Possible Involvement of Ganglionic Blocking Effects of Diltiazem in Regulation of Blood Pressure and Heart Rate of the Rabbit

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Abstract Under pentobarbitone anesthesia, arterial blood pressure and heart rate were recorded in rabbits. The elevation in systemic blood pressure and increased heart rate induced by electrical stimulation of the right thoracic sympathetic nerve trunk were depressed by either an intravenous injection of diltiazem (300 μg/kg) or by placing cotton pledgets soaked in 2 mM diltiazem solution onto the right stellate ganglion. These inhibiting effects of diltiazem were statistically significant with high sympathetic stimulation frequencies (10–20 Hz), but not when lower frequencies (1–5 Hz) were used. On the other hand, hexamethonium (10 mg/kg i.v. or 100 mM applied topically) depressed cardiovascular responses to sympathetic stimulation over a wide range of frequencies (1–20 Hz). Present results reveal a frequency-dependent inhibition of ganglionic transmission by diltiazem, and suggest that diltiazem may depress excessive sympathetic activity without affecting normal ganglionic transmission.

Key words: sympathetic ganglion, stellate ganglion, blood pressure, heart rate, diltiazem.

Diltiazem, a Ca\(^{2+}\)-antagonist, is effective in the therapy of ischemic heart diseases by its vasodilating action on coronary arteries and by preventing an excess of calcium influx that probably occurs in damaged cardiac muscle (Muller and Gunther, 1978; Antman et al., 1980; Stone et al., 1980), it is also utilized for the treatment of hypertension (Wei et al., 1976; Blaustein, 1977; Zoster et al., 1977). Most Ca\(^{2+}\)-antagonists basically have both negative chronotropic and inotropic actions on the heart (Cranefield et al., 1974; Fleckenstein, 1977; Imai et al., 1977; Nawrath et al., 1977; Lathrop et al., 1982). Together with their vasodilating
action (FLECKENSTEIN, 1977), it is easy to predict that these agents decrease systemic blood pressure. However, the detailed mechanisms of their antihypertensive action have not been fully clarified. For precise understanding of the pharmacological and therapeutic effects of these compounds, it is necessary to take into consideration their effects on the autonomic nervous system which plays an important role in the regulation of cardiovascular functions. Only a few reports regarding the effects of Ca$^{2+}$-antagonists on the autonomic nervous system are available.

We have observed that diltiazem induced a frequency-dependent transmission failure in isolated sympathetic ganglia upon preganglionic nerve stimulation, and have concluded that transmission blockade is mainly due to inhibition of Ca$^{2+}$-influx at the preganglionic nerve terminal (ITO and NISHI, 1982; ITO et al., 1984). However, the precise mechanisms underlying the effects of diltiazem on transmitter release still remain unknown. To determine whether the inhibiting effects of diltiazem on ganglionic transmission observed in vitro may also occur in vivo, we designed the present experiments to investigate how diltiazem affects systemic hemodynamics through actions on ganglionic transmission. For this purpose, effects of the drug on arterial blood pressure and heart rate changes produced by electrical stimulation of the thoracic sympathetic nerve trunk were investigated.

**MATERIALS AND METHODS**

Eighteen rabbits were anesthetized with sodium pentobarbitone 30 mg/kg injected intravenously. Periodic supplemental doses of anesthetic (usually 5-10 mg/kg) were given to maintain the anesthesia at a steady level. Blood and fluid loss during the surgical operation were replaced by physiological saline infused intravenously. The trachea was cannulated, and positive pressure respiration was instituted with room air by means of a piston pump (45 strokes/min, 10 ml/kg in tidal volume). The right chest was opened by removing the 3rd and 4th ribs. Body temperature was maintained at 37.0°C by an electric heating element in the operating table.

A polyethylene catheter was passed into the right carotid artery and arterial blood pressure was measured with a pressure transducer (Nihon Kohden, TP-101T) connected to a bio-polygraph (Nihon Kohden, WT-685G). Heart rate derived from electrocardiogram lead II was simultaneously recorded through a cardiotachometer (Nihon Kohden, AT-600G). The right thoracic sympathetic trunk, which mainly includes preganglionic nerve fibers to the stellate ganglion, was cut at the 3rd or 4th rami and stimulated electrically by a pair of platinum electrodes. Nerve stimulation consisted of square wave pulses of 0.1 ms duration and supramaximal voltage ranging from 10 to 20 V at frequencies of 1 to 20 Hz for periods of 1 min. Drugs were applied in two ways: (1) agents were rapidly injected through a catheter previously inserted into the femoral vein in a volume smaller than 1.0 ml (intravenous application); or (2) directly applied to the stellate ganglion by placing cotton pledgets soaked in 2 mM diltiazem or 100 mM hexamethonium onto the...
ganglion (topical application).

The drugs used in the present experiments were diltiazem hydrochloride (Tanabe, Osaka) and hexamethonium bromide (Yamanouchi, Tokyo). Statistical analysis of the data was done by analysis of variance in one-way classifications (SNEDECOR and COCHRAN, 1978) or by Mann-Whitney non-parametric test (SNEDECOR and COCHRAN, 1978) and probability of less than 5% was considered to be significant.

RESULTS

Effects of dissection of vagus, cervical sympathetic nerve trunk, and thoracic sympathetic trunk

Prior to the experiments, both sides of the vagus and sympathetic trunk were dissected and cut below the superior cervical ganglion, and the right thoracic sympathetic trunk was cut at the 3rd or 4th rami to eliminate possible interactions of efferent autonomic nervous activity through these trunks. These operations usually caused alterations of blood pressure and heart rate which are summarized in Table 1, where values were measured 5–10 min after the hemodynamic changes had stabilized.

Effects of intravenous application of diltiazem and hexamethonium

Electrical stimulation of the preganglionic sympathetic nerve elicited an increase in blood pressure and heart rate (Fig. 1). The peak responses to electrical stimulation occurred at frequencies of 10 to 20 Hz. Higher frequencies did not

<table>
<thead>
<tr>
<th>1. Pre-treatment (control)</th>
<th>2. Vagus and cervical sympathetic trunk cut (bilateral)</th>
<th>3. Thoracic sympathetic trunk cut (right)</th>
<th>Statistical significance</th>
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<tr>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>87.5 ± 3.8</td>
<td>102.2 ± 3.1</td>
<td>85.0 ± 3.1</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>57.0 ± 4.0</td>
<td>66.0 ± 3.1</td>
<td>53.8 ± 3.1</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>278.3 ± 3.8</td>
<td>288.1 ± 4.3</td>
<td>268.2 ± 4.2</td>
</tr>
</tbody>
</table>

Values are means of 18 observations and ± S.E.M. Statistics by analysis of variance in a one-way classification. N.S.: not significant.
produce greater responses. Intravenous injections of diltiazem (300 μg/kg) and hexamethonium (10 mg/kg) caused a fall in blood pressure and a decrease in heart rate. These effects, measured approximately 10 min after the injection, are summarized in Table 2. Figure 2 illustrates the typical inhibiting effects of diltiazem (Fig. 2: upper) and hexamethonium (Fig. 2: lower) on changes in systemic blood pressure and heart rate elicited by electrical stimulation (10 Hz, 1 min) of the right thoracic sympathetic trunk. A summary of the effects of diltiazem and hexametho-

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**Fig. 1.** Typical response to electrical stimulation of right thoracic sympathetic trunk at varying frequencies.

**Table 2.** Effects of intravenous injection of diltiazem and hexamethonium on blood pressure and heart rate.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>10 min after drug</th>
<th>Significance</th>
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<tr>
<td><strong>Diltiazem (n = 4); 300 μg/kg</strong></td>
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<td></td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>99.0 ± 4.5</td>
<td>88.8 ± 1.8</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>67.0 ± 7.3</td>
<td>56.5 ± 4.2</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>261 ± 6.2</td>
<td>251 ± 6.1</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td><strong>Hexamethonium (n = 4); 10 mg/kg</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>96.0 ± 1.7</td>
<td>78.8 ± 2.8</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>54.0 ± 3.9</td>
<td>47.5 ± 3.1</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>253 ± 4.7</td>
<td>203 ± 11</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. Statistics by analysis of variance in a one-way classification.
The effects of diltiazem and hexamethonium on changes in systolic blood pressure and heart rate by varying stimulation frequencies are illustrated in Figs. 3 and 4. The illustrations show that the elevation in systolic blood pressure and the increase in heart rate elicited by electric stimulation of the right thoracic sympathetic trunk were depressed by intravenous applications of diltiazem (300 µg/kg) or hexamethonium (10 mg/kg). However, these inhibitory effects were different. The inhibition by diltiazem on the increased systolic blood pressure was significant only during sympathetic stimulation at 10–20 Hz; at lower frequencies (1–5 Hz) no significant inhibition was observed.
H. ITO and M. SAKANASHI

Hexamethonium, on the other hand, depressed the cardiovascular responses to the sympathetic stimulation over a wide range (through 1-20 Hz). A similar pattern of inhibition in heart rate upon the varying stimulation frequencies was also obtained as illustrated in Fig. 4.

Effects of topical application of diltiazem and hexamethonium on the stellate ganglion

After careful exposure of the right stellate ganglion, diltiazem and hexamethonium were applied locally by placing cotton pledgets soaked in high concentrated solutions of the agents (2 and 100 mM, respectively) onto it. These topical applications did not cause any noticeable alterations in systemic blood pressure and heart rate. However, the responses to preganglionic stimulation such as an elevation of blood pressure and an increase in heart rate were diminished. Figure 5 shows the effects of topical applications of diltiazem and hexamethonium. Although quantitative analyses were difficult because the agents acted on the ganglion cells by infiltration and diffusion into tissues, the responses to the electrical stimulation of preganglionic nerve were clearly decreased by either diltiazem or hexamethonium. For complete recovery it was necessary to wait several hours after removal of the drug-soaked cotton pledgets.

Fig. 3. Effects of diltiazem (300 µg/kg, i.v.) and hexamethonium (10 mg/kg, i.v.) on systolic blood pressure. Increase in systolic blood pressure during preganglionic stimulation plotted against frequency of stimulation. Each point represents mean value of 8 observations for control (●), 4 observations for diltiazem (○), and 4 observations for hexamethonium (△). *p < 0.05: significantly different from control by Mann-Whitney test.

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DISCUSSION

Electrical stimulation of the preganglionic fibers to the right stellate ganglion caused a rise in arterial blood pressure and an increase in heart rate which were depressed by intravenous injections of diltiazem. Similar inhibiting actions were observed when diltiazem was topically applied to the stellate ganglion.

Inhibition by diltiazem was significant only when sympathetic stimulation at frequencies of 10–20 Hz was applied. The drug was ineffective when lower frequency stimulations (1–5 Hz) were used. In contrast to diltiazem, hexamethonium, which blocks the action of acetylcholine released from presynaptic nerve endings by stabilizing the postsynaptic membrane, depressed cardiovascular responses to sympathetic stimulation over a wide range (1–20 Hz). Thus, the present study confirms previous observations in vitro (ITO and NISHI, 1982; ITO et al., 1984) where (1) diltiazem blocked ganglionic transmission in excised sympathetic ganglia; and (2) this block was more prominent when presynaptic fibers were stimulated by trains of repetitive pulses (at a frequency of more than 10 Hz). ITO and NISHI (1982) have suggested that ganglionic transmission failure induced by diltiazem might be related, at least partially, to inhibition of Ca\(^{2+}\)-influx in preganglionic nerve
terminals. It is well documented that a key factor in the transmitter release from presynaptic vesicles is the entry of Ca^{2+} ions into the presynaptic terminals (KATZ and MILEDI, 1969; ECCLES, 1977). The present results showing that the inhibiting effects of diltiazem occur only at high frequency stimulations (more than 10 Hz) reveal a frequency-dependent inhibition of ganglionic transmission in vivo by diltiazem. MANNARD and POLOSA (1973) reported that the resting discharge frequency of a single preganglionic neuron, recorded from unanesthetized cat, was in the range of 0.5–4.0 Hz (95\% confidence limits). Thus, the fact that diltiazem had
no significant effect at low stimulating frequencies (1–5 Hz) suggests that diltiazem may depress excessive sympathetic activity without affecting normal ganglionic transmission.

In the present study, an intravenous injection of hexamethonium did not completely abolish the cardiovascular responses induced by preganglionic nerve stimulation. It is possible that nerve fibers running through the stellate ganglion without synapsing (BROWN, 1967; SAKANASHI, 1972) may contribute to the retained responses. These fibers would form synapses in the caudal cervical ganglion or other paravertebral ganglia. As an additional administration of propranolol (1.0 mg/kg i.v.) completely abolished the remaining responses to sympathetic nerve stimulation (data not shown), these responses may be produced via postganglionic adrenergic fibers.

It is well known that there is a considerable asymmetry in the distribution of sympathetic fibers in the left and right sides of the heart (LEVY et al., 1966; FURNIVAL et al., 1973). LEVY et al. (1966) demonstrated chronotropic and inotropic responses to right and left stellate ganglion stimulation in an isolated but innervated dog heart, and proved that the chronotropic effects were more predominant during stimulation of the right side sympathetic nerves, whereas inotropic influences occurred during left side stimulation. In the present study using the rabbit, significant increases in systemic blood pressure and heart rate were observed by stimulation of the right side thoracic sympathetic trunk. This may have occurred because of contralateral distribution of postganglionic fibers from the right stellate ganglion to the left ventricle in the rabbit. Another explanation might be that some fibers from the right stellate ganglion may end on thoracic and brachial visceral arterioles, thus reacting to nerve stimulation by eliciting an increase in blood pressure.

Present in vivo evidence that diltiazem shows a ganglionic blocking action may help us understand the overall effects of Ca\(^{2+}\)-antagonists in clinical use for patients. The present study shows that this agent possesses effects on the autonomic nervous system which regulates cardiovascular functions.

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REFERENCES


