PHARMACOLOGICAL STUDIES ON 8-[(1,4-BENZODIOXAN-2-YL) METHYL]-3-OXO-1-THIA-4,8-DIAZASPIRO-[4,5]-DECANE MALEATE

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Studies have been so far made on the benzodioxane derivatives as a cardiovascular drug which were reported as a peripheral vasodilator, or as a hypotensive agent by Schaper et al. (1) and Green et al. (2). 8-[(1, 4-benzodioxan-2-yl) methyl]-3-oxo-1-thia-4, 8-diazaspiro-[4, 5]-decane maleate (Y-3506) was found desirable as a peripheral vasodilator with low toxicity among the new derivatives synthesized by this laboratory. This report deals with the effects of Y-3506 on the cardiovascular system of the experimental animals in comparison with those of several known vasodilators as reference compounds.

MATERIALS AND METHODS

Compounds: Y-3506 has the following structure:

\[
\begin{align*}
\text{O} & \text{CH}_2\text{N} \quad \text{S} \quad \text{CHCOOH} \\
\text{O} & \text{H} \quad \text{---O} \\
& \text{CHCOOH}
\end{align*}
\]

8-[(1, 4-benzodioxan-2-yl) methyl]-3-oxo-1-thia-4, 8-diazaspiro-[4, 5]-decane maleate (Y-3506), melting point: 197°C. The compound is slightly soluble in water. Acute toxic potencies are: LD\textsubscript{50} of Y-3506: 3700 mg/kg p.o., 650 mg/kg i.p. in mice; 5500 mg/kg p.o., 1100 mg/kg i.p. in rats.

Isoxsuprine hydrochloride, nylidrin hydrochloride, butylsympatol hemisulfate, papaverine hydrochloride and piperoxan hydrochloride were used as reference compounds. These compounds were dissolved in physiological saline immediately before use. Acetylcholine chloride (ACh), epinephrine hydrochloride, norepinephrine hydrochloride, phenoxybenzamine hydrochloride, tolazoline hydrochloride and barium chloride (BaCl\textsubscript{2}) were used as reagents.

Animals: Species were as follows: ddN mice weighing 20 to 25 g (Gunma Jikken Dobutsu), Donryu rats weighing 150 to 250 g, (Ohsawa Shikkujo), guinea pigs weighing 200 to 300 g (Ohsawa Shikkujo) and healthy mongrel dogs weighing 10 to 12 kg (Takeda Kaseijo). All animals were adults of male sex.

Apparatuses: The respirator (made by this laboratory, variable reactance type, Japanese Patent No. 484, 718), the electronic manometer (Nihon Kohden, MP-4T), the electro-
magnetic flow meter (Nihon Kohden tube probe type, MF-2 and constructive probe type, MF-5), the pulse rate tachometer (Nihon Kohden, RT-2), the ink-writing oscillograph (Nihon Kohden, RM-150), the perfusion pump (Sigma motor, Zero Max T6SH), the thermocouple flow meter (crossed thermocouple type, Shinei Denki, Shincorder, CTE-120) and the force-displacement transducer (Nihon Kohden, SB-IT) were used.

Experiments with dogs

Dogs were anesthetized by the administration of sodium secobarbiturate into the vein of the forelimb at the dose of 30 to 35 mg/kg, followed by the maintenance dose of 2 to 5 mg/kg i.v. when necessary. The anesthetized dog was fixed on the operation desk on the back position, and heparinized (300 U/kg, i.v.), followed by the maintenance dose of 100 U/kg i.v. every 60 minutes.

Measurement of the blood flow in the femoral and coronary arteries

The adult male dog was anesthetized, fixed and heparinized by the method as mentioned above. Then, the right femoral artery was perfused with the blood supplied through the tube connected with the left femoral artery, and the left coronary artery was perfused with the blood supplied from the right femoral artery through the special cannula according to the technique of Yago (3). The perfused flow was measured by the electromagnetic flowmeter.

The respiration was measured with the respirometer, the blood pressure in the brachial artery as the systemic blood pressure with the electronic manometer and the heart rate with the pulse rate tachometer.

All of them were recorded with the ink-writing oscillograph. Y-3506 administered through the tube inserted into the left femoral vein.

Per cent increase of the blood flow was calculated by the following formula:

\[
\text{Increase (\%)} = \frac{a-b}{b} \times 100
\]

a: Maximum blood flow after the administration test compound
b: Mean blood flow before the administration

Effective dose (ED) was obtained graphically from the dose response curve as the dose corresponding to 50% increase of the blood flow.

Perfusion pressure in the femoral vessel

In the same technique as in measurement of the blood flow in the femoral artery, the right femoral artery was perfused with the blood supplied through the 4 mm polyethylene tube in diameter connected with the left femoral artery. A perfusion pump was setted in the tube between the probes of the electromagnetic flow meter (MF-2) and the electronic manometer, these being linked through the circuit of the polyethylene tube. Perfusion pressure under working of the pump was adjusted to the pressure before the treatment. Systemic blood pressure was obtained from the brachial artery. The 0.1 ml solution containing Y-3506 at several doses was administered into the femoral artery through the polyethylene tube.
Measurement of the blood flow in the muscle

The muscle gastrocnemius in the right hind-leg of the dog was exposed, and the wire-type probe of the thermocouple flow meter was inserted into the tissue along its muscle fibre at relatively shallow position, then the skin was sutured. The probe was supplied 0.3 A for heating. The femoral arterial blood flow in the same leg of the dog was also measured at the same time with the electromagnetic flow meter (MF-5).

Systemic blood pressure was obtained from the femoral artery. All of these were recorded with the ink-writing oscillograph. The value of the blood flow in the muscle was obtained in \( \mu \text{V} \). The test compound was administered intravenously.

Measurement of the cerebral blood flow

According to the method of Wakisaka et al. (4), the anesthetized dog was laid on its back, and the cervical incision was made widely along the midline. All main arteries and their branches in the cervix were separated from the circumscribed tissues, and ligated except the right and left carotid artery. The circuit was made between the common carotid and internal carotid arteries with the probes of the electromagnetic flow meter (MF-2) and the electromanometer. Y-3506 and reference compounds were injected into the femoral vein through the polyethylene cannula. The result was showed as per cent increase of the blood flow calculated by the same formula as in the femoral blood flow.

Measurement of the renal blood flow

According to the method of McNay and Goldberg et al. (5), the right renal artery of the dog under laparotomy was connected with the probe of the electromagnetic flow meter (MF-5). The test compound was administered into the femoral vein through the polyethylene cannula. The femoral blood flow was also measured simultaneously by means of the electromagnetic flow meter (MF-5). Systemic blood pressure was obtained from the femoral artery.

Experiments with the isolated organs

1. Anti-acetylcholine effect (guinea pig, ileum)

   The isolated ileum was suspended in Tyrode's solution bubbled with air at 32°C. The tension on each preparation was approximately 0.5 g. According to the method of Van Rossum (6), the inhibitory effect of the compounds on the ACh-induced contraction of the ileum represented as \( pA_2 \) value for competitive antagonisms and as \( pD_2^* \) value for non-competitive antagonisms.

2. Anti-norepinephrine and anti-epinephrine effect

   a) Anti-norepinephrine effect (guinea pig, vas deferens)

   The preparation was suspended and tested in the same manner described in the term of Anti-ACh. The inhibitory effects of the compounds on the norepinephrine-induced contraction at the concentration of \( 3 \times 10^{-4} \) g/ml were represented as inhibitory concentration \( (IC_{50}; \mu g/ml) \) corresponding to 50% decrease in the contraction in comparison with the control.

   b) Anti-epinephrine effect (rat, vas deferens)

   The experiment was carried out in the same procedure as mentioned above. Ac-
<table>
<thead>
<tr>
<th>Dose (mg/kg i.v.)</th>
<th>Y-3506</th>
<th>Isosuprine</th>
<th>Piperoxan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Femoral blood flow</td>
<td>Coronary blood flow</td>
<td>Systemic blood pressure</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>31.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>75.8</td>
<td>5.6</td>
<td>0</td>
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<tr>
<td>25</td>
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<td>0</td>
</tr>
<tr>
<td>50</td>
<td>104.6</td>
<td>2.6</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>142.6</td>
<td>14.4</td>
<td>4.2</td>
</tr>
<tr>
<td>250</td>
<td>187.3</td>
<td>14.7</td>
<td>4.7</td>
</tr>
</tbody>
</table>

N*: Number of animals.

<table>
<thead>
<tr>
<th>Dose (mg/kg i.v.)</th>
<th>Nylidrin</th>
<th>Papaverine</th>
<th>Butylsympatol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Femoral blood flow</td>
<td>Coronary blood flow</td>
<td>Systemic blood pressure</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>5.5</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>19.5</td>
<td>11.5</td>
<td>2</td>
</tr>
<tr>
<td>25</td>
<td>25</td>
<td>16.5</td>
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<td>10</td>
<td>43.5</td>
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<td>10</td>
</tr>
<tr>
<td>250</td>
<td>44.5</td>
<td>23</td>
<td>15.5</td>
</tr>
<tr>
<td>500</td>
<td>84.8</td>
<td>67.2</td>
<td>5.2</td>
</tr>
</tbody>
</table>

N*: Number of animals.
According to the cumulative method, the inhibitory effects of the compounds of the epinephrine-induced contraction were shown as $pA_2$ value for competitive antagonisms and as $pD_2^*$ value for non-competitive antagonisms.

3. Anti-epinephrine and anti-barium effect (guinea pig, aorta strip)

According to the method of Furchgott (7), the screw-shaped preparation of the descending aorta was suspended in Tyrode's solution bubbled with air at 37°C. The tension of the preparation was approximately 0.5 g. Epinephrine ($3 \times 10^{-7}$ g/ml) and barium chloride ($5 \times 10^{-4}$ g/ml) were used as the spasmogen. The result was represented as $IC_{50}$ in the same manner described 2.a).

4. Experiments with the atrial preparations

According to the method of Nose et al. (8), the isolated atria of guinea pig weighing about 200 g were suspended in Tyrode's solution at 32°C bubbled with 5% CO$_2$ in oxygen. The tension on each atrium was approximately 0.5 g. Spontaneous beats and isotonic contraction of the preparation were recorded with ink-writing oscillograph through the pulse rate tachometer, and the force-displacement transducer.

RESULTS

1. The effect on the blood flow

a) Effect on the blood flow in the femoral artery

The blood flow in the femoral artery was increased by test and reference compounds administered intravenously (Table 1-a and b, Fig. 1).

Y-3506 showed a remarkable effect to increase the femoral blood flow, which appeared at a dose of 2.5 μg/kg or more. The blood flow was increased immediately after the injection and the effect was dependent on the dose amount (Table 1-a and Fig. 1), and lasted

![Figure 1](image-url)
for 3 to 4 minutes. There were little influences on the respiration, the systemic blood pressure, the coronary blood flow and the heart rate each measured simultaneously (Fig. 2).

As shown in Tables 1-a, b and Fig. 1, isoxsuprine, piperoxan, nylidrin, papaverine and...

![Fig. 2. Effect of Y-3506 on femoral blood flow in an anesthetized dog.](image1)

![Fig. 3. Effect of Y-3506 on peripheral vascular resistance of the femoral artery in an anesthetized dog. The compound was administrated into the femoral artery. Abbreviations are the same as in Fig. 2.](image2)
butylsympatol also increased the blood flow in dependent on the dose amount. These compounds showed a similar pattern of the reaction to Y-3506, while the action of nyliodrin and butylsympatol lasted somewhat longer than that of Y-3506. The effect of isoxsuprine was turned down at doses higher than 50 μg/kg.

In increasing the blood flow in the femoral artery, Y-3506 showed more stable response than any other compounds, and its effect was more potent than that each of nyliodrin, papaverine and butylsympatol.

![Graph showing blood flow response to Y-3506](image)

**FIG. 4.** Effect of Y-3506 on the blood flow measured by thermocouple flowmeter in muscle in an anesthetized dog.

**TABLE 2.** Increasing effects on the blood flow in the muscle gastrocnemius and femoral artery measured simultaneously.

The mean value of each experiment animal is as follows.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose</th>
<th>Femoral blood flow % increase</th>
<th>Blood flow in the muscle gastrocnemius μV increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-3506 (N=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>38.8</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>86.0</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>103.2</td>
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<td></td>
</tr>
<tr>
<td>100</td>
<td>127.9</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>146.7</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Piperoxan (N=2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>114.0</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>137.3</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>130.0</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>16.8</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Isoxsuprine (N=2)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2.5</td>
<td>55.3</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>85.8</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>113.5</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Papaverine (N=3)</td>
<td></td>
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<tr>
<td>500</td>
<td>95.6</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>105.1</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>2500</td>
<td>146.8</td>
<td>5.2</td>
<td></td>
</tr>
</tbody>
</table>
b) Effect of Y-3506 on the perfusion pressure in the femoral vessels

The perfusion pressure was decreased by 20 to 50 mmHg by the administration of Y-3506 to the intrafemoral artery at doses of 0.1 to 0.5 μg/body (Fig. 3). There was no or little influence on the systemic blood pressure at these doses.

c) Effect of Y-3506 on the blood flow in the muscle

Y-3506 increased on the blood flow in the muscle gastrocnemius in a similar pattern of the reaction to the femoral blood flow (Fig. 4).

As shown in Table 2, piperoxan, isoxsuprine and papaverine also increased the muscle blood flow similarly as Y-3506. The response each of these three compounds was the same as in the femoral blood flow measured simultaneously.

d) Effect on the cerebral circulation

The increasing effect on the cerebral blood flow of Y-3506 appeared at dose of 10 to 25 μg/kg, lasting for about 2 minutes (Fig. 5).

Isoxsuprine and papaverine also increased the cerebral blood flow. As shown in Table 3 and Fig. 6, the effect of isoxsuprine was more potent than that of Y-3506, though

<table>
<thead>
<tr>
<th>Dose μg/kg i.v.</th>
<th>Y-3506 % increase</th>
<th>Papaverine % increase</th>
<th>Isoxsuprine % increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dog No. 1 2 3 4 Mean</td>
<td>Dog No. 5 6 7 Mean</td>
<td>Dog No. 8 9 10 Mean</td>
</tr>
<tr>
<td>25</td>
<td>18 15 12 15 11 9 10 12 4 16 10.7</td>
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<tr>
<td>50</td>
<td>46 14 22 30 28 25 15 19 19.7 10 10 8 9.3</td>
<td></td>
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<tr>
<td>100</td>
<td>43 31 34 30 34.5 33 15 31 26.3 15 5 10</td>
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</tr>
<tr>
<td>250</td>
<td>40 40 40 50 30 55 45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5. Effect of Y-3506 on cerebral blood flow in an anesthetized dog.
the potency of isoxsuprine was turned down at doses of 25, 50 and 100 μg/kg.

Nylidrin did not increase on the cerebral blood flow at doses of 2.5 to 25 μg/kg, but decrease by the administration of the compound. Also the hypotensive response of nylidrin was observed simultaneously.

Concerning the systemic blood pressure, the transient hypotension was observed for about 2 minutes by the administration of Y-3506 and papaverine, and the hypotension induced by isoxsuprine and nylidrin lasted longer than that by Y-3506 and papaverine. The hypotensive action of Y-3506 was weaker than that of each nylidrin and isoxsuprine, and stronger than that of papaverine.

e) Effect on the blood flow in the coronary artery

The effect of Y-3506 administered intravenously on the coronary blood flow was less potent than that on the femoral blood flow (Table 1-a, Figs. 2 and 7). Even at a dose of 250 μg/kg, the coronary blood flow was increased transiently only by 14.7 per cent.

Isoxsuprine and piperoxan increased transiently the coronary blood flow at doses of 1.0 or 2.5 μg/kg. Papaverine increased the coronary blood flow at the dose of 50 μg/kg and this effect was similar to or more potent than that on the femoral blood flow. And the effect of papaverine on the coronary blood flow lasted more than that on the femoral blood flow. Nylidrin and butylsympatol increased the coronary arterial blood flow at a dose each of 1.0 and 50 μg/kg, and this effect lasted for 7 to 15 minutes (Table 1-a and b, Fig. 7).

The effect of Y-3506 on the coronary arterial blood flow was the least potent in all the compounds.
Fig. 7. Increasing effects of Y-3506 (---), isoxsuprine (---x---), piperoxane (---○---), nylidrin (---△---), papaverine (---□---) and butylsympathol (---□---) on coronary blood flow in anesthetized dogs.

f) Effect on the renal blood flow

Y-3506 decreased transiently the blood flow in the renal artery at doses of 10 to 100 μg/kg (Fig. 8). Piperoxan and isoxsuprine also decreased the blood flow at doses of 10 to 100 μg/kg and 2.5 to 10 μg/kg, respectively. In all dogs, the hypotensive response by these compounds was accompanied with the decrease in the blood flow. In one case out of the three, papaverine increase the renal blood flow at doses of 500 to 2500 μg/kg after the transient fall of the blood pressure and decrease in blood flow by the treatment.

![Graph of renal blood flow](image-url)

Fig. 8. Effect of Y-3506 on renal blood flow in an anesthetized dog.
2. Effect on the systemic blood pressure

Y-3506 had no effect on the systemic blood pressure at the dose lower than 10 \( \mu \text{g/kg} \). The systemic blood pressure was fall slightly at doses of 50 \( \mu \text{g/kg} \), and at a high dose of 250 \( \mu \text{g/kg} \) it was lowered by 4.7 per cent (Table 1-a, Figs. 2 and 9).

Nylidrin, isoxsuprine and piperoxan decreased the systemic blood pressure at a dose of 2.5 \( \mu \text{g/kg} \). Butylsympatol and papaverine decreased the systemic blood pressure at a dose each of 50 and 100 \( \mu \text{g/kg} \) (Table 1-a and b, Fig. 9).

Hypotensive effect each of Y-3506, piperoxan, isoxsuprine and papaverine administered intravenously was transient, the effect each of nylidrine and butylsympatol lasted for 5 to 10 minutes.

3. Effect on the heart rate

Y-3506 had little effect on the heart rate at the dose lower than 2.5 \( \mu \text{g/kg} \). At doses of 5 to 10 \( \mu \text{g/kg} \), the heart rate was increased slightly and transiently, and was increased by about 20\% at a dose of 250 \( \mu \text{g/kg} \).

Nylidrin, isoxsuprine and piperoxan increased the heart rate at a dose of 1.0 to 2.5 \( \mu \text{g/kg} \). Butylsympatol and papaverine increased the heart rate at the dose of 25 and 50 \( \mu \text{g/kg} \), respectively. Although the increasing effects on the heart rate of Y-3506, piperoxan and isoxsuprine were transient, those of nylidrin, butylsympatol and papaverine lasted for 5 to 10 minutes.

4. Experiment using the isolated organs

a) Anti-acetylcholine effect on the ileum

Y-3506 antagonized non-competitively the ACh-induced contraction of the ileum. The potency was approximately 1/10 as potent as that of papaverine.
Piperoxan and butylsympatol showed $pA_2$, and did not obtain any $pD_2^+$ value on the ileum. Tolazoline did not affect on the ileum up to the concentration $3 \times 10^{-5}$ M/ml.

b) Anti-norepinephrine and anti-epinephrine effects on the vas deferens

Y-3506 had the adrenolytic activity on the vas deferens of the guinea pig and the rat. The potency was almost same to that of tolazoline except in the case of phenoxybenzamine, all compounds tested caused reversible response on the vas deferens.

Papaverine antagonized the norepinephrine-induced contraction of the vas deferens of the guinea pig, and showed the non-competitive antagonism also in the vas deferens of the rat.

c) Anti-epinephrine and anti-barium effects on the descending aorta strip

The anti-epinephrine effect of the adrenolytic agents on the aorta strip coincided with the effect on the preparations of the vas deferens. The effect of Y-3506 was approximately 1/10 as potent as that of piperoxan and isoxsuprine.

The anti-barium effect of Y-3506 was 1/10 as potent as that of papaverine, and 1/3 to 1/4 as potent as that of isoxsuprine. Y-3506 had no activity of contraction of the aorta strip up to the concentration of $10^{-4}$ g/ml. Piperoxan and tolazoline induced the contraction at the concentration of $10^{-4}$ g/ml.

TABLE 4. Effects on the isolated organ preparations. The value of $pA_2$ and $pD_2^+$ were calculated according to the method of Van Rossum (6). $IC_{50}$ represents the inhibitory concentration corresponding to 50\% decrease of the contraction in comparison with control.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Anti-ACh $pA_2$</th>
<th>Anti-norepinephrine $IC_{50}$</th>
<th>Anti-epinephrine $pA_2$</th>
<th>Anti-epinephrine $IC_{50}$</th>
<th>Anti-Barium $IC_{50}$</th>
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</thead>
<tbody>
<tr>
<td>Preparations</td>
<td>Ileum of guinea pig</td>
<td>Vas deferens of guinea pig</td>
<td>Vas deferens of rat</td>
<td>Aorta of guinea pig</td>
<td></td>
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<td>6.2</td>
<td>13</td>
<td>30</td>
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<td>0.9</td>
<td>6.8</td>
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<td>&gt;100</td>
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<td>10</td>
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<td>20</td>
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<td>Phenoxybenzamine</td>
<td>0.003</td>
<td>9.4</td>
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</table>

d) Effects on the atrial preparations

Y-3506 and piperoxan showed negative ino- and chronotropic actions at concentrations of $10^{-5}$ to $10^{-4}$ g/ml. In the case of isoxsuprine, on the contrary, positive ino- and chrono-tropic actions were obtained at concentrations of $3 \times 10^{-7}$ to $10^{-6}$ g/ml.

DISCUSSION

Effects of a new compound, 8 [[(1, 4-benzodioxan-2-yl) methyl]-3-oxo-1-thia-4, 8-diaza-6,6-decan maleate (Y-3506) on the cardiovascular system was studied and compared with several vasodilators by using the anesthetized dog.

The effect of Y-3506 to increase the femoral blood flow was more potent than that
of nylidrin, papaverine and butylsympatol. The potency of Y-3506 on the femoral blood flow was less than that of isoxsuprine and piperoxan, and the influence of Y-3506 on the systemic blood pressure was the least of all the compounds tested. Y-3506 had a remarkable effect to increase the femoral blood flow without any undesirable effects on the systemic blood pressure.

It is known that the effects of isoxsuprine and piperoxan on the systemic blood pressure are weak in general [Goodman (10)]. However, the hypotensive effect of these compounds was more potent than that of Y-3506. The effect of isoxsuprine was weakened at the dose higher that 50 μg/kg, and in piperoxan the dose-response curve became flat at doses of 10 to 100 μg/kg. These phenomena may be attributed to their hypotensive effects in the systemic blood pressure.

There is a close relation between the systemic blood pressure and the peripheral blood flow. The decrease in the systemic blood pressure influences directly on the peripheral blood flow [Garattini (9)]. In this aspect, the dose corresponding to 10% decrease of the systemic blood pressure (A) and the dose corresponding to 50% increase of the femoral blood flow (B) were obtained graphically from each dose-response curve (Figs. 1 and 9), and then the ratio between the two values was calculated. This ratio was expressed as the therapeutic index. As shown in Table 5, therapeutic index of Y-3506 was more than 56.8, and this value was the largest of all the compounds tested. It was suggested that beneficial effect to increase the blood flow was expected in Y-3506.

<table>
<thead>
<tr>
<th></th>
<th>Y-3506</th>
<th>Piperoxan</th>
<th>Isoxsuprine</th>
<th>Nylidrin</th>
<th>Butylsympatol</th>
<th>Papaverine</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>4.4</td>
<td>1.4</td>
<td>1.8</td>
<td>360</td>
<td>230</td>
<td>38.6</td>
</tr>
<tr>
<td>(B)</td>
<td>&gt;250</td>
<td>&gt;250</td>
<td>12</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

The perfusion of the hindlimb of the anesthetized dog with constant flow using the perfusion pump, was performed to see the effect of Y-3506 on the peripheral resistance. The perfusion pressure was decreased remarkably after the administration of Y-3506. The effect of Y-3506 to increase the peripheral flow may be cause to the decrease of peripheral resistance.

The blood flow in the muscle gastrocnemius supplied from the saphenous artery was measured using the crossed thermocouple flow meter, and the result was compared with the effect to increase the femoral blood flow of the same dog using the square wave flow meter. The change of the heat current in the crossed thermocouple flow meter was represented as the change of the direct current potential (μV). The blood flow in the muscle was increased as same as in the femoral blood flow by the systemic administration of Y-3506.

Papaverine increases the cerebral blood flow and decreases cerebrovascular resistance.
in normal subjects, perhaps by its direct vasodilating action on the cerebral blood vessels [Goodman (10)]. Y-3506 also increased the cerebral blood flow of the anesthetized dog, and its effect was more potent than that of papaverine. Isoxsuprine increased the cerebral blood flow at a low dose (2.5-10 μg/kg) more potent than Y-3506, but at a high dose (25-100 μg/kg) its effect was weakened, perhaps by its remarkable hypotensive action on the systemic blood pressure. Although nylidrin was reported to increase the cerebral blood flow in man [Eisenberg (11)], it did not increase the blood flow in this experiment. This may be due to differences in response between man and animal or to experimental conditions.

The coronary and renal blood flows in the anesthetized dog were measured to study the organ vessels selectivity of Y-3506. Y-3506 exhibited slight action on the coronary blood flow and showed little effect on the renal blood flow. The effect each of isoxsuprine, piperoxan and papaverine to increase the coronary blood flow was more potent than of Y-3506. Papaverine, especially, increased markedly the coronary blood flow, and its increasing effect was almost equal to that on the femoral blood flow. Nylidrin and butylsympatol also showed the effect to increase the coronary blood flow, which lasted for relatively long minutes. Y-3506 may possess the higher selectivity on the peripheral blood vessels than these compounds.

Benzodioxan derivatives were reported to possess less potent adrenolytic properties than halokylamines and ergotalkaloids [Shaper et al. (1); O'Leary (12); Fourneau et al. (13); Vleeschhouwer (14)]. In the preparations of the vas deferens of the guinea pig and the rat, Y-3506 showed the adrenergic α-receptor blocking action. The muscular tropic antispasmodic effect of Y-3506 was observed in the preparation of the ileum and the aorta strip like papaverine. This antispasmodic action of Y-3506 was equal to 1/10 of the action of papaverine. Piperoxan constricted the blood vessel at a high concentration in the preparation of the aorta strip. Such constriction of the blood vessel was not seen in Y-3506 at any concentrations. In this point, the pharmacological properties of Y-3506 were different from those of piperoxan. Adrenergic α-receptor blocking actions of Y-3506, piperoxan and isoxsuprine were reversible. They were essentially different from phenoxybenzamine possessing the irreversible property.

From these results, it was concluded that the effect of Y-3506 to increase the peripheral blood flow might be attributable at least to its adrenolytic and papaverine-like actions. Y-3506 had a potent selective peripheral vasodilating activity at the dose which did not affect the systemic blood pressure and the heart rate, and was low in toxicity. Clinical trial of this compound is under way.

**SUMMARY**

The effects of 8-[(1, 4-benzodioxan-2-yl) methyl]-3-oxo-l-thia-4, 8-diazaspiro-[4, 5]decane maleate (Y-3506) were studied on the cardiovascular system of the anesthetized dog.

Y-3506 increased the blood flow in the femoral artery at the dose of 2.5 μg/kg i.v.
or more, and the effective dose corresponding to 50% increase of the blood flow was 4.4 
µg/kg. This compound had the higher potency than nylidrin or papaverine.

The blood flow in the muscle gastrocnemius measured with the crossed thermocouple
flow meter was increased proportionately with the femoral blood flow.

Y-3506 increased the cerebral blood flow at the dose of 10 µg/kg i.v. or more. The
potency was higher than that of papaverine. The activity of this compound to increase
the peripheral blood flow may be due to decrease of resistance of the peripheral vessels.
The compound showed little influence at the effective dose on the coronary blood flow,
on the systemic blood pressure or on the heart rate.

In the preparation of the isolated organs, Y-3506 had an antispasmodic action like
papaverine and an α-adrenergic receptor blocking activity, and had negative ino- and
chronotropic actions in the preparation of the isolated atria.

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