MECHANISM OF CHRONOTROPIC AND INOTROPIC EFFECTS OF PHENYLEPHRINE

Shigetoshi CHIBA
Department of Pharmacology, Faculty of Medicine, Shinshu University, Matsumoto 390, Japan
Accepted April 21, 1977

Abstract—The effects of phenylephrine and methoxamine were studied on pacemaker activity in the sinoatrial node and on atrial and ventricular contractility in isolated, blood-perfused atrial and ventricular preparations of dogs. Each drug was administered directly into the cannulated sinus node artery of an isolated atrium or the anterior descending branch of the isolated left ventricle over a period of 4 sec. Two response patterns to phenylephrine were observed in atrial preparations: monophasic positive chronotropic and inotropic effects; double peaked effects, positive effects were temporarily interrupted by negative effects. Phenylephrine-induced positive effects were blocked by an adrenergic beta-blocker, alprenolol or carteolol, but not by desipramine or phentolamine. Phenylephrine-induced negative effects were inhibited by atropine, tetrodotoxin or phentolamine. In ventricular preparations, phenylephrine usually induced a monophasic positive isotropic effect which was completely blocked by a beta-blocker but not by phentolamine. On the other hand, methoxamine usually induced negative chronotropic and inotropic effects in both preparations, and these negative effects were not blocked by atropine, tetrodotoxin and phentolamine. These results suggest that phenylephrine produces a cholinergic excitation which may occur through an adrenergic alpha-mechanism in parasympathetic nerve terminals, in addition to its adrenergic beta-stimulating effect.

In 1968, James et al. (1) reported that sinus deceleration with the adrenergic alpha-stimulant, methoxamine was prevented by phenoxybenzamine but not by atropine. These workers had directly perfused the sinus node artery of the dog heart in situ (2). They concluded that this phenomenon was “evidence for alpha receptor depressant activity”. The negative chronotropic effect was not however, observed when phenylephrine was administered. In 1971, Hashimoto et al. (3) using a modified direct perfusion method of the canine sinus node artery demonstrated that phenylephrine induced not only sinus acceleration but also sinus deceleration (4). They concluded that phenylephrine causes a cholinergic excitation, which may occur through interaction of phenylephrine with terminal synapses of the vagus nerve as the sinus deceleration induced by phenylephrine was blocked by atropine, hexamethonium, tetracetylatammonium, phentolamine and phenoxybenzamine. However, even in the in situ SA node preparations, a complete isolation from extracardiac factors which may influence pacemaker activity of the SA node via neurogenic reflexes is most difficult to attain. Recently Chiba et al. (5, 6) developed a completely isolated, blood-perfused atrial preparation of the dog, which allows for a well controlled study of such local mechanisms and perfusion is carried out only through the sinus node artery.

In the present study, the isolated canine atrial preparation was employed to determine
the effects of phenylephrine and methoxamine on SA nodal pacemaker activity and atrial contractility. The isolated left ventricular preparation (7) was also utilized in several experiments to determine effects of phenylephrine and methoxamine on ventricular contractility.

MATERIALS AND METHODS

The experimental methods of atrial (5, 6) and ventricular preparations (7) have been previously described. Briefly, thirty-eight mongrel dogs were anesthetized with sodium pentobarbital, 30 mg/kg, i.v. After intravenous injection of sodium heparin (500 units/kg), the heart was quickly excised, and the isolated right atrium or left ventricular muscle along the anterior descending branch of the left coronary artery were dissected in saline solution at about 4–10°C. The right atrium or the ventricular muscle was perfused via the dorsal right atrial artery (= so-called sinus node artery) or the anterior descending branch of the left coronary artery with blood which was pumped from a carotid artery of a heparinized support dog also anesthetized with sodium pentobarbital. Perfusion was begun within 5 to 15 min after excision of the heart. A pneumatic resistance was placed in parallel with the perfusion system in order to maintain a constant perfusion pressure of 100 mm Hg. The atrium or ventricle was suspended in a bath filled with blood at a constant temperature of 37°C. Sinus rate was recorded with a tachometer (Nihon-Kohden RT-5) which was triggered by the action potential wave of atrial electrograms, and isometric tension development of the atrial or ventricular muscle was measured with a force displacement transducer (Grass FTO3B). The ventricular muscle was electronically stimulated at a rate of 1.5–2 Hz through platinum electrodes, with rectangular pulses of 1–5 msec duration and voltage double the threshold by use of an electronic stimulator (Nihon-Kohden MSE-3).

The volume of drug solution injected was 0.01–0.03 ml in a period of 4 sec. Drugs used in this study were phenylephrine hydrochloride (Kowa), methoxamine hydrochloride (Nippon Shinyaku), norepinephrine hydrochloride (Sankyo), tetrodotoxin (kindly provided by Sankyo Central Lab.), phentolamine hydrochloride (Ciba), atropine sulphate (Takeda), alprenolol hydrochloride (AB Hassel), carteolol hydrochloride (kindly provided by Otsuka Pharm. Co.) and desipramine hydrochloride (Fujisawa).

RESULTS

Chronotropic and inotropic effects of phenylephrine and methoxamine given into the sinus node artery

When phenylephrine was injected into the cannulated sinus node artery, the positive chronotropic and inotropic effects were produced. A relatively small dose of phenylephrine, 0.3 μg, produced only a monophasic positive chronotropic and inotropic effect. With increasing doses over 1 μg, a biphasic chronotropic and inotropic effect or a double peaked chronotropic and inotropic effect was frequently observed: i.e., there was a brief negative chronotropic and inotropic effect, followed by a positive effect, or there was a primary positive chronotropic and inotropic effect, followed by a brief negative effect, after
Fin. 1. Chronotropic and inotropic effects of increasing doses of phenylephrine on a spontaneously beating dog atrium.

which there was a sustained positive effect. Fig. 1 shows the chronotropic and inotropic responses to increasing doses of phenylephrine in a representative experiment. With small doses of 0.1 and 0.3 μg, phenylephrine induces a double peaked effect. Larger doses of phenylephrine produced increased degrees of either positive or negative effects. A specified dose caused a similar double peaked response repetitively in the same atrium, even though the degree varied somewhat in successive trials. The duration of the negative effects of phenylephrine was within 1 min even with increasing doses. Table 1 shows the frequency of incidence of negative chronotropic and inotropic responses in the response course of positive responses induced by different doses of phenylephrine. With 10 μg, negative effects were produced in 56 percent of all 18 atria. Table 2 shows summarized data of positive chronotropic and inotropic responses to phenylephrine.

When another adrenergic alpha-stimulant, methoxamine was administered into the

![Image](image_url)

**Fig. 1.** Chronotropic and inotropic effects of increasing doses of phenylephrine on a spontaneously beating dog atrium.

**TABLE 1.** Frequency of incidence of negative chronotropic and inotropic responses in the course of positive responses induced by different doses of phenylephrine given into the sinus node artery.

<table>
<thead>
<tr>
<th>Dose of phenylephrine (μg)</th>
<th>0.3</th>
<th>1.0</th>
<th>3.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of NCE and NIE</td>
<td>0/23</td>
<td>4/23</td>
<td>6/22</td>
<td>10/18</td>
</tr>
</tbody>
</table>

NCE: Negative chronotropic effect, NIE: Negative inotropic effect.

**TABLE 2.** Positive chronotropic and inotropic effects of increasing doses of phenylephrine on the isolated dog atrium.

<table>
<thead>
<tr>
<th>Dose of drug (μg)</th>
<th>No. of exps.</th>
<th>(A) Maximum increase in sinus rate (%)</th>
<th>(B) Maximum increase in tension developed (%)</th>
<th>Ratio (B)/(A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylephrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>23</td>
<td>4.6 ± 0.8</td>
<td>22.4 ± 4.2</td>
<td>4.9</td>
</tr>
<tr>
<td>1.0</td>
<td>23</td>
<td>15.6 ± 3.3</td>
<td>48.9 ± 7.1</td>
<td>3.1</td>
</tr>
<tr>
<td>3.0</td>
<td>22</td>
<td>26.5 ± 5.3</td>
<td>87.5 ± 9.8</td>
<td>3.3</td>
</tr>
<tr>
<td>10.0</td>
<td>18</td>
<td>39.9 ± 4.8</td>
<td>131.7 ± 12.3</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Values represent the percentage increases from the initial sinus rate (102 ± 2.0 beats/min in 23 preparations) and the developed tension.
sinus node artery, negative chronotropic and inotropic effects were dose-relatively induced in all 5 experiments. Fig. 2 shows chronotropic and inotropic effects of increasing doses of norepinephrine, phenylephrine and methoxamine. As shown in this figure, methoxamine induces only negative chronotropic and inotropic effects at any administered dose, although norepinephrine and phenylephrine produce positive effects. Summarized data on effects of methoxamine are shown in Table 3.

**Effect of adrenergic beta-receptor blocking agents, alprenolol and carteolol, a non-depressant beta-blocker, on phenylephrine-induced responses**

Phenylephrine-induced positive chronotropic and inotropic effects were completely

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**Table 3. Negative chronotropic and inotropic responses to increasing doses of methoxamine on five isolated dog atria**

<table>
<thead>
<tr>
<th>Dose of methoxamine (µg)</th>
<th>(A) Maximum decrease in sinus rate (%)</th>
<th>(B) Maximum decrease in tension developed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1.3±0.3</td>
<td>7.0±0.6</td>
</tr>
<tr>
<td>10</td>
<td>5.3±0.3</td>
<td>19.0±5.6</td>
</tr>
<tr>
<td>30</td>
<td>9.0±1.0</td>
<td>42.6±14.0</td>
</tr>
<tr>
<td>100</td>
<td>14.6±3.1</td>
<td>57.3±11.4</td>
</tr>
<tr>
<td>300</td>
<td>21.6±2.8</td>
<td>76.6±10.1</td>
</tr>
</tbody>
</table>

The mean control sinus rate was 115 beats/min in five isolated preparations.

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**Fig. 3. Blocking effect of alprenolol on phenylephrine (PE)-and norepinephrine (NE)-induced positive chronotropic and inotropic actions.**
blocked by treatment with an adrenergic beta-receptor blocking agent, alprenolol or carteolol in all 5 experiments. Fig. 3 shows that phenylephrine- and norepinephrine-induced positive effects were blocked by treatment with alprenolol. After 40 min, all responses were restored to the control level. These phenylephrine-induced positive effects were not influenced by treatment with phenolamine in all five preparations. In cases of double peaked responses to phenylephrine, phenylephrine produced only negative effects after treatment with a non-depressant beta-blocker, carteolol, as shown in Fig. 4.

Absence of blocking effect of desipramine on phenylephrine-induced positive chronotropic and inotropic responses

When desipramine at doses of 10–100 μg was injected into the sinus node artery, initially brief negative chronotropic and inotropic effects followed by long-lasting positive effects were produced as previously reported (8, 9). These dose levels of desipramine significantly inhibited tyramine-induced positive chronotropic and inotropic responses as previously reported (8, 9). In three experiments, positive chronotropic and inotropic responses to 3–10 μg of phenylephrine were not suppressed by 10–100 μg of desipramine.

Effects of atropine, tetrodotoxin and phentolamine on phenylephrine-induced negative chronotropic and inotropic responses

When atropine was administered into the sinus node artery, slight positive chronotropic and inotropic responses were induced. After treatment with atropine, phenylephrine-induced double peaked responses disappeared and only monophasic positive chronotropic and inotropic responses were produced in all three cases. Fig. 5 shows a typical experiment of the effect of atropine on phenylephrine-induced responses.

When tetrodotoxin was injected into the sinus node artery, negative chronotropic and
inotropic responses were produced (5). After treatment with 3–10 μg of tetrodotoxin, phenylephrine-induced double peaked responses to 10 μg disappeared in all three experiments. Fig. 6 shows an example of the effect of tetrodotoxin on the phenylephrine-induced responses.

When phentolamine was injected into the sinus node artery, positive chronotropic and inotropic responses were temporarily produced (10). After phentolamine treatment (30–100 μg), double peaked responses to phenylephrine (3–10 μg) became monophasic in all three experiments. Fig. 7 demonstrates the effect of phentolamine on phenylephrine-induced double peaked responses.

Absence of blocking effect of atropine, tetrodotoxin or phentolamine on methoxamine-induced negative chronotropic responses

Methoxamine-induced negative chronotropic responses with 3–10 μg were not blocked by treatment with adequate doses of atropine, 10–100 μg, tetrodotoxin, 3–10 μg or phentolamine, 30–100 μg, in two cases each.

Effects of phenylephrine and methoxamine on the isolated left ventricle

When phenylephrine was given into the isolated, left ventricle via the anterior descending branch of the left coronary artery, positive inotropic effects were dose-relatedly induced in all preparations. Fig. 8 shows an experiment of the effect of increasing doses of phenylephrine on a paced ventricular muscle. These positive inotropic effects were blocked by adequate doses of alprenolol and carteolol, but were not modified by a large amount of phentolamine (100–300 μg). On the other hand, methoxamine usually caused only negative inotropic effects at doses of 10 to 300 μg in all three preparations. These negative inotropic effects were not influenced by atropine or phentolamine.
DISCUSSION

In 1968, Govier (11) reported that phenylephrine produced a positive inotropic response in guinea pig atria, and Benfey (12) and Schumann et al. (13) also described a similar effect of phenylephrine on rabbit atria and cat papillary muscles. Since this response was at least partially blocked by phentolamine, it was considered an alpha-adrenergic response. In the present study, the spontaneously beating atrial preparation of the dog was used, and there was no evidence of the positive inotropic response to phenylephrine and methoxamine via an adrenergic alpha-mechanism. Even in the ventricular muscle, phenylephrine induced a positive inotropic effect only, through an adrenergic beta-mechanism. Furthermore, since phenylephrine-induced positive effects were not suppressed by an uptake blocker, desipramine, these effects may not be due to a release of catecholamine from adrenergic nerve terminals. Therefore, in both atrial and ventricular muscle of the dog, phenylephrine-induced positive inotropic effect may be produced through only adrenergic beta-receptors.

On the other hand, in atrial preparations, phenylephrine occasionally caused temporarily negative inotropic and chronotropic effects. It is known that sinus deceleration causes an decrease in the developed tension. In a previous paper (14), the frequency-force relationship of the canine atrium was studied, and it was noted that a 20% change in sinus rate resulted in approximately a 7% change in the tension developed within a range of 100 to 200 beats/min. In the present study, however, it was shown that phenylephrine caused a clear negative inotropic effect with a slight negative chronotropic effect. Therefore, it is considered that the negative inotropic response to phenylephrine may be not due to changes in sinus rate. Since these negative effects were completely inhibited by atropine, it is considered that phenylephrine caused a release of acetylcholine from cholinergic nerve terminals. As these negative responses to phenylephrine were blocked by tetrodotoxin, phenylephrine appears to cause an excitation of parasympathetic nerve fibers. It is well known that tetrodotoxin blocks selectively the sodium carrier system of excitable tissue, particularly nerve fibers (15, 16), and Hashimoto and Chiba (17) demonstrated that tetrodotoxin when injected into the sinus node artery blocks transmitter release caused by nerve excitation on the SA node area. Moreover, as phenylephrine-induced negative effects were blocked by an adrenergic alpha-receptor blocking agent, phentolamine, it may be concluded that an adrenergic alpha-mechanism exists in cholinergic nerve terminals. The present results differ from previously reported results in isolated guinea-pig, rabbit and cat hearts (11-13). This may be due to species differences. Endoh and Schümmer (18) reported frequency-dependence of the positive inotropic effect of methoxamine and naphazoline in the rabbit papillary muscle and Mugelli et al. (19) found that the alpha-receptor-mediated positive inotropic effect could be frequency-dependent, because phentolamine antagonized the effect of low concentrations of epinephrine in guinea pig ventricle strips driven at 1 Hz, but did not modify the curve of the agonist at the higher stimulation rate. Evidence for the role of alpha-adrenoceptors in the production of a positive inotropic effect has been chiefly obtained in heart preparations driven at a low rate (11-13, 20-22). In blood-perfused canine atrium preparations, the spontaneously beating rate is approximately 100 beats/min which is similar
to physiological heart rates of conscious dogs. Thus, it may be difficult to detect the alpha-adrenergic component of the positive inotropic effect.

James et al., using the direct perfusion method of the canine sinus node artery reported that methoxamine produced only a negative chronotropic effect (1). They also stated that atropinization had no effect on methoxamine-induced sinus deceleration, but that the deceleration could be reversed with either phenotolamine or phenoxybenzamine. They concluded that the negative chronotropic action of methoxamine is due to alpha-receptor stimulation. They did not, however, demonstrate a negative chronotropic effect of any given dose of phenylephrine, a potent alpha-stimulant. On the other hand, in 1975, Rabinowitz et al. (23) reported that in cat papillary muscle, methoxamine produced a dose-related increase in force development that was selectively blocked by alpha-adrenergic antagonists. In the present study, methoxamine induced negative chronotropic and inotropic effects in atrial and ventricular muscle preparations. These negative effects were not inhibited by either atropine or phenotolamine, which completely blocked phenylephrine-induced negative responses. Thus, methoxamine-induced negative effects may be due to direct cardiac depressant properties of the drug as reported previously (24, 25), and not mediated by an adrenergic alpha-mechanism.

Regarding chronotropism, Hashimoto et al. (3, 26, 27) and Chiba et al. (28) reported that an adrenergic alpha-mechanism may exist in cardiac cholinergic nerve terminals as determined when a modified direct perfusion of the sinus node artery was carried out (4). However, neurogenic reflex mechanisms could not be ruled out as extracardiac factors may have been involved. The results of the present work suggest that phenylephrine induces a release of acetylcholine locally from cholinergic nerve terminals through an adrenergic alpha-mechanism.

Acknowledgements: Gratitude is due to Miss M. Tsukada and Mrs. Y. Itoh for technical assistance.

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