Prolongation of the Life Span of Cardiomyopathic Hamster by the Adrenergic $\beta_1$-Selective Partial Agonist Denopamine

Hideo Kurosawa, Hiroshi Narita, Minako Kaburaki, Hideo Yabana, Hisayoshi Doi, Emiko Itogawa and Masahito Okamoto

"Lead Optimization Research Laboratory and "Pharmaceutical Development Research Laboratory, Tanabe Seiyaku Co., Ltd., 2-2-50 Toda, Saitama 335, Japan"

Received May 24, 1996 Accepted September 11, 1996

ABSTRACT—Influence of cardiotonic agents on the prognosis of heart failure depends on the individual therapeutic agents, and favorable and unfavorable effects of these agents have been reported in clinical trials. We studied the effect of the cardiotonic agent denopamine on the life span of cardiomyopathic hamsters (BIO 14.6 strain) in the heart failure period. Non-treated hamsters started to die at 40 weeks of age, and their survival rate decreased to 23.8% at the age of 65 weeks. Hamsters treated with denopamine (400 ppm in diet) from 36 weeks of age did not die until the age of 52 weeks, except in cases of accidental death. The survival rate of this group at 65 weeks of age was about 40%. Survival rates of these 2 groups were significantly different (P<0.05) when animals with accidental death were excluded. To elucidate the mechanism of the effect of denopamine, we performed several experiments after dietary treatment with denopamine for 4 to 6 weeks from 37 weeks of age. Denopamine treatment lowered plasma levels of noradrenaline and dopamine (P<0.05), but affected neither the cardiac contractility nor the $\beta$-adrenoceptor density. In summary, denopamine significantly decreases the mortality of cardiomyopathic hamsters. Its effect to lower the plasma catecholamine levels may be responsible for the beneficial effect of denopamine.

Keywords: Denopamine, Cardiomyopathic hamster, Prognosis, Congestive heart failure, Plasma catecholamine level

Angiotensin converting enzyme inhibitors have been established as standard remedies for the treatment of patients with chronic congestive heart failure, since several large scale clinical studies, such as the SOLVD study (1) and VHeFT-II study (2) demonstrated the effect of these agents on the long-term prognosis of patients. On the contrary, the efficacy of novel cardiotonic agents on the long-term prognosis of patients with chronic heart failure has been considered to be doubtful because of negative findings with phosphodiesterase III (PDE III) inhibitors, including milrinone (3) and the adrenergic $\beta_1$-selective partial agonist xamoterol (4). However, venesarnone that induces the positive inotropic effect predominantly by inhibition of PDE III but also has effects on K$^+$ (5) and Na$^+$ (6) channels improved morbidity and mortality of patients with heart failure (7). An animal study also showed that pimobendan with PDE III inhibitory action and calcium sensitizing action prolonged life span of cardiomyopathic hamsters, when the treatment had been started at quite a young age (8). Thus, the influence of cardiotonic agents on the life span of patients with heart failure has been controversial and appears to be much dependent on the individual therapeutic agents.

Denopamine was introduced to the Japanese market in 1988 as an orally active cardiotonic agent with adrenergic $\beta_1$-selective partial agonist action (9). Denopamine elicited a less positive chronotropic effect than isoprenaline for a given increase in force of contraction (10), had a weaker action to increase the cardiac oxygen consumption than isoprenaline (11), was less arrhythmogenic than other sympathomimetic amines (12), and did not suffer from tolerance with effective doses (13).

Denopamine improved the cardiac function in patients with congestive heart failure (14, 15), but its effect on the prognosis of patients is yet unknown. To get insight into this point, we studied the effect of denopamine on the life span of cardiomyopathic hamsters, one of the representative models of congestive heart failure (16) in the heart.
failure stage. We further studied the mechanism involved in the effect of denopamine to prolong the survival in this animal model.

MATERIALS AND METHODS

Animals

Male cardiomyopathic hamsters of the BIO 14.6 strain were purchased from Charles River Japan (Tokyo). Hamsters were individually housed in our animal room for more than a week before use under a 12-hr light, 12-hr dark cycle (6:00–18:00) at 24±1°C. Powdered diet (CE-7; Nihon Clea, Tokyo) and tap water were given ad libitum.

Experiments to investigate the effect of denopamine on life span were started at the age of 36 weeks. Hamsters were divided into 2 groups of 21 animals each by their body weight, and the diet for the denopamine group was changed to one containing denopamine at a concentration of 400 ppm.

Experiments to analyze the mechanism responsible for the denopamine-induced effect were started at 37 weeks of age, and the duration of treatment was 4 to 6 weeks. The treatment with denopamine was the same as described above.

Protocol to study the effect of denopamine on survival rate

Denopamine was administered to hamsters as described above. Numbers of deaths of the hamsters were determined daily, and the body weight and food consumption were measured once a week. Postmortem examination was performed throughout the experimental period.

The experiment was terminated when the survival rate of the control group reached 25% of the initial number of animals. Surviving animals were sacrificed in the morning of the day after termination of the experiment and examined macroscopically under deep ether anesthesia. Blood samples were obtained with a syringe containing a small amount of heparin Na solution for measurement of the plasma level of denopamine in the denopamine group. Plasma was separated by centrifugation (850 x g, for 10 min at 0–4°C) and stored at −80°C until assayed. Plasma catecholamine levels were determined at SRL Co., Ltd. (Tokyo) by high-performance liquid chromatography with the trihydroxyindole reaction (17).

Analysis of the mechanism of action of denopamine

After the dietary administration of denopamine for 4 to 6 weeks, the following parameters were assessed. Control animals were fed a normal diet as described previously.

Determination of plasma catecholamine levels

Each of 10 animals was used in this series of experiments. A 3-ml blood sample was obtained from the abdominal aorta under ether anesthesia and transferred rapidly to a chilled tube containing 6 mg of EDTA Na. The blood was then centrifuged (1,200 x g, for 10 min at 0–4°C) immediately, and the plasma was separated and stored at −80°C until assayed. Plasma catecholamine levels were determined at SRL Co., Ltd. (Tokyo) by high-performance liquid chromatography with the trihydroxyindole reaction (18).

Measurement of cardiac function

Cardiac function was examined in 7 control and 6 denopamine-treated animals under urethane anesthesia (1 g/kg, i.p.). Responsiveness to denopamine was also assessed by intravenous injection of denopamine after the measurement of basal cardiac function.

Under urethane anesthesia, a polyethylene tubing that was connected to a pressure transducer (TP400T; Nihon Kohden, Tokyo) was inserted into the right carotid artery to measure blood pressure and heart rate (preamplifiers: AP621G and AT620G, Nihon Kohden). The left ventricle was punctured by a 22G needle that was connected to another pressure transducer (TP300T, Nihon Kohden) via a polyethylene tubing to measure the left ventricular pressure (preamplifier: AP621G, Nihon Kohden). The first derivative of left ventricular pressure (LV dp/dt) was obtained by means of a differentiator (EQ621G, Nihon Kohden) as an index of cardiac function. Another polyethylene tubing with a 27G needle was inserted into the femoral vein for intravenous injection of the drug. Changes in all parameters were recorded simultaneously on a lineacorder (WR-3301; Graphtec, Tokyo).

After an equilibration period of about 30 min, baseline values were obtained. Thereafter, increasing doses of denopamine (3 to 3000 µg/kg) were injected intravenously at an interval of 3 min.

Isolated left ventricular papillary muscle

Immediately after the decapitation, the heart was removed, and the left ventricular papillary muscle was excised in chilled physiological salt solution (PSS). The composition of PSS was as follows: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM KH2PO4, 25 mM NaHCO3, 1.2 mM MgSO4, 11 mM glucose, pH 7.4.

The papillary muscle was suspended in an organ bath containing 10 ml of PSS that was maintained at 30°C and was aerated with 95% O2−5% CO2 gas. One end of the muscle was fixed at the bottom of the organ bath and the other end was connected to a strain gauge transducer (UL20G, Minebea, Tokyo) with a thread. Isometric tension developed was recorded on a lineacorder (WR-3701,
Graphtec). After loading a resting tension of ca. 0.5 g, the muscle was stimulated with rectangular pulses of 5-msec in duration with a voltage of 1.5 times the threshold at 1 Hz by means of an electric stimulator (SEN-3301, Nihon Kohden). PSS was exchanged twice at an interval of 30 min during the equilibration period. After the muscle contraction became stabilized, denopamine at 10^{-8}-10^{-4} M was added to the bathing solution in a cumulative manner. The positive inotropic effect of denopamine was expressed as a percent of the maximum increase induced by 10 mM Ca^{2+}.

**β-Adrenoceptor binding assay**

In another series of experiments, 5 hamsters in both groups, in which plasma catecholamine levels had been determined, were used for examination of β-adrenoceptor density in the left ventricle and septum. The heart was excised immediately after the blood sampling and then rinsed in chilled 0.9% NaCl solution. The atria and the right ventricular free wall were removed. The left ventricular free wall and septum were blotted, weighed and stored in liquid nitrogen until the assay of β-adrenoceptor density.

The sample was kept on ice, added with 20 vol. Tris-HCl (pH 7.4) and homogenized with a polytron homogenizer (setting 6, 10 sec × 3) (Type PT10/35; Kinematica, Luzern, Switzerland). The homogenate was centrifuged for 10 min at 28,000 × g and the pellet was washed once more by repeating the same procedure as described above. The washed pellet was suspended with 1 ml Tris-HCl/7 mg wet weight of the ventricular muscle, and this suspension was used for the β-adrenoceptor binding assay. [125I]-Iodocyanopindolol (ICYP) was used as a radioactive ligand. β-Adrenoceptor density (B_max) and affinity of ICYP (K_d) were determined by saturation binding, and the β1/β2 ratio was evaluated by competition binding to ICYP with (+)-bisoprolol. The incubation was performed in a total volume of 300 μl with 50 μl of each ligand and the drug solution. Ligand binding was assessed by a γ-autowell counter (COBRA II/Auto-Gamma; Packard, Downers Grove, IL, USA), and the BCA method (19) was used for protein assay.

**Measurement of heart weight**

Heart weights of hamsters used in this study except those used in the life-span study were measured: i.e., heart weights of hamsters treated with the denopamine mixed diet or normal diet for 4 to 6 weeks were measured. Hearts were rinsed in 0.9% NaCl solution or PSS at the end of the experiments or after the sampling and then blotted on filter paper. They were divided into the left ventricular free wall and septum, right ventricular free wall and atria. Absolute and relative weight to body weight were determined by weighing each part of the heart.

**Drugs**

Denopamine (Tanabe Seiyaku Co., Ltd., Osaka) was used as the free base form mixed in the diet or used as the HCl salt form in other experiments. [125I]-Iodocyanopindolol was purchased from Amersham (Buckinghamshire, UK) and (±)-bisoprolol was kindly donated by E. Merck (Darmstat, Germany). Other reagents of the commercially available best quality were purchased.

**Statistical analyses**

Results are expressed as means ± S.E.M. The method of statistical analysis used in the life-span study was the log-rank test by the Kaplan-Meire method. One way analysis of variance of randomized block design by the Dunnett method or Student's t-test was applied in other experiments. The difference was considered to be significant when the P value was less than 0.05.

**RESULTS**

**Effects of denopamine on the rate of survival**

Chronological changes in survival rate are shown in Fig. 1A. Three animals in the denopamine group died from the intestinal disease that has been known to occur in rodents (20) or due to broken incisors caused by abnormal growth caused by the powdered diet, whereas none in the control group died from non-cardiac causes. Therefore, the survival rate from which these non-cardiac deaths were deleted is also presented in Fig. 1B.

As shown in Fig. 1A, animals in the control group started to die at 40 weeks of age, and the survival rate reached 23.8% at 65 weeks of age. On the contrary, animals in the denopamine group did not die until 52 weeks of age, except one animal that died from a non-cardiac problem soon after starting the experiment. Thereafter, animals in the denopamine group died one by one until the final survival rate at 65 weeks of age reached about 40%. Statistical analysis of the survival rate in Fig. 1A revealed no significant differences. However, when the survival rate was corrected for non-cardiac death (Fig. 1B), denopamine significantly prolonged the life span (P <0.05).

Body weight tended to decrease in both groups during the experimental period, although there were large variations due to death and systemic edema in animals with severe heart failure. Denopamine consumption that was calculated from food consumption and body weight was about 38 mg/kg/day initially, but it decreased to about 25 mg/kg/day by the end of the experiment. The plasma level of denopamine in the surviving animals was 4.98 ±
0.39 ng/ml (n = 8).

Table 1 shows the major macroscopic findings. The majority of the cardiomyopathic hamsters in both groups showed ascites, hydrothorax, myocardial calcification and fibrosis, and atrial dilatation. There were no obvious differences between the control and denopamine-treated groups in these parameters, and this remained true when the animals were divided into surviving and dead cases.

**Plasma catecholamine levels**

Table 2 summarizes the data on plasma catecholamine levels. Treatment with denopamine for 4 to 6 weeks did not affect the plasma level of adrenaline, but significantly decreased both the plasma noradrenaline and dopamine levels compared with the levels in the control group.

**Cardiac function and response to denopamine**

Table 3 represents the baseline cardiac function and Fig. 2 shows the dose-response relationship of

| Table 1. Gross pathological findings after autopsy and/or necropsy of cardiomyopathic hamsters (BIO 14.6 strain) |
|---------------------------------------------------------|
| **Findings** | **Group** | **Control** | **Denopamine** |
|-------------|----------|------------|---------------|
| Ascites     | 10/21    | 10/21      |
| Hydrothorax | 15/21    | 12/21 (11/18) |
| Myocardial calcification | 17/21 | 15/21 (13/18) |
| Myocardial fibrosis | 18/21 | 18/21 (16/18) |
| Atrial dilatation | 20/21 | 19/21 (16/18) |

Numbers indicate observed cases/whole animals used. Numbers in parentheses indicate the same index correction with non-cardiac death.

| Table 2. Influence of chronic treatment with denopamine on the plasma catecholamine levels of cardiomyopathic hamsters (BIO 14.6 strain) |
|----------------------------------------------------------------------------------------------------------------------------------|
| **Group** | **Number** | **Plasma catecholamine level (pg/ml)** |
|-----------|------------|---------------------------------------|
|           |            | Adrenaline | Noradrenaline | Dopamine |
| Control   | 10         | 4362.9±806.4 | 7164.4±787.9 | 135.4±16.4 |
| Denopamine| 10         | 4958.0±798.2 | 5343.0±340.2* | 87.1±7.7* |

*P<0.05 vs the control group by means of Student's t-test.

| Table 3. Baseline value of cardiac function in cardiomyopathic hamsters (BIO 14.6 strain) |
|------------------------------------------------------------------------------------------------|
| **Group** | **Number** | **MBP (mmHg)** | **HR (beats/min)** | **LV dp/dt max (mmHg/sec)** |
|-----------|------------|----------------|-------------------|-----------------------------|
| Control   | 7          | 59.0±2.9 | 411.4±9.2 | 7495±563 |
| Denopamine| 6          | 62.5±5.7 | 456.3±19.9 | 7470±909 |

Data presented are means±S.E.M. MBP, mean blood pressure; HR, heart rate.
Denopamine-induced changes in cardiac function. Baseline values for mean blood pressure, heart rate and LV dp/dt max were not affected by treatment with denopamine (Table 3). In the control group, intravenously administered denopamine clearly increased LV dp/dt max at doses of 30 pg/kg and higher (30-300 pg/kg, P<0.01) with concomitant increases in heart rate (≥30 pg/kg, P<0.01). These changes peaked at doses of 300 or 1000 pg/kg and then decreased. Mean blood pressure tended to decrease dose-dependently at doses of 100 pg/kg and higher, and this effect reached a significant level at the dose of 3000 pg/kg (P<0.01). These hemodynamic effects of denopamine were also not affected by treatment with denopamine (Fig. 2).

**Effect of denopamine on isolated left ventricular papillary muscle**

Figure 3 represents concentration-response curves for the positive inotropic effect of denopamine in left ventricular papillary muscles isolated from cardiomyopathic hamsters. Denopamine caused a positive inotropic effect in both groups at concentrations of 3×10⁻⁸ M and higher (≥3×10⁻⁶ M, P<0.01), and this effect reached about 60% of the response to 10 mM Ca²⁺ at 3×10⁻⁵ M. Thus, the positive inotropic effect of denopamine was not affected by the treatment of animals with denopamine.

**β-Adrenoceptor density**

The β-adrenoceptor density in the membrane prepara-
tion of the left ventricular muscle of cardiomyopathic hamsters is shown in Table 4. The values for $B_{\text{max}}$, $K_d$, $\beta_1/\beta_2$ ratio and Hill slope in the control group were 32.7 fmol/mg protein, 17.1 nM, 60/40 and close to 1, respectively, which were not significantly different from those in the denopamine-treated group.

**Heart weight**

Table 5 shows the heart weight of cardiomyopathic hamsters. Absolute as well as relative left ventricular weight, ventricular weight and heart weight of both groups were not significantly different from each other, indicating that denopamine administered orally for 4–6 weeks did not affect the cardiac weight.

**DISCUSSION**

van Meel et al. reported that the PDE III inhibitor pimobendan, one of the cardiotonic agents, prolonged the life span in cardiomyopathic hamsters of the CHF 146 strain (8). However, since they started treating the animals at quite a young age (30- to 40-days-old) when cardiac lesion is not obvious, it is difficult to judge whether or not pimobendan is effective when administration is initiated after occurrence of cardiac lesions. In addition, it is not clear whether the positive inotropic effect or other actions such as vasodilating and antiplatelet actions contributed to its long-term effect (8).

**Table 4. $\beta$-Adrenergic receptors in heart membrane of cardiomyopathic hamsters (BIO 14.6 strain)**

| Group     | Number | $K_0$ (pM) | $B_{\text{max}}$ (fmol/mg protein) | $\beta_1/\beta_2$ (%) | $K_i$ ($\beta_1$) (nM) | $K_i$ ($\beta_2$) (nM) |
|-----------|--------|------------|-----------------------------------|-----------------------|------------------------|------------------------|
| Control   | 5      | 17.2±1.0   | 32.7±1.9                          | 60.2/39.8             | 67.4±11.5              | 6761±761               |
| Denopamine| 5      | 17.1±0.9   | 33.1±1.5                          | 59.8/40.2             | 66.6±5.7               | 6036±576               |

ICYP: iodocyanopindolol.

**Table 5. Influence of chronic treatment with denopamine on the heart weight of cardiomyopathic hamsters (BIO 14.6 strain)**

| Group     | Number | Body weight (g) | Heart weight (mg) | Heart weight (mg) | Heart weight (mg) |
|-----------|--------|-----------------|-------------------|-------------------|-------------------|
|           |        |                 | Left ventricle    | Ventricle         | Whole heart       |
| Control   | 18     | 119.6±2.5       | 394.8±8.5         | 498.6±12.1        | 548.2±19.0        |
| Denopamine| 18     | 123.2±2.2       | 388.1±8.9         | 487.8±10.6        | 526.6±11.9        |

| Relative heart weight (mg/g) | Left ventricle | Ventricle | Whole heart |
|------------------------------|----------------|-----------|-------------|
| Control                      | 3.34±0.13      | 4.22±0.18 | 4.65±0.25   |
| Denopamine                   | 3.15±0.03      | 3.96±0.06 | 4.28±0.07   |

Relative heart weight was calculated from the heart and body weight.
Denopamine and Life Span of Cardiomyopathic Hamster

In carrying out the present study, we tried to overcome the disadvantages of previous studies by mimicking the clinical situation. Namely, we studied the effect of denopamine on the life span in aged cardiomyopathic hamsters when the animals suffered from heart failure (16) with impaired cardiac function (21) and down-regulated \( \beta \)-adrenoceptors (22). Under the present experimental conditions, denopamine significantly prolonged the life span when the non-cardiac deaths were excluded. It should be noted that the significant difference between groups was first obtained when accidental deaths were excluded because the number of animals we used was not sufficiently large. Nevertheless, the plasma level of denopamine in the present study is comparable with the lowest level observed in the clinical setting (23), implying some extent of practical relevance. Among the parameters examined to elucidate the mechanism of action of denopamine, the sole positive finding was the decreased plasma catecholamine levels in the denopamine-treated group.

Catecholamine release, especially release of noradrenaline, is considered to increase to compensate for low cardiac output in congestive heart failure (24-26). Plasma catecholamine levels were elevated depending on the severity of heart failure, and a significant correlation between the plasma noradrenaline level and the prognosis of heart failure patients has been reported (27). Thus, the denopamine-induced decrease in plasma noradrenaline level seems to be important for the prognosis of cardiomyopathic hamsters.

It is well-known that catecholamines cause cardiac lesion (28) and have arrhythmogenicity (29). These cardio-toxicities of catecholamines are considered to be due to their excessive physiological and pharmacological effects. Contraction band necrosis, calcification and fibrosis of the heart occur as catecholamine-induced histological changes (28), and single or consecutive administration of catecholamines could be used to prepare animal models of cardiomyopathy (30-32). On the other hand, arrhythmogenicity of catecholamines is considered to be due, in part, to their cardiovascular effects, such as the \( \beta \)-adrenoceptor-mediated chronotropic effect and \( \alpha \)-adrenoceptor-mediated vasoconstriction (12, 33, 34), and catecholamines could be used to prepare a model of arrhythmia with halothane anesthesia (35, 36).

Although denopamine is a \( \beta \)-adrenergic agonist, it lacks a catechol moiety in its chemical structure (37). When compared with naturally-occurring catecholamines like adrenaline, noradrenaline and dopamine, denopamine is selective to \( \beta_1 \)-adrenoceptors and possesses partial agonistic action with quite high intrinsic activity (38). The \( \beta_1 \)-selectivity and partial agonistic property of denopamine are assumed to be responsible for characteristics of this compound such as less development of tolerance (13) and less arrhythmogenicity (12). In addition, denopamine causes less \( \alpha \)-adrenoceptor-mediated vasoconstriction (13, 39). In this connection, it is noteworthy that denopamine did not affect the heart weight and cardiac \( \beta \)-adrenoceptor density because denopamine at the same dose significantly decreased cardiac death in cardiomyopathic hamsters.

Denopamine elicited a positive inotropic effect in anesthetized hamsters and in isolated papillary muscle, although the effect was not pronounced. Therefore, when denopamine was administered chronically, it is possible that the release of noradrenaline from the sympathetic nerve terminals was decreased due to the effect of denopamine on cardiac contractility. In addition, denopamine may directly decrease noradrenaline release from sympathetic nerve terminals due to its \( \beta_2 \)-adrenoceptor-blocking action (38, 40). Therefore,cardio-toxicity and arrhythmogenicity of noradrenaline may be alleviated by denopamine because it is a partial agonist having no \( \alpha \)-adrenoceptor-stimulating action (13).

However, the finding that dietary administered denopamine did not affect cardiac function seems to be inconsistent with our assumption. It is likely that the decreased noradrenaline release may offset the positive inotropic effect of denopamine. To elucidate the reason for the discrepancy, a further study using younger hamsters with larger doses of denopamine for longer duration is required.

Xamoterol, another \( \beta_1 \)-adrenoceptor partial agonist, has been reported to deteriorate the prognosis of patients with heart failure (4). The difference between xamoterol and denopamine lies in their intrinsic activities (38): i.e., xamoterol has an intrinsic activity of 0.4, which is lower than that of denopamine (0.9), and this difference may be responsible for the different chronotropic responses to these agents (4, 41). However, little is known at the moment about the pharmacological characteristics of \( \beta_1 \)-partial agonists in pathophysiological situations, and more experimental evidence is required to understand the clinical significance of this class of agents.

The influence of denopamine on the prognosis of patients with chronic heart failure has been currently studied by an open study, and it has been reported that at least it does not shorten the life span (9). However, this report has its own limits because they did not include a placebo group. Thus, the influence of denopamine on the prognosis of patients with heart failure should be studied by a more precise large scale clinical trial in the future.

In summary, our study revealed that denopamine at a dose giving a comparative plasma denopamine level to clinical doses prolonged the life span of cardiomyopathic hamsters, a model of congestive heart failure. The
decrease in plasma catecholamine levels induced by denopamine may be responsible for the beneficial effect of the compound as one of the underlying mechanisms.

REFERENCES

1. The SOLVD Investigators: Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. N Engl J Med 325, 293–302 (1991)
2. Cohn JN, Johnson G, Ziesche S, Cobb F, Francis G, Tristani F, Smith R, Dunkman WB, Loeb H, Wong M, Bhat G, Goldman S, Fletcher RD, Doherty J, Hughes CV, Carson P, Cintron G, Shabetai R and Haakenson C: A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure. N Engl J Med 325, 303–310 (1991)
3. DiBianco R, Shabetai R, Kostuk W, Moran J, Schlant RC and Wright R: A comparison of oral milrinone, digoxin, and their combination in the treatment of patients with chronic heart failure. N Engl J Med 320, 677–683 (1989)
4. The Xamoterol in Severe Heart Failure Study Group: Xamoterol in severe heart failure. Lancet 336, 1–6 (1990)
5. lijima T and Taira N: Membrane current changes responsible for the positive inotropic effect of OPC-8212, a new positive inotropic agent, in single ventricular cells of guinea pig heart. J Pharmacol Exp Ther 240, 657–662 (1987)
6. Lathrop DA and Schwartz A: Evidence for possible increase in sodium channel open time and involvement of Na/Ca exchange by a new positive inotropic drug: OPC-8212. Eur J Pharmacol 117, 391–392 (1985)
7. Feldman AM, Bristow MR, Parmley WW, Carson PE, Pepine CJ, Gilbert EM, Strobeck JE, Hendrix GH, Powers ER, Bain RP and White BG: Effects of venesinine on morbidity and mortality in patients with heart failure. N Engl J Med 329, 149–155 (1993)
8. van Meel JCA, Mauz ABM, Wienen W and Diederen W: Dimobendan increases survival of cardiomypathic hamsters. J Cardiovasc Pharmacol 13, 508–509 (1989)
9. lizuka M, Kimata S, Sasayama S, Kumada T and Hirota Y: Long-term administration of denopamine, an oral positive inotropic agent, in chronic heart failure: a multicenter open trial. Jpn Pharmacol Ther 21, 2251–2266 (1993)
10. Nagao T, Ikeo T, Murata S, Saito 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 the anesthetized dog and isolated guinea pig heart. Jpn J Pharmacol 35, 415–422 (1984)
11. Ikeo T and Nagao T: Effects of denopamine (TA-064), a new positive inotropic agent on myocardial oxygen consumption and left: ventricular dimension in anesthetized dogs. Jpn J Pharmacol 39, 179–189 (1985)
12. Narita H, Yabana H, Kikkawa K, Miyazaki K, Ikeo T and Nagao T: Weak arrhythmogenic property of the new cardiotoxic agent denopamine in dogs: comparison with catecholamines. Jpn J Pharmacol 41, 335–344 (1986)
13. Yabana H, Naito K and Nagao T: Effect of chronic administration of denopamine (TA-064), a new positive inotropic agent, on cardiac response of rats to denopamine. Jpn J Pharmacol 42, 87–97 (1986)
14. Kino M, Hirata Y, Yamamoto S, Moriguchi M, Kotaka M, Kubo S and Kawamura K: Cardiovascular effects of a newly synthesized cardiotonic agent (TA-064) on normal and diseased hearts. Am J Cardiol 51, 802–810 (1983)
15. Thormann J, Kramer W, Kindler M, Neuss H, Bahawar H and Schlepper M: Analysis of the efficacy of the new cardiotonic agent TA-064. Am Heart J 110, 436–438 (1985)
16. Gertz EW: Cardiomyopathic Syrian hamster: A possible model of human disease. Prog Exp Tumor Res 16, 242–260 (1972)
17. Tagawa K, Ueki T, Mizobe M, Noda K and Samejima M: Determination of denopamine in human and dog plasma by high-performance liquid chromatography with electrochemical detection. J Chromatogr 529, 500–506 (1990)
18. Minami M, Sano M, Tanoshi H, Endo T, Saito I, Nomura A, Saito H, Nakamura N, Kurimoto F, Sakurai H and Yasuda H: The factors affecting plasma catecholamine concentration in rats and man. Folia Pharmacol Jpn 83, 17–31 (1984) (Abstr in English)
19. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goekem NM, Olson BJ and Klenk DC: Measurement of protein using bicinchoninic acid. Anal Biochem 150, 76–85 (1985)
20. Handler AH and Chesherman FC: Spontaneous diseases. In The Golden Hamster. Its biology and use in Medical Research. Edited by Hoffman RA, Robinson PF and Malagahe H, pp 209–214, Iowa State University Press, Ames (1968)
21. Awad SS and Welty JD: Comparisons of hemodynamics throughout the life span of the B14.6 cardiomyopathic with the F1B normal hamster. Comp Biochem Physiol 97A, 487–491 (1990)
22. Hori M, Kagiya T and Inoue M: Adrenergic receptors and calcium ion channels in cardiomyopathic Syrian hamsters. Saishin Igaku 42, 977–981 (1987) (Abstr in English)
23. Nagao T and Nakajima H: Denopamine. Cardiovasc Drug Rev 7, 310–315 (1989)
24. Francis GS, Goldsmith SR and Cohn JN: Relationship of exercise capacity to resting left ventricular performance and basal plasma norepinephrine levels in patients with congestive heart failure. Am Heart J 104, 725–731 (1982)
25. Lehmann M, Wybitul K, Kapp R, Spielberger B and Keul J: Correlations of hemodynamic parameters with plasma catecholamines in patients with congestive cardiomyopathy. Clin Cardiol 5, 493–499 (1982)
26. Levine TB, Francis GS, Goldsmith SR, Simon AB and Cohn JN: Activity of the sympathetic nervous system and renin-angiotensin system assessed by plasma hormone levels and their relationship to hemodynamic abnormalities in congestive heart failure. Am J Cardiol 49, 1659–1666 (1982)
27. Cohn JN, Levine TB, Olivi MT, Garveng B, Luna D, Francis GS, Simon AB and Rector T: Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med 311, 819–823 (1984)
28. Haft JI: Cardiovascular injury induced by sympathetic catecholamines. Prog Cardiovasc Dis 17, 73–86 (1974)
29. Turtell RR and Mills J: Dobutamine. Development of a new catecholamine to selectively increase cardiac contractility. Circ Res 36, 185–196 (1975)
30. Fleckenstein A, Janke J, Doring HJ and Pachinger O: Ca overload as the determinant factor in the production of catecholamine-induced myocardial lesions. In Recent advances in studies on cardiac structure and metabolism: Cardio-
