# Anti-Hypotensive Effects of M6434, an Orally Active $\alpha_1$-Adrenoceptor Agonist, in Rats

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ABSTRACT — The anti-hypotensive effects of M6434 were evaluated and compared with those of other orally active sympathomimetics in rats. Oral administration of M6434 (0.5–2.0 mg/kg) and midodrine (1.0–5.0 mg/kg) also produced a dose-related increase in mean arterial pressure in normotensive rats. The pressor effect of M6434 was about 4 times more potent than that of midodrine. Both M6434 and midodrine caused a dose-dependent decrease in heart rate. The pressor effect of M6434 (1.0 mg/kg) did not diminish after its repeated administration for 7 days. The pretreatment with M6434 (0.5–1.0 mg/kg) and midodrine (2.0–5.0 mg/kg) improved the orthostatic index in the experimental model of postural hypotension in rats. The effect of M6434 on postural hypotension was about 5 times more potent than that of midodrine. Intravenously injected M6434 (3–300 $\mu$g/kg) produced a dose-dependent increase in the blood pressure of pithed rats. These results suggest that M6434 possesses a potent anti-hypotensive activity which is superior to that of midodrine, and M6434 may be useful in the treatment of essential and postural hypotension.

*Keywords:* M6434, $\alpha_1$-Agonist, Midodrine, Postural hypotension, Orthostatic index

M6434, 2-[(5-chloro-2-methoxyphenyl)azo]-1*H*-imidazole, is an $\alpha_1$-adrenoceptor agonist (1). It has been demonstrated that M6434 exerted beneficial effects on the survival rate and hemodynamic parameters in various experimental shock models (2). The mechanisms involved in the anti-shock effect of M6434 have been elucidated to be as follows: 1) the improvement of venous blood pooling was observed in canine hemorrhagic shock (3), and 2) the redistribution of the organ blood flow was confirmed in hemorrhagic shocked rats (4). On the other hand, Ohnishi et al. (1) reported that M6434 also showed the pressor effects in conscious rats when it was given by the oral route. Therefore, to evaluate this compound as an orally active anti-hypotensive agent, we examined the following points: 1) the hypertensive effect in normotensive conscious rats in comparison to the effect of midodrine, an orally active anti-hypotensive agent (5), 2) its effects on an experimental model of postural hypotension in reserpine-pretreated rats, and 3) its hypertensive effect in pithed rats. Since other $\alpha_1$-agonists such as methoxamine and phenylephrine are usually used by injection, in the present study, we compared M6434 with midodrine and dihydroergotamine which are both orally administered in clinical therapy.

## MATERIALS AND METHODS

### *Effects on blood pressure and heart rate in normotensive rats*

Male Sprague-Dawley rats weighing 260–350 g, fasted overnight with free access to tap water, were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). One end of the polyethylene tube (PE-50, Clay Adams) filled with heparin (Heparin-Na, Mochida) solution (1000 unit/ml) was inserted via the right carotid artery to the aortic arch. The other end of the tube was plugged with short stainless steel wire, and this end of the tube was led through subcutaneous fatty tissue and fixed to the nape. On the day after the operation, the end of the tube in the nape was connected to the pressure transducer (MPU-0.5A, Nihon Kohden) and blood pressure was monitored with a pressure amplifier (AP-620G, Nihon Kohden) in the conscious unrestrained condition. Heart rate (HR) was counted by a tachometer (AT-620G, Nihon Kohden) triggered by the blood pressure pulse wave. After an equilibration period of 60 min, the drugs were administered orally.

In the case of the single administration, hemodynamic monitoring was continued for 6 hours after the drug administration. In the case of repeated administration, M6434 or midodrine was given once a day for 7 consecutive days (day 1 – 7) and 3 days after the last administration (day 10). Mean arterial pressure (MAP) was measured on days 1, 3, 5, 7 and 10.

*Effects on postural hypotension in reserpine-pretreated rats*

Male Sprague-Dawley rats weighing 260 – 300 g were pretreated with reserpine (Apopron®, Daiichi) intraperitoneally at a dose of 3 mg/kg. Under light ether anesthesia, the animals were restrained in a prone position on a tilting table (6). The tilting table permits movement of the body from the horizontal to the vertical position within 0.5 sec. A polyethylene tube was inserted into the right femoral artery, and blood pressure was recorded continuously throughout the experiment. After recovering from ether anesthesia, the rats were tilted 90° to the head-up position for 2 min, and the changes of blood pressure were observed. The tilting operation was performed at 15 and 30 min after the drug administration. The severity of postural hypotension was judged by the method of Sponer et al. (7). The two parameters, orthostatic reaction (OR) and orthostatic index (OI) were calculated by the following equation:

$$OR = \frac{\text{Maximal drop in MAP in response to tilting}}{\text{MAP before tilting}}$$

$$OI = \frac{\text{OR after drug administration}}{\text{OR before drug administration}} \times 100\ (\%)$$

*Effect on blood pressure in pithed rats*

Male Sprague-Dawley rats weighing 240 – 380 g, fasted overnight with free access to tap water, were anesthetized with sodium-pentobarbital (50 mg/kg, i.p.) and ventilated artificially with room air (60 strokes/min, 20 ml/kg). Polyethylene tubes were inserted into femoral artery and jugular vein for monitoring blood pressure and injecting the drug solution, respectively. The animals were pithed by inserting a sharp stainless steel needle into the spinal channel via the orbit. After an equilibration period of 30 min, drugs were injected into the jugular vein in a cumulative manner. The pressor responses in normal rats without spinal damage were also examined in the same protocol.

*Drugs*

In the case of oral administration, M6434 (supplied by Hodogaya Chemical Co., Ltd., Tokyo, Fig. 1), midodrine HCl (Metridine®, Taisho) and dihydroergotamine (Sigma) were suspended in 5% arabic gum solution and administered in a volume of 5 ml/kg. For intravenous injection, M6434 was dissolved in 0.5 N HCl at a concentration of 30 mg/ml and diluted with saline and injected at a volume of 1 ml/kg.

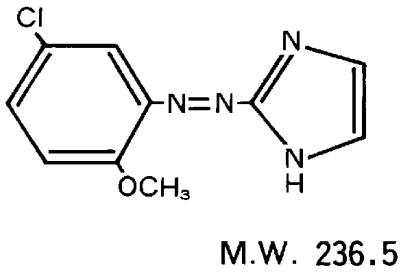


**Fig. 1.** Chemical structure of M6434.

*Statistical analysis*

The changes of MAP in normotensive rats were evaluated by analysis of variance followed by Scheffe's multiple comparison test. In the experiment on postural hypotension, statistical analysis was performed by analysis of variance followed by Dunnett's multiple comparison test.

## RESULTS

*Effects on blood pressure in normotensive rats*

*Single administration:* Oral administration of M6434 at doses ranging from 0.5 to 2.0 mg/kg increased MAP in a dose-dependent manner. The hypertensive effect of M6434 at a dose of 2.0 mg/kg reached its peak at 10 min and lasted for more than 60 min after the drug administration (Fig. 2A). Midodrine at doses ranging from 1.0 to 5.0 mg/kg produced an increase in MAP, and the effects peaked at 30 min after the drug administration. The hypertensive effects of midodrine lasted for about 60 min (Fig. 2B). In all cases, the hypertensive actions of both drugs were accompanied by a decrease in HR (Fig. 2, A and B). The doses of M6434 and midodrine at which they increased MAP by 20% were 0.61 and 2.59 mg/kg, respectively.

*Repeated administration:* In the control group, MAP and HR did not show noticeable change over a 7-day period. Oral administration of M6434 at a dose of 1.0 mg/kg induced the significant elevation of MAP, and the hypertensive effects of M6434 did not diminish during the repeated administration of 7 consecutive days. The hypertensive action of midodrine at the dose of 5.0 mg/kg showed a moderate diminution on the 3rd day of administration (day 3) and the initial hypertensive activity remained unchanged on day 5 and day 7. Three days after the last administration (day 10), the

hypertensive activities of both drugs were not changed in comparison with the response of day 1 (Fig. 3).

*Effects on postural hypotension in reserpine-treated rats*

In the control group, pretreatment of reserpine lowered MAP from the basal value of 117 ± 3 mmHg to 94 ± 3 mmHg. Oral administration of M6434 at the doses of 0.5 and 1.0 mg/kg increased MAP before the tilting operation in a dose-dependent manner. M6434 at a dose of 0.5 mg/kg increased OI slightly; and at 1.0 mg/kg, it induced a significant increase in OI (Fig. 4). Midodrine at the doses of 2.0 and 5.0 mg/kg showed the hypertensive effect. Midodrine increased OI at these doses, but these effects were not statistically significant. Dihydroergotamine, at a dose of 20 mg/kg, slightly reduced OI, and no hypertensive action was observed (Table 1).

*Effects on blood pressure in pithed rats*

Intravenously injected M6434 induced a dose-depend-

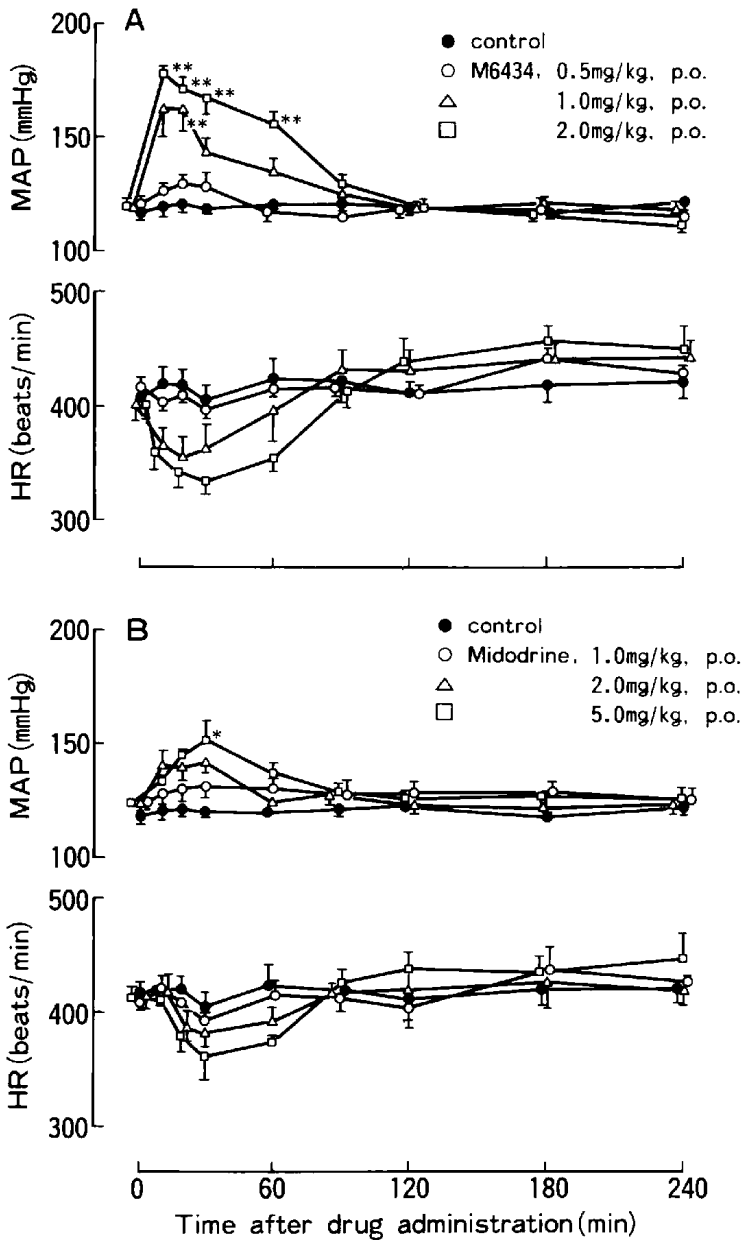


**Fig. 2.** Effects of M6434 (A) and midodrine (B) on mean arterial pressure (MAP) and heart rate (HR) in conscious unrestrained rats. Each point represents the mean ± S.E.M. of 5–6 animals. $^{*}P < 0.05$, $^{**}P < 0.01$: Significantly different from the control group (Scheffe's multiple comparison test).

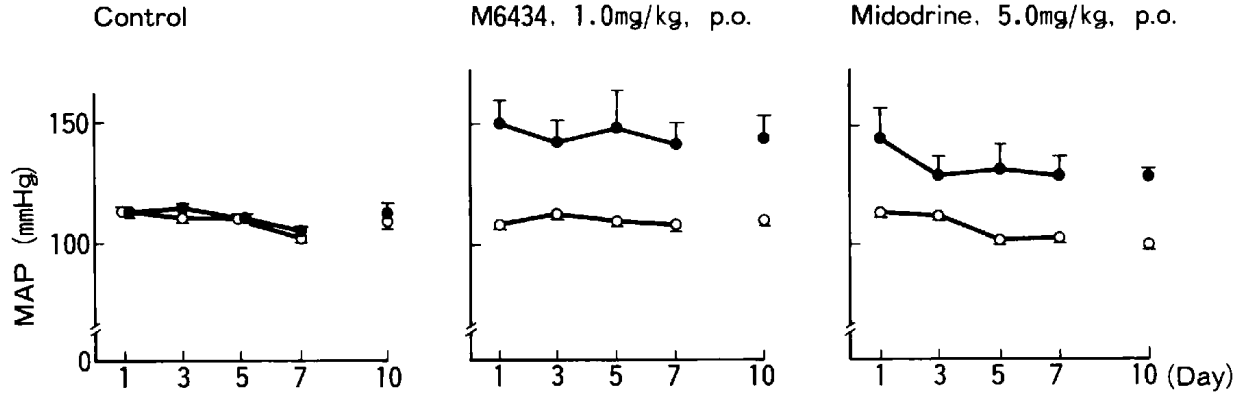


**Fig. 3.** Effects of daily repeated administration of M6434 and midodrine on mean arterial pressure (MAP) in conscious unrestrained rats. The drugs were administered orally from days 1 to 7 and day 10. Each value represents the mean ± S.E.M. of 5 animals. ○: before drug administration, ●: 30 min after drug administration.

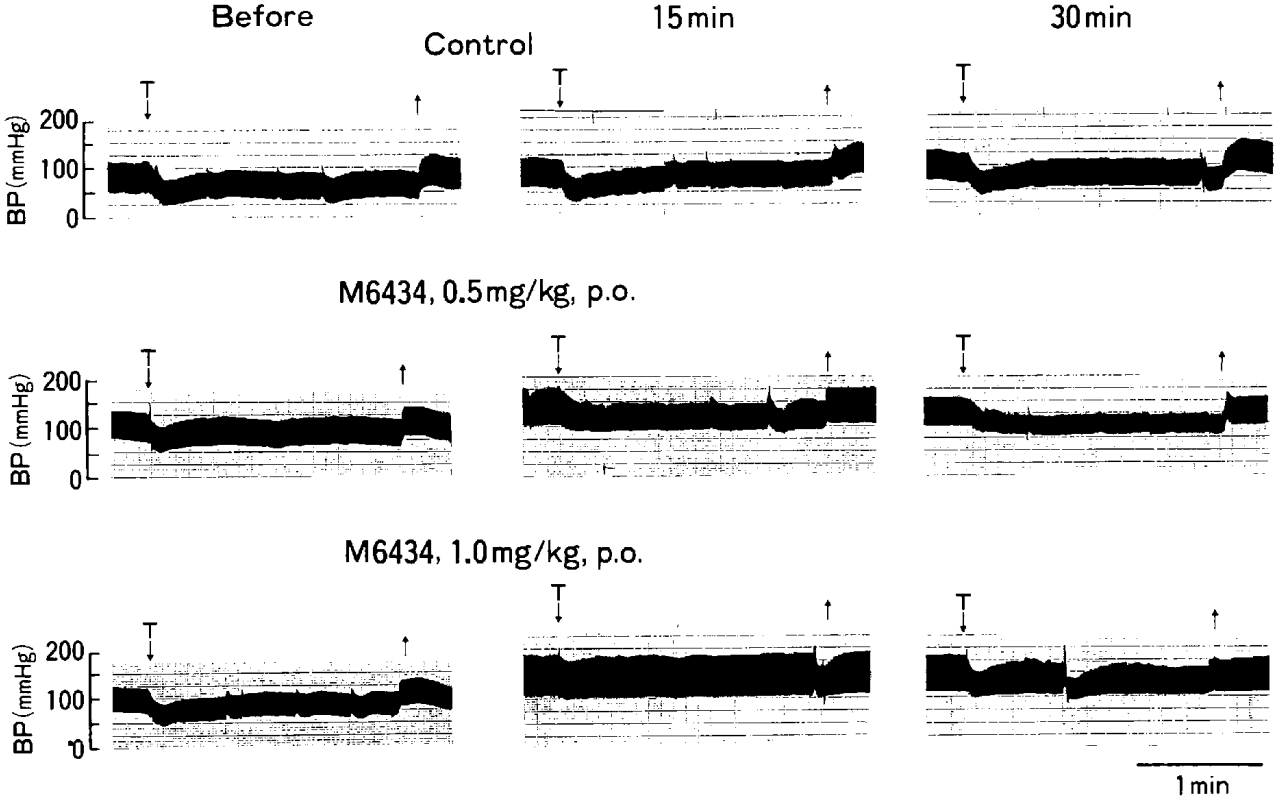


**Fig. 4.** Representative example demonstrating the effects of M6434 on the tilt induced changes in blood pressure (BP). T represents the tilt. ↓: change to vertical position, ↑: change to horizontal position. The tilt responses were observed before and 15 min and 30 min after drug administration. Upper: Control; Middle: M6434 0.5 mg/kg, p.o.; Bottom: M6434, 1.0 mg/kg, p.o.

ent increase in diastolic arterial pressure in pithed rats over the dose range of 3 to 300 $\mu$g/kg. In the rats without spinal damage, M6434 also induced a dose-dependent increase in MAP over the dose range of 3 to 100 $\mu$g/kg, but the hypertensive effects of M6434 were weaker than those in pithed rats and became saturated at doses higher than 100 $\mu$g/kg (Fig. 5).

## DISCUSSION

M6434 produced a dose-dependent increase in MAP in normotensive conscious rats. Comparison of the

**Table 1.** Effects of M6434, midodrine and dihydroergotamine (DHE) on orthostatic index (OI) and mean arterial pressure (MAP) in reserpine-pretreated rats

| Drugs | Dose (mg/kg, p.o.) | OI (%) 15 min | OI (%) 30 min | MAP (mmHg) before | MAP (mmHg) 15 min | MAP (mmHg) 30 min |
|---|---|---|---|---|---|---|
| Control | | 93 ± 4 | 94 ± 5 | 94 ± 3 | 97 ± 5 | 93 ± 3 |
| M6434 | 0.5 | 100 ± 4 | 99 ± 3 | 93 ± 1 | 125 ± 5* | 112 ± 5 |
| | 1.0 | 112 ± 5* | 108 ± 5 | 93 ± 3 | 147 ± 8** | 136 ± 9** |
| Midodrine | 2.0 | 105 ± 6 | 100 ± 4 | 93 ± 3 | 108 ± 4 | 105 ± 3 |
| | 5.0 | 110 ± 5 | 106 ± 5 | 96 ± 3 | 145 ± 9** | 144 ± 4** |
| DHE | 20.0 | 82 ± 5 | 91 ± 7 | 94 ± 3 | 88 ± 3 | 96 ± 4 |

OI before drug administration = 100%. *$P < 0.05$, **$P < 0.01$: Significantly different from the control group (Dunnett's multiple comparison test). Each value represents the mean ± S.E.M. of 5 animals.

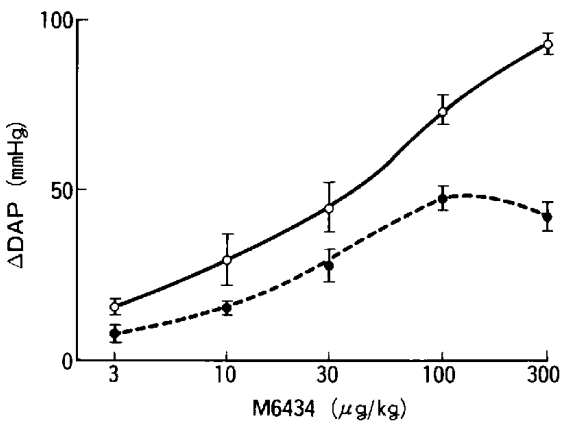


**Fig. 5.** Pressor effects of M6434 in normal (—○—) and pithed (- -●- -) rats. The drugs were injected intravenously in a cumulative manner. Each value represents the mean ±S.E.M. of 5 animals. DAP: diastolic arterial pressure.

doses that induced a 20% elevation of MAP revealed that the hypertensive effect of M6434 was 4 times more potent than that of midodrine. The duration of the hypertensive effect of M6434 was almost the same as that of midodrine. These hypertensive effects were accompanied by a decrease in HR, and it showed a time course similar to the changes of MAP. A previous study showed that the hypertensive effect of M6434 was more potent than those of an $\alpha_1$-adrenoceptor agonist like phenylephrine and methoxamine (1). Furthermore, intraduodenal administrations of both midodrine and methoxamine have been shown to elevate MAP to the same degree (8). These investigations suggest that M6434 has more potent hypertensive action than other $\alpha_1$-agonists as well as midodrine. The repeated administration of M6434 did not affect the potency of the hypertensive effect. Although the pressor effect of midodrine at a dose of 5 mg/kg was reduced slightly on day 3, the effect remained unchanged on day 5 and day 7. These results suggest that M6434 and midodrine do not induce tachyphylaxis at the doses that caused significant elevations of MAP. Pittner et al. (8) reported that midodrine reduced the hypertensive effect by chronic administration at a higher dose than that used in our experiment. Hornykiewics and Obenaus (9) reported that the sympathomimetic amine induced tachyphylaxis, and it can be attributed to the down regulation of adrenergic receptors. From these investigations, there is the possibility that tachyphylaxis may be induced when larger doses of both compounds are used. In the experiment on postural hypotension, M6434 significantly increased OI which indicates the severity of the postural hypotension. The improving effects of M6434 was 5 times more potent than that of midodrine. Humphrey and McCall (10) reported that reserpine-pretreatment produced significant hypotension and orthostasis in rats. In our experiment, the orthostatic reactions were obtained similarly by reserpine-pretreatment, and the reactions were suppressed by the treatment of M6434. It is well-known that the hemodynamic changes that occur during a postural change from a supine to a standing position cause a reduction of blood pressure, cardiac output and cerebral blood flow because of the blood pooling within the venous vascular beds (11–13). Uemura et al. (3) reported that M6434 increased the venous return in the dogs with cardiopulmonary bypass under normal and shocked states. It seems, therefore, that the blood pooling within the venous vascular beds during the standing position may be reduced by the treatment of M6434, and the hemodynamic derangement caused by a postural change may be quickly compensated. Furthermore, M6434 has been shown to have the ability to increase myocardial contractility of rat myocardium through an $\alpha$-adrenoceptor (14, 15). The preventive effect of

M6434 in postural hypotension may be partially due to the positive inotropic effect of M6434.

Dihydroergotamine did have any anti-hypotensive effect on postural hypotension in reserpine-pretreated rats. There may be two possible reasons why dihydroergotamine has no effect in this model. One is that the vasopressor effect of dihydroergotamine is partially due to the release of endogenous catecholamine from nerve granules (16); the hypertensive effect of dihydroergotamine may be attenuated in reserpine-pretreated rats. Another is that orally administered dihydroergotamine has the low bioavailability (17).

In pithed rats, M6434 produced a dose-dependent increase in blood pressure. This result indicates that the hypertensive effect of M6434 is not caused by an action on the central nervous system but due to an action on the peripheral vessels (18).

In conclusion, M6434 showed potent hypertensive effect in conscious rats without tachyphylaxis after repeated oral administration. M6434 showed a preventive effect on postural hypotension in reserpine-pretreated rats, and the effect was more potent than that of midodrine. Although further studies are necessary to clarify the mechanisms of the effect on postural hypotension, M6434 is expected to be a potential antihypotensive agent.

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