THE PARTIAL AGONIST ACTIVITY OF XAMOTEROL (ICI 118,587) STUDIED BY HEART RATE RESPONSE IN PITHED RATS

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The partial agonist activity of xamoterol was evaluated by measuring changes in heart rate (HR) in pithed rats. Xamoterol showed dose-dependent positive chronotropic effects in catecholamine-depleted pithed rats (HR: 199 ± 6 beats/min) and dose-dependent negative chronotropic effects in sympathetic nerve-stimulated pithed rats (HR: 325 ± 16 beats/min). In contrast, isoproterenol exerted dose-dependent positive chronotropic effects in either condition (HR: 189 ± 7 and 332 ± 5 beats/min) and propranolol exerted dose-dependent negative chronotropic effects in either condition (HR: 209 ± 5 and 344 ± 19 beats/min). When exogenous noradrenaline was continuously infused into catecholamine-depleted pithed rats, xamoterol showed dose-related positive chronotropic effects, with noradrenaline at $0.5 \mu\text{g}/(\text{kg min})$ (HR: 235 ± 4 beats/min), virtually no effects at $1.5 \mu\text{g}/(\text{kg min})$ (HR: 297 ± 11 beats/min) and dose-related negative chronotropic effects at $5 \mu\text{g}/(\text{kg min})$ (HR: 330 ± 5 beats/min). During continuous infusion of xamoterol [$100 \text{ ng}/(\text{kg min})$] into pithed rats, the control HR before stimulation was increased and the maximum increase produced by the sympathetic nerve stimuli at 0.25 to 4 Hz decreased. The maximum increase in HR brought about by xamoterol was about 71% of that induced by isoproterenol, and those by pindolol and practolol were about 40% and 21% respectively. It is concluded that xamoterol is a partial agonist with a strong agonist action.

XAMOTEROL is a new selective β_1 -adrenoceptor partial agonist. When given intravenously, xamoterol has a long duration of effect (about 2 h)¹. It is also active when given orally². Digitalis or catecholamines have been used in the treatment of cardiac failure. It is well known that digitalis has a small safety margin, particularly in the elderly where special care is needed to avoid possible intoxication. On the other

hand, catecholamines cannot be orally administered, and are rapidly inactivated after parenteral administration. Xamoterol overcomes these problems, and there are great expectations for the drug as a new treatment for cardiac failure. However, the drug has been reported to exert an antagonist action under certain conditions because of its pharmacological features as a partial agonist^{3,4}.

Though the cardiovascular effects of xamoterol have been extensively studied in dogs³⁻⁵ and humans^{1,6,7} there have been no studies of the partial agonist activity of xamoterol in rats. Also there have been no comparative studies of

Key words:

Xamoterol
Partial agonist
 β -blocker
Isoproterenol
Pithed rat

(Received October 23, 1986; accepted July 17, 1987)

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Fig.1. Dose-response curves of blood pressure changes induced by intravenous injection of tyramine in control and reserpine pretreated rats. Each point represents mean $\pm$ S.E.M.

xamoterol and other partial agonists, pindolol and practolol. Use of pithed rats allows controlled electrical stimulation of the sympathetic nervous system, with continuous monitoring of heart rate (HR), and it is possible to change HR by altering the frequency of stimulation.

The purpose of the present study, therefore, was to compare the partial agonist activity of xamoterol and other partial agonists and to obtain more detailed information about the partial agonist activity of xamoterol by using pithed rat preparations.

MATERIALS AND METHODS

1) Experiments in catecholamine-depleted pithed rats

Male rats of the Wistar strain, weighing 220 to 450g, which had received reserpine at 5 mg/kg by intraperitoneal injection 24 hours before, were used in the experiments. In a preliminary study, tyramine was given to rats which had received reserpine and control rats which had not received reserpine, and the dose-response curve of blood pressure changes plotted. The maximum increase in blood pressure induced by tyramine in the reserpinized rats was about 20% of that in the control rats (Fig. 1). The results of this preliminary study demonstrated that the rats which had received reserpine were catecholamine depleted.

Reserpine pretreated rats were anesthetized

by intraperitoneal injection of thiopental at 50 to 60 mg/kg, and atropine sulfate at 1 mg/kg by intravenous injection, followed by bilateral vagotomy. The right carotid artery was ligated; then a polyethylene cannula inserted into the left carotid artery and connected to a transducer for the measurement of blood pressure. Pulse waves were amplified by a DC amplifier to operate a pulse rate tachometer which recorded HR. The left jugular vein was ligated, and a polyethylene cannula was inserted into the right jugular vein for drug administration.

Following the method of Shipley and Tilden,⁸ a pithing rod (1.5 mm in external diameter and 19 cm long) was inserted from the left orbit and was advanced through the foramen magnum to the spinal column. After this procedure, the cannula which had been previously inserted into the trachea was immediately connected to a respirator (TB-101, Takashima Shoten). Respiratory rates were adjusted to 80 to 90 strokes/min, and tidal volume to 0.8 to 1 ml/100g (excluding the dead space). The animals were left for about 30 to 60 minutes until their condition stabilized, and then the experiment began. The animals were warmed with water mats at about 37°C during the experiment. Noradrenaline was continuously infused into the unilateral femoral vein using an infusion pump.

2) Electrical stimulation of the sympathetic nerves in pithed rats

Fig.2. Frequency-response curves of HR changes induced by repeated sympathetic nerve stimuli at 0.25 to 4 Hz (10 to 30V of 0.5 ms, for 30 s). Each point represents mean $\pm$ S.E.M.

Male rats of the Wistar strain weighing 250 to 450g were used. Procedures from anesthesia up until immediately before insertion of the pithing rod were as described above. The subsequent procedures were those of Gillespie et al.⁹ A trocar (2.5 mm in external diameter and 11 cm long) was inserted from the orbit to the spinal column around the level of C₆, and a pithing rod was inserted through the trocar. Then, the animals were immediately connected to a respirator, and respiration was controlled as above. After removing the pithing rod, the stimulating electrode, which was completely insulated apart from the 2 mm tip, where the indifferent electrode was exposed, was inserted so that the tip reached the level of C₇ to Th₁. d-Tubocurarine was given at 1 to 2 mg/kg by intravenous injection to prevent muscle twitching due to the electrical stimulation. The experiment began after the condition of the animals was stabilized. Body temperature of the animals was maintained as described in the previous section.

In the experiment with continuous stimulation, stimuli (monophasic square-wave pulses of 0.5 ms duration) fixed at 20V in the frequency of 1 Hz were given by a stimulator (MSE-3, Nihon Kohden). When the frequency-response curve was obtained, stimuli of 10 to 30V of 0.5 ms were given at 0.25 to 4 Hz for 30 s. Voltage was adjusted so that the increase in HR at 0.25 Hz was about 20 to 25 beats/min. The same voltage was maintained in each rat through-

out the experiment. If repeated stimuli were given, the next stimulation was given after the response to the previous stimulation had disappeared. Repeated stimuli at 0.25 to 4 Hz were defined as one course of stimulation. The preliminary study demonstrated that the response was reproducible only for two runs, and that the response to the 3rd run of stimuli was clearly diminished (Fig. 2). Therefore, xamoterol was given between the 1st and 2nd runs of stimuli.

The drugs used included xamoterol, 1-isoproterenol hydrochloride, dl-propranolol hydrochloride, pindolol, practolol, dl-noradrenaline hydrochloride, reserpine, thiopental sodium, atropine sulfate, d-tubocurarine chloride and tyramine hydrochloride.

RESULTS

1) Effects of xamoterol, isoproterenol, pindolol, practolol and propranolol on HR in catecholamine-depleted and sympathetic nerve-stimulated pithed rats

When xamoterol (0.1 to 10 μ g/kg) was given cumulatively to catecholamine-depleted pithed rats by intravenous injection, the HR increased in a dose-related manner. However, the drug (0.001 to 1 mg/kg) decreased the HR when the sympathetic activity was elevated by continuous electrical stimulation of the spinal cord in pithed rats. When isoproterenol (0.01 to 3 μ g/kg) was injected, there was a dose-related increase in HR in either condition. Following propranolol (0.01 to 1 mg/kg), on the other hand, HR decreased in a dose-related manner, under either condition. Pindolol and practolol caused dose-related increases in HR in catecholamine-depleted pithed rats. The maximum increase in HR following xamoterol was about 71% of that induced by isoproterenol, and those following pindolol and practolol were about 40% and 21% respectively (Fig. 3). In catecholamine-depleted pithed rats, the control values of HR before injection of xamoterol, isoproterenol, pindolol, practolol and propranolol were 199 ± 6 , 189 ± 7 , 198 ± 28 , 213 ± 7 and 209 ± 5 beats/min respectively. In sympathetic nerve-stimulated pithed rats, the control values of HR before stimulation and injection of xamoterol, isoproterenol and propranolol were $218 \pm 13 \rightarrow 325 \pm 16$, $209 \pm 8 \rightarrow 332 \pm 5$ and $219 \pm 18 \rightarrow 344 \pm 19$ beats/min, respectively.

2) Effect of xamoterol on HR during continu-

Fig.3. Dose-response curves of HR changes induced by cumulative administrations of xamoterol, isoproterenol, pindolol, practolol and propranolol in catecholamine-depleted (solid lines) and sympathetic nerve-stimulated (dotted lines) pithed rats. Each point represents mean $\pm$ S.E.M.

Fig.4. Dose-response curves of HR changes induced by xamoterol during infusion of noradrenaline (Nor.) at 0.5, 1.5 and 5 $\mu\text{g}/(\text{kg min})$. Each point represents mean $\pm$ S.E.M.

ous infusion of noradrenaline

The subsequent experiments were conducted by continuous infusion of noradrenaline into

catecholamine-depleted pithed rats. When HR increased slightly by continuous infusion of noradrenaline at 0.5 $\mu\text{g}/(\text{kg min})$ ($195 \pm 9 \rightarrow$

Fig.5. Frequency-response curves of HR changes induced by repeated sympathetic nerve stimuli at 0.25 to 4 Hz (10 to 30V of 0.5 ms, for 30 s) before and during infusion of xamoterol. Each point represents mean $\pm$ S.E.M.

235 $\pm$ 4 beats/min), xamoterol produced a further dose-related increase in HR. However, when HR increased moderately by infusion of noradrenaline at 1.5 μ g/(kg min) (203 $\pm$ 18 $\rightarrow$ 297 $\pm$ 11 beats/min), HR was virtually unaffected by xamoterol. Lastly, noradrenaline was given at 5 μ g/(kg min) to increase HR greatly (204 $\pm$ 6 $\rightarrow$ 330 $\pm$ 5 beats/min); xamoterol then induced a dose-related decrease in HR (Fig. 4).

3) Effect of xamoterol on the frequency-response curve of HR changes produced by the sympathetic nerve stimuli

In the experiment to obtain response curve of HR in relation to the frequency of stimulation, the frequency-response curve was first obtained with stimuli at 0.25 to 4 Hz. Thereafter, continuous infusion of xamoterol at 100 ng/(kg min) was started. The second frequency-response curve was obtained after the HR was stabilized. During infusion of the drug, the control HR before stimulation was increased and the maximum increase produced by the sympathetic nerve stimuli decreased (Fig. 5). The control value of HR before stimulation was 207 $\pm$ 3 beats/min.

DISCUSSION

The first purpose of the pithed rat preparation was to assay pressor substances⁸. The reason was that this animal preparation was very sensitive to pressor substances because of the very

low sympathetic activity. Another rat preparation of decreased sympathetic activity is that catecholamine depleted by pretreatment with syrosingopine or reserpine. This preparation has frequently been used to evaluate the intrinsic sympathomimetic activity of β -blockers.^{10,11} In the present study, catecholamine-depleted rats were pithed to ensure the decrease in sympathetic activity, and the partial agonist activity of xamoterol was evaluated in these rats. The different sympathetic activity was simulated by continuous infusion of exogenous noradrenaline or sympathetic stimulation of pithed rats without catecholamine depletion, and the effect of xamoterol at varying levels of sympathetic activity was investigated.

The present study showed that xamoterol acted as an agonist at lower levels of HR, and as an antagonist at higher levels of HR following electrical stimulation. This means that the drug had both agonist and antagonist properties. In contrast, isoproterenol always acted as an agonist, and propranolol always as an antagonist, whether HR was elevated or not. These results demonstrated that xamoterol was a partial agonist, isoproterenol was a full agonist, and propranolol was a full antagonist. When HR was altered by infusion of noradrenaline, xamoterol acted in three different ways, depending on the dose levels of noradrenaline. It sometimes acted as an agonist, sometimes showed virtually no effect, and sometimes acted as an antagonist. Similar results were found in humans. Detry et al.⁷ injected xamoterol at 0.2 mg/kg intravenously into 10 patients with cardiac diseases to evaluate the hemodynamic effect of the drug. The results showed that xamoterol increased HR at rest from 75 to 88 beats/min, while there was no significant change during exercise of low intensity (control HR: 103 beats/min). However, the drug decreased HR by 10 and 24 beats/min during exercise of moderate and heavy intensity (control HR: 121 and 149 beats/min). Both the results of the present study and those of Detry et al. demonstrated that the effects of xamoterol were related to the control levels of HR. The results of the more detailed experiment are shown in Fig. 5. As shown in this figure, following infusion of xamoterol, the control HR was increased but the maximum increase produced by the sympathetic nerve stimuli decreased. These results are consistent with those of Nuttall and Snow,³ who gave electrical stimuli at 0.2 to 20 Hz to the right ansa subclavia of dogs to depict

frequency-response curves. When xamoterol was injected at 5, 50 and 250 $\mu\text{g/kg}$, then the control HR was increased and the maximum increase obtainable by sympathetic nerve stimuli decreased in a dose-related manner. The curve became almost flat following the maximum dose of 250 $\mu\text{g/kg}$. They concluded that the overall effect of the compound was to stabilize cardiac response almost at a value which depended on the intrinsic activity of the compound. In their experiment, the effect of the three dose levels of xamoterol was examined continuously in the same individual dogs. However, the results of our preliminary study demonstrated that the frequency-response curve was reproducible only in two experiments in each single rat. For this reason, the effect of xamoterol was tested only at one dose in each rat.

The present study demonstrated that the maximum agonist effect of xamoterol was about 71% of the maximum level of isoproterenol, and those of the other partial agonists, pindolol and practolol, were about 40% and 21% respectively. This order of the strength of the agonist activity of the latter two drugs was the same as that presented by Barrett and Carter.¹⁰ This difference in maximum agonist effect between xamoterol and pindolol justifies the use of xamoterol as a β -stimulant, and pindolol as a β -blocker. Nuttall and Snow³ also evaluated the maximum agonist effect of xamoterol in dogs. They compared only two drugs, isoprenaline and xamoterol, and reported that the maximum agonist effect of xamoterol was about 43% the level of isoprenaline. This level is clearly lower than that presented here. It is not known if this difference was related to an animal species difference or a difference in experimental conditions.

In conclusion, xamoterol is a partial agonist with strong agonist activity of about 71% the level of isoproterenol. However, there is a critical point for the effect of the drug, and it

works as an agonist or antagonist, depending on the state of the sympathetic activity.

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