Abstract—The effects of beta-adrenergic receptor blocking agents administered i.v. on the blood pressure in conscious spontaneously hypertensive rats (SHR), renal hypertensive rats (RHR) and normotensive Wistar strain rats (NR) were studied. dl-Propranolol and dl-YB-2, 1 mg/kg i.v., caused a sustained rise in blood pressure in SHR and RHR. The maximum response of each beta-blocking agent after phentolamine, 10 mg/kg i.v., in SHR and RHR was significantly larger than that in NR. The potency ratio for the hypertensive activities of the l- and d-isomers of propranolol and YB-2 was similar to the ratio of their beta-blocking activities. The pressor effects of the beta-blocking agents after phentolamine were significantly inhibited by adenalec- tomy, reserpination and pretreatment with hexamethonium. The results suggest that the pressor effect of the beta-blocking agents may be due to their beta-blocking activities and the unmasking of alpha-receptor activities of the blood vessels. Furthermore, the greater pressor effect of the agents observed in hypertensive rats is attributed to a greater activity of the sympathetic nervous system in these rats as compared to normotensive rats.

The antihypertensive activity of propranolol and other beta-adrenergic receptor blocking agents in certain hypertensive patients has been reported (1–8), but the activity has been observed only after prolonged oral administrations in relatively large doses. However, exact mechanism involved in the antihypertensive activity of the agents is not clear. The activity of beta-blocking agents has not been confirmed in commonly used hypertensive animals (9, 10). Recently, Roba et al. reported that beta-blocking agents administered orally caused a significant fall in blood pressure in unanesthetized spontaneously hypertensive rats and the order of hypotensive potency was unrelated to the beta-blocking activity (11).

In the present study, the effects of intravenously administered beta-blocking agents on blood pressure and heart rate in conscious spontaneously hypertensive rats and renal hypertensive rats were investigated utilizing the direct cannulation method into the abdominal aorta via the median coccygeal artery and the results were compared with those observed in normotensive Wistar strain rats. The difference in the cardiovascular responsiveness to beta-blocking agents, and that in the sympathetic nervous activity to the cardiovascular system between hypertensive and normotensive rats was also studied.
MATERIALS AND METHODS

Male spontaneously hypertensive (SHR), renal hypertensive (RHR) and normotensive Wistar strain rats (NR) were used. SHR originated in the Okamoto strain, F24 generation and were about 4 months old. RHR were prepared by the following procedures; the right renal artery of male Wistar strain rats weighing 130-150 g was clamped with a silver clip and the rats were contralaterally nephrectomized one week later. The RHR were used for the experiments a month after the surgery. The SHR and RHR which had mean arterial blood pressure higher than 140 mmHg were used for the experiments.

Arterial blood pressure in conscious rats was measured by a direct cannulation method; under light ether anesthesia a polyethylene catheter was inserted into the abdominal aorta via the median coccygeal artery. Blood pressure was measured through the catheterized artery by a pressure transducer (Nihon Kohden, MPU-0.5) and heart rate by a cardiotachometer (Nihon Kohden, RT-2) triggered by the pulse of blood pressure. The recordings were made on an ink-writing oscillograph (Nihon Kohden, WI-260). Another polyethylene catheter was inserted into the caudal vein for the injection of drug solutions, which were administered at least one hour after the cannulation when the ether anesthetized animals were conscious.

In some experiments, reserpine, 3 mg/kg/day s.c., was administered for two days prior to the experiments and the depletion of catecholamines was verified by the administration of tyramine, 200 μg/kg i.v.

Rats were adrenalectomized by removing the bilateral adrenal glands and were provided 1% NaCl solution in tap water for drinking. These rats were also used for experiments more than one week after the surgery.

Drugs used were as follows; dl-L-(7-indenyloxy)-3-isopropylaminopropane-2-ol hydrochloride (dl-YB-2) and its optical isomers (Yamanouchi Pharmaceutical Co.), dl-propranolol hydrochloride and its optical isomers (Sumitomo Chemical Co.), dl-practolol hydrochloride (Sumitomo Chemical Co.), phentolamine mesylate (CIBA-Geigy Ltd), reserpine (Yamanouchi Pharmaceutical Co.), hexamethonium chloride (Yamanouchi Pharmaceutical Co.), and tyramine hydrochloride (Tokyo Kasei Kogyo Co.).

These drugs were freshly dissolved in 0.9% NaCl solution and injected through a polyethylene catheter which had been inserted into the caudal vein. For flushing, 0.02 ml of physiological saline was used. All doses of the drugs are expressed in terms of the salt.

RESULTS

Effects of beta-blocking agents on blood pressure in conscious hypertensive rats

The changes in blood pressure induced by i.v. administered beta-blocking agents in conscious SHR and RHR were compared with those in each control group given physiological saline.

In SHR dl-propranolol, 1 mg/kg i.v., (N = 6) and dl-YB-2, 1 mg/kg i.v., (N = 5) caused a sustained rise in mean blood pressure, which lasted at least for 30 min, as shown in Fig. 1A. The changes in mean blood pressure induced by these agents were significantly greater
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The effects of beta-blockers on the mean arterial blood pressure in conscious spontaneously hypertensive (SHR) and renal hypertensive rats (RHR) were studied. DL-YB-2, 1 mg/kg i.v., and DL-propranolol, 1 mg/kg i.v., induced maximum changes of 36.4 ± 8.7 (S.E.M.) and 28.0 ± 4.2 mmHg, respectively, 24 min after the injections of each agent.

In RHR, DL-propranolol, 1 mg/kg i.v., (N=6) caused a sustained rise in mean blood pressure, which was significantly greater (p<0.05) than those in the control group (N=6) for 20 min after injection, as shown in Fig. 1B. The maximum change induced by DL-propranolol, 1 mg/kg i.v., was 33.1 ± 7.9 mmHg 12 min after the injection of the agent.

Phentolamine, 10 mg/kg i.v., was injected before the treatment with beta-blocking agents. Phentolamine caused a marked fall in blood pressure and an increase in heart rate. The depressor effect of the agent on SHR was greater than that on NR. The pressor effect of DL-propranolol, 1 mg/kg i.v., was potentiated by the treatment with phentolamine and was greater in SHR than in NR. The pressor response was induced faster and a higher blood pressure level was maintained than that observed when phentolamine was not given. Typical recordings in NR and SHR are shown in Fig. 2.
Since the beta-blockade appeared to play an important role in the pressor effect of the beta-blocking agents, the pressor activities of the optical isomers of the agents were compared in SHR, RHR and NR. The cumulative doses of l- and d-propranolol, 0.0001-10 and 0.001-10 mg/kg i.v., respectively, were administered after phentolamine, 10 mg/kg.

**Fig. 3.** Effects of l- and d-propranolol (A and B, respectively) on heart rate and arterial blood pressure after treatment with phentolamine in conscious spontaneously hypertensive rats.

**Fig. 4.** Dose-response curves for changes in mean arterial blood pressure induced by l- and d-propranolol in conscious spontaneously hypertensive (SHR), renal hypertensive (RHR) and normotensive rats (NR).

Abscissa: the doses administered in mg/kg i.v. and ordinate: the changes in mean arterial blood pressure in mmHg. Each curve represents data from six rats. Vertical bars represent standard errors of mean values.
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i.v., and caused a rise in blood pressure and a decrease in heart rate. Injections of l- and d-propranolol in doses as high as 2-7 mg/kg i.v. caused initial depressor responses. Fig. 3 illustrates typical recordings for the effect of l- and d-propranolol on blood pressure and heart rate in SHR. The dose-response curves for the changes in mean blood pressure induced by the agents in SHR, RHR and NR are shown in Fig. 4. The maximum rise in blood pressure induced by l-propranolol was as large as that by d-propranolol and the threshold dose for pressor effect of the d-isomer was higher than that of the l-isomer. The pressor response to d-propranolol in low doses tended to be more transient than that to the l-isomer.

No significant differences between the maximum pressor responses in SHR and RHR to the l- and d-isomers, 10 mg/kg i.v., were obtained. These responses of SHR and RHR to the agents were significantly greater than those of NR (p<0.001). The results obtained from the experiments on the optical isomers of YB-2 in SHR, RHR and NR were similar to those from the isomers of propranolol, as shown in Fig. 5.

![Fig. 5. Dose-response curves for changes in mean arterial blood pressure induced by l- and d-YB-2 in conscious spontaneously hypertensive (SHR), renal hypertensive (RHR) and normotensive rats (NR). Each curve represents data from six rats. Other notations as in Fig. 4.](image)

![Fig. 6. Effects of dl-practolol on heart rate and arterial blood pressure after treatment with phentolamine in a conscious normotensive rat.](image)
Cumulative doses of dl-practolol, 0.001–7 mg/kg i.v., were administered to NR after the treatment with phentolamine, 10 mg/kg i.v. The pressor response to dl-practolol was observed after administrations in doses higher than those with d-propranolol and d-YB-2. Fig. 6 illustrates typical recordings for the effect of dl-practolol on heart rate and blood pressure in a NR. The dose-response curve for the changes in mean blood pressure induced by the agent with those of the optical isomers of propranolol and YB-2 is shown in Fig. 7.

In order to compare the pressor activities of these beta-blocking agents, the negative logarithmic values of the doses required to produce half of the maximum pressor responses were obtained, as shown in Table 1. From the results, the pressor activity of the isomers of propranolol and YB-2 was similar to each corresponding isomer in each rat group. dl-Practolol showed the weakest pressor activity.

**Influence of adrenalectomy, reserpinization and hexamethonium-treatment on the pressor effects of beta-blocking agents**

To investigate the role of endogenous catecholamines and that of the sympathetic nervous system in the pressor effects of beta-blocking agents, the pressor effects of dl-propranolol in bilaterally adrenalectomized NR and reserpinned NR were compared with those in non-treated control NR. Furthermore, the effects of d- and l-YB-2 in NR treated with hexamethonium were also examined.
Conscious adrenalectomized rats and reserpinized rats were given dl-propranolol, 1 mg/kg i.v., after the treatment with phentolamine, 10 mg/kg i.v., as shown in Fig. 8. Phentolamine caused a depressor effect and an increase in heart rate in adrenalectomized rats, whereas the agent caused a pressor effect and a decrease in heart rate in reserpinized rats. After the treatment with phentolamine, dl-propranolol caused a pressor effect and a decrease in heart rate in adrenalectomized rats and reserpinized rats, as summarized in Table 2, in which the pressor effect of dl-propranolol was significantly smaller than in the control animals (p<0.05 and p<0.001, respectively). Furthermore, the effect in adrenalectomized rats was significantly smaller than that in reserpinized animals (p<0.05).

The cumulative doses of l- and d-YB-2, 0.0001-10 mg/kg i.v., were administered after phentolamine, 10 mg/kg i.v. As shown in Fig. 9, pretreatment with hexamethonium significantly inhibited the pressor effects of l- and d-YB-2; the maximum pressor effects of

![Fig. 8. Effects of dl-propranolol on heart rate and arterial blood pressure in adrenalectomized (A) and reserpinized normotensive rats (B).](image)

**Table 2. Comparison of the changes in mean arterial blood pressure induced by dl-propranolol, 1 mg/kg i.v., after treatment with phentolamine in conscious reserpinized and adrenalectomized normotensive rats.**

| Treatment               | No. of expts. | Change in blood pressure (mmHg) |
|-------------------------|---------------|---------------------------------|
| Control                 | 5             | 41.4 ± 0.8                      |
| Reserpinized            | 5             | 28.5 ± 4.4*                     |
| Adrenalectomized        | 5             | 12.9 ± 1.9***†                  |

* : significantly different from the control (p<0.05) and *** : (p<0.001).
† : significantly different from the reserpinized rats, (p<0.05).
DISCUSSION

The present study shows that propranolol and YB-2 consistently caused a sustained rise in blood pressure in conscious hypertensive rats. This result appears to coincide with those reported by Tabei et al. (9) and Farmer and Levy (10). The pressor effects were potentiated by the treatment with phentolamine. This observation suggests that the pressor effect may not be due to the direct stimulation of alpha-adrenergic receptors, and confirmed the results obtained by Yamamoto and Sekiya (12), Dasgupta (13), and Regoli (14) in anesthetized NR. Consequently, in the following experiments the pretreatment with phentolamine was utilized in order to evaluate the effects of beta-blocking agents. Yamamoto and Sekiya suggested that the result may be explained in part by the predominant beta-receptor tone after alpha-blocking agents and thus the pressor effect of propranolol came out markedly (12). From the results with antagonistic action between the alpha- and beta-blocking agents, Olivares et al. suggested that beta-blocking agents "unblocked" the alpha-receptor which had been previously blocked by the alpha-blocking agent (15). On the other hand, it has been suggested that in tissues with alpha- and beta-receptors, the beta-blockade subsequent to alpha-blockade results in unmasking of residual alpha-adrenergic activity of the agonists with mixed activity, not previously blocked by the alpha-blocking agent (16-19).

The threshold doses of the I-isomers of propranolol and YB-2 required to elevate blood pressure were lower than those of the d-isomers in SHR, RHR and NR. The doses required to produce half of the maximum response to the I-isomers of the agents were far smaller than those of the d-isomers given to all rat groups. The relative potencies for beta-blocking activity as reported for the isomers of propranolol (20, 21) and YB-2 (22, 23) may explain these results. This certainly appears to be the case with practolol; dose of the agent required to produce half of the maximum response was larger than that of d-propranolol and d-YB-2 in NR. The dose of practolol which caused a decrease in
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heart rate, i.e. 0.1 mg/kg i.v., showed no sustained rise in blood pressure. It has been reported that practolol has a cardioselective beta-blocking activity and a weaker blocking activity than propranolol (24, 25).

Roba, et al. reported that beta-blocking agents administered orally caused a fall in blood pressure in conscious SHR and the order of hypotensive potency was unrelated to their beta-blocking activities (11). The discrepancy between their results and those in the present study may be due to the different route of administration. However, we could not confirm their results in a preliminary experiment of our own.

Lydtin and Sommerfeldt reported that d-propranolol elevated blood pressure more effectively than the l-propranolol when chronically administered to conscious DOCA-hypertensive rats (26), these results being inconsistent with those in the present study. Differences are attributed to the route of administration, the type of hypertension and methodology of measuring blood pressure.

The large doses of propranolol and YB-2, 2-7 mg/kg i.v., caused an initial fall in blood pressure, presumably due to their non-specific inhibitory effect on the heart and blood vessels. Shanks reported that the vasodilating effects of beta-blocking agents may result from their local anesthetic activities (27).

Yamamoto and Sekiya observed a marked inhibition of sustained pressor effect of propranolol and pronethalol after adrenalectomy or hexamethonium treatment in anesthetized rats and in spinal rats (12). The present result confirmed their observations; in conscious adrenalectomized, reserpinized and hexamethonium treated rats given propranolol after the treatment with phentolamine, a significantly smaller pressor effect was seen as compared to the control animals. The depletion of catecholamines by reserpine has been reported to develop more slowly and to be less complete in the adrenal medulla than in the other tissues including the heart and blood vessels (28). The vasoconstriction caused by propranolol in anesthetized dog hind limb has been reported to be due to catecholamines released from the adrenal medulla (29, 30).

The pressor effects of propranolol and YB-2 after phentolamine in hypertensive rats were significantly larger than that in the control NR. This was true in both cases with the d- and l-isomers of the agents. Dasgupta observed that pithing or reserpination prevented the pressor response to propranolol in anesthetized rats, and suggested that the pressor response may be maintained in part through a central sympathetic mechanism (13).

Yamori et al. investigated the catecholamine metabolism in the brain and the effects of its modification on blood pressure in SHR, and suggested that a deficiency of norepinephrine metabolism in the brainstem of SHR might be related to an elevation of the peripheral sympathetic nervous activity (31). The present results indicate that sympathetic nervous activity in hypertensive rats (SHR and RHR) is higher than that in NR.

Folkow et al. showed that a medial thickening in the resistance vessels of SHR resulted in raising the wall/lumen ratio and the maximum contractile strength, nevertheless the vascular sensitivity appeared largely unchanged (32). In the present study, the threshold dose of each isomer of propranolol and YB-2 required to produce the pressor effect in
hypertensive rats appears to be comparable to that in NR. Thus it would appear that sensitivity for the pressor effect of beta-blocking agents in hypertensive rats does not differ from that in NR.

Consequently, the sympathetic nervous system or sympatho-adrenal activity appears to play an important role in the pressor effect of beta-blocking agents.

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