11), and only limited data are available on the low affinity component (12–15). Thus, in the model of right ventricular hypertrophy induced by MCT in rats, we focused primarily on changes in the high affinity binding properties of the radioligands; i.e., (+)-[^{3}H]isradipine (PN200-110), a DHP Ca^{2+} channel ligand, and [^{3}H]glyburide, a sulfonylurea receptor ligand, in membranes obtained from cardiac right and left ventricles as well as in those from lungs and brain. Our results indicate that the maximum density of binding sites (B_{max}) for both DHP Ca^{2+} and K_{ATP} channel ligands was reduced in hypertrophied right ventricle, whereas that for the DHP Ca^{2+} channel ligand in the lungs of MCT-treated rats was significantly increased.

MATERIALS AND METHODS

Treatment with monocrotaline

Male Sprague-Dawley rats (Japan SLC, Shizuoka), 5-week-old and having a body weight of about 150 g at the start of treatment, were used. The rats were treated with a single s.c. injection of MCT (40 mg/kg) or saline (2 ml/kg) 3 weeks before the experiments. The rats were fed in our authorized animal house following the guidelines for animal care and experiments established by The Japanese Pharmacological Society. The general condition and body weight of the rats were checked every day.

Measurements of circulatory parameters

Rats were anesthetized by i.p. administration of α-chloralose (80 mg/kg) and urethane (0.8 g/kg). After the induction of anesthesia, tracheotomy was performed; and artificial ventilation was then started with an artificial respirator (Model SN-480-7, Shinano Seisaku Sho, Tokyo). A polyethylene catheter was placed in the left common carotid artery for direct monitoring of continuous arterial pressure and heart rate. After thoracotomy, the right ventricular systolic pressure (RVSP), a measure of pulmonary arterial pressure (16), was also recorded via a hypodermic needle inserted into the right ventricle. Afterwards, blood samples for the measurement of hematocrit value and hemoglobin concentration were taken from the abdominal aorta; and the animals were then exsanguinated from the common carotid arteries. Following the removal of the pulmonary artery and aorta, the heart and lungs were isolated and weighed to determine the ratio of the weight of the free wall of the right ventricle to the that of the left ventricle plus interventricular septum (RV/(LV+S)) (17).

Increases in RVSP and RV/(LV+S) were taken as indications of pulmonary hypertension and right ventricular hypertrophy, respectively. Furthermore, the ratio of lung wet weight to dry weight and hematocrit value were measured for indications of lung edema and polycythemia, respectively.

Histological experiments

In a separate series of experiments, lungs and hearts of rats (MCT-treated, n=7; sham, n=6) were examined histologically by light microscopy. These organs were fixed in 10% neutral-buffered-formalin, dehydrated and embedded in paraffin. The paraffin block was cut and stained with hematoxylin-eosin.

Membrane preparations

When the ventricular hypertrophy began to be initiated at about 1.5 weeks (10 days) or was established 3 to 4 weeks after the administration of MCT, the drug-treated and the age matched control animals were decapitated; and the hearts, forebrains (whole brain minus brain stem and cerebellum), and lungs were dissected rapidly and placed in ice-cold 50 mM Tris-HCl buffer, pH 7.4, after removal of blood by washing. After right and left atria had been removed, the right ventricle was separated from the left ventricle and septum. The ventricles were then minced with scissors and disrupted 3 times with a Polytron (Physcotron homogenizer, Model NS-60; Nichi-on, Tokyo) (12,000 rpm for 15 s each time) and then homogenized with a glass-Teflon tissue homogenizer (MJ1-315-01; Iuchi, Osaka) in 15 vol of ice-cold Tris-HCl buffer. Forebrains were also minced with scissors and disrupted 2 times with a Polytron (12,000 rpm for 15 s each time) and then homogenized in a glass-Teflon tissue homogenizer in 15 vol of ice-cold Tris-HCl buffer. Suspensions of both tissues were filtered through a double layer of cheesecloth, and the filtrates were centrifuged 2 times at 45,000×g for 45 min. The pellets were resuspended in incubation buffer (50 mM Tris-HCl) and used for radiobinding assays.

The membrane fraction of lung tissue was also prepared. Main trunks of pulmonary arteries and veins were removed, and the lung tissue was minced with scissors and disrupted 5 times with a Polytron (12,000 rpm for 15 s each time), with subsequent homogenization in 7 vol of ice-cold Tris-HCl buffer in a glass-Teflon tissue homogenizer. The tissue suspension was centrifuged for 10 min (500×g), and the supernatant was filtered through a double layer of cheesecloth. The filtrate was centrifuged at 10,000×g for 45 min, and the supernatant was further centrifuged 2 times at 45,000×g for 45 min each time. The pellet was resuspended in ice-cold Tris-HCl buffer and used for radiobinding assays. Protein in tissues used was determined by the method of Lowry et al. (18) with bovine serum albumin as a standard.

(+)-[^{3}H]PN200-110 (isradipine) binding

(+)-[^{3}H]PN200-110 binding to heart, brain and lung membranes was determined by our previously reported method in which membranes of porcine coronary artery smooth muscles (19) and canine skeletal muscles (20) were used. Tissue membrane fragments (80−150 μg protein) were incubated with (+)-[^{3}H]PN200-110 (15−500 pM) in 50 mM Tris-HCl buffer, pH 7.4. Incubation was ordinary performed in the dark with a sodium lamp for 60 min at 37℃. The reaction was terminated by rapid filtration under vacuum (Cell Harvester, model M-24R; Brandel, Gaithersburg, MD, USA) through Whatman GF/B glass fiber filters, and the filters were rinsed 3 times with 4 ml ice-cold Tris-HCl buffer. Tissue-bound radioactivity was extracted from the filters overnight into scintillation cocktail, and the radioactivity was determined in a liquid scintillation counter. Specific (+)-[^{3}H]PN200-110 binding was determined from the difference between counts with and without 3 μM nifedipine as a displacer. All assays were performed in triplicate. Specific binding to the membranes represented 70−90% of total binding at 37℃.

[^{3}H]Glyburide binding

In order to identify high-affinity binding of [^{3}H]glyburide to the membrane preparations, the radioligand concentration range (20−600 pM) was examined, and 0.1 μM glyburide was used as a displacer. Since freezing has been reported to deteriorate the affinity and density of [^{3}H]-glyburide binding (21), only freshly-prepared membranes were used in this study. Briefly, heart (250−500 μg protein) and brain (350−500 μg protein) membranes were incubated with [^{3}H]glyburide (20−600 pM) in Tris-HCl buffer at pH 7.4. Incubation was performed for 60 min at 25℃. The reaction was terminated by rapid filtration under vacuum through glass fiber filters, and the filters were rinsed 3 times with 4 ml ice-cold Tris-HCl buffer. Tissue-bound radioactivity was extracted from the filters overnight into scintillation cocktail, and the radioactivity was determined in a liquid scintillation counter. Specific [^{3}H]glyburide binding was determined from the difference between counts with and without 0.1 μM glyburide as a displacer. All assays were performed in triplicate.

Data analyses

The equilibrium dissociation constant (K_d) and B_{max} for each ligand were estimated by linear regression analysis after Scatchard analysis of the saturation binding data. Hill slopes for saturation data of radioligand and inhibition by antagonists were obtained by Hill plot analysis. All data, including measurements of cardiac functions, were reported as the mean±S.E.M. Statistical analyses were made by the paired or unpaired Student's *t*-test. To determine whether responses differed significantly between groups, we performed Tukey's multiple range tests after analysis of variance (ANOVA). Differences were considered significant when the P value was less than 0.05.

Drugs

(+)-[^{3}H]PN200-110 (isradipine) (3.18 Tbq/mmol) and [^{3}H]glyburide (1,883.3 Gbq/mmol) were purchased from Dupont-New England Nuclear (Boston, MA, USA). Bovine serum albumin, glyburide and nifedipine were obtained from Sigma (St. Louis, MO, USA). MCT was obtained from Wako (Osaka). Other drugs of reagent grade were used. MCT was dissolved in 1 N HCl and neutralized with 1 N NaOH to pH 7.4.

RESULTS

General and hemodynamic characteristics

At the start of the experiments, the body weight was similar between MCT-treated and the age-matched sham groups (MCT, 145±3.0 g vs sham, 152.7±5.8 g; each n=6). However, after treatment with MCT for 3 weeks, the body weight of the rats increased more slowly in comparison with that of the sham group (MCT, 251.5±9.4 g vs sham, 298.0±13.5 g; $P<0.01$, each n=6); and at 4 weeks, the weight of the rats treated with MCT was rather decreased (See Table 1).

Figure 1 shows the time-course of changes in cardiac parameters, including RVSP and mean arterial pressure (MAP), and the indication of ventricular hypertrophy as shown by the increased weight ratio of right ventricle to left ventricle plus septum (RV/(LV+S)). Although the MAP was similar between MCT- and saline-treated rats after 4 weeks, the heart rate was decreased in the MCT-treated rats (MCT, 382±31 beats/min vs sham, 438±14 beats/min; $P<0.05$, each n=6). RVSP in the MCT-treated rat was significantly increased 3 to 4 weeks after

Table 1. Weight of right ventricle (RV), left ventricle + septum (LV+S), lung, forebrain and body weight 3−4 weeks after treatment with monocrotaline or saline

	Control	MCT
RV (g)	0.17±0.01	0.32±0.02**
LV+S (g)	0.64±0.02	0.58±0.02
Lung (g)	1.02±0.04	1.56±0.09**
Forebrain (g)	1.08±0.01	1.06±0.02
Body weight (g)	317.3±11.7	250.8±9.0**

Values are the mean±S.E.M. of 9−12 experiments. **$P<0.01$, as compared with the corresponding control value.

administration of MCT. The weight ratio of right ventricle to left ventricle plus septum (RV/(LV+S)) was also significantly increased at 3 to 4 weeks, and RV/(LV+S) well correlated with RVSP.

Table 1 summarizes the chronic effect of MCT on heart, lung, as well as forebrain. The wet weight of the total heart and right ventricle was significantly increased, whereas that of the left ventricle was not. The maximum free wall thickness of the right ventricle was also significantly increased in the MCT-treated rats (MCT, 1.93 ± 0.12 mm vs sham, 1.18 ± 0.06 mm; each $n=7$, $P<0.01$), whereas there was no significant difference in those properties of the left ventricle between the drug-treated and sham groups (MCT, 2.97 ± 0.28 mm vs sham, 2.98 ± 0.26 mm; each $n=7$). Interestingly, the dry weight of the lungs isolated from the MCT-treated rat was significantly increased when compared with that of the sham rats (MCT, 0.31 ± 0.02 g vs sham, 0.21 ± 0.01 g; each $n=9$, $P< 0.01$).

Animals with a RV/(LV+S) value over 0.5 after administration of MCT for 3 to 4 weeks were used as MCT-induced pulmonary hypertensive rats, as were aged-matched sham rats for the radioligand binding study.

Histopathologic changes occurred in right ventricles, including ventricular wall, septum of right side and papillary muscles, and lungs as well as pulmonary arteries isolated from MCT-treated rats. In the right ventricles, mononuclear cell infiltration, swelling and vacuolic change of muscle fibers were observed. In the lungs, infiltration of mononuclear cells into the adventitia and fibrinnoid degeneration of the intrapulmonary arteries were occasionally observed. In addition to these changes, edema and accumulation of the foam cells were observed in the lung parenchyma.

Radioligand binding to cardiac, brain and lung membranes

(+)-[^{3}H]PN200-110 binding: (+)-[^{3}H]PN200-110 binds specifically and with high affinity to cardiac, brain and

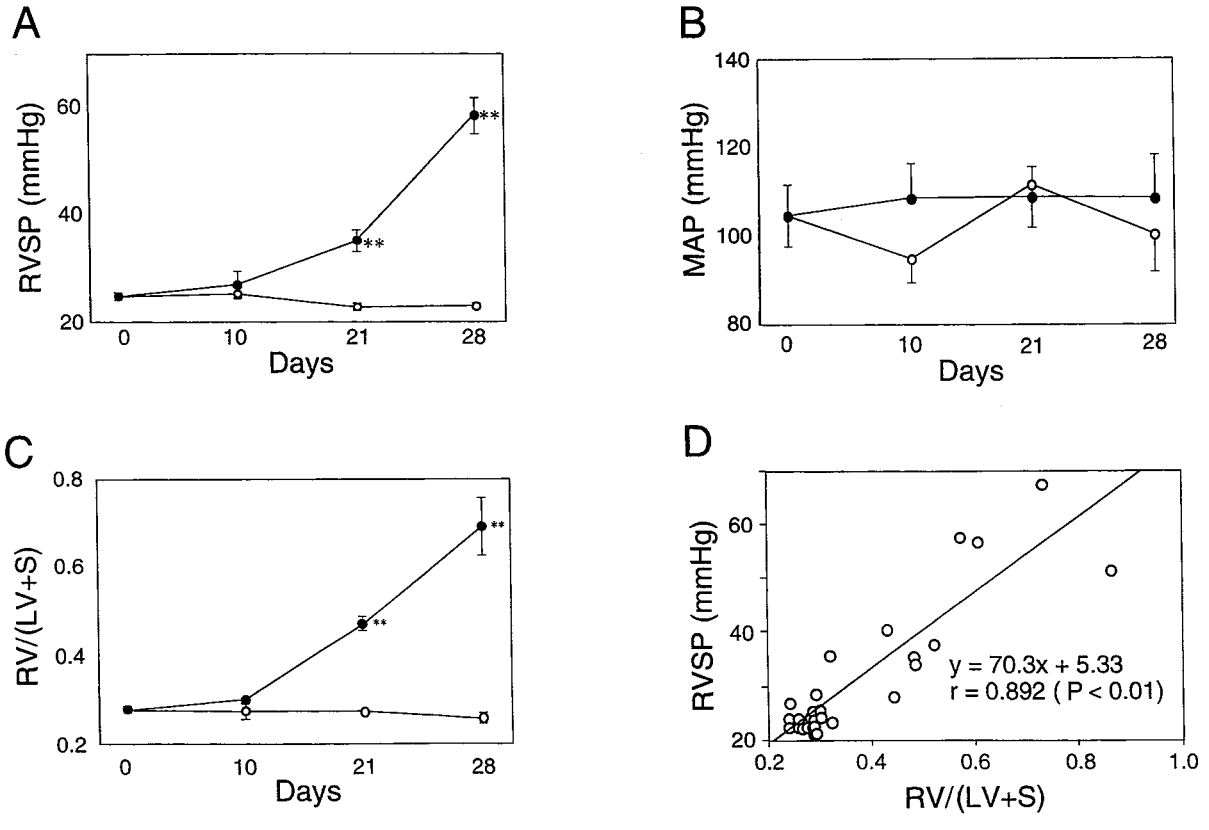

Fig. 1. Time-course of changes in hemodynamic parameters after treatment with a single subcutaneous injection of MCT (40 mg/kg). Changes in RVSP (right ventricular systolic pressure, mmHg: panel A), MAP (mean arterial pressure, mmHg: panel B), and weight ratio of RV (right ventricle) to LV (left ventricle) plus septum (S) (panel C) are shown as is the relationship between RVSP and RV/(LV+S) (panel D). Open and closed symbols are age-matched control and MCT-treated animals, respectively. Each point with bars represents the mean±S.E.M. of 5–6 animals. A significant difference from the vehicle-treated group is shown (**$P<0.01$).

lung membranes. A single population of (+)-[^{3}H]PN200-110 binding sites was detected in cardiac ventricle, brain and lung membranes within the ligand concentration range studied (15–400 pM). Nonspecific binding of (+)-[^{3}H]PN200-110 ranged from 10% to 20% of total binding in both cardiac and brain preparations, whereas it reached about 30% in the lung membranes. The nonspecific binding was independent of the status of pulmonary hypertension and right hypertrophy induced by MCT.

Figure 2 shows a typical curve for the specific (+)-[^{3}H]PN200-110 binding to right ventricular membranes, as a function of increasing concentrations of the ligand, from the rats treated with vehicle (saline) (panel A) or with MCT for 3 to 4 weeks (panel B), and the Scatchard analysis of the data. For both membranes, the specific binding at 37℃ was saturable, thus forming a plateau in the concentration ranges of the radioligand used (15–400 pM). Nonspecific binding, measured at half saturation, was about 20% of the total binding.

Scatchard analysis of the saturation curves at the observed concentration range showed a linear plot, suggesting a single population of binding sites (Fig. 2, panel C). Hill slopes (nH) were not significantly different from unity, as revealed by saturation analysis of right ventricles from both the MCT-treated and the age-matched control rats, within the concentration range examined (nH, MCT, 1.002±0.025 vs sham, 0.966±0.025; each n=5).

Table 2 summarizes values of K_d and B_{max} for (+)-[^{3}H]PN200-110 in various tissue membranes obtained from animals treated or not treated with MCT. The B_{max} for the right ventricle treated with vehicle for 1.5 (10 days) and 3 to 4 weeks was in a range of about 140–150 fmol/mg protein, and the K_d of (+)-[^{3}H]PN200-110 was 60–70 pM. Scatchard analysis of the data obtained from the rats treated with MCT for 3–4 weeks and the age-matched sham rats revealed that the regression lines were parallel, indicating that there was no significant difference between the values of K_d, whereas B_{max} for binding to right ventricular muscle membranes obtained from rats treated with MCT for 3 to 4 weeks was significantly decreased, about 20% as compared with the age-matched sham group (Fig. 2 and Table 2). However, the values of K_d and B_{max} for (+)-[^{3}H]PN200-110 binding to right ventricular muscle membranes from the rats treated with MCT for 10 days were not significantly different from

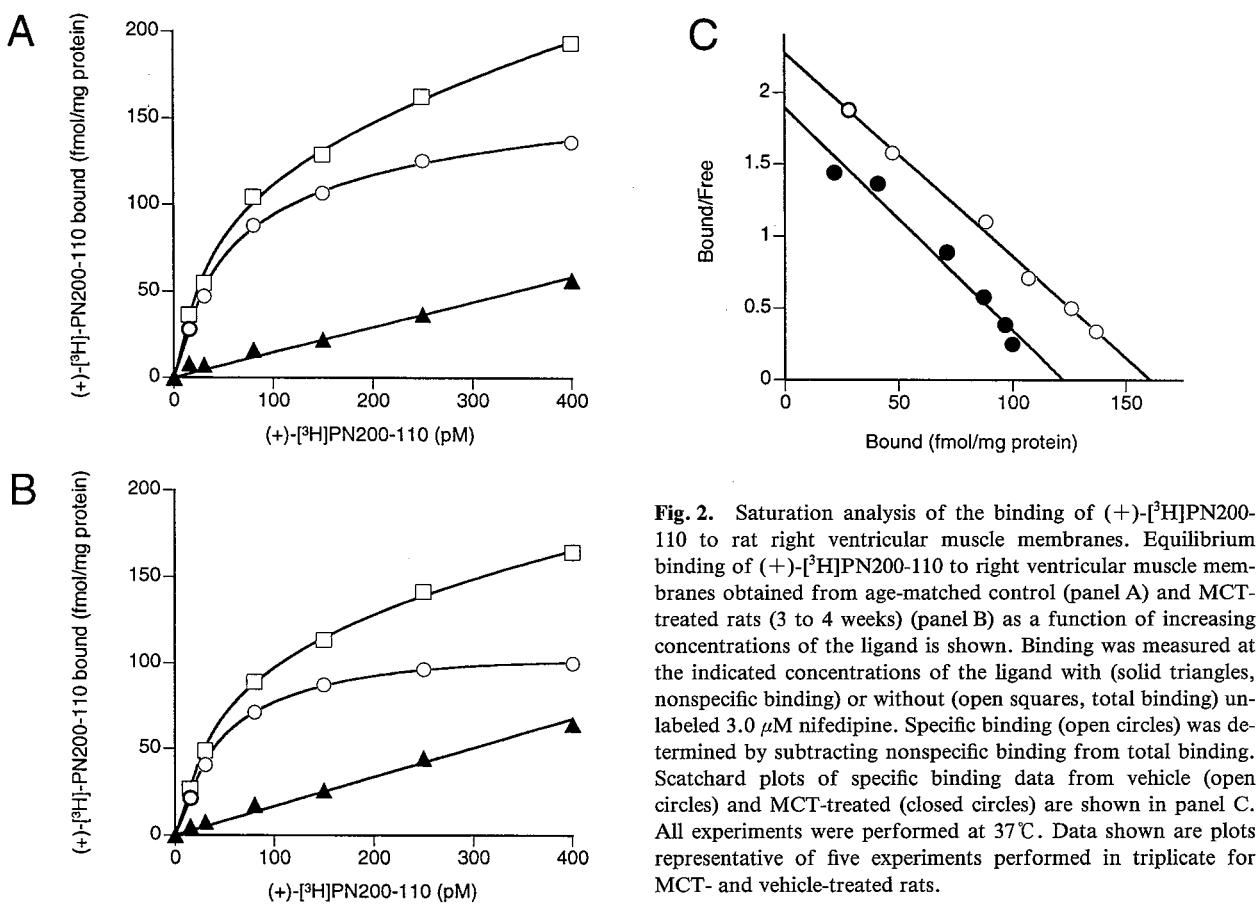

Fig. 2. Saturation analysis of the binding of (+)-[^{3}H]PN200-110 to rat right ventricular muscle membranes. Equilibrium binding of (+)-[^{3}H]PN200-110 to right ventricular muscle membranes obtained from age-matched control (panel A) and MCT-treated rats (3 to 4 weeks) (panel B) as a function of increasing concentrations of the ligand is shown. Binding was measured at the indicated concentrations of the ligand with (solid triangles, nonspecific binding) or without (open squares, total binding) unlabeled 3.0 μM nifedipine. Specific binding (open circles) was determined by subtracting nonspecific binding from total binding. Scatchard plots of specific binding data from vehicle (open circles) and MCT-treated (closed circles) are shown in panel C. All experiments were performed at 37℃. Data shown are plots representative of five experiments performed in triplicate for MCT- and vehicle-treated rats.

those of the age-matched sham group. In the left ventricle plus septum, no significant change occurred in either K_d or B_{max} for (+)-[^{3}H]PN200-110, irrespective of whether the animal was treated with MCT or not (Table 2).

Interestingly, an enormous increase in B_{max}, about 40% of (+)-[^{3}H]PN200-110 binding, was noted in lung membranes obtained only from rats treated with MCT for 3 to 4 weeks, whereas the value of K_d was not significantly altered in the MCT-treated and age-matched sham groups (Table 2). Saturation analysis performed using the brain membranes showed no significant change in the K_d and B_{max} values for the control and MCT-treated animals (Table 2). Hill slopes were unity in tissues also from the lungs, forebrain and left ventricle of MCT-treated and the age-matched sham rats (data not shown).

[^{3}H]Glyburide binding: [^{3}H]Glyburide bound specifically and with high affinity to cardiac and brain membranes, whereas the ligand did not show specific binding with a high affinity to lung membranes, in both MCT-treated and the age-matched sham groups. The specific binding of the ligand to cardiac and brain membranes represented 70–80% of the total binding, whereas that to lung membranes was less than 50% of the total binding and non-saturable. Scatchard analysis of the saturation curves at the observed concentration range showed a linear plot, indicating a single population of binding sites in cardiac and brain membranes, when the radioligand concentration range of 20–600 pM was examined and 0.1 μM glyburide used as a displacer.

Figure 3 shows a typical curve for the specific [^{3}H]glyburide binding to right ventricular membranes as a function of increasing concentrations of the ligand, from rats treated with vehicle (saline) (panel A) or MCT (panel B), and the Scatchard analysis of the data. Table 3 summarizes values of K_d and B_{max} for [^{3}H]glyburide for cardiac and brain membranes obtained from animals treated or not treated with MCT.

The B_{max} for binding to right ventricular muscle membranes obtained from rats treated with MCT for 3 to 4 weeks was significantly decreased, about 40% in comparison with that of the age-matched control (Table 3). The K_d, for the right ventricle, however, did not change significantly between MCT-treated and control groups (Table 3). The values of B_{max} and of K_d were not altered significantly for membranes of either the brain or the left ventricle plus septum obtained from animals treated with MCT for 3 to 4 weeks in comparison with those of the sham group. No significant change in K_d or B_{max} could be observed for the right ventricle or the left ventricle and septum obtained from rats treated with MCT for 10 days, when compared to the sham group (Table 3). Hill slopes were unity by saturation analysis in tissues, including right and left ventricles and forebrain isolated from the MCT-treated and sham groups (data not shown).

DISCUSSION

The present study provides evidence for the reduction in the B_{max} values for both DHP Ca^{2+} and K_{ATP} channels, as determined by the binding of radiolabeled [^{3}H]PN200-110 and [^{3}H]glyburide, respectively, in the right ventricles of MCT-treated rats at the established stage of pulmonary hypertension. An increase in the B_{max} of DHP Ca^{2+} channels over 40% was also observed in the lung tissue isolated from the pulmonary hypertensive rats. In contrast, there was no significant difference in the B_{max} values

Table 2. (+)-[^{3}H]PN200-110 bindings to cardiac, forebrain and lung membranes in monocrotaline-induced pulmonary hypertensive rats

		10 days			3–4 weeks		
		n	K_d (pM)	B_{max} (fmol/mg protein)	n	K_d (pM)	B_{max} (fmol/mg protein)
RV	Control	4	62.6±6.5	142.1±3.5	5	70.3±6.4	144.2±5.4
	MCT	4	58.4±2.6	147.0±3.5	5	59.4±3.5	113.2±6.5**
LV+S	Control		N.D.		4	74.6±15.6	151.1±11.3
	MCT		N.D.		4	79.3±12.4	150.4±11.4
Lung	Control	4	62.8±4.3	53.5±3.9	5	82.9±9.2	47.7±3.4
	MCT	4	59.3±5.9	61.4±4.7	5	81.1±8.6	68.7±5.4*
Forebrain	Control		N.D.		6	58.5±1.9	176.4±3.0
	MCT		N.D.		6	57.7±4.4	190.6±12.7

RV, right ventricle; LV+S, left ventricle plus septum; MCT, monocrotaline-treated rats; N.D., not determined. Values are the mean±S.E.M. of the indicated number (n) of experiments. *$P<0.05$, **$P<0.01$, as compared with the corresponding control value.

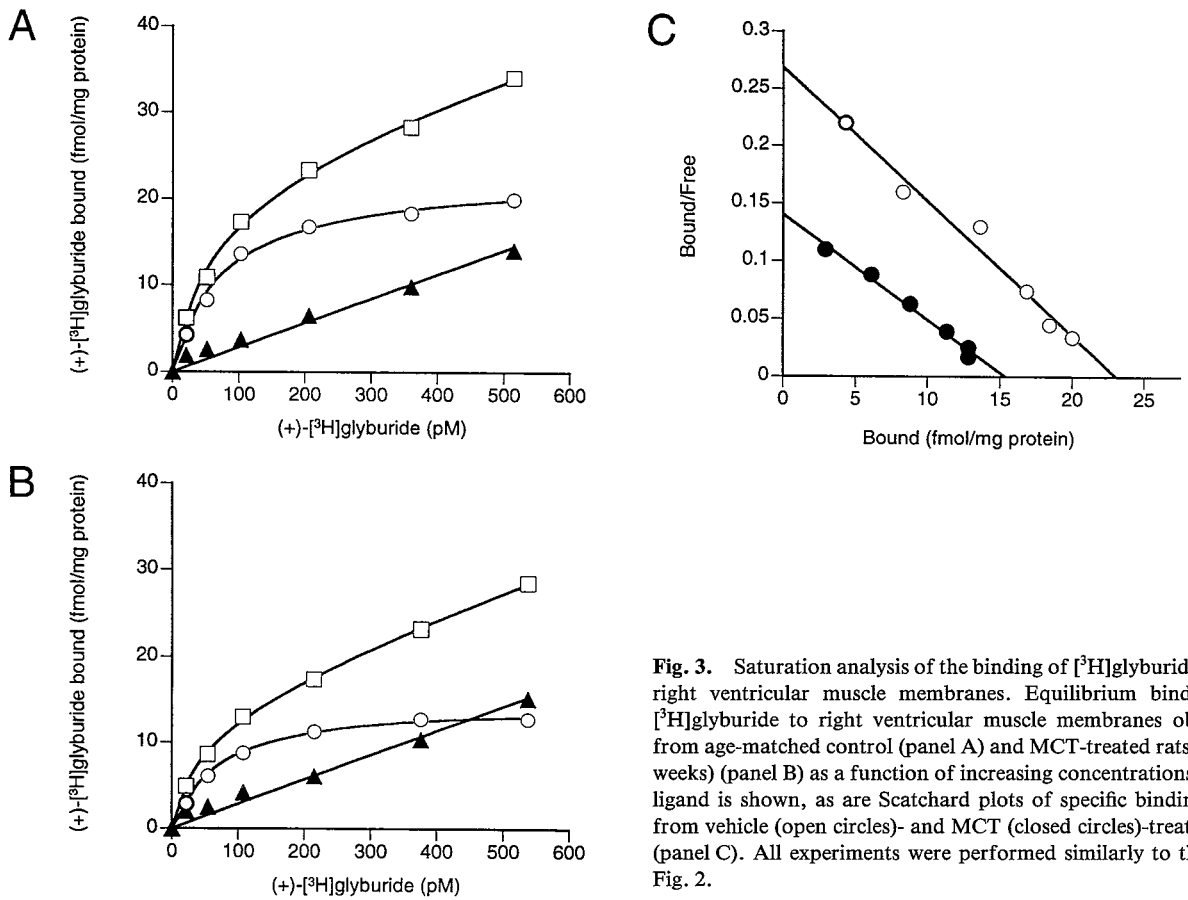

Fig. 3. Saturation analysis of the binding of [^{3}H]glyburide to rat right ventricular muscle membranes. Equilibrium binding of [^{3}H]glyburide to right ventricular muscle membranes obtained from age-matched control (panel A) and MCT-treated rats (3 to 4 weeks) (panel B) as a function of increasing concentrations of the ligand is shown, as are Scatchard plots of specific binding data from vehicle (open circles)- and MCT (closed circles)-treated rats (panel C). All experiments were performed similarly to those in Fig. 2.

Table 3. [^{3}H]Glyburide bindings to cardiac, forebrain and lung membranes in monocrotaline-induced pulmonary hypertensive rats

		10 days			3–4 weeks		
		n	K_d (pM)	B_{max} (fmol/mg protein)	n	K_d (pM)	B_{max} (fmol/mg protein)
RV	Control	5	83.9±5.6	20.6±0.9	5	91.9±5.2	22.6±1.9
	MCT	4	91.9±5.2	19.7±1.3	5	83.7±4.6	14.5±1.0*
LV+S	Control	4	86.4±8.2	23.9±0.9	4	86.9±6.2	25.4±0.3
	MCT	5	90.2±8.8	20.4±0.8	5	82.2±2.2	22.5±1.1
Lung	Control		N.D.			N.D.	
	MCT		N.D.			N.D.	
Forebrain	Control		N.D.		4	105.4±8.3	96.8±7.1
	MCT		N.D.		4	99.2±5.6	94.9±6.7

RV, right ventricle; LV+S, left ventricle plus septum; MCT, monocrotaline-treated rats; N.D., not determined. Values are the mean±S.E.M. of the indicated number (n) of experiments. *$P<0.05$, as compared with the corresponding control value.

of both DHP Ca^{2+} and K_{ATP} channels between rats treated with MCT for 10 days, at which no appreciable symptom of pulmonary hypertension was noticed, and age-matched sham rats. It is likely, therefore, that the changes in the B_{max} values of DHP Ca^{2+} and K_{ATP} channels in the right ventricle are correlated with the progression of pulmonary hypertension.

It has been reported that a significant increase in the

DNA and RNA concentrations in the right ventricle occurs in the first or second week after the injection of MCT. While the DNA concentration of the right ventricle of MCT-treated rats becomes comparable to that of the control rats 3 to 4 weeks after the injection, i.e., at the established stage of pulmonary hypertension, the RNA concentration is maintained at a higher level, indicating the possible augmentation of cardiac protein synthesis (22). A significant increase in total collagen content has been demonstrated in the hypertrophied right ventricle of MCT-treated rats (23). It is likely, therefore, that the increase in wet weight exclusively observed in the right ventricle after the injection of MCT in the present study might be a secondary change caused by preceding pulmonary circulatory disorders, and this primarily may be attributable to hypertrophy; i.e., abnormal enlargement of cardiac cells including contractile elements and other fibrotic elements.

The lung weight was significantly increased in MCT-induced pulmonary hypertensive rats when assessed in terms of both wet and dry tissue mass. The B_{max} value for DHP Ca^{2+} channels in the lung was also increased. Our previous study has shown that endothelium-dependent relaxing responses to acetylcholine of the intrapulmonary artery isolated from rats treated with MCT for 10 days is impaired and that the artery has a spontaneous vascular tone and is hyperactive to Ca^{2+} and various vasoconstrictors, including endothelin-1, angiotensin II and 5-hydroxytryptamine (24). The increased B_{max} of DHP Ca^{2+} channels may account for this enhanced tone and these responses in the MCT-induced pulmonary hypertensive rats. In the present study, we could not detect specific [^{3}H]glyburide bindings in the lung tissue, which may be attributable to a weak expression of mRNA encoding K_{ATP} channels (i.e., Kir 6.2) (25).

In the present study, we recognized only the high affinity binding site for [^{3}H]glyburide in the cardiac and forebrain membranes preparations. High and low affinity binding sites for the sulfonylurea/[^{3}H]glyburide have been identified only when examinations were carried out over a wider radioligand concentration range up to 5 nM (11, 13). Furthermore, the low affinity component was detectable only with a high concentration (over 10 μM) of glyburide, but not with other sulfonylureas, as a displacer (11). Glyburide is known to partition into the membrane (26), and at the high concentrations needed to saturate the low affinity component, non-specific effect of glyburide is likely to occur (11). Thus, the high affinity component referred to as a sulfonylurea receptor (10) has been considered to play a more important role in the cardiovascular system (11).

As to the mechanism responsible for the reduced densities of DHP Ca^{2+} and K_{ATP} channels in the right ventricle of MCT-induced pulmonary hypertensive rats, several possibilities can be considered. Firstly, the increased surface area of plasma membrane due to hypertrophy of each cardiac cell may account for the apparent reduction of densities of both channels, if it is assumed that the total number of channels located in the right ventricular cell membrane are unaltered in both MCT-treated and control groups. Cardiac myocytes have been shown to undergo hypertrophy without any increase in cell number in the development of cardiac hypertrophy, including MCT-induced right ventricular hypertrophy (27). Secondly, there may be a decrease in protein synthesis, including channel components. It has also been reported that severe metabolic blockades such as inhibition of glycolysis combined hypoxia or with the inhibition of oxidative phosphorylation impairs the binding of K_{ATP} channel ligands (11). Further studies are required to elucidate how the densities of DHP Ca^{2+} and K_{ATP} channels do change as pulmonary hypertension becomes more severe.

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