ANALYSIS OF HYPOTENSIVE MECHANISMS OF PINDOLOL, A β -ADRENOCEPTOR BLOCKING DRUGS IN RATS

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Abstract—The hypotensive mechanisms of pindolol in anesthetized and conscious rats were investigated. Pindolol caused a fall in blood pressure in anesthetized, conscious and spinal rats, though in conscious rats a higher dose of the drug was required to produce such a response. This hypotension with pindolol in anesthetized and conscious rats was markedly inhibited by pretreatment with the drug itself or other β -adrenoceptor blocking drugs. A similar phenomenon also occurred when isoproterenol was injected intravenously in anesthetized and conscious rats. The relationship between the hypotensive actions of four β -adrenoceptor blocking drugs in anesthetized rats and their intrinsic β -sympathomimetic actions in isolated catecholamine-depleted tracheal preparations was determined. Order of hypotensive potencies was the same as that of their intrinsic β -sympathomimetic action, namely, pindolol > carteolol > bufetolol $\approx$ propranolol ($p < 0.05$). These results suggest that the hypotension with pindolol is mediated through a decrease in the peripheral vascular resistance due to an intrinsic β -sympathomimetic action of the drug.

Since Prichard and Gillam (1) reported that propranolol was most effective in the clinical management of hypertensive patients, the attention of many investigators has been focused on the hypotensive mechanisms of β -adrenoceptor blocking drugs including propranolol. Actually, other β -blockers have been prescribed for hypertensive patients, even though the exact hypotensive mechanisms have not been elucidated. With propranolol, however, the following hypotensive mechanisms have been suggested; 1) suppression of the renin-angiotensin system (2), 2) reduction in cardiac output (3, 4), 3) the centrally mediated hypotensive effect (5, 6), 4) the resetting of the baroreceptors at a low level (1, 7, 8), etc..

Pindolol is a safe and potent hypotensive drug which has been recently introduced for clinical use in the management of hypertensive patients (9-12). The drug is a strong, potent and useful β -blocker in cases of angina pectoris, and the myocardial depressant effect is much weaker than that of propranolol (13).

The present study was undertaken to clarify the hypotensive mechanism of pindolol in both anesthetized and conscious rats.

MATERIALS AND METHODS

Measurement of blood pressure and heart rate in anesthetized rats

Male Wistar rats weighing 250-350 g were anesthetized with α -chloralose (80 mg/kg i.v.)-urethane (500 mg/kg i.v.). Arterial blood pressure was recorded from the right common carotid artery via a pressure transducer (Nihon Kohden, MPU-0.5) on a polygraph (San-ei

Instrument, 8S), and simultaneously, heart rate was recorded using a pulse rate tachometer (San-ei Instrument, 2130) triggered by the pulse of the blood pressure. All drugs were dissolved in physiological saline, and the solution (0.5 ml/kg) were injected i.v. through a cannula inserted into the left jugular vein.

Measurement of blood pressure and heart rate in conscious rats

Male Wistar rats weighing 250–350 g were used. Arterial blood pressure was recorded from the abdominal aorta according to the method of Weeks and Jones (14), using the above described instruments. The recording of arterial blood pressure was begun 3–4 days after the operation.

Measurement of blood pressure and heart rate in spinal rats

Male Wistar rats weighing 250–350 g were used. Under ether anesthesia, spinal rats were prepared by sectioning of the spinal cord at the C₁–C₂ level and bilateral cervical vagal nerves. Artificial ventilation was maintained throughout the experiment using a respirator appropriate for a small animal. Arterial blood pressure and heart rate were measured by the same methods as those used for anesthetized rats.

Measurement of tension of the isolated guinea-pig trachea

Guinea pigs of both sexes, weighing 300–400 g, were sacrificed by a blow on the head, and the trachea was excised and set in a 20 ml organ bath filled with Krebs-Henseleit bicarbonate solution at 37°C and aerated with 95% oxygen + 5% carbon dioxide gas mixture. The inner pressure of the isolated intact trachea was recorded via a pressure transducer (Nihon Kohden, LPU-0.1) on a polygraph (Nihon Kohden, RM-85) according to the method of Jamieson (15) with slight modification by the present authors.

The guinea pig was treated with reserpine (2.5 mg/kg s.c.) 24 hr before use, since the depletion of catecholamines of the nerve terminals before the assessment of partial agonist activity is necessary to obviate the blockade to endogenous norepinephrine by partial agonists. The muscle relaxing values of β -adrenoceptor blocking drugs, propranolol, bufetolol, carteolol and pindolol, are expressed as percent of the maximum relaxing response (100%) with *l*-isoproterenol (1×10^{-8} g/ml). All drugs were dissolved in distilled water.

Drugs used were pindolol hydrochloride (Sandoz), propranolol hydrochloride (Sumitomo Chemicals), carteolol hydrochloride (Otsuka), *l*-isoproterenol hydrochloride (Nikken Chemicals), reserpine (Dai-ichi), α -chloralose (Nakarai Chemicals) and urethane (Nakarai Chemicals).

Statistical analysis was done using the Student's *t*-test.

RESULTS

Effect of pindolol on blood pressure and heart rate in anesthetized rats

Pindolol (2 μ g, 20 μ g, 1 mg/kg i.v.) caused a dose-dependent sustained fall in blood pressure and a bradycardia in anesthetized rats as shown in Fig. 1. In a smaller dose of the drug, a delayed tachycardia was observed following an initial bradycardia. The hypotension with pindolol (1 mg/kg i.v.) disappeared 2–3 hr after the injection of the drug, but

FIG. 1. Time courses of changes in mean blood pressure (MBP) and heart rate (HR) of anesthetized rats after pindolol given i.v. Each point and vertical bar indicate mean $\pm$ S.E.. $\triangle$: pindolol 2 μ g/kg ($n=3$), $\times$: pindolol 20 μ g/kg ($n=8$), $\circ$: pindolol 1 mg/kg ($n=6$).

FIG. 2. Blood pressure responses to *l*-isoproterenol (*l*-Iso) and pindolol after treatment with pindolol in anesthetized rats. BP: blood pressure (mm Hg), HR: heart rate (beats/min).

FIG. 3. Effects of propranolol and bufetolol on the blood pressure responses to *l*-isoproterenol (*l*-Iso) and pindolol in anesthetized rats. BP: blood pressure (mm Hg), HR: heart rate (beats/min).

the response to *l*-isoproterenol (0.1 μ g/kg i.v.) remained to be blocked, and in addition, a hypotension with the second injection of pindolol (1 mg/kg i.v.) was not observed (Fig. 2). The hypotension with pindolol (1 mg/kg i.v.) was also markedly inhibited by pretreatment with the other β -blocker, propranolol (500 μ g/kg i.v.) or bufetolol (500 μ g/kg i.v.) as shown in Fig. 3.

Effect of pindolol on blood pressure and heart rate in spinal rats

Pindolol (20 μ g/kg i.v.) caused a sustained fall in blood pressure and a gradual but relatively long lasting tachycardia (Fig. 4).

Effect of pindolol on blood pressure and heart rate in conscious rats

Contrasting to the responses with pindolol in anesthetized rats, in conscious rats pindolol (2 μ g, 20 μ g, 1 mg/kg i.v.) caused an initial prompt rise in blood pressure, but did not cause

FIG. 4. Blood pressure responses to *l*-isoproterenol (*l*-Iso) and pindolol in spinal rats. BP: blood pressure (mm Hg), HR: heart rate (beats/min).

FIG. 5. Time courses of changes in mean blood pressure (MBP) and heart rate (HR) of conscious rats after pindolol given i.v. Each point and vertical bar indicate mean $\pm$ S.E., $\triangle$ — $\triangle$: pindolol 2 μ g/kg ($n=5$), $\times$ — $\times$: pindolol 20 μ g/kg ($n=5$), $\circ$ — $\circ$: pindolol 1 mg/kg ($n=5$).

a significant fall in blood pressure at least within 2 hr after the injection of the drug (Fig. 5). A smaller dose of pindolol (2 μ g, 20 μ g/kg i.v.) had no influence on heart rate, but a durable tachycardia following an initial prompt bradycardia was observed after the injection of pindolol (1 mg/kg i.v.). Thus, although it was found difficult to observe a hypotension with pindolol within a shorter time after the injection of the drug in conscious rats, a higher dose of pindolol (5 mg/kg i.p.) caused a gradual but significant sustained fall in blood pressure reaching the maximum in 6 hr after the injection of the drug ($p<0.01$), as shown in Fig. 6. The hypotension with pindolol (5 mg/kg i.p.) was significantly inhibited by pre-treatment with propranolol (5 mg/kg i.p.) ($p<0.05$). This dose of propranolol produced hypertension, not hypotension in conscious rats.

As above described results indicate that the hypotension with pindolol in anesthetized and conscious rats is mainly due to the β -sympathomimetic action of the drug, the following experiments were carried out.

Comparison between the blood pressure responses to isoproterenol in anesthetized and conscious rats

l-Isoproterenol (0.03–1 μ g/kg i.v.) caused a dose-dependent fall in blood pressure in anesthetized and conscious rats. However, the responses, duration and hypotensive intensity, to *l*-isoproterenol in conscious rats were significantly shorter and weaker than those

FIG. 6. Effect of propranolol on the blood pressure response to pindolol in conscious rats. Pindolol was injected at the 0-time and propranolol at 30 min prior to pindolol. $\circ-\circ$: saline i.p. ($n=12$, mean of mean blood pressure ($\bar{x}$)=122 mm Hg, mean of heart rate $y=415$ beats/min), $\square-\square$: pindolol 5 mg/kg i.p. ($n=8$, $\bar{x}=128$ mm Hg, $y=409$ beats/min), $\triangle-\triangle$: propranolol 5 mg/kg i.p. ($n=3$, $\bar{x}=117$ mm Hg, $y=387$ beats/min), $\times-\times$: pindolol 5 mg/kg i.p. after treatment with propranolol 5 mg/kg i.p. ($n=4$, $\bar{x}=124$ mm Hg, $y=377$ beats/min). a: significantly different from saline at 4 hr ($p<0.05$), b: significantly different from saline at 6, 8, 10 hr ($p<0.01$), c: significantly different from pindolol at 6 hr ($p<0.05$), d: significantly different from pindolol at 8, 10 hr ($p<0.01$).

FIG. 7. Comparison between the blood pressure responses, duration and hypotensive potency, to *l*-isoproterenol in anesthetized and conscious rats. Each point and vertical bar indicate mean $\pm$ S.E. ($n=5$). $\triangle$: anesthetized rats, $\circ$: conscious rats. *significantly different from conscious rats ($p<0.05$), ** significantly different from conscious rats ($p<0.01$).

in anesthetized rats, as shown in Fig. 7. The mean of the initial blood pressure levels of anesthetized and conscious rats was 108 ± 12.2 and 125 ± 3.9 mm Hg ($n=5$), respectively. On the other hand, in contrast to changes in blood pressure of anesthetized and conscious rats after the injection of *l*-isoproterenol, the tachycardia with *l*-isoproterenol in conscious rats was more remarkable than that in anesthetized rats. The mean of the changes in heart rate (Δ HR) of anesthetized and conscious rats after injections of *l*-isoproterenol (0.03, 0.1, 0.3 and 1 μ g/kg i.v.) corresponded to 23 ± 8.5 , 49 ± 13.9 , 53 ± 12.3 , 59 ± 11.0 and 18 ± 18.0 , 137 ± 4.3 , 189 ± 11.6 , 207 ± 13.2 beats/min, respectively.

TABLE 1. Effects of β -adrenoceptor blocking drugs on mean blood pressure (MBP) and heart rate (HR) in anesthetized rats

Drugs	Dose (mg/kg i.v.)	Before administration		After administration	
		MBP (mm Hg)	HR (beats/min)	MBP (mm Hg)	HR (beats/min)
Pindolol (7)	1	115 $\pm$ 2.9	390 $\pm$ 27	73 $\pm$ 3.8*	370 $\pm$ 24
Carteolol (5)	1	114 $\pm$ 3.3	364 $\pm$ 18	91 $\pm$ 1.8**	334 $\pm$ 19
Bufetolol (5)	1	119 $\pm$ 6.4	402 $\pm$ 54	101 $\pm$ 1.6 NS	411 $\pm$ 45
Propranolol (7)	1	120 $\pm$ 2.1	353 $\pm$ 16	109 $\pm$ 7.7	279 $\pm$ 21

Data in the table are expressed as mean $\pm$ S.E. Figures in parentheses indicate number of animals. *Significantly different from Carteolol ($p < 0.01$). **Significantly different from Bufetolol ($p < 0.01$). NS: Not significantly different from Propranolol ($p > 0.1$).

TABLE 2. Relaxing actions of β -adrenoceptor blocking drugs and *l*-isoproterenol in isolated guinea pig tracheal preparations treated with reserpine

Drug	Concentration (g/ml)	Number of tracheae	Percentage of relaxation (%)
<i>l</i> -Isoproterenol	1×10^{-8}		100
Pindolol	1×10^{-7}	3	41.3 $\pm$ 7.0*
Carteolol	1×10^{-7}	5	24.8 $\pm$ 2.4**
Bufetolol	1×10^{-7}	4	11.7 $\pm$ 5.5 NS
Propranolol	1×10^{-7}	4	1.2 $\pm$ 1.2

Data in the table are expressed as mean $\pm$ S.E. The minus value indicates contraction.

*Significantly different from Carteolol ($p < 0.05$). **Significantly different from Bufetolol ($p < 0.05$). NS: Not significantly different from Propranolol ($p > 0.05$).

Relationship between the hypotensive actions in anesthetized rats and the intrinsic β -sympathomimetic actions in isolated guinea pig tracheal preparations induced by certain β -adrenoceptor blocking drugs

Some of most potent β -adrenoceptor blocking drugs, pindolol, carteolol, bufetolol and propranolol, were used in the present experiment. As shown in Table 1, all these drugs, in a dose of 1 mg/kg i.v., caused a fall in blood pressure in anesthetized rats, and the order of their hypotensive potencies was as follows; pindolol > carteolol > bufetolol $\approx$ propranolol ($p < 0.01$). On the other hand, these drugs, in a concentration of 1×10^{-7} g/ml, caused a relaxation of the isolated guinea pig trachea pretreated with reserpine (2.5 mg/kg s.c. 24 hr prior to these drugs) except for propranolol, as shown in Table 2. The order of their relaxing potencies was as follows; pindolol > carteolol > bufetolol $\approx$ propranolol ($p < 0.05$). The relaxation of the isolated trachea induced by pindolol, carteolol or bufetolol (1×10^{-7} g/ml) was inhibited by pretreatment with propranolol (1×10^{-7} g/ml).

DISCUSSION

Pindolol causes a significant fall in blood pressure in anesthetized and conscious rats, though in the latter cases a higher dose of the drug is required to produce such a response. This response to pindolol is inhibited by pretreatment with the drug itself or propranolol,

and in addition, the response to pindolol is observed even in spinal rats, such as is the case with isoproterenol. Therefore, it is considered that the hypotension with pindolol in anesthetized or conscious rats is due to a direct stimulation of β -adrenoceptors, and as a result, a decrease in the peripheral vascular resistance. Such a finding parallels those reported by other workers (16, 17). This finding is also deduced from the result that the order of the hypotensive potencies between four β -blockers, pindolol, carteolol, bufetolol and propranolol, in anesthetized rats has a good correlation with that of their intrinsic β -sympathomimetic activities in isolated catecholamine-depleted tracheal preparations. The reason for a considerable difference between the hypotensive potencies of pindolol in anesthetized and conscious rats may be due to the fact that the hypotensive actions of β -sympathomimetic agents are more effectively exerted in a state of the lower peripheral sympathetic tonus, since even a relatively lower dose of pindolol causes a fall in blood pressure in anesthetized and spinal rats. In addition, this phenomenon is also observed in the case of isoproterenol. However, the difference between the hypotensive potencies of isoproterenol in anesthetized and conscious rats is much less than that in the case of pindolol. On the other hand, pindolol causes a positive chronotropic effect in anesthetized, conscious and spinal rats. In the latter two cases, however, a more prominent positive chronotropic effect is observed. It may be that pindolol has a more marked tendency to cause a negative effect in anesthetized rats. As shown in conscious rats, the positive chronotropic effect with pindolol is not inhibited by pretreatment with propranolol. Pindolol may have a much higher affinity than propranolol to the cardiac β -adrenoceptors, even though such is not the case with those of other sympathetic organs.

As regards the hypotensive mechanism of propranolol the following explanations have been given; 1) inhibition of the renin secretion (2), 2) reduction in cardiac output (3, 4), 3) centrally mediated hypotensive effect (5, 6), 4) resetting of the baroreceptors at a low level (1, 7, 8), etc.. However, pindolol does not cause any change or conversely causes a progressive rise in the plasma renin activity (18, 19) and its myocardial depressant effect is much weaker than that of propranolol despite producing a considerable fall in blood pressure (13). In addition, it has been demonstrated that the order of the hypotensive potencies of certain β -adrenoceptor blocking drugs has no correlation with that of their β -adrenoceptor blocking potencies (20). Thus, in the case of pindolol, decrease in the peripheral vascular resistance due to an intrinsic β -sympathomimetic action of the drug is a probable explanation. However, the fact that propranolol produces hypertension rather than hypotension in conscious rats, as shown in the present experiments, indicates that results obtained from animal experiments do not always correlate well with those from clinical studies. Other mechanisms should be considered when attempting to assess data in clinical experiments.

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