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Characteristics of Cardiac β-Adrenoceptors in *Suncus murinus*

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Characteristics of cardiac β-adrenergic responses in *Suncus murinus* (suncus) were examined in comparison with those of the rat. In both species, isoproterenol produced positive inotropic and chronotropic responses in spontaneously beating right atria and positive inotropic response in electrically driven papillary muscles. However, the effects of β-adrenoceptor stimulation in suncus papillary muscles were considerably smaller than in the rat. Tyramine, an indirect sympathomimetic amine, did not affect the contraction of ventricular muscle in suncus. The affinity and density of specific [^{3}H]dihydroalprenolol binding sites in suncus atrium were close to those in rat atrium. On the other hand, specific [^{3}H]dihydroalprenolol binding sites in suncus ventricle were distinctly lower both in affinity and in density than those of rat ventricle. These results suggest that ventricular β-adrenergic responses in the suncus are markedly smaller than those in the rat, probably due to lower density and affinity of β-adrenoceptors.

Keywords——*Suncus murinus*; heart; β-adrenergic response; [^{3}H]dihydroalprenolol binding; isoproterenol

Suncus murinus (suncus) belongs to the family Soricidae of the order Insectivora, regarded as the direct ancestors of the Primates in the phylogenetic system.[1] Recent studies from our laboratory have shown that the suncus is a unique experimental animal model for various researches, such as studies on nerve growth factor,[2] alcohol-induced hepatitis[3] and emesis.[4] However, the pharmacological properties of the cardiovascular system of the suncus have not yet been clarified. Therefore the present study was undertaken to characterize cardiac β-adrenergic responses in the suncus by using pharmacological and radioligand binding techniques. The results were compared with those in rats, whose β-adrenoceptor characteristics are well understood.

Materials and Methods

Radioligand and Drugs——l-[^{3}H]Dihydroalprenolol (specific activity, 110 Ci/mmol) was purchased from Amersham International (Amersham, Bucks., England), stored at −20 °C and protected from light. The following drugs were used: forskolin (Sigma, St. Louis, MO, U.S.A.), (±)-isoproterenol hydrochloride (Sigma), DL-propranolol hydrochloride (Sigma) and tyramine hydrochloride (Wako, Osaka, Japan). All other chemicals were of reagent grade.

Animals——The animals (*Suncus murinus*), originally introduced from the Central Institute for Experimental Animals (Kawasaki, Japan), were bred in our experimental animal institute. Male adult suncus (50—70 g) were used in all experiments, and female adult suncus (40—55 g) were used only for the preparation of atrial membranes because few were available. In preliminary experiments, no sex difference was observed in the reactivity of suncus atrium to β-adrenergic agents. Male Wistar rats (250—350 g) were used for comparison with the suncus.

Recordings of Mechanical Responses of Isolated Preparations——After animals were sacrificed, the whole hearts were quickly isolated and placed in a dish filled with oxygenated (95% O_2–5% CO_2) physiological salt solution (PSS) of the following composition (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 1.8, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose

11.1; pH 7.4. The right atrium and the papillary muscle of the left ventricle were carefully removed. Each preparation was vertically mounted in a 20 ml organ bath containing PSS which was continuously oxygenated (95% O_2–5% CO_2) and maintained at 37°C. Isometric contraction was measured with a force displacement transducer (Nihon Kohden, TB-612T), and recorded on ink-writing oscillographs (Nihon Kohden, WI 621-G). The papillary muscle was stimulated electrically at the frequency of 1 Hz (pulse duration, 0.3 ms; voltage, two-fold threshold) through a pair of platinum plate electrodes. The resting tension was adjusted so that myocardial contraction in the absence of drugs was the maximum. Concentration–response curves (CRCs) were obtained by the cumulative addition of each agent.

Preparation of Membrane Fraction——The isolated whole hearts were immediately place in ice-cold sucrose buffer (sucrose 0.25 M, $MgCl_2$ 1 mM, Tris 5 mM; pH 7.4). Each preparation was roughly minced with scissors, filtered through two layers of gauze to remove blood, and then homogenized with a Polytron homogenizer (Kinematica; setting 8, 5 s × 5) in 10 ml of ice cold sucrose buffer. The homogenate was first centrifuged at 3000 × *g* for 10 min. The pellet was suspended in 10 ml of the sucrose buffer and centrifuged again under the same conditions. The supernatants of two centrifugations were mixed well and further centrifuged at 10000 × *g* for 15 min. The resulting supernatant was centrifuged at 100000 × *g* for 60 min. The final supernatant was discarded, and the remaining membrane pellet was resuspended in ice-cold 50 mM Tris–hydrochloride buffer (pH 7.4). All the above procedure was done at 4°C. Protein concentration in the microsome was determined by the method of Lowry *et al.*[5]

Binding Studies——The microsomal suspension (atria, 0.25 mg/ml protein; ventricles, 0.50 mg/ml protein) was incubated with [^{3}H]dihydroalprenolol at 25°C for 30 min. The incubation was terminated by filtration through Whatman GF/F glass fiber filters, which were rinsed twice with 3 ml of the ice-cold Tris buffer. The filters were dried and radioactivities were counted with a liquid scintillation counter (Aloka, LSC-700). All assays were performed in duplicate. Amounts of [^{3}H]dihydroalprenolol bound in the absence and presence of 10 μM propranolol were regarded as total and nonspecific bindings, respectively.

Statistical Analysis——The pD_2 values were calculated as described in our previous paper.[6] The maximum binding capacity (B_{max}) and the equilibrium dissociation constant (K_D) were calculated from Scatchard analysis of the binding data.[7] Data were expressed as the mean ± S.E.M. of n independent observations unless otherwise mentioned. Significance of differences was determined by using Student's t-test for unpaired observations. Regression lines were drawn by the least-squares methods.

Results

Basal heart rates of the isolated right atria from suncus and rat were 267.5 ± 14.7 beats/min (n=4) and 278.0 ± 4.8 beats/min (n=4), respectively. Isoproterenol (10^{-10} to 3×10^{-7} M) and tyramine, an indirect sympathomimetic amine, increased both the heart rate and the contractile force of suncus and rat atrium in a concentration-dependent manner (Fig.

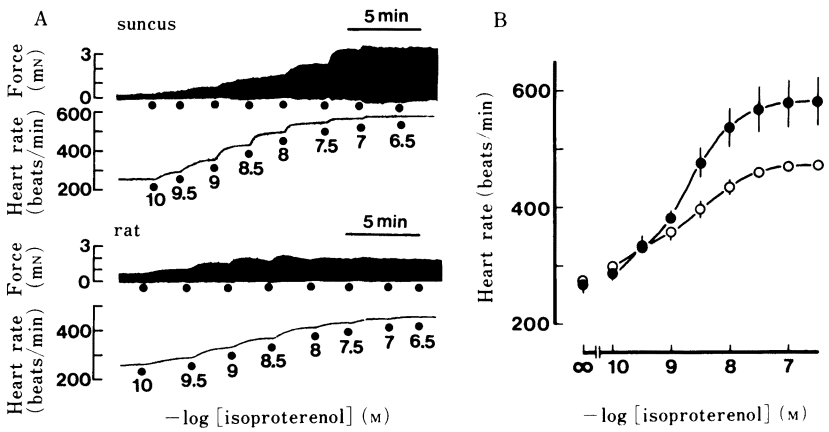

Fig. 1. Positive Inotropic and Chronotropic Effects of Isoproterenol in Spontaneously Contracted Right Atria of Suncus and Rat

A: Typical tracings showing effects of isoproterenol on force (upper stair) and heart rate (lower stair) of suncus (upper panel) and rat (lower panel) right atria. Isoproterenol was added cumulatively at the dots. B: Concentration–response curves for positive chronotropic effects of isoproterenol in suncus (●) and rat (○) atria. ∝ on the abscissa indicates absence of isoproterenol. Symbols and vertical bars indicate the mean ± S.E.M. of four experiments.

TABLE I. pD_2 Values and Maximal Effects of Isoproterenol and Tyramine in Right Atria and Papillary Muscles of Suncus and Rat

	Isoproterenol		Tyramine	
	pD_2	Maximum[a]	pD_2	Maximum[a]
Chronotropic effect (Right atria)				
Suncus	8.85±0.11 (4)	582.0±43.0 (4)[b]	5.60±0.19 (3)	605.7±29.9 (3)[b]
Rat	8.84±0.18 (4)	473.5± 8.3 (4)	5.52±0.08 (3)	457.3±15.8 (3)
Inotropic effect (Papillary muscles)				
Suncus	7.42±0.20 (4)	162.6± 9.9 (4)[b]	—	—
Rat	7.67±0.19 (4)	244.2±24.4 (4)	<4	>143

Values are expressed as the mean±S.E.M. of the number of experiments shown in parentheses. *a*) Maximal heart rate in right atria or maximal contractile force expressed as a percentage of the basal contraction in papillary muscles. *b*) Significantly different from rat ($p<0.05$).

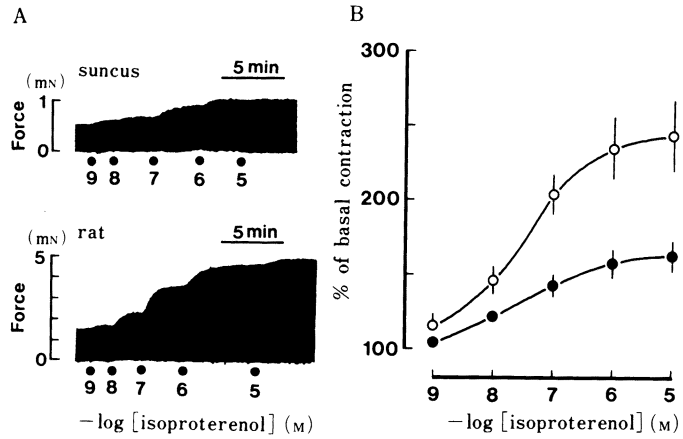

Fig. 2. Positive Inotropic Effects of Isoproterenol in Electrically Driven Papillary Muscles of Suncus and Rat

A: Typical tracings showing the effects of isoproterenol on contractile force of suncus (upper panel) and rat (lower panel) papillary muscles. B: Concentration–response curves for positive inotropic effects of isoproterenol in suncus (●) and rat (○) papillary muscles. The magnitude of the contractile force is expressed as a percentage of the basal contraction. Symbols and vertical bars indicate the mean±S.E.M. of four experiments.

1, Table I). Although there was no difference in sensitivities to isoproterenol and tyramine between suncus and rat, the maximal effects of the drugs were always greater in suncus. In most cases, especially in rat, the increased contractile force had declined before the heart rate reached a steady state, possibly reflecting the negative staircase phenomenon,[8] *i.e.*, an increase in beating frequency decreases contractility. Therefore, positive inotropic effects of isoproterenol and tyramine in atria were not analyzed quantitatively.

Isoproterenol (10^{-9} to 10^{-5} M) increased the contractile force of the isolated papillary muscle from suncus and rat in a concentration-dependent manner (Fig. 2, Table I). Contrary to the results in atrium, the maximal effect of isoproterenol in suncus ventricle was significantly smaller than that in rat. Tachyphylaxis was not observed either in suncus or in rat, even when isoproterenol was applied repeatedly. The contraction pattern of suncus papillary muscle was considerably changed by application of isoproterenol. The initial

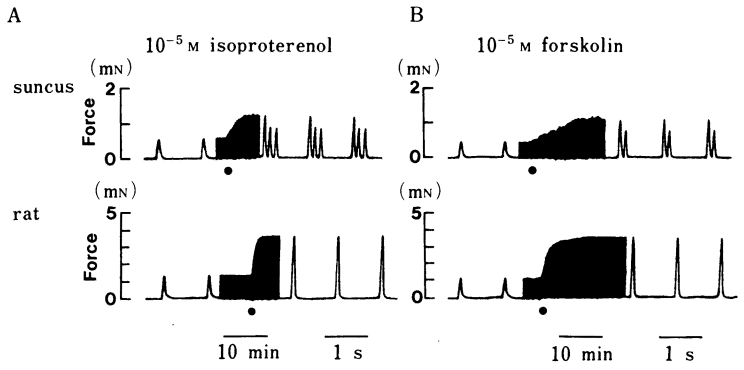

Fig. 3. Effects of 10^{-5} M Isoproterenol (A) and 10^{-5} M Forskolin (B) on the Contractile Response of Suncus (Upper Panel) and Rat (Lower Panel) Papillary Muscles

The drugs were applied at the dots. Before and after application of drugs, the tracings were recorded at a faster sweep speed to observe changes in contraction pattern.

TABLE II. Characteristics of Specific [^{3}H]Dihydroalprenolol Binding Sites in Atria and Ventricles of Suncus and Rat

	Atria		Ventricles	
	K_D	B_{max}	K_D	B_{max}
Suncus	0.91 ± 0.13	106.2 ± 9.3	1.60 ± 0.17[a)]	62.2 ± 9.2[a)]
Rat	0.61 ± 0.15	116.8 ± 9.7	0.45 ± 0.09	124.4 ± 19.3

Values are expressed as the mean ± S.E.M. of three experiments. K_D, dissociation constant (nM). B_{max}, maximum binding capacity (fmol/mg protein). *a*) Significantly different from rat ($p<0.05$).

contraction was potentiated, but one to four repetitions arrhythmic contractions appeared subsequently (Fig. 3A). Such an arrhythmogenic response was never observed in rat papillary muscle (Fig. 3A). The time to reach the maximal effects was considerably longer in suncus. Tyramine (10^{-4} M) had little effect on the contractile force of suncus papillary muscle, whereas the contractile force of rat papillary muscle was increased to 143.1 ± 5.6% ($n=3$) of the basal contraction by the same concentration of tyramine.

Application of forskolin (10^{-5} M), a direct activator of adenylate cyclase, increased the contractile force of suncus and rat papillary muscle, but the subsequent arrhythmic contraction was observed only in suncus (Fig. 3B).

Specific [^{3}H]dihydroalprenolol bindings to suncus and rat atrial membranes were saturable and of high affinity (Fig. 4A). The Scatchard plots of the binding data were linear in both species, indicating the existence of a single class of binding sites (Fig. 5, broken lines). The K_D and B_{max} values of the specific [^{3}H]dihydroalprenolol binding sites were similar in suncus and rat atria (Table II).

A similar single component of specific binding was observed in rat ventricular membranes (Fig. 4B). However, the K_D value of the specific binding sites in suncus ventricles was distinctly greater than that in rat and the B_{max} value was smaller in suncus ventricles than in rat (Fig. 5, solid lines; Table II).

Discussion

A physiological response common to many mammals is that stimulation of β-adrenocep-

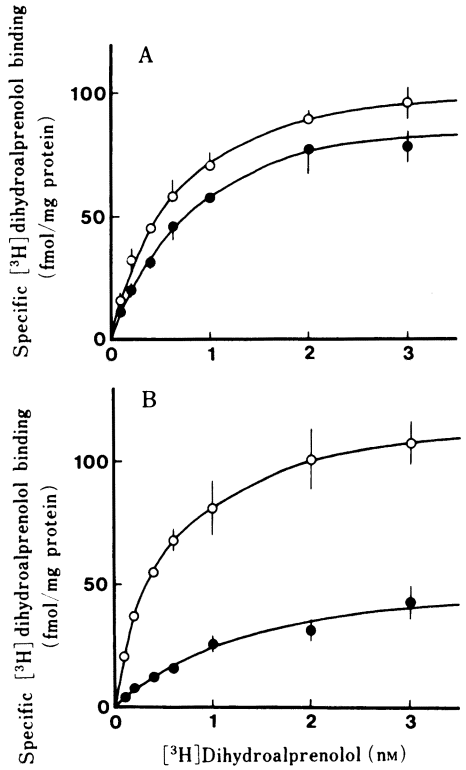

Fig. 4. Saturation Curves of Specific [^{3}H]Dihydroalprenolol Binding to Atrial (A) and Ventricular (B) Membranes of Suncus (●) and Rat (○)

Symbols and vertical bars indicate the mean ± S.E.M. of three experiments.

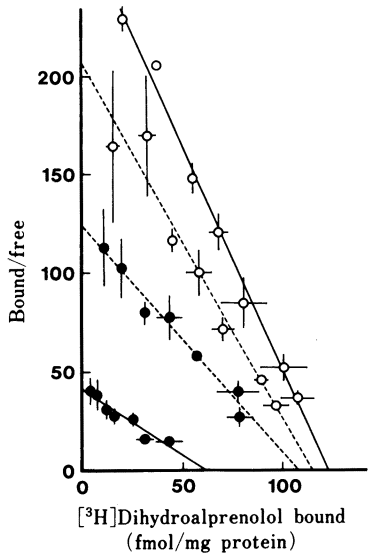

Fig. 5. Scatchard Plots of Specific [^{3}H]Dihydroalprenolol Binding to Atrial (Broken Lines) and Ventricular (Solid Lines) Membranes of Suncus (●) and Rat (○)

Symbols and bars indicate the mean ± S.E.M. of three experiments.

tors by catecholamine causes augmentation of cardiac contractility and beating rate. The β-adrenergic effects are considered to be mediated by the increase in intracellular adenosine 3′:5′-cyclic monophosphate (cyclic AMP).[9] In the present study, isoproterenol produced positive inotropic and chronotropic responses in suncus heart. The contractile force of suncus papillary muscle was also potentiated by forskolin, a direct activator of adenylate cyclase. These data show that the β-adrenoceptor–cyclic AMP system also exists in suncus heart. However, the effects of β-adrenoceptor stimulation in the suncus heart were quantitatively different from those in the rat.

The pD_2 values for positive chronotropic effects of isoproterenol were almost the same in suncus and rat atria, but the maximal effect was larger in suncus. Tyramine caused positive inotropic and chronotropic responses in suncus right atria in a similar manner to isoproterenol. This indicates that suncus right atrium is sympathetically innervated as densely as rat right atrium and is controlled by endogenous norepinephrine. On the other hand, the maximal positive inotropic effect of isoproterenol in suncus papillary muscle was considerably smaller than in the rat, although the pD_2 values were similar. The time to reach the maximal effects of isoproterenol and forskolin were longer in the suncus. The contractile force of suncus papillary muscle was not significantly affected by 10^{-4} M tyramine, which produced a distinct positive inotropic response in rat papillary muscle. Therefore, the regulation of contractility through the sympathetic nerve-β-adrenoceptor system is not well developed in suncus

ventricle. One probable reason is that either the density of β-adrenoceptors or the affinity to β-agonists, or both, are low in suncus heart.

The receptor binding studies using [^{3}H]dihydroalprenolol showed that both the K_D and B_{max} values of the specific binding sites in suncus atrium were close to those in rat atrium. These results seem to accord with the similarity of contractile responses of suncus and rat atria to β-stimulation. However, the [^{3}H]dihydroalprenolol binding sites in suncus ventricular membranes showed a larger K_D value and considerably smaller B_{max} value than those in rat ventricular membranes, which corresponded well with the contractile effects of isoproterenol. Therefore, low affinity and number of β-adrenoceptors are probably responsible for the inferior reactivity of suncus papillary muscle to β-stimulation.

The regional variations in β-adrenoceptor density of canine myocardium were reported in detail.[10] The sinoatrial (SA) node, left ventricles and Purkinje fibers of the canine sections contain higher receptor density, while the right ventricle, septum, atrium and the atrioventricular (AV) node contain lower β-adrenoceptor density. It is possible that these variations of β-adrenoceptor density are closely connected with specialized cell function. The present data that atrial β-adrenoceptors were greater in density and affinity than ventricular β-adrenoceptors in suncus may indicate functional superiority of the atrium to the ventricle. It is possible that an unknown factor other than the sympathetic nerve–β-adrenoceptor system regulates the ventricular contractility in suncus.

In mammalian ventricular myocardium, catecholamine-induced arrhythmias are observed in pathological conditions such as ischemia, preparations depolarized by high-potassium solution containing low concentrations of barium[11] or by electrical current.[12] In the present study, isoproterenol or forskolin induced arrhythmic contraction in suncus papillary muscle. However, the same conditions never induced arrhythmia in rat heart. The suncus heart may be more susceptible to catecholamine-induced arrhythmia.

Positive inotropic effects mediated by β-adrenoceptors are attributed to the increase in the availability of Ca channels.[13] Our preliminary study showed that an increase in extracellular concentration of Ca_2^+ did not significantly augment the contraction of suncus ventricular muscle. Therefore, plasma membrane of suncus myocardial cells may have different ionic permeabilities compared to other species. It is necessary to clarify their electrophysiological properties and excitation–contraction coupling mechanisms.

In conclusion, the present results suggest that ventricular β-adrenergic responses in suncus were markedly smaller than those in rat, and this is attributable to low affinity and density of β-adrenoceptors. The physiological significance of the deficiency in ventricular β-adrenoceptors is not clear, but suncus ventricle may be a model of certain cardiac diseases.[14] It would be interesting to study physiological factor(s) that regulate the contractility of suncus ventricle.

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