An Analysis of Blood Pressure Effects of Nipradilol and Prizidilol in Normotensive and Spontaneously Hypertensive Rats

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Abstract—Nipradilol and prizidilol are \( \beta \)-adrenoceptor blocking drugs with vasodilator action. These drugs lowered blood pressure (BP) in spontaneously hypertensive (SHR) rats acutely (24 hr) and subacutely (3 weeks) at doses of 10 and 20 mg/kg per day, p.o., respectively. Nipradilol decreased plasma renin concentration in acute and subacute studies, whereas it was unchanged with prizidilol treatment. Paradoxical effects of these drugs on BP were analyzed further: BP determined indirectly at the tail was slightly higher in SHR rats than the control, whereas BP determined directly through an aortic cannula without anesthesia, restraint, or prewarming was lower. We found that the discrepancy between BP values determined directly and indirectly was due to the increase in BP by prewarming stress during the determination by the tail cuff method.

Nipradilol and prizidilol are unique drugs designed to have two distinct and mutually beneficial actions: \( \beta \)-adrenoceptor blockade and arterial vasodilation in a single molecule (1-7). The drugs may be classified into the fourth generation of \( \beta \)-adrenoceptor (\( \beta \)-) blocking drugs (8).

We have studied their effects on blood pressure (BP) and heart rate (HR) and have investigated relationships between plasma drug concentrations and \( \beta \)-blocking or vasodilator activities in rabbits (9).

During the preclinical examination reported in this paper on BP effects of these drugs in rats, we observed discrepancies in BP values obtained by direct and indirect methods. BP determination in rats by an indirect method at the tail requires restraint and prewarming of the rat, which are known to affect BP values (10, 11). We analyzed this problem using a newer apparatus, which determines maximum BP values more accurately.

The purposes of this paper are to report effects of nipradilol and prizidilol on BP, HR and plasma renin concentration (PRC) in normotensive and spontaneously hypertensive (SHR) rats, and to analyze the discrepancies in BP values obtained by direct and indirect methods. In this analysis, we also used several antihypertensive drugs that act by different mechanisms, propranolol, hydralazine, phenoxybenzamine, prazosin, and captopril.

Materials and Methods

Experimental design: The study consists of two major parts: (A) Acute and subacute effects of nipradilol and prizidilol on BP, HR and PRC and (B) an analysis of paradoxical effects on BP, by comparing direct and indirect BP values.

In part A, a total of 6 experimental groups, 5-10 SHR rats each were used. In part B, effects of prewarming on BP values were studied in 32 groups of 8 rats each, different
combinations of normotensive or SHR rats, and 7 antihypertensive drugs or untreated control.

Scheffe’s S and LSD tests were used for statistical analyses.

Animals: SHR rats were F40–41 from the colony of the Department of Pharmacology, Jichi Medical School. In part A, the rats were 16 or 20 weeks of age, weighing 280–320 g. In part B, SHR rats were 10–13 weeks of age, weighing 210–290 g. Age matched normotensive HOS®: Donryu strain (DON) rats, weighing 200–250 g, were also used.

Determination of blood pressure by direct cannulation of the abdominal aorta: The mean BP was determined directly without anesthesia or restraint through a cannula inserted into the abdominal aorta via the femoral artery 2–10 days before (12) by an electronic system (Century Technology CP-01, Star Medical PA-011 and Natsume KN-260).

Indirect determinations of blood pressure and heart rate at the tail: BP and HR were determined by a manometer and tachometer system (Natsume KN-209) as reported previously (13), once a week immediately before and for 2 weeks after the drug treatments had started in part A. Prewarming the rat at 50°C for 3 min was used throughout the experiments. Intervals between the drug administrations and the determinations were 3–6 hr and randomized in each rat.

In part B, tail BP was determined by a new system (Natsume KN-210). It detects BP when the pulses disappear during the cuff inflation, as reported by Leenen and de Jong (14). By this device, the determination became considerably easier than before, because the rats do not move during the manipulation.

The new system (KN-210) measures the maximum BP more accurately than the ordinary one (KN-209), which detects BP when the pulses reappear during the cuff deflation. Theoretically, either system should measure the maximum BP indirectly. However, the values obtained by the ordinary systems were rather near the mean BP (13, 15, 16) because of the time lag between reappearance of the pulses and its mechanical detection.

The new system gave relatively higher values, which accords the maximum BP (17). As reported previously (17), we found that differences between indirect and direct BP obtained under anaesthetized and anaesthetized conditions are approximately equal to 2/3 of the pulse pressure determined at the abdominal aorta of DON and SHR rats under pentobarbital anesthesia (50 mg/kg, i.p.) after laparotomy.

Drugs and doses: Drugs used were prazidilol-2HCl-H2O (Smith Kline & French), nipradilol (Kowa), propranolol hydrochloride (ICI Pharma), hydralazine hydrochloride (Yamanouchi), phenoxybenzamine hydrochloride (Tokyo Kasei), prazosin hydrochloride (Pfizer Taito) and captopril (Sankyo). Prazidilol and nipradilol were suspended in 0.5% carboxymethyl cellulose solution. Phenoxybenzamine and prazosin were dissolved in distilled water after dissolution with a few drops of ethyl alcohol. Propranolol, hydralazine and captopril were dissolved in distilled water.

The drug solutions were administered orally by a gastric tube at a volume of 5 ml/kg body weight. The following doses for each drug were selected to lower BP by about 30 mmHg or near maximal but less than 30 mmHg: 30 mg/kg prazidilol (2), 10 mg/kg nipradilol (7), 100 mg/kg propranolol (13), 4 mg/kg hydralazine (15), 100 mg/kg phenoxybenzamine (13), 30 mg/kg prazosin (18) and 100 mg/kg captopril (19). The doses were in terms of the free bases.

Determination of plasma renin concentration: Through the aortic cannula, a blood sample of 0.5 ml was obtained following BP determination at the 5th hr in the acute studies and at the 3rd week in the subacute studies. PRC was determined by the modified method of Carvalho et al. (12, 20).

Results

Effects of nipradilol and prazidilol on blood pressure and plasma renin concentration in SHR rats

1) Acute effects (Fig. 1, Table 1): Mean BP of SHR rats before the drug treatments was 170–190 mmHg. BP changed only slightly in the control group throughout the observation period of 5 hr and after 24 hr.
Nipradilol and prizidilol treatments decreased BP by 25–30 and 20–30 mmHg, respectively. After 24 hr, BP returned almost to the initial values, although a significant difference from the control group existed after nipradilol treatment. PRC decreased appreciably by nipradilol, but the difference was statistically not significant. Prizidilol treatment had no effect on PRC in the acute study.

2) Subacute effects (Figs. 2 and 3, Table 2): The body weight of each of the three groups of SHR rats increased gradually at an almost equal rate. Both drugs decreased HR markedly. Tail BP of the control group continued to increase during the experiment. Mean BP value of this group determined directly at the 3rd week was almost equal to the indirect one determined at the tail by the ordinary system (Natsume KN-209). Tail BP in both treated groups was slightly higher against the control, whereas mean BP at the 3rd week was appreciably lower. The discrepancy between indirect and direct values were examined further, as shown in the following section.

One day after drug withdrawal, BP increased only slightly, indicating that no rebound phenomenon existed after nipradilol and prizidilol treatments. After administration of nipradilol for 3 weeks, PRC decreased significantly against the control. Prizidilol treatment decreased PRC only slightly.

An analysis of paradoxical effects on blood pressure (Figs. 4–7)

As shown in the previous section, we obtained higher tail BP after nipradilol and prizidilol treatments, whereas mean BP obtained directly through a cannula inserted into the abdominal aorta was lower than the control. This paradoxical effect was analyzed further.

The essential differences in the procedures between direct and indirect BP
determinations are prewarming and restraint in the holder of the rat. Therefore, effects of prewarming at 50°C for 3 min in the holder were investigated. The new system (Natsume KN-210) was used in this part of the studies.

The left panels of Figs. 4 and 5 show the acute effects of 7 different antihypertensive drugs 2 hr after administration in unaesthetized and unrestrained DON and SHR rats, respectively. The right panels show the effects of the same drugs after prewarming the rats in the holders at 50°C for 3 min. Both the directly and indirectly determined BP values are plotted.

In SHR rats, treatments with nipradilol and prizidilol decreased mean BP significantly under the unrestrained condition, whereas it was about the same after prewarming compared to the control values obtained without prewarming. Tail BP determined at the same time were 31.0 and 29.4 mmHg higher than the direct values, respectively. Propranolol resulted in the same tendency.

Under the same condition, differences in mean and tail BP were 29.4–37.1 mmHg on the average after various drug treatments in SHR rats, confirming the previous result (17) that BP values determined by the new apparatus (Natsume KN-210) was approximately equal to the maximum. These differences in DON rats were 16.6–28.3 mmHg.

Good correlations were shown when linear least regression analyses were made between simultaneously determined tail and mean BP values in DON (Fig. 6) and SHR (Fig. 7) rats, resulting in correlation coefficients of 0.891 (n=62, P<0.01) and 0.901

**Fig. 2.** Subacute effects of nipradilol and prizidilol on heart rate in SHR. *P<0.025 and **P<0.005, compared to the control. Other details are the same as in Fig. 1.

**Fig. 3.** Subacute effects of prizidilol and nipradilol on blood pressure. Details are the same as in Fig. 1.
BP Effects of Nipradilol and Prizidilol

Discussion

Nipradilol (10 mg/kg, p.o.) and prizidilol (20 mg/kg, p.o.) lowered BP in SHR rats acutely and subacutely as indicated by the observations for 24 hr and 3 weeks, respectively. HR was also decreased markedly as shown in the subacute study, suggesting that these drugs are basically β-adrenoceptor blocking (1–9). These results on BP and HR are in accord with previous reports (2, 7).

Nipradilol decreased PRC appreciably in the acute study for 5 hr and decreased it significantly in the subacute one for 3 weeks in SHR rats. This agrees with the renin suppressing effects of β-blocking drugs in general (21). Prizidilol did not suppress PRC significantly in either the acute or the subacute study. This can be explained by its relatively stronger vasodilating activity, which is known to release renin (22). Plasma renin

Fig. 4. Effects of prewarming on blood pressure after antihypertensive drug treatments in DON rats. *P<0.05 and **P<0.01, when compared to the control values by the same determining conditions. Details are the same as in Fig. 1.

Fig. 5. Effects of prewarming on blood pressure after antihypertensive drug treatments in SHR rats. *P<0.05 and **P<0.01 different from the control. Details are the same as in Fig. 1.

(n=62, P<0.01), respectively. This indicates that treatments with 7 different antihypertensive drugs had the least effects on pulse pressure and that tail (maximum) and mean BP proportionately changed.
activity was reported to decrease only slightly with 150–600 mg of prizidilol in 8 patients with essential hypertension (23).

Tail BP, which is approximately equal to the maximum value, was slightly higher than the control in SHR rats after nipradilol and prizidilol treatments in the subacute study, whereas mean BP was appreciably lower. Two possible reasons can be considered for this discrepancy: prewarming stress during the determination increased tail BP or these drugs affected pulse pressure extraordinarily.

The answer was that prewarming stress caused a BP rise in SHR rats specifically with $\beta$-blocking drugs, nipradilol and prizidilol. Propranolol showed the same effect. Mean BP determined directly after prewarming was not lower than the control after the treatment with $\beta$-blocking drugs in SHR rats. This is explained by vasoconstricting effects of these drugs by vascular $\beta_2$-blockade.

The effects of other antihypertensive drugs: hydralazine, captopril, phenoxym benzamine, or prazosin were not influenced by prewarming, probably because these drugs do not act on vascular $\beta_2$-receptors.

Mean and tail BP values determined directly and indirectly after prewarming were parallel with each other after various drug treatments. Changes in pulse pressure, estimated by the difference between mean and tail BP, were not large enough to explain this discrepancy.

We have devised an apparatus to warm the rats under milder conditions (21) and have resolved the paradoxical effects of $\beta$-blocking drugs on BP, caused by direct and indirect determinations.

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