PHARMACOLOGIC ANALYSIS OF THE RESPONSE TO THYMOXAMINE (6-ACETOXYTHYMOXY-ETHYLDIMETHYLAMINE) ON THE SINOATRIAL NODE OF THE DOG HEART

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Abstract—Using in situ constant pressure perfusion of the SA node artery at 100 mmHg, the effect of thymoxamine, which is a salt of (6-acetoxythymoxy) ethyldimethylamine, was investigated in 7 vagotomized dogs weighing 9 to 15 kg. Thymoxamine injected into the SA node artery induced a biphasic chronotropic response, i.e. a deceleration of sinus rate followed by an acceleration. The threshold dose for induction of sinus deceleration by thymoxamine was 3 to 10 μg. This deceleration response was not influenced by atropine treatment. The threshold dose for induction of sinus acceleration by thymoxamine was 10 to 30 μg. This accelerated response was inhibited by propranolol or tetrodotoxin. These results indicate that thymoxamine induces direct depressive action on the SA node pacemaker and the acceleration due to catecholamine release accompanying excitation of sympathetic nerve fibers in the SA node region.

Greef and Schümann (1) first described (6-acetoxythymoxy) ethyldimethylamine, thymoxamine, as a sympathicolytic agent to reduce or abolish the rise in blood pressure by an i.v. injection of norepinephrine in anesthetized cats. Birmingham et al. (2, 3) showed that thymoxamine is a competitive antagonist at adrenergic alpha-receptors in the in vivo and in vitro experiments.

Although alpha-adrenergic blocking agents induce commonly reflex tachycardia caused by peripheral blockade of vasomotor tone, i.v. administration of thymoxamine frequently induced a deceleration of heart rate followed by an acceleration. While heart rate is frequently modified by the direct effect of drug, it is also markedly influenced by adrenergic and cholinergic mechanisms, as the sinoatrial (SA) node area is densely innervated by sympathetic and parasympathetic nerve fibers (4). A selective administration of the drug into the SA node artery is a very useful method for investigating the direct effect of drugs on the pacemaker of SA node and also to perform pharmacological analysis on the mechanism. Thus, using an in situ blood-perfused SA node preparation of the dog heart (5–7), the effect of thymoxamine on the SA node pacemaker activity was investigated herein.

METHODS

Seven mongrel dogs of both sexes weighing 9 to 15 kg were anesthetized with sodium pentobarbital i.v. (30 mg/kg). The SA artery was perfused directly with blood led from
the femoral artery. Details of the preparation have been described in previous papers (6, 7). Flow rate in the SA node artery at 100 mmHg was \(2.8 \pm 0.6\) ml/min (mean \pm S.E.) in 6 experiments. Heart rate was continuously recorded using a cardiotachograph (Nihon Kohden, RT-2), triggered by the R wave of lead II. Both vagi were cut at the mid-cervical level.

Drug solution was injected in a volume of 0.01 to 0.03 ml for a period of 4 sec by a microinjector into the perfusion system to the SA node artery. Drugs used were thymoxamine hydrochloride (Kohjin), dl-norepinephrine hydrochloride (Sankyo), atropine sulphate, dl-propranolol hydrochloride (Sumitomo Chemicals) and tetrodotoxin (Sankyo), which were dissolved in 0.9% saline.

RESULTS

1) Effects of thymoxamine injected into the sinus node artery (Figs. 1, 2 and 3, Table 1)

When a single dose of thymoxamine was injected into the SA node artery, sinus rate was initially depressed and then accelerated. Vasodilation was usually observed when thymoxamine was injected at doses above 1 \(\mu\)g. The threshold dose for induction of sinus deceleration was 3 to 10 \(\mu\)g and sinus acceleration was approx. 10 to 30 \(\mu\)g. At the dose level of 100 \(\mu\)g to 300 \(\mu\)g, a biphasic response was usually observed. The duration of the negative chronotropic response to thymoxamine was approx. 20 sec to 1 min at the dose range of 100 \(\mu\)g to 1 mg and positive response was approx. 3 to 5 min at the same dose range. A larger dose of thymoxamine depressed SA node pacemaker activity and sinus rhythm was replaced by nodal rhythm in one out of 4 cases at a dose of 1 mg. Sinus rate was completely depressed with 3 mg of thymoxamine and nodal rhythm appeared in 2 out of 3 cases. Typical responses to increasing doses of thymoxamine are

![Fig. 1. Response patterns of the SA node to increasing doses of thymoxamine injected into the canine sinus node artery. SBP: systemic blood pressure. HR: heart rate. AVNR: AV nodal rhythm.](image-url)
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FIG. 2. Percent changes in sinus rate by increasing doses of thymoxamine.

FIG. 3. Repetition of the biphasic chronotropic response to the same dose of thymoxamine, 1 mg, injected into the sinus node artery. SBP: systemic blood pressure. HR: heart rate.

TABLE 1. Chronotropic responses of the SA node to thymoxamine.

<table>
<thead>
<tr>
<th>Dose</th>
<th>No. of dogs</th>
<th>Initial heart rate (beats/min)</th>
<th>Chronotropic responses to thymoxamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Minimum heart rate</td>
</tr>
<tr>
<td>3 µg</td>
<td>6</td>
<td>134±7.1</td>
<td>133±7.2</td>
</tr>
<tr>
<td>10 µg</td>
<td>6</td>
<td>134±7.1</td>
<td>125±7.6</td>
</tr>
<tr>
<td>30 µg</td>
<td>6</td>
<td>134±7.1</td>
<td>119±8.2</td>
</tr>
<tr>
<td>100 µg</td>
<td>6</td>
<td>134±7.1</td>
<td>106±7.8</td>
</tr>
<tr>
<td>300 µg</td>
<td>6</td>
<td>128±6.0</td>
<td>85±10.5</td>
</tr>
<tr>
<td>1 mg</td>
<td>4</td>
<td>125±6.4</td>
<td>70±12.2*</td>
</tr>
<tr>
<td>3 mg</td>
<td>3</td>
<td>123±3.7</td>
<td>61±9.7**</td>
</tr>
</tbody>
</table>

* Nodal rhythm was induced in one out of 4 cases.
** Nodal rhythm was induced in 2 out of 3 cases.
Results are given as mean±S.E.
shown in Figs. 1 and 2. A certain dose of thymoxamine caused a similar biphasic response repetitively in the same animal, even though the degree varied somewhat in successive trials. Fig. 3 shows an experiment with 1 mg of thymoxamine, in which tachyphylaxis was not seen during repetitive intra-arterial injection with 10 to 30 min interval. Sinus deceleration induced by thymoxamine was not influenced by bilateral vagotomy. Atropine (30 μg) did not prevent sinus deceleration induced by thymoxamine 30 μg to 1 mg. The data are summarized in Table 1.

2) Effect of propranolol on thymoxamine-induced acceleration of sinus rate (Fig. 4, Table 2)

Sinus acceleration with norepinephrine was inhibited by propranolol injected into the sinus node artery. Thymoxamine-induced acceleration was also blocked by propranolol. These blocking effects of propranolol disappeared after 20 min. Typical responses are shown in Fig. 4. The summarized data are in Table 2.

3) Effect of tetrodotoxin on thymoxamine-induced acceleration of sinus rate (Fig. 5, Table 3)

Injection of tetrodotoxin into the SA node artery blocked the SA nodal responses to electrical stimulation of either the right vagus or the right stellate ganglion (8). Tetro-

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**Table 2. Effect of propranolol on the positive chronotropic responses to norepinephrine and thymoxamine.**

<table>
<thead>
<tr>
<th>Dose of compounds</th>
<th>No. of dogs</th>
<th>Initial heart rate (beats/min)</th>
<th>Positive chronotropic response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>0.1 μg</td>
<td>3</td>
<td>137±3.3</td>
<td>31±15.6</td>
</tr>
<tr>
<td>Thymoxamine</td>
<td></td>
<td>137±3.3</td>
<td>12±4.2</td>
</tr>
</tbody>
</table>

Results are given as mean±S.E.
dotoxin in doses of 1 to 3 µg injected into the SA node artery caused a deceleration of the sinus rate. The accelerated response to 300 µg-1 mg of thymoxamine was inhibited by treatment with 1 to 3 µg of tetrodotoxin. Norepinephrine-induced acceleration of sinus rate was never inhibited by tetrodotoxin. The typical responses are shown in Fig. 5. After treatment with 3 µg of tetrodotoxin injected into the SA node artery, the positive chronotropic responses to 300 µg and 1 mg of thymoxamine were completely blocked. That to 0.1 µg of norepinephrine was never inhibited by tetrodotoxin.

DISCUSSION

It was demonstrated that thymoxamine induced a biphasic response, i.e. an initial deceleration followed by an acceleration of sinus rate, using an in situ direct perfusion technique of the canine SA node artery (5-7). The initial deceleration response to thymoxamine was not blocked by a large dose of atropine, indicating that thymoxamine exerts direct depressive action of the SA node pacemaker activity. On the other hand, the positive chronotropic response to thymoxamine was inhibited by both an adrenergic beta-blocking agent, propranolol, and tetrodotoxin. Hashimoto and Chiba (8) have demonstrated that a small dose of tetrodotoxin, 1 to 3 µg, completely blocked the effects of electrical stimulation of either the vagus (deceleration) or stellate ganglion (acceleration), where-

![Fig. 5. Effect of tetrodotoxin on norepinephrine- and thymoxamine-induced acceleration of sinus rate. SBP: systemic blood pressure. HR: heart rate.](image-url)
as the SA node responded to either acetylcholine or norepinephrine as usual. Thus, when an adequate dose was used, tetrodotoxin was found to be a selective blocking agent of nerve stimulation in the sinoatrial preparation. Tetrodotoxin has neither anti-cholinergic nor anti-adrenergic properties. Furthermore, it does not block catecholamine release induced by tyramine (8). The blocking effect of tetrodotoxin is therefore selectively limited to the peripheral nerve excitation. Recently, Chiba et al. (9, 10) reported that the positive chronotropic response to nicotine was blocked by treatment with tetrodotoxin but the dimethylphenylpiperazinium (DMPP)-induced positive chronotropic effect was not blocked by tetrodotoxin. This suggested that the positive chronotropic action of DMPP is different from that of nicotine on the SA node and is similar to that of tyramine. In the present study, it was found that the positive chronotropic response to thymoxamine was blocked by treatment of tetrodotoxin into the sinus node artery. It is therefore suggested that an acceleration of sinus rate induced by thymoxamine is due to catecholamine release from the SA node region being accompanied by excitation of sympathetic nerve fibers. The mechanism of the positive chronotropic response to thymoxamine is similar to that of nicotine and different from that of tyramine or DMPP.

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REFERENCES
4) James, T.N.: Anat. Record 143, 251 (1962)