Anti-Nicotinic and Anti-Muscarinic Actions of Eperisone in the Isolated Canine Atrium

Kimiaki Saegusa, Yasuyuki Furukawa, Kunio Akahane, Masayuki Haniuda and Shigetoshi Chiba *
Department of Pharmacology, Shinshu University School of Medicine, Matsumoto 390, Japan
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ABSTRACT—The effects of eperisone, an antispastic agent, on the chronotropic and inotropic responses to acetylcholine, nicotine or stimulation of intracardiac autonomic nerves were evaluated in isolated, blood-perfused canine atrium. Eperisone (10–300 μg) injected into the sinus node artery of the isolated atrium produced dose-related negative chronotropic and inotropic effects, which were not affected by atropine. In the same doses, eperisone inhibited the negative chronotropic and inotropic responses to an injection of acetylcholine and intracardiac parasympathetic stimulation. Eperisone also suppressed the negative followed by positive cardiac responses to nicotine, but did not modify the positive responses to intracardiac sympathetic stimulation or nor-epinephrine. The inhibitory effect persisted much longer for the responses to nicotine or parasympathetic stimulation than for those to acetylcholine. These results suggest that eperisone at doses that induce direct cardiac depressant effects exerts its blocking action on nicotinic receptors at parasympathetic ganglia and sympathetic nerve terminals and on muscarinic receptors at the effector cells in the dog heart.

Eperisone hydrochloride (4'-ethyl-2-methyl-3-piperidinopropiophenone hydrochloride) is an antispastic agent. Actions of eperisone on several organs have been characterized by the following: an inhibition of mono- and multisynaptic reflexes in relation to the inhibitory action on alpha- and gamma-efferent neurons in the spinal cord and supra-spinal structures (1), a reduction of the number of spikes elicited by electrical nerve stimulation in the vestibular nucleus neurons (2), and an elevation of the electrical threshold required for generation of the action potential and a Ca antagonistic action in the smooth muscle cells of the basilar artery (3). However, no studies have been carried out to investigate the action of eperisone on the cardiac autonomic nervous system.

In the present study, we have observed the effects of eperisone on pacemaker activity and myocardial contractility by using isolated, blood-perfused canine atrial preparations (4, 5). Furthermore, to clarify the mechanisms by which eperisone exerts its neural action, we examined whether eperisone modified the chronotropic and inotropic responses to acetylcholine, nicotine, or electrical stimulation of the intracardiac autonomic nerves.

MATERIALS AND METHODS

Preparation of the isolated, blood-perfused dog atrium

Thirty-three donor dogs weighing 9–21 kg were anesthetized with 30 mg/kg, i.v. of sodium pentobarbital. Each dog was artificial-
ly ventilated through a cuffed endotracheal tube with room air using a Harvard respirator (model 607). Heparin, 500 USP units/kg, was administered intravenously at the beginning of the perfusion, and 200 USP units/kg was added at 1-hr intervals. Isolated right atrial preparations were obtained from 33 other mongrel dogs weighing 6–14 kg. Each dog was anesthetized with sodium pentobarbital, 30 mg/kg, i.v. After treatment with 200 USP units/kg of sodium heparin, the right atrium was excised and plunged into a cold Tyrode solution of 4°C. The wet weight of the atrial preparations varied from 6 to 12 g. The sinus node artery was cannulated via the right coronary artery and perfused with heparinized blood from the common carotid artery of the donor dog using a peristaltic pump (Harvard Apparatus, model 1210). A pneumatic resistance was placed in parallel with the perfusion system so that the perfusion pressure could be maintained constant at 100 mmHg. The blood flow rate to the isolated atrium was 3–9 ml/min.

The ventricular margin of the isolated atrium was fixed to a stainless steel bar and placed in a cup-shaped glass container kept at 37°C. The atrium was stretched to a resting tension of 2 g. The isometric tension was recorded on a thermo-writing rectigraph (Nihon Kohden, WT 685G). Two bipolar silver electrodes, with an inter-electrode distance of 1.5 mm, were brought into contact with two sites on the atrial epicardium. The first pair of electrodes was placed on the atrial free wall for recording the electrogram. The spontaneous atrial rate was derived from the atrial electrogram with a cardiotachometer (Nihon Kohden, AT600G). The second pair of electrodes was placed on the atrial free wall for recording the electrogram. The spontaneous atrial rate was derived from the atrial electrogram with a cardiotachometer (Nihon Kohden, AT600G). The second pair of electrodes was placed on the posterior portion in the caval margin for activation of the intracardiac parasympathetic nerve fibers or intracardiac parasympathetic and sympathetic nerve fibers (6, 7). Electrical stimulation was performed with rectangular pulses by means of an electrical stimulator through an isolation unit (Nihon Kohden, SEN 7103). Intracardiac autonomic nerves were stimulated at a frequency of 30 Hz for 5 sec. Stimulation with a narrow pulse duration (less than 0.1 msec) induces negative cardiac responses (6, 8) and that with a wide pulse duration (usually 1 msec) induces negative followed by positive cardiac responses (6, 7). After atropine, stimulation with a wide pulse duration induces only the positive cardiac responses. The voltage was adjusted to obtain 20% changes in chronotropic response to nerve stimulation and subthreshold for pacemaker cells and atrial muscles. The mean voltage used in the present experiments was 3.6 V. Details of this preparation have been described previously (4–6).

Protocols

We carried out two series of experiments. In the first series, we examined the effects of eperisone on the sinoatrial pacemaker activity and atrial contractility in 6 isolated atria and analyzed the negative cardiac effects of eperisone before and 2 min after treatment with atropine (3 μg) to investigate whether the cardiac responses to eperisone were mediated by muscarinic receptors in 4 isolated atria. In the second series, we investigated whether eperisone inhibited the cardiac responses to intracardiac parasympathetic nerve stimulation and acetylcholine in 5 isolated atria and the cardiac responses to nicotine, intracardiac sympathetic and parasympathetic nerve stimulation, and norepinephrine (NE) in 4–5 isolated atrial preparations. We also studied the effects of eperisone on the cardiac responses to nicotine in 4 isolated atria and to sympathetic and parasympathetic nerve stimulation in 1 of 4 experiments after 10 μg of atropine. The responses to each intervention were studied before and at least 2 min after the drug administration. In most cases, after the direct effects of eperisone returned to near the pre-drug levels, the responses to the intervention was observed. The order of drug injection was randomized.

Drugs

Chemicals used in this study were eperisone hydrochloride (4'-ethyl-2-methyl-3-piperidino-propiophenone hydrochloride, Eisai, Tokyo,
Japan), acetylcholine chloride (ACh, Daiichi, Tokyo, Japan), atropine sulfate (Wako Pure Chemical, Osaka, Japan), nicotine (base, Yamanouchi, Tokyo, Japan), d,l-norepinephrine hydrochloride (NE, Sankyo, Tokyo, Japan), and d,l-propranolol hydrochloride (Sigma, St. Louis, MO, U.S.A.). Each drug was dissolved in physiological saline before starting the experiment. Drugs were injected into the sinus node artery of the isolated atrium through a rubber tube with a microsyringe (Terumo). The amount of drug solution injected was 0.01–0.03 ml in a period of 4 sec.

Statistical analysis

Data are given as percentage changes in a maximum positive or negative direction from each basal control level before a drug injection or nerve stimulation. Data collected before and after treatment with a drug were analyzed by the paired t-test.

RESULTS

Effects of eperisone on sinoatrial nodal pacemaker activity and atrial contractility

When eperisone in doses ranging 10–300 μg was injected directly into the cannulated sinus node artery of the isolated atrium, negative chronotropic and inotropic effects were induced in a dose-related manner. Figure 1 shows the dose-response curves for the chronotropic and inotropic effects of eperisone at doses of 10–100 μg in 6 isolated atria. After treatment with atropine (3 μg), the negative chronotropic and inotropic effects of acetylcholine (ACh) were inhibited significantly (P < 0.05), while the negative cardiac responses to 100 μg of eperisone were not affected (Fig. 2).

Effects of eperisone on responses to acetylcholine and intracardiac parasympathetic stimulation

ACh injected to the SA node artery of the isolated atrium and stimulation of intracardiac parasympathetic nerves decreased atrial rate and contractile force. Eperisone at doses of 10 to 100 μg inhibited the negative chronotropic and inotropic responses to ACh or parasympathetic nerve stimulation. Figure 3 shows summarized data of the inhibition of the negative cardiac responses to ACh and parasympathetic stimulation by eperisone at a dose of 100 μg in 5 isolated atria. When the responses to ACh were completely restored to the control responses at 20 ± 3 (mean ± S.E.M., n = 5) min after eperisone treatment, the responses to parasympathetic stimulation were not yet completely restored. We did not further study the complete recovery of the responses to parasympathetic stimulation.

Effects of eperisone on the responses to nicotinic, intracardiac sympathetic and parasympathetic stimulation, and norepinephrine

To investigate the effects of eperisone on the responses mediated through nicotinic re-
Fig. 2. Effects of atropine (3 μg) on percentage changes in chronotropic and inotropic responses to eperisone (100 μg) and acetylcholine (ACh, 0.3 μg) in 4 isolated atria. Vertical lines show S.E.M. ns means no statistical significance (P > 0.05). □ control, □ atropine, 3 μg.

Fig. 3. Effects of eperisone on percentage changes in chronotropic and inotropic responses to acetylcholine (ACh, 0.3 μg) and electrical parasympathetic stimulation in 5 isolated, perfused dog atria. ns means no statistical significance. Control levels of atrial rate and contractile force in 5 isolated atria were 106 ± 5 (mean ± S.E.M.) beats/min and 3.1 ± 0.7 g, respectively. □ control, □ eperisone, 100 μg.

Fig. 4. Effects of eperisone on atrial rate and contractile force responses to nicotine and norepinephrine (NE) in an isolated, blood-perfused canine atrium.
ceptors in the heart, we studied the cardiac responses to nicotine, intracardiac sympathetic and parasympathetic nerve stimulation and norepinephrine before and after treatment with eperisone (10–300 μg). Eperisone (100 μg) inhibited the negative followed by positive chronotropic and inotropic responses to nicotine (3 μg) but not the positive cardiac responses to norepinephrine (Figs. 4 and 5). Moreover, when intracardiac sympathetic and parasympathetic stimulation induced the negative followed by positive responses, eperisone (100–300 μg) inhibited the negative but not the positive responses (Fig. 5).

After atropine (10 μg), nicotine (3 μg) induced only positive cardiac responses. Eperisone (10 μg) significantly (P < 0.05) inhibited the increases in atrial rate and contractile force in response to nicotine from 22.4 ± 7.1% to 5.9 ± 2.4% and from 88.2 ± 29.4% to 41.2 ± 14.7%, respectively, in 4 experiments. Positive chronotropic and inotropic responses to stimulation of the intracardiac sympathetic nerve fibers were not attenuated by 300 μg of eperisone in an isolated atrium after atropine was given.

DISCUSSION

In the present study, injection of the antispastic agent eperisone (10–300 μg) into the sinus node artery of the isolated atrium produced negative chronotropic and inotropic effects, which were not affected by treatment with the muscarinic antagonist atropine. Therefore, it is postulated that eperisone has direct depressant properties on the pacemaker activity and the atrial contractility. Eperisone acts as a calcium antagonist on smooth muscle tissues of the guinea pig basilar artery and is an antispastic agent (3). Eperisone is also a calcium antagonist in the snail neuron (9). Calcium antagonists induce the negative chronotropic and inotropic effects, which are not affected by atropine, in the isolated, blood-perfused dog heart preparations (10–13). Thus, calcium antagonistic properties may partly explain the direct negative cardiac effects of eperisone in the dog heart.

In the same range of doses that induced cardiac depressant effects, eperisone inhibited the negative chronotropic and inotropic responses to ACh. This indicates that eperisone blocks muscarinic receptors at the effector site. This antimuscarinic effect of eperisone has been recently reported in isolated dog saphenous arteries and veins (14). Some antispasmodics such as trihexyphenidyl and benztropine exert their antispasmodic effect, at least in part.
through blockade of muscarinic receptors (15). These antispasmodics possess an atropine-like action on the peripheral autonomic nervous system as well as the central nervous system. Thus, it is likely that the antispastic effect of eperisone may be partly due to the atropine-like action of this drug.

Eperisone inhibited the positive chronotropic and inotropic responses to an injection of nicotine in isolated atria treated with or without atropine. The positive cardiac effects of nicotine are caused by a release of NE, which is attributed to stimulation of nicotinic receptors at sympathetic nerve terminals (4, 16, 17). In the present experiments, the positive chronotropic and inotropic responses to stimulation of intracardiac sympathetic nerves or NE injection were not inhibited by eperisone. Thus, the inhibitory effect of eperisone on nicotine-induced positive cardiac responses seems to be due to blockade of nicotinic receptors at the presynaptic sympathetic nerves, but not due to blockade of nerve excitation or blockade of beta-adrenergic receptors at the effector cells.

Eperisone inhibited the negative chronotropic and inotropic responses to intracardiac vagus stimulation. However, eperisone's inhibitory effect on the response to nerve stimulation persisted for much longer than its inhibition of the response to injected ACh. This means that eperisone might inhibit the release of ACh by parasympathetic stimulation from nerve endings, in addition to its temporal atropine-like action. In the blood-perfused sinoatrial node preparation, the release of ACh elicited by direct electrical stimulation mainly results from excitation of parasympathetic preganglionic fibers as reported previously (6, 18, 19). Recently, Noma et al. (2) reported that iontophoretic application of eperisone in lower doses of 25–100 nA produced a dose-dependent inhibition of spike generation upon electrical nerve stimulation with little effect on the spike height, while a higher dose of 200 nA reduced the spike height in the vestibular nucleus neurons. Their results indicate that only at high doses, eperisone exerts its inhibitory effect on nerve excitation by reducing fast sodium inward current similar to the activity of the local anesthetic lidocaine (20, 21). In the present study, the negative chronotropic and inotropic responses to nicotine were also depressed by treatment with eperisone at the low dose of 10 μg, which was the threshold dose for producing the inhibitory effect on the negative cardiac responses to parasympathetic nerve stimulation. Furthermore, the positive cardiac responses to intracardiac sympathetic stimulation were not inhibited by eperisone. It has been well-recognized that hexamethonium blocks nicotinic receptors not only at parasympathetic ganglia but also at sympathetic nerve terminals (4). This suggests that the nicotinic receptors of parasympathetic ganglia are qualitatively similar to those of the sympathetic nerve terminals. Therefore, it appears that the inhibitory effect of eperisone on the negative cardiac responses to preganglionic vagus stimulation is not due to reduction of fast sodium inward current in the cholinergic neurons, but is rather due to suppression of impulse propagation through blockade of nicotinic receptors at parasympathetic ganglia. However, it can not be ruled out completely that at higher doses, a blocking action of eperisone on fast inward channels in the autonomic nerves contributes to the inhibition of neurotransmitter release from nerve endings.

In conclusion, our results indicate that eperisone has anti-nicotinic and anti-muscarinic effects and that these effects are accompanied with slight but obvious cardiac depressions in the isolated, blood-perfused canine atrium.

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