PREVENTION OF MYOCARDIAL DAMAGE IN BIO 14.6 STRAIN OF CARDIOMYOPATHIC HAMSTERS BY DENOPAMINE

TOSHIO SUTANI
First Department of Internal Medicine, Nara Medical University
Received September 27, 1995

Abstract: Denopamine, which is an orally effective cardiotonic agent and a beta-1-receptor-selective agonist, reportedly prolongs the survival of the BIO 14.6 strain of cardiomyopathic Syrian hamsters (BIO). The latter is an animal model of human idiopathic cardiomyopathy. The purpose of the present experiment was to investigate the effects of denopamine on myocardial damage in BIO hamsters. They were divided into two groups: one that received denopamine treatment (1 mg/kg/day) from 2 months of age, and a control (untreated) group. Morphological studies of the myocardium, assays for beta-adrenergic receptors, and measurements of myocardial adenylate cyclase (AC) activity and cAMP concentration were performed at 1, 3, and 7 months of age in all animals. It was observed that denopamine: 1) inhibited the progression of disease from the stage of hypertrophy to that of congestive failure that was demonstrated in the control BIO hamster, 2) inhibited the down-regulation of beta-1-adrenergic receptors in the myocardium of the control BIO hamsters at 7 months of age, and 3) prevented an increase in myocardial AC activity and cAMP concentration that was seen in control BIO hamsters at 3 months of age (stage of early hypertrophy). In conclusion, denopamine may prevent myocardial damage in BIO hamsters by inhibiting the down-regulation of beta-1-adrenergic receptors, thus preventing an increase in myocardial AC activity and cAMP concentration

Index Terms

BIO 14.6 hamster, denopamine, beta-adrenergic receptor, adenylate cyclase, cAMP

INTRODUCTION

The BIO 14.6 strain of cardiomyopathic Syrian hamster (BIO) is a useful model of the idiopathic cardiomyopathy that occurs in humans. This strain develops spontaneous cardiomyopathy with myocardial necrosis, fibrosis, and calcification at 1 or 2 months of age, followed by cardiac hypertrophy and congestive heart failure. This disorder is transmitted as an autosomal recessive trait [1)2)3)4)]. Sole et al.[5)6)] reported that the rate of cardiac norepinephrine synthesis and metabolism was increased in the BIO hamster, and that dopamine accumulated in the middle layer of the ventricular muscle. Karliner et al.[7)] reported the number of alpha-1 and beta-adrenergic receptors to be higher in the BIO hamsters than in the control animals. These reports suggest that sympathetic nervous system abnormalities may be linked to the development of cardiomyopathy in the BIO hamster.

Denopamine ((-)-α-(3,4-dimethoxyphenethylaminomethyl)-4-hydroxybenzylalcohol) is a phenylethanolamine derivative that is orally administered as a cardiotonic agent. Clinical and experimental studies suggest that denopamine exerts a positive inotropic action in doses that

do not significantly influence the blood pressure or pulse rate[8)9)10)]. Denopamine is a selective agonist of the beta-1-receptor[11)]. Its administration has been shown to prolong the survival of BIO hamsters (personal communication from Tanabe Seiyaku Co., Ltd., Osaka). We therefore hypothesized that denopamine may prevent myocardial damage in the BIO hamster via its involvement with the beta-adrenergic receptors. Accordingly, we investigated the effects of denopamine on myocardial damage by studying the morphology of the myocardium, the density of the beta-1-adrenergic receptors on the cardiac membrane, the activity of myocardial adenylate cyclase (AC), and the concentrations of cAMP in BIO hamsters.

MATERIALS AND METHODS

BIO hamsters (Shizuoka Laboratory Animal Co., Ltd., Shizuoka) were utilized. Two animals were placed in each cage and were divided into treated and control groups. Begining at the age of two months, the denopamine-treated hamsters were fed laboratory chow (Oriental Yeast Co., Ltd., Tokyo) that contained denopamine, at a daily adjusted dose of 1 mg/kg body weight. Control animals received chow without denopamine. Ten animals were sacrificed by exsanguination via an intra-aortic catheter and ventricles were excised at 1, 3, and 7 months of age respectively.

Morphological studies

The ventricles were separated from the atria and fatty tissues and weighed. Staining with hematoxylin-eosin (HE), Azan-Mallory, and p-aminosalicylic acid (PAS) was performed routinely using sections of the left ventricle in a half trans-sectional specimen. The sectional area of the left ventricular lumen, the percent area of myocardial fibrosis (area of fibrosis/myocardium) and diameter of the myocyte were each measured with a color image processor (SPICCA II, Avionics Japan Co., Ltd., Tokyo).

Membrane preparation

Membrane fractions were prepared according to Van et al.[12)], with some modifications. Briefly, ventricles were homogenized in an ice-cold 5 mM Tris-HCl buffer (pH 7.4) that contained 1 mM $MgCl_2$, 0.25 M sucrose, and 1 mM phenylmethyl sulfonyl floride (PMSF) by using a Bio Mixer (Nihonseiki Co., Ltd., Tokyo). The homogenate was centrifuged at 400× g for 10 min and the supernatant was then centrifuged at 105,000×g for 40 min at 4℃. The resulting pellets were resuspended in ice-cold 50 mM Tris-HCl buffer (pH 7.4) that contained 10 mM $MgCl_2$ and 1 mM PMSF, and recentrifuged 105,000×g for 20 min at 4℃. The final pellets were resuspended in 50 mM Tris-HCl buffer as described above. Each of the above steps was performed at 4℃. Isolated membranes were used for receptor binding assays and AC activity assays.

Assay for beta-adrenergic receptors

Beta-adrenergic receptors were evaluated by the radioligand binding assay described by Williams et al.[13)], with some modifications. Homogenates of the cardiac membrane were incubated with a range of concentrations of [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) (New Research Products, Boston, MA, USA) (0.25-6 nM [^{3}H]-DHA in a final volume of 200 μl) at 37℃ for 10 min. Each incubation was terminated by adding 15 ml of cold buffer (1 ml/sec for 15 sec), followed by rapid vacuum filtration through fiber glass filters (Whatman, GF/C, Whatman Internatinal Ltd., Maidstone, England). The radioactivity of the membrane-bound

[^{3}H]-DHA was measured by liquid scintillation counting method. Nonspecific binding was determined by performing parallel assays in the presence of 1 μM propranolol. Specific binding to beta-adrenergic receptors was defined as the difference between the total and nonspecific binding. Specific binding data were analyzed by Scatchard plots to determine the number of beta-adrenergic receptors present[14].

Measurement of cAMP concentration

The concentration of cAMP was determined according to a previously described method[15)16)]. Approximately 20 mg of heart muscle was frozen in liquid nitrogen immediately after excision. Frozen tissue was homogenized in approximately 100 volumes of ice-cold 6% trichloroacetic acid (TCA) by using a Bio Mixer. Homogenates were centrifuged at 3000×g for 10 min at 4 ℃. The resulting supernatants were centrifuged three times at 3,000×g for 5 min at 4℃. Prior to each centrifugation, supernatants were mixed with ether-saturated water. A volume of 50 μl of the final supernatant was succinylated by mixing with 45 μl of succinic anhydride in dioxane and 5 μl of triethylamine for 10 min at room temparature. Next, 400 μl of 0.3 M imidazole buffer was added to this preparation. Following derivatization, 100 μl of each sample was incubated with 100 μl of ^{125}I-succinyl cAMP tyrosine methylester and 100 μl of cAMP antiserum for 36 hours on ice. Any remaining ^{125}I-succinyl cAMP tyrosine methylester that did not bind to the cAMP antiserum was removed by passage through dextran-coated charcoal. Radioactivity was measured by using a gamma counter. Parallel assays with standard solutions of cAMP were performed to obtain the standard curve. Concentrations of cAMP in the myocardium were quantitated by reference to this standard curve. The RIA kit used in these experiments was purchased from Yamasa Co., Ltd. (Chiba).

Adenylate cyclase activity assay

Adenylate cyclase activity was determined according to the methods of Koji et al.[17] and Abe et al.[18] with some modifications. Briefly, 90 μg of the membrane fractions were incubated with 50 mM Tris-HCl buffer (pH 7.5) that contained 5 mM $MgCl_2$, 1 mM Tris-EGTA, 2.5 mM Na_2-adenosine triphosphate (ATP), and with or without 10^{-3}M isoproterenol at 37℃ for 5 min. The fractions were then centrifuged at 6,000×g for 20 min at -4℃. Concentrations of cAMP in the supernatants were measured as described above. The amount of cAMP synthesized from ATP was considered to represent the adenylate cyclase activity in membrane fractions.

Statistical analysis

Data are expressed as the mean±SD. Differences between groups were analyzed by the Mann-Whitney method. A level of $p<0.05$ was considered statistically significant.

RESULTS

Morphological studies

The weight of heart at 3 months of age significantly exceeded that at 1 month of age. However, no appreciable change in the weight of the heart was observed from 3 months of age to 7 months of age. The weight of the heart did not differ in the denopamine-treated vs. the control groups at 3 and 7 months of age (Table 1).

The cross-sectional area of the left ventricular lumen at 3 months of age significantly exceeded that at 1 month of age in both groups. There were no significant differences in the cross-sectional area of the left ventricular lumen in both groups at 3 months of age. The cross

-sectional area of the left ventricular lumen did not change between 3 and 7 months of age in the denopamine-treated group. The control group exhibited a significant increase in lumen area in the same period. Thus, the cross-sectional area of the left ventricular lumen in the control group significantly exceeded that of the denopamine-treated group at 7 months of age (Table 1).

The diameter of the myocyte increased in the denopamine-treated and the control groups between 1 and 3 months of age. no appreciable changes in myocyte diameter occurred in the denopamine-treated group until 7 months of age. However, decreases in myocyte diameter were observed until the age of 7 months in the control group. Thus, the diameter of the myocyte in the control group was significantly less than that of the denopamine-treated group at 7 months (Table 2).

The percent area of fibrosis increased significantly between 1 and 3 months of age in the denopamine-treated and control groups. However, at 7 months of age, this parameter increased in the control group, but did not change in the denopamine-treated group. Thus, at 7 months of age, the percent area of fibrosis was significantly greater in the control group relative to that in the denopamine-treated group (Table 2).

Desity of beta-adrenergic receptors

The density of the beta-adrenergic receptors was significantly higher at 3 months of age than that at 1 month of age in the denopamine-treated and control groups. The density of the beta -adrenergic receptors significantly decreased in both groups between 3 and 7 months of age, with a more pronounced decrease occurring in the control group. Thus, at 7 months of age, the receptor density was lower in the control group than in the denopamine-treated group (Table 3).

Table 1. Heart weight and left ventricular lumen area

	Heart weight (g)			Left ventricular lumen area (mm^2)		
Age	1M	3M	7M	1M	3M	7M
D		0.78 ± 0.08^a	0.80 ± 0.07^a		5.68 ± 0.32^a	4.32 ± 1.81^a
C	0.40 ± 0.04	0.77 ± 0.05^a	0.79 ± 0.08^a	2.27 ± 1.70	4.54 ± 1.93^a	13.80 ± 2.41^b

M, month of age.
D, denopamine-treated BIO 14.6 cardiomyopathic hamster.
C, control BIO 14.6 cardiomyopathic hamster.
a) vs. C (1M), $p<0.05$, b) vs. C (1M), $p<0.01$, vs. C (3M) and D (7M), $p<0.05$.
Data are expressed as the mean±SD of 10 hamsters.

Table 2. Myocyte diameter and area of fibrosis/myocardium ratio

	Myocyte diameter (μm)			Area of fibrosis/myocardium ratio (%)		
Age	1M	3M	7M	1M	3M	7M
D		16.51 ± 0.70^a	16.71 ± 0.93^a		3.09 ± 1.63^a	2.64 ± 0.59^c
C	11.31 ± 0.70	17.00 ± 1.86^a	9.30 ± 0.47^b	0.45 ± 0.26	4.54 ± 1.82^a	4.91 ± 1.55^a

M, month of age.
D, denopamine-treated BIO 14.6 cardiomyopathic hamster.
C, control BIO 14.6 cardiomyopathic hamster.
a) vs. C (1M), $p<0.01$, b) vs. C (3M) and D (7M), $p<0.01$,
c) vs. C (1M), $p<0.01$, vs. C (3M) and D (7M), $p<0.05$.
Data are expressed as the mean±SD of 10 hamsters.

Concentration of cAMP

At 3 months of age, the control group exhibited cAMP concentrations that were higher than at 1 month of age. However, the concentrations in the denopamine-treated group showed no appreciable changes from 1 to 3 months of age. Collectively, the cAMP concentrations in the control group were higher than those in the denopamine-treated group. Compared to the data at 3 months of age, the cAMP concentration was significantly increased in the denopamine-treated group, and significantly decreased in the control group at 7 months of age. Taken together, at 7 months of age, the concentrations of cAMP in the control group were significantly lower than those in the denopamine-treated group (Table 3).

AC activity

In the absence and presence of isoproterenol, AC activity in cardiac membranes was significantly higher at 3 months of age than at 1 month of age in the control group. However, in the denopamine-treated group, the AC activity was significantly lower at 3 months of age than at 1 month of age with or without isoproterenol. The AC activity in the absence and presence of isoproterenol at 3 months of age was significantly higher in the control group vs. the denopamine-treated group. Regardless of isoproterenol, AC activity was decreased between 3 and 7 months of age in the control group, and showed no changes from its 3 month value in the denopamine-treated group. Thus, AC activity at 7 months of age was lower in the control group vs. the denopamine-treated group (Table 4).

Table 3. Density of beta-adrenegric receptors and cAMP concentration

	Density of beta-adrenergic receptors (fmol/mg of protein)			cAMP concentration (nmol/g of wet tissue)		
Age	1M	3M	7M	1M	3M	7M
D		20.0±2.4[a]	11.7±1.7[b]		2.02±0.30	3.31±0.93[e]
C	14.8±2.8	22.4±5.2[a]	6.9±2.4[c]	2.27±0.21	3.36±0.25[d]	2.52±0.88

M, month of age.
D, denopamine-treated BIO 14.6 cardiomyopathic hamster.
C, control BIO 14.6 cardiomyopathic hamster.
a) vs. C (1M), $p<0.01$, b) vs. C (1M), $p<0.05$, D (3M), $p<0.01$,
c) vs. C (3M), $p<0.01$, d) vs. C (1M), C (7M) and D (3M), $p<0.05$,
e) vs. C (1M), C (7M) and D (3M), $p<0.05$.
Data are expressed as the mean±SD of 10 hamsters.

Table 4. Adenylate cyclase activity

	Baseline activity (pmol/mg/min)			Isoproterenol-stimulated activity (pmol/mg/min)		
Age	1M	3M	7M	1M	3M	7M
D		31.1±6.7[a]	33.7±6.2[c]		72.5±10.0[a]	69.0±13.9[c]
C	41.2±2.0	47.4±7.7[b]	21.6±3.5[a]	82.2±13.3	137.9±11.9[b]	57.2±10.9[a]

M, month of age.
D, denopamine-treated BIO 14.6 cardiomyopathic hamster.
C, control BIO 14.6 cardiomyopathic hamster.
a) vs. C (1M), $p<0.05$, b) vs. C (1M), $p<0.05$, C (7M) and D (3M), $p<0.01$,
c) vs. C (1M) and C (7M), $p<0.05$.
Data are expressed as the mean±SD of 10 hamsters.

DISCUSSION

In this study, it was observed that the cardiac muscle of the control BIO hamster was hypertrophic at 3 months of age and exhibited evidence of severe heart failure at 7 months of age. It was also observed that the cardiac muscle of the denopamine-treated BIO hamster was hypertrophic at 3 and 7 months of age. This indicates that denopamine administration suppressed the disease progression from the stage of hypertrophy to that of congestive failure during cardiomyopathy in BIO hamsters.

Previous studies have shown that sympathetic nervous system abnormalities may be associated with the development of cardiomyopathy in the BIO hamster[5)6)7)]. Kobayashi et al.[19)] described increases in the number of beta-adrenergic and alpha-adrenergic receptors in BIO hamsters prior to hypertrophy. In addition, Ikegawa et al.[20)] showed increases in the number of these receptors before and during the early stage of hypertrophy. In this study, we observed the same changes in the beta-adrenergic receptor level at 3 months of age and a decrease in number at 7 months of age in both the denopamine-treated and the control BIO hamsters. However, there was a greater decrease in receptor number in the control BIO hamsters relative to those treated with denopamine. These data suggest that denopamine inhibits the down-regulation of the myocardial beta-adrenergic receptors in BIO hamsters.

This study demonstrated that, regardless of isoproterenol stimulation, AC activity and cAMP concentration were higher in control BIO hamsters than in denopamine-treated BIO hamsters at 3 months of age. This illustrates that denopamine suppresses the increase in AC activity and cAMP concentration that parallels the changes in beta-adrenergic receptor number seen in the early hypertrophy of this animal model. Taira et al.[21)] reported that in the isolated canine myocardium, the cAMP concentration required to stabilize cardiac muscle contraction was lower during the administration of denopamine than of isoproterenol. Moreover, by using canine myocardium as well, Bing et al.[22)] demonstrated that denopamine-stimulated AC activity was one-quarter of that obtained by equal concentrations of isoproterenol. These studies show that denopamine suppresses the increase in AC activity and cAMP concentration produced by isoproterenol. Stadel et al.[23)] reported that cAMP phosphorylates the beta-adrenergic receptors via a cAMP-dependent protein kinase. These authors also found that AC activity was correlated with the phosphorylation of the beta-adrenergic receptors in turkey erythrocytes. Benovic et al.[24)] further corroborated this by showing a positive correlation between AC activity and beta-adrenergic receptor kinase catalysis. It is possible that denopamine may prevent the down-regulation of beta-adrenergic receptors in the cardiac cell membranes in 7 month-old BIO hamsters by suppressing the elevation of AC activity and cAMP concentration. The prevention of the down-regulation of the beta-adrenergic receptors by denopamine may preserve the myocardial contractile response to catecholamines in the BIO hamster, even at 7 months of age. This may be a mechanism by which denopamine suppresses the progression from hypertrophy to congestive failure in cardiomyopathic BIO hamsters.

The elevated calcium levels found in the myocardium of pre-hypertrophic BIO hamsters are thought to be involved in various cardiac dysfunctions in these animals[7)19)]. cAMP-dependent protein kinase A regulates L-type calcium channels, phospholamban, and troponin I. Protein kinase A can cause a calcium overload by influencing these calcium-regulatory molecules in the

myocyte [25)27)]. Therefore, this study suggests that denopamine may prevent an increase in calcium concentrations by inhibiting the increase in cAMP concentrations early in the stage of cardiac hypertrophy in the BIO hamster.

In this study, it was concluded that denopamine may prevent myocardial damage in BIO hamsters by inhibiting the down-regulation of beta-1-adrenergic receptors, thereby preventing an increase in myocardial AC activity and cAMP concentration. Thus, denopamine may be a benefical treatment for idiopathic cardiomyopathy

Acknowledgement: The author is sincerely grateful to Prof. K. Dohi for pertinent advice and encouragement, and to Prof. Y. Hiasa and Prof. T. Nakashima for critically reading the manuscript. The author is also grateful to Tanabe Seiyaku Co., Ltd., Osaka for the generous supply of denopamine.

REFERENCES

1) **Bajusz, E.**: Hereditary cardimyopathy: a new disease model. Am. Heart J. **77**: 686-696, 1969.
2) **Homburger, F.**: Myopathy of hamster dystrophy: history and morphologic aspects. Ann. NY Acad. Sci. **317**: 2-17, 1979.
3) **Jasmin, G.** and **Eu, H. Y.**: Cardiomyopathy of hamster dystrophy. Ann. NY Acad. Sci. **317**: 46-58, 1979.
4) **Bajusz, E.** and **Lossnitzer, K.**: A new disease model of congestive heart failure: studies on its pathogenesis. Trans. NY Acad. Sci. **30**: 939-948, 1968.
5) **Sole, M. J., Kamble, A. B.** and **Hussain, N.**: A possible change in the rate-limiting step for cardiac norepinephrine synthesis in the cardiomyopathic Syrian hamster. Circ. Res. **41**: 814-817, 1977.
6) **Sole, M. J., Helke, C. J.** and **Jacobowitz, D. M.**: Increased dopamine in the failing hamster heart: transvesicular transport of dopamine limits the rate of norepinephrine synthesis. Am. J. Cardiol. **49**: 1682-1690, 1982.
7) **Karliner, J. S., Alabaster, C., Stephens, H., Barnes, P.** and **Dollery, C.**: Enhanced noradrenaline response in cardiomyopathic hamsters: possible relation to changes in adrenoceptors studied by radioligand binding. Cardiovasc. Res. **15**: 296-304, 1981.
8) **Nagao, T., Ikeo, T., Murata, S., Sato, M.** and **Nakajima, H.**: Cardiovascular effects of a new positive inotropic agent, (-)-(R)-1-(p-hydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)amino] ethanol (TA-064) in anesthetized dog and isolated guinea pig heart. Jpn. J. Pharmacol. **35**: 415-423, 1984.
9) **Kino, M., Hirota, Y.** and **Yamamoto, S.**: Cardiovascular effects of a newly synthesized cardiotonic agent (TA-064) on normal and disease hearts. Am. J. Cardiol. **51**: 802-810, 1983.
10) **Inamatsu, M., Tosuka,T., Morita, T.** and **Takeyama, S.**: Effects of (-)-(R)-1-(p-hydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)amino] ethanol (TA-064), a new cardiotonic agent, on circulating parameters of carbohydrate and lipid metabolism in the rat. Biochem. Pharmacol. **33**: 2171-2177, 1984.
11) **Naito, K., Nagao, T., Otsuka, M., Harigaya, S.** and **Nakajima, H.**: Studies on the affinity and selectivity of denopamine (TA-064), a new cardiotonic agent, for β-adrenergic receptors. Jpn. J. Pharmacol. **38**: 235-241, 1985.
12) **Van, A. E., Burch, R. M., Knickelbein, R. G., Hungerford, E. J., Gower, E. J., Webb, J. G., Poe, S. L.** and **Lindenmayer, G. E.**: Isolation of sealed vesicles highly enriched with sarcolemma markers from canine ventricle. Biochim. Biophys. Acta **602**: 131-143, 1980.
13) **Williams, L. T., Snyderman, R.** and **Lefkowitz, R. J.**: Identification of β-adrenergic receptors in human lymphocytes by (-) [^{3}H]-alprenolol binding. J. Clin. Invest. **57**: 149-155, 1976.
14) **Scatchard, G.**: The attractions of proteins for small molecules and ions. Ann. NY Acad. Sci. **251**: 660

-672, 1949.

15) **Steiner, A. L., Parker, C. W.** and **Kipnis, D. M.**: Radioimmunoassay for cyclic nucleotides I. preparation of antibodies and iodinated cyclic nucleotides. J. Biol. Chem. **247**: 1106-1113, 1972.

16) **Homma, M., Satoh, T., Takezawa, J.** and **Ui, M.**: An ultraselective method for the simultaneous determination of cyclic AMP and cyclic GMP in small-volume sample from blood and tissue. Biochem. Med. **18**: 257-273, 1977.

17) **Koji, T.** and **Terayama, H.**: In vivo and in vitro effects of proteases upon adenylate cyclase activity of liver cells and plasma membranes. Biophys. Acta **633**: 10-21, 1980.

18) **Abe, M., Yamazaki, N., Suzuki, Y., Kobayashi, A.** and **Ohta, H.**: Effect of palmitoylcarnitine on Na^+, K^+, -ATPase and adenylate cyclase activity of canine myocardial sarcolemma. J. Mol. Cell. Cardiol. **16**: 239-245, 1984.

19) **Kobayashi, A., Yamashita, T., Kaneko, M., Nishiyama, T., Hayashi, H.** and **Yamazaki, N.**: Effects of verapamil on experimental cardiomyopathy in the Bio 14.6 Syrian hamster. J. Am. Coll. Cardiol. **10**: 1128-1134, 1987.

20) **Ikegawa, T., Nishiyama, T., Kobayashi, A.** and **Yamazaki, N.**: Role of α-adrenergic receptors and the effect of bunazosin on the histopathology of cardiomyopathic Syrian hamsters of strain BIO 14.6. Jpn. Circ. J. **52**: 181-187, 1988.

21) **Yokoyama, H., Yanagisawa, T.** and **Taira, N.**: Details of mode and mechanism of action of denopamine, a new orally active cardiotonic agent with affinity for β_1-receptors. J. Cardiovasc. Pharmacol. **12**: 323-331, 1988.

22) **Bing. R. J., Sasaki, Y., Burger, W.** and **Chemnitius, J. M.**: Cardiac inotropic response of a new β-1-agonist (TA-064) with low sarcolemmal adenylate cyclase activation. Curr. Therap. Res. **36**: 1127-1144, 1984.

23) **Stadel, J. M., Nambi, P., Shorr, R. G. L., Sawyer, D. F., Caron, M. G.** and **Lefkowitz, R. J.**: Catecholamine-induced desensitization of turkey erythrocyte adenylate cyclase is associated with phosphorylation of the β-adrenergic receptor. Proc. Natl. Acad. Sci. USA. **80**: 3173-3177, 1983.

24) **Benovic, J. L., Staniszewski, C., Mayor, F., Caron, M. G.** and **Lefkowitz, R. J.**: β-adrenergic receptor kinase. J. Biol. Chem. **263**: 3893-3897, 1988.

25) **Yatani, A., Imoto, Y.** and **Goto, M.**: The effects of caffeine on the electrical properties of isolated, single rat ventricle cells. Jpn. J. Physiol. **34**: 337-349, 1984.

26) **Tada, M., Kirchberger, M. A.** and **Katz, A. M.**: Phosphorylation of a 22,000-dalton component of the cardiac sarcoplasmic reticulum by adenosine 3′: 5′ -monophosphate-dependent protein kinase. J. Biol. Chem. **250**: 2640-2647, 1975.

27) **Wilkinson, J. M.** and **Grand, R. J. A.**: Comparison of amino acid sequence of troponin I from different striated muscles. Nature **271**: 31-35, 1978.