Effects of 2-[(5-Chloro-2-Methoxyphenyl)azo]-1H-Imidazole (M6434) on Alpha Adrenergic Receptors

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Abstract—The effects of a newly synthesized compound, 2-[(5-chloro-2-methoxyphenyl)azo]-1H-imidazole (M6434) on α-adrenergic receptors were investigated by using the atria of normal and hypothyroid rats, rat vasa deferentia and canine arteries. M6434 showed a positive inotropic effect on rat left atria, which was suppressed by phentolamine but not by propranolol and reserpine. M6434 also showed a positive chronotropic effect on rat right atria. These positive inotropic and chronotropic effects of M6434 were enhanced in propylthiouracil-induced hypothyroid rats. M6434 caused contraction of rat vas deferens and increased its spontaneous movement. These effects on vas deferens were suppressed by phentolamine. M6434 induced contraction of canine arteries. The pD2 values for vasoconstrictive effects of M6434 on the aorta, pulmonary artery, renal artery and femoral artery were about equal to those of phenylephrine, and the intrinsic activity of M6434 was somewhat lower than that of phenylephrine. These results suggest that M6434 is an adrenergic α-agonist which is about as potent as phenylephrine and that M6434 has neither a β-stimulating activity nor a catecholamine-releasing one.

A newly synthesized compound, 2-[(5-chloro-2-methoxyphenyl)azo]-1H-imidazole (M6434), was shown to induce pressor effects in dogs after intravenous injection (1); and the pressor effects of M6434 were thought to be caused by its adrenergic α-stimulating activity.

The present experiments were undertaken to clarify the α-agonistic action of M6434 using the atria of normal and hypothyroid rats, which have been shown to possess α-adrenergic receptors (2–4), rat vasa deferentia and canine arteries.

Materials and Methods

Drugs and animals

M6434 (Mochida Pharmaceutical Co., Ltd.), an orange crystalline powder relatively insoluble in aqueous solution, was dissolved in 0.1 N hydrochloric acid in 50% ethylene glycol. Phenylephrine hydrochloride (Tokyo Kasei), reserpine malate (Apoplon®, Daiichi Pharmaceutical Co.), phentolamine hydrochloride (Regitin®, Ciba-Geigy), d,l-propranolol hydrochloride (Inderal®, ICI) and 6-propyl-2-thiouracil (PTU, Nakarai) were used.

Male Wistar rats, weighing 140 to 160 g, were divided into two groups. One of which was rendered hypothyroidic by feeding them a diet containing 15% PTU for 3 months. Mongrel dogs of both sexes, weighing 8–14 kg, were used.

Methods

Rats were killed by a blow on the head and exanguination from the carotid arteries and dogs by exanguination under pentobarbital anesthesia, and the objective organs were removed. Rat atria, rat vasa deferentia and canine arteries were suspended in organ baths containing Tyrode’s solution maintained at 30°C, 30°C and 37°C, respectively. Tyrode’s solution was prepared by dissolving 8.0 g NaCl, 0.2 g KCl, 0.2 g CaCl2, 0.21 g MgCl2·6H2O, 0.06 g NaH2PO4·2H2O, 1.0 g NaHCO3 and 1.0 g glucose in 1 liter of distilled water, and the solution was bubbled with 95% O2 – 5% CO2. A force displacement
transducer (Nihon Kohden, SB-1T) was used to measure the contractile force of the organs which was recorded by a pen recorder. Drug solutions were added cumulatively to the organ bath.

**Effect on rat left atria:** The left atria of normal and hypothyroid rats were driven by square wave pulses of 3 msec duration at a frequency of 2.5 Hz through platinum electrodes and at a voltage about twice threshold which was generated by an electronic stimulator (Nihon Kohden, S-3039) under a resting tension of 0.5 g. The isometric contraction was recorded, and the effect of the drug on the contractile force was determined. The effect on M6434 on the left atria obtained from 24 hr after an intraperitoneal injection of 5 mg/kg reserpine was also examined.

**Effect on rat right atria:** The beating rate of the spontaneously beating right atria of normal and hypothyroid rats was measured under a resting tension of 0.3 g from the contractile wave with a tachometer (Nihon Kohden, RT-2), and the effect of the drug on the beating rate was determined.

**Effect on rat vas deferens:** Changes in the contractile force and spontaneous movement of the vas deferens of rats induced by the drug were recorded under a resting tension of 0.3 g. The spontaneous movement was expressed as the sum of contractile forces recorded in the spontaneous contractions occurring over a 10-min period.

**Effect on canine arteries:** Under a resting tension of 2.0 g, the effect of drug on the contractile forces of 3 mm x 25 mm spiral strips of the aortae (8.0–9.0 mm in diameter), pulmonary arteries (10.0–13.0 mm in diameter), renal arteries (2.0–2.4 mm in diameter) and the femoral arteries (1.8–2.0 mm in diameter) were recorded. The pD2 values were determined, and the intrinsic activity of M6434 was compared with that of phenylephrine.

**Statistical analysis:** Statistical significances were examined by Student’s t-test. All values were expressed as the mean±S.E.

**Results**

**Effect on rat left atria:** The contractile responses were expressed as the percentage of each response to the maximal response to M6434. M6434 showed a dose-dependent positive inotropic effect on the rat left atria at the doses ranging from $3 \times 10^{-7}$ M to $3 \times 10^{-4}$ M, the maximal response being 371±38 mg. This effect of M6434 was diminished in the presence of $1 \times 10^{-6}$ M of phentolamine, but not influenced by $3 \times 10^{-7}$ M of propranolol (Fig. 1). M6434 also showed a positive inotropic effect on the left atria obtained from rats pretreated with reserpine (5 mg/kg, i.p.) 24 hr before the experiment (Fig. 2). The maximal responses of the atria to M6434 in the control and reserpinized rats were 594±67 mg and 821±125 mg, respectively. The basal tension in normal and hypothyroid atria were 342±30 mg and 295±55 mg, respectively, and the percent changes of the tension from that observed immediately before the addition of M6434 were determined. The increases in contractile force induced by M6434 in hypothyroid atria were greater than those in normal atria. The dose response curve for the inotropic effect of M6434 on the left atria showed a shift to the left in hypothyroid rats (Fig. 3). The maximal responses of the normal and hypothyroid atria to M6434 were 565±67 mg and 821±125 mg, respectively.

![Fig. 1. Effects of phentolamine and propranolol on the positive inotropic effect of M6434 in isolated rat left atria. Ordinate: effects as percentage of maximal response to M6434. Abscissa: M6434 concentration, log M. ○: M6434, n=4. ●: M6434+phentolamine, $10^{-6}$ M, n=4. △: N6434+propranolol, $3\times10^{-7}$ M, n=4. Each point indicates a mean value±S.E.](image-url)
Effect of M6434 on α-Adrenergic Receptor

Effect on rat right atria: The basal rates of the spontaneous beating of normal and hypothyroid atria were 145±5 beats/min and 98±4 beats/min, respectively. The percent changes of the beating from that observed immediately before the addition of M6434 were determined. M6434 showed a dose-dependent positive chronotropic effect on the rat right atria at doses ranging from $3\times10^{-7}$ M to $3\times10^{-4}$ M. The increases in beating rate induced by M6434 in hypothyroid atria were greater than those in normal atria. The dose response curve for the positive chronotropic effect of M6434 on the right atria showed a shift to the left in hypothyroid rats. The maximal responses of normal and hypothyroid atria to M6434 were 167±2 beats/min and 133±11 beats/min, respectively (Fig. 4).

Effect on rat vas deferens: The changes in contractile responses and spontaneous movements were expressed as the percentage of each response to the maximal response to M6434. M6434 induced contractile responses and increased the spontaneous movements of rat vas deferens in a dose-dependent manner at doses ranging from $3\times10^{-8}$ M to $3\times10^{-6}$ M. The maximal contractile responses and spontaneous movements of vas deferens were 0.63±0.21 g and 35.2±7.9 g/10 min, respectively. These effects were abolished by pretreatment with $1\times10^{-6}$ M of phentolamine (Fig. 5).

**Effect on rat vas deferens:** The changes in contractile responses and spontaneous movements were expressed as the percentage of each response to the maximal response to M6434. M6434 caused contractile responses and increased the spontaneous movements of vas deferens in a dose-dependent manner at doses ranging from $3\times10^{-8}$ M to $3\times10^{-6}$ M. The maximal contractile responses and spontaneous movements of vas deferens were 0.63±0.21 g and 35.2±7.9 g/10 min, respectively. These effects were abolished by pretreatment with $1\times10^{-6}$ M of phentolamine (Fig. 5).

Effect on canine arteries: The contractions were expressed as the percentage of each response to the maximal response to phenylephrine. M6434 induced contraction of the aortae and the pulmonary, renal and
femoral arteries in a dose-dependent manner at doses ranging from $1 \times 10^{-7}$ M to $1 \times 10^{-4}$ M. The pD$_2$ values of M6434 on the aortae, pulmonary arteries, renal arteries and femoral arteries were 5.60±0.37, 5.87±0.15, 5.64±0.07 and 5.98±0.11, respectively. Phenylephrine, at doses of $1 \times 10^{-7}$ M to $3 \times 10^{-4}$ M, induced dose-dependent contractions of these arterial strips. The maximal responses of the aortae, pulmonary arteries, renal arteries and femoral arteries to phenylephrine were 0.81±0.42 g, 1.54±0.56 g, 1.64±0.26 g and 2.67±1.04 g, respectively. The pD$_2$ values of phenylephrine on the aortae, pulmonary arteries, renal arteries and femoral arteries were 5.24±0.23, 5.74±0.91, 5.31±0.22 and 5.60±0.23, respectively. The intrinsic activities of M6434 on the aortae, pulmonary arteries, renal arteries and femoral arteries were 0.64±0.04, 0.72±0.12, 0.73±0.08 and 0.73±0.14, respectively (Fig. 6).

**Discussion**

It has been biochemically shown by the binding assay method (5–8) as well as physiologically shown (2–4, 9–14) that $\alpha$-receptors as well as $\beta$-receptors exist in the heart. The authors have previously reported...
the existence of α-receptors on rat atria and its positive inotropic and chronotropic effects caused by their stimulation (3, 4). Further, the sensitivity to α-stimulants has been reported to be elevated in a hypothyroid state (2–4).

M6434 induced a positive inotropic effect on rat left atria which could be suppressed by phentolamine but not by propranolol. Because of the possibility that this positive inotropic effect is an indirect one attributable to the release of catecholamines, rats were pretreated with reserpine and subjected to the same procedure, but no significant effect was noted. M6434 also induced a positive chronotropic effect on rat right atria which is similar to those previously found for phenylephrine or methoxamine on rat atria (3, 4). Positive inotropic and chronotropic effects of M6434 on the left and right atria were potentiated in PTU-induced hypothyroid rats, and these effects were similar to those of phenylephrine and methoxamine (3, 4). These findings suggest that M6434 has an α-stimulating activity.

M6434 caused contraction and increased spontaneous movements of rat vas deferens, which is known to contain α-receptors. Phentolamine suppressed this contractile response and the increased spontaneous movements, supporting the α-stimulating action of M6434.

M6434 induced contraction of canine arteries, and in this experimental system, the intrinsic activity of M6434 was somewhat lower than that of phenylephrine. The pD₂ value of M6434 was about equal to that of phenylephrine in inducing contractile responses of the aorta, pulmonary artery, renal artery and femoral artery. On the other hand, the efficacy of M6434 was more potent than phenylephrine with regard to the inotropic effects on rat atria. It has been reported that the inotropic effect of phenylephrine can be attributed to an α-stimulating action (15), a β-stimulating action (16) and also to an indirect action mediated by the release of catecholamines (17). The inotropic effect of M6434 was unaffected by treatment with propranolol or by reserpinization. indicated that M6434 appears to have neither a β-stimulating action nor catecholamine-releasing activity. M6434 differs from phenylephrine in these two points.

The above data suggest that M6434 is an α-agonist which is about as potent as phenylephrine and that M6434 has neither a β-stimulating activity nor a catecholamine-releasing one.

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