Chronotropic, Dromotropic and Inotropic Effects of Dilazep in the Intact Dog Heart and Isolated Atrial Preparation

Shigetoshi CHIBA, M.D., Miyoharu KOBAYASHI, M.D.,
Masahiro SHIMOTORI, M.D., Yasuyuki FURUKAWA, M.D.,
and Kimiaki SAEGUSA, M.D.

SUMMARY

When dilazep was administered intravenously to the anesthetized donor dog, mean systemic blood pressure was dose dependently decreased. At a dose of 0.1 mg/Kg i.v., the mean blood pressure was not changed but a slight decrease in heart rate was usually observed in the donor dog. At the same time, a slight but significant decrease in atrial rate and developed tension of the isolated atrium was induced. Within a dose range of 0.3 to 1 mg/Kg i.v., dilazep caused a dose related decrease in mean blood pressure, bradycardia in the donor dog, and negative chronotropic, dromotropic and inotropic effects in the isolated atrium. At larger doses of 3 and 10 mg/Kg i.v., dilazep caused marked hypotension, frequently with severe sinus bradycardia or sinus arrest, especially in isolated atria.

When dilazep was infused intraarterially at a rate of 0.2–1 μ g/min into the cannulated sinus node artery of the isolated atrium, negative chrono- and inotropic effects were dose dependently induced. With respect to dromotropism, SA conduction time (SACT) was prolonged at infusion rates of 0.2 and 0.4 μ g/min. But at 1 μ g, dilazep caused an increase or decrease of SACT, indicating a shift of the SA nodal pacemaker.

It is concluded that dilazep has direct negative chrono-, dromo- and inotropic properties on the heart at doses which produced no significant hypotension.

Additional Indexing Words:

Dilazep Isolated dog atria Chronotropism SA conduction time Atrial contraction

From the Department of Pharmacology and Research Laboratory for Cardiovascular Diseases, Shinshu University School of Medicine, Matsumoto 390, Japan.

Received for publication November 1, 1983.

DILAZEP has been used clinically as a antianginal agent. The pharmacological properties of dilazep are also well known. It is a potent coronary vasodilator and seems to act by enhancing the coronary vasodilator effects of adenosine.¹⁾⁻³⁾ It also provides effective myocardial protection during ischemic arrest and reperfusion in the heart.⁴⁾

In intact animals, direct cardiac effects of the drug are usually modified by indirect components such as reflex mechanisms. Since dilazep has a potent vasodilating action, it is thought that its cardiac effects may be influenced by its hypotensive effects.

In the present study, we attempted to investigate whether dilazep has direct cardiac action in doses which induced slight hypotensive changes in intact animals, using an isolated and blood-perfused dog atrial preparation perfused with donor's blood which was developed by Chiba et al.^{5),6)} Moreover, to investigate the direct effects of dilazep on SA nodal pacemaker activity, SA conductivity and atrial contractility, dilazep was administered into the cannulated sinus node artery of the isolated atrium.

METHODS

Twenty-four mongrel dogs of either sex weighing 9 to 21 Kg were anesthetized with sodium pentobarbital, 30 mg/Kg intravenously. The right atrium ($n=12$) was quickly excised and immersed in Tyrode's solution at 4 to 10°C. The isolated atrium was perfused with arterial blood through the cannulated sinus node artery. The blood was introduced from the carotid artery of the heparinized donor dog under a constant perfusion pressure of 100 mmHg by aid of a peristaltic pump (Harvard Apparatus 1210). The atrium was suspended in a bath filled with blood at a constant temperature of 37°C. The spontaneously rate of the isolated atrium was recorded by a tachometer (Nihon Kohden RT-2) which was triggered by the atrial electrogram. A bipolar stimulating electrode was attached to the atrial epicardium near the upper part of the sulcus terminalis. A recording electrode was placed on the caudal portion of the epicardium at a distance of 1.5 cm from the stimulating electrode. SACT was assessed during constant atrial pacing consisting of a train of 8 consecutive beats at a rate of 10 beats/min faster than the control atrial rate.⁷⁾ SACT was calculated by subtracting the sinus cycle length (AA) from the interval between the last paced atrial electrogram (Ap) and atrial electrogram of the first escape sinus node cycle (A). This interval (ApA minus AA) represents the total conduction time into and out of the sinus node with intact automaticity. SACT was digitized by a pulse counter (Nihon Kohden ET612J) and a printer (Citizen BM Co). Pacing stimuli were pro-

vided by an electric stimulator (Nihon Kohden SEM 7103) at an intensity of about twice diastolic threshold and a duration of 2 msec. This procedure was repeated 5 times within 2 min before and during the infusion of dilazep into the sinus node artery or after intravenous administration of dilazep to the donor dog. SACT was then obtained using a 20% trimmed mean. The upper part of the sulcus terminalis of the isolated atrium was connected directly to the force displacement transducer (Grass FTO3B) by a silk thread and isometric developed tension was constantly measured.

The donor dogs ($n=12$) were anesthetized with sodium pentobarbital, 30 mg/Kg intravenously, and ventilated through a cuffed endotracheal tube with room air and additional oxygen (Harvard Respirator, Model 607). Sodium heparin (500 units/Kg, i.v.) was given before cannulation of the carotid artery and then hourly thereafter (200 units/Kg, i.v.). The heart rate of the donor dog was also measured by a tachometer which was triggered by the R

Fig. 1. Effects of increasing doses of dilazep, administered intravenously to a donor dog, on mean systemic blood pressure (SBP) and heart rate (HR) in a donor dog, and on atrial rate (AR) and developed tension (DT) in an isolated, blood-perfused atrium.

waves of the electrocardiograph. The mean systemic blood pressure of the donor dog was continuously recorded. Details of the cross-circulated cardiac muscle preparation are described in previous papers.^{5),6),8),9)}

RESULTS

Effects of dilazep, injected intravenously into the donor dog, on systemic blood pressure and heart rate in the donor dog and on atrial rate and developed tension in the isolated atrium

When dilazep was administered intravenously to the donor dog, a de-

Fig. 2. Effects of relatively large doses of dilazep injected intravenously to a donor dog on mean systemic blood pressure (SBP) and heart rate (HR) in a donor dog, and on atrial rate (AR) and developed tension (DT) in an isolated atrium. In an isolated atrium, 3 mg/Kg of dilazep causes sinus arrest following a severe decrease in atrial rate.

crease in mean arterial blood pressure and heart rate in the donor dog and atrial rate and developed tension in the isolated atrium were usually observed. However, at a relatively small dose, dilazep had only a cardiac depressant action. As shown in Fig. 1, 0.1 and 0.3 mg/Kg of dilazep never produced a significant decrease in systemic blood pressure, but cardiac effects on the intact dog and the isolated atrial preparation were clearly observed. As shown in Fig. 2, a relatively large dose of dilazep induced greater depressant effects on the SA nodal pacemaker activity. In this case, 3 mg/Kg of dilazep caused severe sinus bradycardia in the donor dog and sinus arrest in the isolated atrium. Summarized data are shown in Fig. 3. As shown in Fig. 3, dilazep in a relatively small dose caused slightly greater depressant effects on the SA node and atrial contraction than on systemic blood pressure. Com-

Fig. 3. Effects of increasing doses of dilazep and nicardipine, administered intravenously to donor dogs, on mean systemic blood pressure (SBP) and heart rate (HR) in donor dogs and on atrial rate (AR) and developed tension (DT) in isolated atria. Control SBP is 103 ± 12 mmHg (mean $\pm$ SE, $n=6$), HR 137 ± 13 beats/min ($n=6$), AR 109 ± 4 beats/min ($n=6$) and DT 1.4 ± 0.1 g ($n=6$).

pared to the effects of nicardipine in the same preparations, which have already been reported,¹⁰⁾ dilazep has 1/10 the potency of nicardipine, although their response patterns are very similar.

Effects of intravenously administered dilazep on systemic blood pressure and heart rate in the donor dog, and chrono-, dromo- and inotropic effects on the isolated atrium

As shown in Fig. 4, dilazep injected into the donor dog had predominantly cardiac depressant effects. In the isolated atrium, prolongation of the sinus cycle length (SCL) and SACT and a decrease in developed tension were clearly observed. At 3 mg/Kg i.v., dilazep did not cause a dose-related prolongation of SACT in this case.

Effects of dilazep in the isolated atrial preparation when infused into the cannulated sinus node artery

Fig. 4. Effects of increasing doses of dilazep injected intravenously to a donor dog on mean systemic blood pressure (SBP) and heart rate (HR) in a donor dog, and on sinus cycle length (SCL), sinoatrial conduction time (SACT) and developed tension (DT) in an isolated atrium.

When dilazep was continuously infused into the cannulated sinus node artery in a dose range of 0.2–1 $\mu\text{g}/\text{min}$, negative chronotropic and inotropic effects were usually induced in a dose related manner. With respect to dromotropism, dilazep in a relatively small dose caused a prolongation of SACT in a dose related manner. In the case shown in Fig. 5, dilazep caused negative chrono-, dromo- and inotropic effects. In the case shown in Fig. 6, negative chrono- and inotropic effects were dose dependently induced, but SACT was rather shortened at all examined doses. In some cases dilazep caused both an increase and decrease of SACT as shown in Fig. 7, although pacemaker and contractile activities were dose dependently suppressed. Summarized data are shown in Fig. 8. Compared to the effects of propranolol which have been published by us,¹¹⁾ dilazep appears to have different effects on SACT. At a relatively large dose, dilazep caused a sudden shortening of SACT but propranolol usually produced a prolongation of SACT in a dose

Fig. 5. Effects of increasing doses of dilazep continuously infused into the cannulated sinus node artery of an isolated atrium on sinus cycle length (SCL), sinoatrial conduction time (SACT) and developed tension in an isolated atrium.

Fig. 6. Effects of intraarterially injected increasing doses of dilazep on sinus cycle length (SCL), sinoatrial conduction time (SACT) and developed tension (DT) in an isolated atrium.

related manner.

DISCUSSION

Dilazep had been well known as a coronary vasodilator.¹⁾⁻³⁾ However, it has rather complex pharmacologic actions. In 1972, Lenke et al¹⁾ reported that dilazep exerted strong, persistent and specific action on myocardial blood flow. Furthermore, they suggested that dilazep has a slight positive inotropic effect in intact animals and that the action of dilazep on myocardial blood flow can be neutralized by oxyethyltheophylline. In 1974, Sano²⁾ reported that dilazep potentiated the coronary blood flow response to adenosine. Saito et al¹²⁾ also described an enhancement of myocardial reactive hyperemia by dilazep. It has been also reported that dilazep potentiates the negative chrono- and inotropic effects of adenosine on guinea pig atria.^{13),14)} Kobayashi et al¹⁵⁾ have reported that the mechanism of the coronary vasodilating

Fig. 7. An example of chrono-, dromo- and inotropic effects of intra-arterial dilazep on sinus cycle length (SCL) and developed tension (DT) in an isolated atrium.

action of dilazep might be associated with an increase in cyclic AMP in the coronary artery of the dog. In this study, we administered dilazep intravenously to the donor dog or intraarterially to the isolated dog atria using cross-circulated preparations. With neither administration route did we observe positive chronotropic and inotropic effects in the isolated atria. It has been recognized that an increase in tissue cyclic AMP causes positive chronotropic and inotropic effect.¹⁶⁾ Thus, the doses of dilazep we used might not have induced production of adequate amounts of cyclic AMP in the heart in situ. The positive inotropic effect observed by Lenke et al¹⁾ might be due to indirect action on the heart.

In the present experiments, we clearly demonstrated direct cardiac effects of dilazep by use of an isolated and blood-perfused atrial preparation. Since a small dose of dilazep, which caused no hypotension, induced slight negative chrono-, dromo- and inotropic effects, it may exert a direct depressant action on the heart in a dose related manner. With respect to dromotropism, a relatively large dose of dilazep frequently induced a shortening of

Fig. 8. Summarized data of the effects of increasing doses of dilazep on sinus cycle length (SCL), sinoatrial conduction time (SACT) and developed tension (DT) in isolated atrial preparations into which dilazep was infused via the cannulated sinus node artery of the isolated atrium. Control SCL is 627 ± 36 msec (mean $\pm$ SE, $n=6$), SACT 89 ± 9 msec ($n=6$) and DT 3 ± 1 g ($n=6$), respectively.

SACT. On the other hand, as reported previously,¹¹⁾ propranolol did not induce such shortening of SACT. A large dose of dilazep may cause a pacemaker shift, because dilazep has no beta-adrenoceptor blocking properties and never inhibits the effects of circulating catecholamines.

Although their potencies in inducing cardiac depression are different, the response patterns of dilazep and nicardipine, a calcium antagonist, are very similar. The calcium channel blocking action of dilazep may contribute to its cardiovascular actions. Tamura et al.¹⁷⁾ reported that dilazep at a concentration of 3×10^{-6} M showed relaxant effects on potassium induced contracture in guinea pig taenia coli, suggesting a Ca antagonistic effect. It is reported that adenosine inhibits the slow inward Ca channel in heart muscle.¹⁸⁾ As dilazep potentiated adenosine-induced effects, endogenous adenosine may participate in the cardiovascular action of dilazep.

ACKNOWLEDGMENT

We are grateful to Kowa Co. Ltd., Japan, for a supply of dilazep.

REFERENCES

1. Lenke D, Brock N, Zechel HJ: Zur Pharmacologie von 1,4-Bis-[3-(3,4,5-trimethoxybenzoyl-oxy)-propyl]-perhydro-1,4-diazepin(Dilazep I.N.N.), einer neuen koronaraktiven Substanz. *Arzneim-Forsch (Drug Res)* **22**: 639, 1972
2. Sano N: Enhancement of coronary vasodilating action of adenosine by dilazep and dipyridamole in the dog. *Jpn J Pharmacol* **24**: 471, 1974
3. Hensel I, Bretschneider HJ, Kettler D, Knoll D, Kochsiek K, Reploh HD, Spieckermann PG, Tauchert M: Die Wirkung von 1,4-Bis-[3-(3,4,5-trimethoxybenzoyl-oxy)-propyl]-perhydro 1,4-diazepin-dihydrochlorid auf Herzstoffwechsel, Haemodynamik, Koronar- und Nierendurchblutung. *Arzneim-Forsch (Drug Res)* **22**: 652, 1972
4. Shida H, Kobayashi M, Chiba S: Myokardschutz mit Dilazep bei myokardialer Ischämie und Reperfusion. *Arzneim-Forsch (Drug Res)* **31**: 1902, 1981
5. Chiba S, Kimura T, Hashimoto K: Muscarinic suppression of the nicotinic action of acetylcholine on the isolated, blood-perfused atrium of the dog. *Naunyn-Schmiedeberg Arch Pharmacol* **289**: 315, 1975
6. Chiba S, Yabuuchi Y, Hashimoto K: Comparison of the effects of norepinephrine and acetylcholine between intraarterial and extravascular administration of the isolated, blood-perfused canine atrium. *Jpn J Pharmacol* **25**: 433, 1975
7. Narula OS, Shantha N, Vasquez M, Towne WD, Linhart JW: A new method for measurement of sinoatrial conduction time. *Circulation* **58**: 706, 1978
8. Kobayashi M, Shimotori M, Chiba S: Direct effects of digoxin and deslanoside on sinoatrial conduction in isolated, blood-perfused dog atria. *Jpn Heart J* **24**: 649, 1983
9. Kobayashi M, Shimotori M, Chiba S: The effects of aminophylline on adenosine and ATP actions on sinoatrial conduction in the isolated, blood-perfused dog atrium. *Europ J Pharmacol* **91**: 261, 1983
10. Watanabe H, Furukawa Y, Iwatsuki K, Chiba S: Effects of nicardipine on the cross-perfused canine atrium. *Jpn J Pharmacol* **31**: 725, 1981
11. Shimotori M, Kobayashi M, Chiba S: Effects of 4 antiarrhythmic drugs on sinoatrial conduction time in isolated and blood-perfused dog atria. *Arch internat Pharmacodyn Ther* **267**: 67, 1984
12. Saito D, Kusachi S, Nagashima H, Haraoka S: Enhancement of myocardial reactive hyperemia and adenosine-induced coronary vasodilation by dilazep, a new adenosine potentiator. *Arzneim-Forsch (Drug Res)* **32**: 1029, 1982
13. Buyniski JP, Losada M, Bierwagen ME, Gardiner RW: Cerebral and coronary vascular effects of a symmetrical N,N-disubstituted hexahydrodiazepine. *J Pharmacol Exp Ther* **181**: 522, 1972
14. Fujita S, Ishida Y, Isumi K, Moritoki H, Ohara M, Takei M: Potentiation by dilazep of the negative inotropic effect of adenosine on guinea pig atria. *Br J Pharmacol* **68**: 343, 1980
15. Kobayashi A, Ogawa K, Yamazaki N: The effects of dilazep and SG 75 on cyclic nucleotides in the coronary artery. *Jpn Heart J* **21**: 85, 1980
16. Chiba S: Effects of dibutyryl cyclic AMP on sinus rate and atrial contractile force in the isolated atrium of the dog. *Tohoku J Exp Med* **115**: 81, 1975
17. Tamura H, Shirasawa Y, Hori H, Kondo S: Effects of dilazep on isolated smooth muscle. *Folia Pharmacol Japon* **71**: 757, 1975
18. Schrader J, Rubio R, Berne RM: Inhibition of slow action potentials of guinea pig atrial muscle by adenosine. A possible effect on Ca^{2+} influx. *J Mol Cell Cardiol* **7**: 427, 1975