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Functional and Metabolic Effects of Nicardipine and Diltiazem in the Rat Heart-lung Preparation

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Abstract

The functional and metabolic effects of nicardipine and diltiazem have been examined in the rat heart-lung preparation. Forty-eight Wistar-ST rats were anesthetized with isoflurane and prepared for the lung-heart models. The heart was perfused with Krebs-Ringer buffer solution containing with blood cells at a cardiac output of 30 $m\ell \cdot min^{-1}$ and systolic blood pressure of 10.7 kPa. Nicardipine (200 $ng \cdot m\ell^{-1}$, 400 $ng \cdot m\ell^{-1}$) or diltiazem (45 $ng \cdot m\ell^{-1}$, 450 $ng \cdot m\ell^{-1}$, 4500 $ng \cdot m\ell^{-1}$) was administered five minutes after the start of perfusion except in the control group. High energy phosphates (ATP, ADP and AMP), lactate and glycogen in the myocardium were measured after the thirty minutes perfusion. Nicardipine, 400 $ng \cdot m\ell^{-1}$, and diltiazem, 4500 $ng \cdot m\ell^{-1}$ depressed cardiac output and heart rate significantly. The therapeutic concentration of nicardipine is about 3.5-100 $ng \cdot m\ell^{-1}$ and that of diltiazem is about 30-135 $ng \cdot m\ell^{-1}$. Therefore, the depressant effect of nicardipine seems to be more potent than that of diltiazem because 400 $ng \cdot m\ell^{-1}$ of nicardipine is closer to its therapeutic range than 4500 $ng \cdot m\ell^{-1}$ of diltiazem. However, there were no significant differences in myocardial ATP, ADP, AMP, lactate and glycogen levels among the all groups. Although there were no adverse effects on myocardial metabolism, the margin of safety of nicardipine may be narrower than that of diltiazem in their direct depressant effects on heart.

Key words : Cardiac function, Cardiac metabolism, Nicardipine, Diltiazem, Rat heart

Introduction

Nicardipine is a dihydropyridine calcium antagonist chemically closely related to the calcium ion inhibitor nifedipine[1]. There are many reports that it is a potent systemic and coronary vasodilator without cardiodepressed effects[2~5]. Diltiazem is a benzothiazepine derivative with notable coronary vasodilating activity[6]. It also exhibits a weak depressive effect, in a large dose, on the beating rate and contractile force of the dog heart[7]. Most studies were performed about nicardipine and diltiazem at their therapeutic concentrations.

Therefore, it is interesting to examine effects of nicardipine and diltiazem on cardiac function at higher doses than their therapeutic concentrations in the rat heart-lung preparations. This method can eliminate any confounding neurohumoral effects of in vivo studies and determine functional effects of drugs[8].

Methods

The experiment was performed in accordance with Guidelines for Animal Experiments, Yamanashi Medical University. The techniques used were almost similar to those used in an earlier

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study[8]. Briefly, 48 male Wistar-ST rats (300-320 g) were anesthetized with 5 % isoflurane. Tracheotomy was performed, and intermittent positive ventilation was instituted with air. The chest was opened and flooded with ice-cold saline and the heart arrested. Cannulae were inserted into the aorta and the superior and inferior venae cavae. The cannula in the superior vena cava was used for monitoring right atrial pressure. The heart-lung preparation was perfused with a solution containing red blood cells collected from another rat and Krebs-Ringer bicarbonate buffer, with hematocrit and pH of 25 % and 7.4, respectively. The concentrations (mM) of the buffer consituents were : NaCl 127, KCl 5.1, $CaCl_2$ 2.2, KH_2PO_4 1.3, $MgSO_4$ 2.6, $NaHCO_3$ 15 and glucose 5.5. The perfusate blood (total volume is 25 mℓ) pumped from the aorta passed through a pneumatic resistance and collected in a reservoir kept at 37℃ and then returned to the inferior vena cava. In this model, no other organ except heart and lung was perfused, cardiac output was determined by the inflow, provided the heart did not fail,and systolic arterial pressure was regulated by the pneumatic resistance. Heart rate was recorded with a bioelectric amplifier (Nihonkohden AB-621G, Tokyo, Japan) and cardiac output was measured with an electromagnetic blood flowmeter (MFV-1200 Nihonkoden). Arterial pressure and right atrial pressure were measured with transducers (TP-101T and LPU-0.1A Nihonkoden) and carrier amplifiers (AB-601G Nihonkoden).

All hearts were perfused initially with a cardiac output of 30 $m\ell \cdot min^{-1}$ and systolic arterial pressure of 10.7 kPa. Five min after the start of perfusion, nicardipine or diltiazem was added to the reservoir except in the control group. Groups were divided into 6 according to the concentrations of drugs as follows: 1) Control group; no drug. 2) N200 group; 200 $ng \cdot m\ell^{-1}$ of nicardipine. 3) N400 group; 400 $ng \cdot m\ell^{-1}$ of nicardipine. 4) D45 group; 45 $ng \cdot m\ell^{-1}$ of diltiazem. 5) D450 group; 450 $ng \cdot m\ell^{-1}$ of diltiazem. 6) D4500 group; 4500 $ng \cdot m\ell^{-1}$ of diltiazem.

When right atrial pressure increased over 8 kPa, the inflow from reservoir to right atrium was diminished. It was estimated as cardiac failure that cardiac output was decreased under 20 $m\ell \cdot min^{-1}$. Thirty min after the start of the experiment, the hearts were freeze-clamped and freeze-dried for 6 days. An aliquot was eztracted with perchloric acid and centrifuged at 3000 g. Myocardial high energy phosphates (ATP, ADP and AMP) were measured by the high liquid performance chromatography[9]. Lactates were determined spectrophotometrically by standard techniques[10]. Another piece of freeze-dried sample was placed in 30 % KOH and digested at 100 ℃. Tissue glycogen was extracted, hydrolyzed and assayed as glucose equivalents[11]. The values are expressed as μ $mole \cdot g^{-1}$.

Statistical analysis used one way analysis of variance followed by the Dunnett test for comparison with the control values. The incidences of A-V block and cardiac failure were analyzed using the chi-square test. A probability of $P < 0.05$ was regarded as statistically significant. The date were given as means ± S. D.

Results

The incidences of cardiac failure in the N200,

Table 1. Incidences of cardiac failure

	Drug adminisstration ↓ 0	5	10	15	20	25	30 min
CONTROL	0/8	0/8	0/8	0/8	0/8	0/8	0/8
N 200	0/8	0/8	3/8 *	3/8 *	2/8	0/8	0/8
N 400	0/8	0/8	6/8 **	6/8 **	6/8 **	6/8 **	6/8 **
D 450	0/8	0/8	0/8	0/8	0/8	0/8	0/8
D 450	0/8	0/8	1/8	1/8	1/8	1/8	1/8
D 4500	0/8	0/8	4/8	5/8	5/8	5/8	5/8

*$p<0.05$, **$p<0.01$ vs control

N400 and D4500 groups were significantly higher than that in the control group (Table 1). In addition, cardiac output in the N400 and D4500 groups were decreased significantly at 10, 15, 20, 25 and 30 min after the drug administration (Fig. 1). Ventricular rate also decreased significantly in the N400 and D4500 groups (Fig. 2). The incidences of A-V conduction block in the N400 and D4500 groups were significantly higher than that in the control group (Table 2). However, there were no significant differences in myocardial ATP, ADP, AMP, lactate and glycogen levels among the groups (Fig 3, 4).

Dicussion

Nicardipine and diltiazem are popular calcium entry blockers used in patients with hypertension during operation. Many previous studies indicated that nicardipine had no cardio-depression[12] or only slight negative inotropic effects[13~17]. Another study also indicated that diltiazem had almost no cardio-depressive effect at therapeutic concentrations[18~20], and that diltiazem had depressed cardiac function dose-dependently[7,21~23]. However, most studies were performed on nicardipine and diltiazem at their therapeutic concentrations. In our study, 45 and 450 ng·mℓ$^{-1}$ of diltiazem hardly depressed cardiac function and 200 ng·mℓ$^{-1}$ of nicardipine slightly showed negative inotropism. However, both 400 ng·mℓ$^{-1}$ of nicardipine and 4500 ng·mℓ$^{-1}$ of diltiazem depressed cardiac function profoundly and their depressive effects on cardiac output were almost same. The therapeutic concentration of nicardipine is about 3.5-100 ng·mℓ$^{-1}$ [24,25] and that of diltiazem is about 30-135 ng·mℓ$^{-1}$ [26~28]. Therefore, the depressant effect of nicardipine seems to be more potent than that of diltiazem because 400 ng·mℓ$^{-1}$ of nicardipine is closer to its therapeutic range than 4500 ng·mℓ$^{-1}$ of diltiazem. These results suggest that the high dose administration of nicardipine may have more cardiodepres-

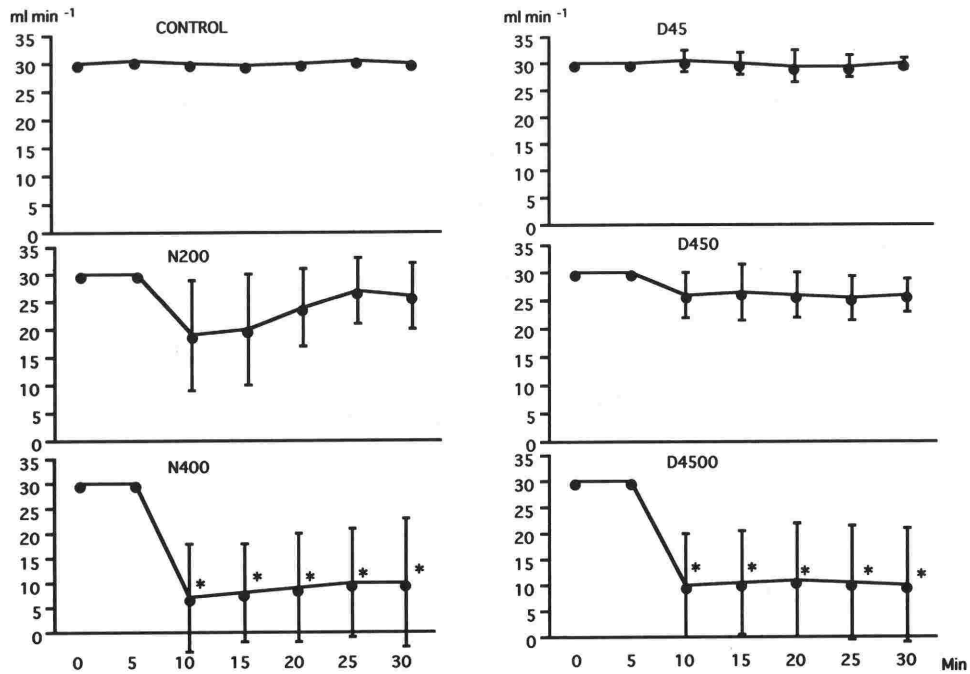

Figure 1 Changes in cardiac output over time after administration of nicardipine or diltiazem. Data are presented as mean ± SD. *P<0.05 vs. values at 5 min.

sive effect than that of diltiazem.

Hypotension induced by calcium entry blockers is known to activate baroreceptor mediated autonomic reflexes which, besides causing tachycardia, mask their negative inotropic effects. The fall in blood pressure with nifedipine is more rapid than diltiazem, resulting in a greater stimulation of arterial baroreceptors[29]. As nicardipine has similar hemodynamic effects to nifedipine[2], it may induce a more pronounced reflex activation in the sympathe-

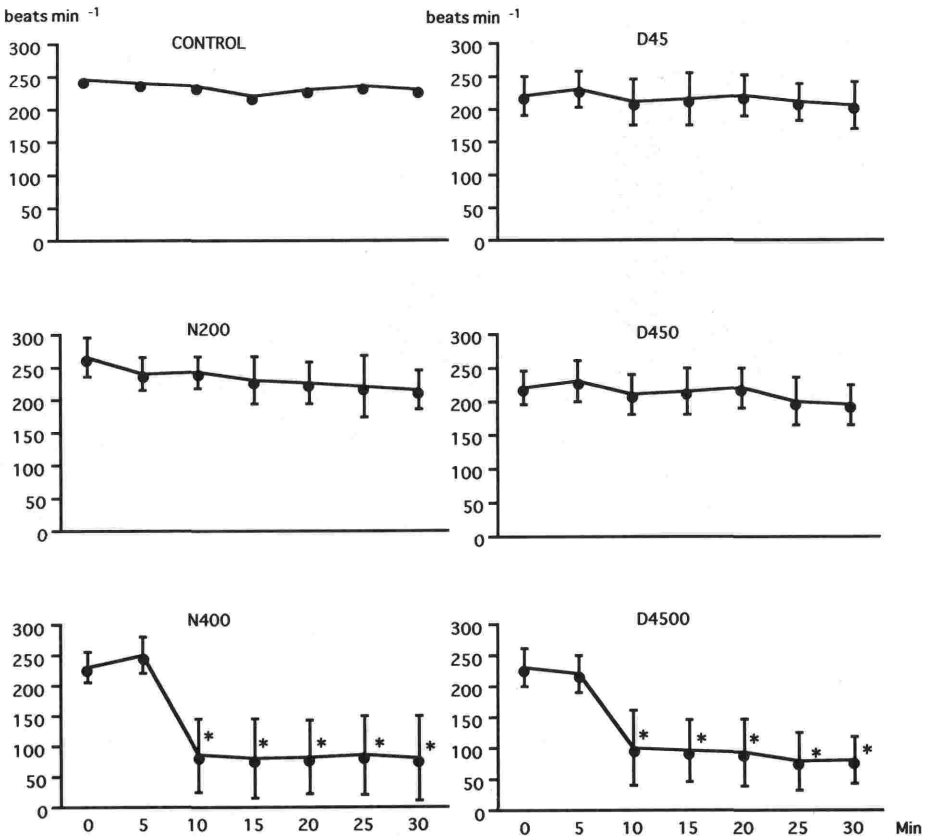

Figure 2 Changes in ventricular rate over time after administration of nicardipine or diltiazem. Data are presented as mean ± SD. *P<0.05 vs. values at 5 min.

Table 2. Incidences of A–V conduction block

	Drug adminisstration ↓ 0	5	10	15	20	25	30	min
CONTROL	0/8	0/8	0/8	0/8	0/8	0/8	0/8	
N 200	0/8	0/8	0/8	0/8	1/8	0/8	0/8	
N 400	0/8	0/8	6/8*	6/8*	6/8*	6/8*	6/8*	
D 45	0/8	0/8	0/8	0/8	0/8	0/8	0/8	
D 450	0/8	0/8	1/8	1/8	1/8	1/8	1/8	
D 4500	0/8	0/8	5/8*	6/8*	6/8*	7/8*	7/8*	

*p<0.05, **p<0.01 vs control

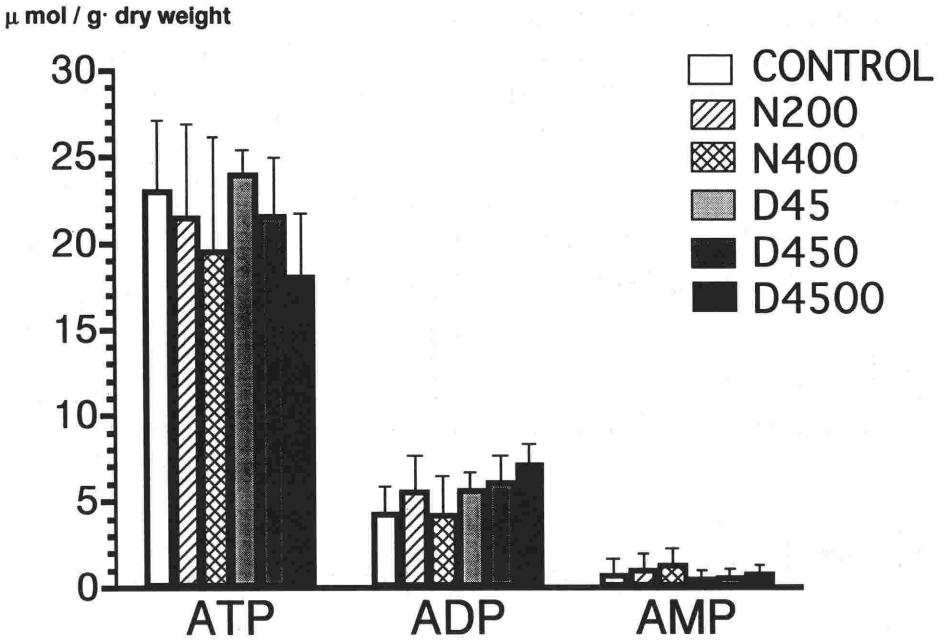

Figure 3 Concentrations of ATP, ADP and AMP in myocardium. There are no significant differences among the groups. Data are presented as means ± SD.

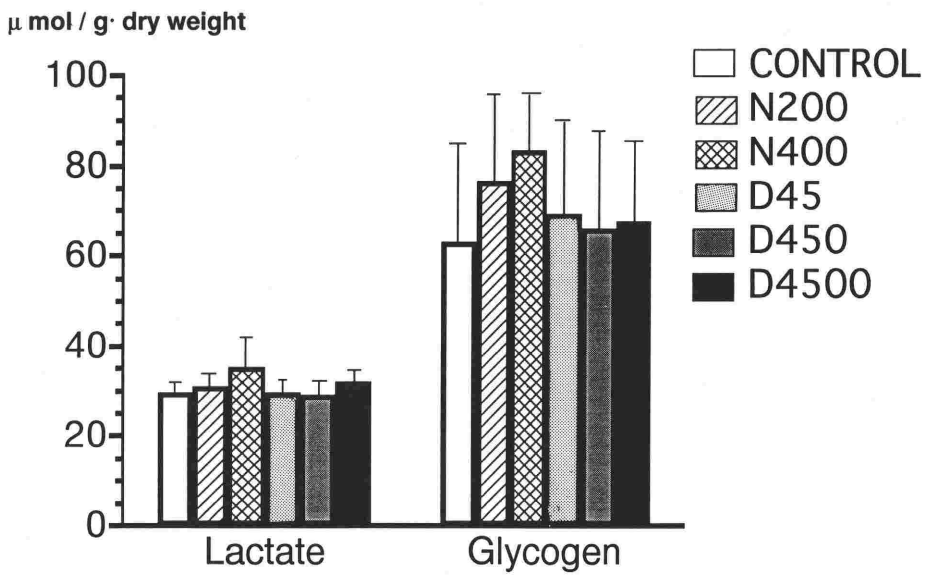

Figure 4 Concentrations of lactate and glycogen in myocardium. There are no significant differences among the groups. Data are presented as means ± SD.

tic system than diltiazem. However, our heart-lung preparation is not affected by central nervous system, so that sympathetic and parasympathetic reflexes can not occur and the peripheral resistance is constant. Therefore, the reason for less cardiac depression by nicardipine in clinical situations may be due to autonomic reflexes than by diltiazem.

Although 400 ng·mℓ$^{-1}$ of nicardipine and 4500 ng·mℓ$^{-1}$ of diltiazem depressed cardiac function, there were no significant differences in myocardial metabolites among the groups. It is likely that nicardipine and diltiazem might increase coronary blood flow which induced the increase of oxygen delivery. In addition, they might decrease oxygen demand by means of decreasing myocardial contractility and the consumption of intracellular ATP. These effects may have attributed to the inprovement of oxygen demand-supply relationship. Another possible explanation is that the depression of cardiac function was not so great because the heart was working, which might maintain myocardial metabolism.

In conclusion, 400 ng·mℓ$^{-1}$ of nicardipine and 4500 ng·mℓ$^{-1}$of diltiazem decreased cardiac output and ventricular rate significantly in the rat heart-lung preparation. Although there were no adverse effects on myocardial metabolism, the margin of safety of nicardipine may be narrower than that of diltiazem in their direct depressant effects on heart.

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