α -Blockade and Vasodilatation Induced by Nipradilol, Arotinolol and Labetalol in Pithed Rats

Yoshito NAKAGAWA, Hajime NAKAHARA, Wan-Pao CHIN
and Shoichi IMAI

Department of Pharmacology, Niigata University School of Medicine,
Niigata 951, Japan

Accepted August 21, 1985

Abstract—In pithed rats two recently-introduced β -blockers, nipradilol and arotinolol, as well as labetalol shifted the pressor dose-response curve for phenylephrine to the right. Labetalol and arotinolol did not modify the pressor dose-response curve for clonidine, while nipradilol induced a definite rightward shift. These results indicate that labetalol and arotinolol are selective α_1 -blockers, while nipradilol is a non-selective one. In addition, all the three β -blockers produced complex changes in the blood pressure in pithed rats. A fall of the diastolic blood pressure induced by labetalol and nipradilol was preceded by a slight rise, while arotinolol produced a fall at lower doses and a rise at higher ones. The hypotension by labetalol was abolished after propranolol, while the hypertension was suppressed by prazosin, indicating that labetalol has an intrinsic β - and α_1 -sympathomimetic effect. The hypertension and the hypotension produced by nipradilol and arotinolol persisted even in the presence of propranolol and prazosin or propranolol and yohimbine.

Numerous β -blockers are now being used in ischemic heart diseases, arterial hypertension and cardiac arrhythmias. Among them labetalol is unique in that it possesses both β - and α -blocking activities (1, 2). According to Blakely and Summers (3), it is a more potent blocker of α_1 -adrenoceptors than of α_2 -receptors.

In this study, selectivity of the α -blocking actions of the two recently-introduced β -blockers with α -blocking activities, i.e., nipradilol (3,4-dihydro-8-(2-hydroxy-3-isopropylaminopropoxy)-3-nitroxy-2H-1-benzopyran, K-351) (4) and arotinolol (dl-2-(3'-t-butylamino-2'-hydroxypropylthio)-4-(5'-carbamoyl-2'-thienyl)thiazole hydrochloride, S-596) (5) was studied in comparison with that of labetalol. The inhibition of the pressor responses to phenylephrine and clonidine in pithed rats was used as measures of α_1 - and α_2 -blocking activities. In our previous experiment (6), we had demonstrated that phenylephrine and clonidine were selective α_1 - and α_2 -agonists

in the pithed rat. As the pithed rat preparation was a suitable model for exploring the vasodilatory action of drugs, we also examined the vasodilatory action of these compounds.

Materials and Methods

1. Preparation of pithed rats: Male Wistar-Ikumachi rats weighing between 235–340 g were anesthetized with sodium thiopental (60–70 mg/kg) administered intraperitoneally, and bilateral adrenalectomy was performed through two lateral incisions. The animals were artificially ventilated with room air via tracheal cannula connected to a positive pressure pump (Takashima-Shoten TB-101) (tidal volume 0.8 ml/100 g, 70–80 strokes/min). To insure a good oxygenation of the arterial blood, oxygen gas was blown to the inspiratory tube. For drug injections, the right femoral vein was cannulated with a polyethylene tubing. The left carotid artery was cannulated with a polyethylene tubing (PE 50) filled with heparin (200 U/ml)-

saline solution, and the blood pressure was measured via a pressure transducer (Gould P-50) connected to a carrier amplifier (San-ei 1236). Heart rate was monitored with a cardi tachometer (San-ei 2130) triggered by the arterial pressure pulse. Both parameters were recorded on a linear recorder (Watanabe Mark V). Pithing of the rats was made according to the method of Shipley and Tilden (7). After injection of atropine (1 mg/kg, i.v.), bilateral vagus nervi were cut. A trocar (O.D.=2.5 mm, 11 cm long) was introduced through the left orbit into the spinal cord (C₆) after ligation of the right carotid artery. Using the trocar as a guide, a pithing rod (O.D.=1.5 mm, 19 cm long) was inserted to destroy the spinal cord completely. During the experiment, body temperature was maintained at 37°C using a thermostatically controlled heating pad. After a 30 min period of equilibration, during which the cardiovascular parameters were allowed to stabilize, experiments were performed.

2. Studies on the selectivity of the α -blocking action: The pressor responses to phenylephrine and clonidine were determined from the changes in the diastolic blood pressure. Phenylephrine was administered as a single injection, while clonidine was administered in a cumulative fashion. In experiments in which the effects of labetalol, nipradilol and arotinolol on the pressor effects of phenylephrine and clonidine were tested, the pressor responses to the agonists were determined 5 min after administration of the three β -blockers. All the experiments were performed in the presence of propranolol (1 mg/kg). The drugs were injected via the polyethylene cannula inserted into the right femoral vein without flushing. Volumes of single injection of drugs ranged 0.01–0.1 ml per 100 g.

3. Statistics: Results were expressed as the mean $\pm$ S.E. The data were evaluated using Student's *t*-test and *P*-values less than 0.05 were regarded as significant.

4. Drugs: The following drugs were used: 1-phenylephrine hydrochloride (Kowa), clonidine hydrochloride (Nihon Boehringer), labetalol hydrochloride (Shinnihonjitsugyo), nipradilol (K-351, Kowa), arotinolol (S-596,

Sumitomo Chemicals), prazosin hydrochloride (Taito Pfizer), propranolol hydrochloride (ICI) and atropine sulfate (Wako).

Results

1. Studies on the vasodilatory effects:

The initial mean values of the diastolic blood pressure and the heart rate of the pithed rats were 48.8 ± 1.2 mmHg and 265 ± 12 beats/min, respectively ($n=15$). The three β -blockers with α -blocking action produced complex changes in the blood pressure when administered to the pithed rats in a cumulative fashion. Labetalol and nipradilol induced an initial slight rise of the blood pressure followed by a fall, while arotinolol produced a fall of the diastolic blood pressure at lower doses and a rise at higher doses (Fig. 1). The hypotensive effect of labetalol was abolished after treatment of the preparation with propranolol (Fig. 2), while the initial hypertension was suppressed by prazosin (Fig. 3). Both the hyper- and hypotensive effects of nipradilol and arotinolol persisted even in the presence of α - and β -blockers. Figure 4 depicts the effects of treatment of the preparation with prazosin or yohimbine on the hypertension produced by arotinolol in the presence of propranolol.

2. Studies on the selectivity of the α -blocking action: To evaluate the selectivity of the α -blocking effects, log dose-response curves for the pressor effect of phenylephrine (α_1 -agonist) and clonidine (α_2 -agonist) obtained in the presence of the three β -blockers, labetalol, nipradilol and arotinolol, were compared with those obtained in the absence of the β -blockers (Fig. 5). All the three compounds used produced a shift to the right of the pressor dose-response curves for phenylephrine, while the shift to the right of the pressor dose-response curves for clonidine was observed only with nipradilol (Fig. 5).

Discussion

In the present study, labetalol did not modify the pressor dose-response curve for clonidine, but shifted to the right the pressor dose-response curve for phenylephrine, indicating that the α -adrenoceptor blocking effect of this β -blocker was restricted to the

Fig. 1. Representative record of the blood pressure changes induced by intravenous administration of labetalol, nipradilol and arotinolol in pithed rats.

Fig. 2. Effects of propranolol on the hypotensive responses of the pithed rats to labetalol. DBP: changes in the diastolic blood pressure. ○ control ($n=7$), ● after treatment of the animals with propranolol (1 mg/kg) ($n=7$). ** $P<0.01$, *** $P<0.001$, compared with the change in DBP in the absence of propranolol.

α_1 -adrenoceptor. Blakely and Summers (3) have also reported that the α_2 -blocking effect of labetalol is extremely weaker than the α_1 -blocking effect. Arotinolol behaved in a similar manner to labetalol, while nipradilol produced an inhibition of the pressor effects not only of phenylephrine but also of

Fig. 3. Effects of prazosin (0.03 mg/kg) on the hypertensive response of the pithed rats to labetalol. To eliminate the ensuing hypotensive phase, the animals were pretreated with propranolol (1 mg/kg). ○ control ($n=7$), △ after treatment of the animals with prazosin (0.03 mg/kg) ($n=7$). *** $P<0.001$, compared with the change in diastolic blood pressure (DBP) in the absence of prazosin.

clonidine. Thus, it may be concluded that arotinolol is a β -blocker with α_1 -blocking activity, while nipradilol does not have such a selective α -blocking action.

It was reported by Brittain and Levy (8)

that labetalol did not possess an intrinsic activity. However, it has later been reported that labetalol can induce positive inotropic and chronotropic effects in the isolated guinea

pig atria and in the heart-lung preparation supported by a donor dog (9) and vasodilation in the perfused femoral artery (10) and hindlimb of dogs (11). In our present study conducted in the pithed rat, labetalol induced a hypotensive response and tachycardia, which were abolished after treatment of the preparation with propranolol. Thus, the existence of the intrinsic sympathomimetic activity was further substantiated. The hypotension by labetalol was always preceded by a transient phase of hypertension. This hypertensive phase, which became manifest after propranolol, was suppressed by prazosin, indicating the participation of the α_1 -stimulation in the observed rise of the blood pressure. Nipradilol produced a biphasic blood pressure response. Arotinolol produced a hypotension at lower doses and a hypertension at higher doses. Both the hyper- and hypotensive effects of these two compounds (arotinolol and nipradilol) persisted even in the presence of propranolol and prazosin or propranolol and yohimbine, indicating that

Fig. 4. Effects of prazosin and yohimbine on the hypertensive responses of the pithed rat to arotinolol. The animals were pretreated with propranolol (1 mg/kg). ○ control (n=4), △ after prazosin (0.03 mg/kg) (n=4), ▲ after yohimbine (1 mg/kg) (n=4).

Fig. 5. Effects of labetalol (1 mg/kg, i.v.), nipradilol (3 mg/kg, i.v.) and arotinolol (3 mg/kg, i.v.) on the pressor responses of the pithed rat to phenylephrine (a) and clonidine (b). DBP: diastolic blood pressure. ○ control (n=7 for phenylephrine and n=4 for clonidine), ● after labetalol (n=4 for phenylephrine and n=5 for clonidine), △ after arotinolol (n=6 for phenylephrine and n=5 for clonidine), ▲ after nipradilol (n=5 for phenylephrine and n=4 for clonidine). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the change in DBP in the absence of the β -blocker (○).

these two substances have direct vaso-constrictor and vasodilator effects of unknown mechanisms.

Acknowledgements: The authors wish to thank Miss R. Nakagawa and Miss M. Sato for their help in preparing the manuscript.

References

- 1 Farmer, J.B., Kennedy, I., Levy, G.P. and Marshall, R.J.: Pharmacology of AH5158: A drug which blocks both α - and β -adrenoceptors. *Br. J. Pharmacol.* **45**, 660–675 (1972)
- 2 Kennedy, I. and Levy, G.P.: Combined α - and β -adrenoceptor blocking drug AH5158: Further studies on α -adrenoceptor blockade in anesthetized animals. *Br. J. Pharmacol.* **53**, 585–592 (1975)
- 3 Blakely, A.G.H. and Summers, R.J.: The effects of labetalol (AH 5158) on adrenergic transmission in the cat spleen. *Br. J. Pharmacol.* **59**, 643–650 (1977)
- 4 Uchida, Y., Nakamura, M., Shimizu, S., Shirasawa, Y. and Fujii, M.: Vasoactive and β -adrenoceptor blocking properties of 3,4-dihydro-8-(2-hydroxy-3-isopropylamino)propoxy-3-nitroxy-2H-1-benzopyran (K-351), a new antihypertensive agent. *Arch. Int. Pharmacodyn. Ther.* **262**, 132–149 (1983)
- 5 Miyagishi, A., Nakahara, H., Hara, Y. and Nakatani, H.: Effects of the new β -adrenoceptor blocking agent, S-596 on the peripheral autonomic nervous system and smooth muscles. *Arch. Int. Pharmacodyn. Ther.* **261**, 222–237 (1983)
- 6 Nakagawa, Y., Chin, W.-P. and Imai, S.: Effects of guanfacine on pre- and postsynaptic α -adrenoceptors studied in comparison with those of clonidine. *Folia Pharmacol. Japon.* **79**, 431–439 (1982) (Abs. in English)
- 7 Shipley, R.E. and Tilden, J.H.: A pithed rat preparation suitable for assaying pressor substances. *Proc. Soc. Exp. Biol. Med.* **64**, 453–455 (1947)
- 8 Brittain, R.T. and Levy, G.P.: A review of the animal pharmacology of labetalol, a combined α - and β -adrenoceptor blocking drug. *Br. J. Clin. Pharmacol.* **3**, Supp. 681–694 (1976)
- 9 Katano, Y., Takeda, K., Nakagawa, Y., Hashimoto, T., Nakazawa, M., Tsukada, T., Otorii, T. and Imai, S.: Alpha- and beta-adrenoceptor blocking actions of labetalol and effects on the myocardial function, coronary circulation and myocardial energy metabolism thereof. *Folia Pharmacol. Japon.* **74**, 819–832 (1978) (Abs. in English)
- 10 Hashimoto, K., Hashimoto, T., Iizuka, H., Tsukada, T., Matsuda, H. and Imai, S.: Hypotensive and vascular effects of labetalol in the normotensive rat and dog. *Japan. Heart J.* **19**, 762–774 (1978)
- 11 Sweet, C.S., Solar, J. and Gaul, S.L.: Peripheral vasodilator and β -adrenoceptor blocking properties of several β -adrenoceptor antagonists. *Clin. Exp. Hypertension* **1**, 449–472 (1979)