The studies on structure and action of 3-substituted sydnonimines and their ring-opened derivatives (1, 2) indicated that hypotensive action induced by the compounds was closely related to the chemical stabilities of their sydnonimine rings and ring-opened derivatives. That is, a ring-opened derivative of 3-morpholinosydnonimine hydrochloride (SIN-1), N-nitroso-N-morpholinoaminoacetonitrile (SIN-1A), is unstable but a rapid-acting and very potent vasodilator comparable to nitroglycerin. On the other hand, N-nitroso-N-cyclohexylaminoacetonitrile is stable and proved to be inactive. SIN-1 has less rapid but longer hypotensive action than SIN-1A. The former is chemically stable to acid but suffers ring-opening in alkali, leading to the formation of the latter and to the release of NO ion (3). It was also revealed that N-acylation of imino group of sydnonimine greatly increased chemical stability (4) and resulted in gradually developing and prolonged hypotensive action as well as in decreased toxicity.

This paper mainly reports hemodynamic action of N-ethoxycarbonyl-3-morpholinosydnonimine SIN-10, one of the least toxic, mild- and long-acting N-acylated sydnonimines. SIN-10 is a white, crystalline substance with a molecular weight of 242.2 and a melting point of 140 to 141°C. It is soluble in water as a 1.45% solution at room temperature and has the following structural formula.

\[
\text{O} \quad \text{N} \quad \text{N} \quad \text{-CH} \\
\text{N} \quad + \quad \text{C} \quad = \quad \text{N} \quad \text{-CO} \cdot \text{OC}_2 \cdot \text{H}_5
\]

**METHODS**

1. Blood pressure, heart rate and respiration in anesthetized animals

Cats of either sex, weighing from 2 to 4 kg, were anesthetized with chloralose (40 mg/kg i.v.) and urethane (250 mg/kg i.v. and 250 mg/kg i.p.). Mongrel dogs of either sex, weighing from 8 to 13 kg, were anesthetized with sodium pentobarbital 30 mg/kg i.v. and supplemented with doses of 2.5 to 5 mg/kg i.v. whenever necessary during the experiment. Blood pressure was recorded from cannulated femoral or carotid artery after inserting an endotracheal tube. The arterial cannula was filled with heparinized saline.
solution and attached to a pressure transducer (Nihon Kohden LPU-0.5). The transducer was connected to an E & M or Nihon Kohden carrier preamplifier and blood pressure was recorded with an E & M or Nihon Kohden polygraph. Heart rate was monitored continuously with an ECG-triggered Nihon Kohden tachograph. Respiratory movement and/or rate was recorded by an E & M impedance pneumograph or from a thermocouple inserted into a tracheal cannula.

2. Coronary circulation and left ventricular dp/dt in anesthetized, open-chest dogs

Under artificial respiration with room air the chest was opened through the fifth left intercostal space. After heparinization (1,000 units/kg i.v.), the circumflex branch of the left coronary artery was proximally ligated and the distal segment immediately cannulated with a bent polyethylene cannula and perfused with the blood flowing through an electromagnetic flowmeter (Nihon Kohden MF-2) from the left carotid artery. In constant flow perfusion experiments of the coronary artery, a constant flowpump (Sigmamotor Model T-8) was inserted in series with the flowmeter. Before activating the pump, the rate of the blood flow entering the cannulated coronary artery was obtained and then the rate was kept constant by the pump. Coronary perfusion pressure was recorded from a Nihon Kohden transducer inserted between the pump and the coronary artery.

Left ventricular pressure pulse was measured with a polyethylene catheter introduced into the ventricle through the left auricle and the rate of rise of the ventricular pressure pulse (LV dp/dt) was recorded through a differential electrical circuit with time constant of 2.5 msec.

3. Femoral circulation in anesthetized dogs

After heparinization, blood flow through the femoral artery was measured by interposing the flowmeter between the proximal and distal cut ends of the artery. Perfusion experiments of innervated or denervated (5) hind limb consisted of similar cannulation of the femoral artery but instead of the flowmeter, the constant flowpump was inserted to supply the peripheral femoral artery with blood from the iliac artery. The rate of blood flow was adjusted at the beginning of each experiment to give a perfusion pressure similar to the mean systemic arterial pressure in innervated limbs or 100 to 120 mmHg in denervated limbs. Perfusion pressure was measured from a side arm in the tubing between the pump and the perfused artery.

4. Blood pressure and heart rate in unanesthetized normotensive and spontaneously hypertensive rats

Experiments were carried out in female spontaneously hypertensive (Okamoto and Aoki) (6) and normotensive Wistar rats weighing 200 to 260 g (23 to 27 weeks of age). Systolic blood pressure was measured in unanesthetized animals using Nakao et al.'s tail cuff method (7). Heart rate was calculated from the amplified pulse waves taken during the blood pressure measurement. The measurements were carried out at one hour before and at 1, 3 and 5 hours after drug administration.

5. Nictitating membrane in anesthetized cats

Nictitating membrane responses from anesthetized cats were recorded with a force-displacement transducer (Nihon Kohden SB-1T) and the Nihon Kohden polygraph.
Resting tension on the membrane was approximately 3 g. Preganglionic stimulation of cut end of the cervical sympathetic nerve was maintained for 10-second period with square wave pulses of submaximal voltage (2.5 to 5 V) at a frequency of 20 per second and duration of 0.2 msec.

6. Isolated, spontaneously beating guinea pig atria

Atria were dissected from the heart of guinea pig (300 to 450 g body weight) killed by a blow on the head. They were suspended in a 40 ml bath containing oxygenated Locke solution maintained at 37°C. The amplitude of isometric contractions (resting tension of 0.5 g) and the rate of spontaneous contraction were recorded utilizing the Nihon Kohden force-displacement transducer and tachograph.

7. Guinea pig ventricular strip

The right ventricular wall was dissected from the heart of guinea pig (250 to 350 g body weight) killed by a blow on the head. The ventricular strips were suspended in a 40-ml bath containing oxygenated Locke solution maintained at 37°C. Electrical stimulation was provided by a Nihon Kohden electronic stimulator (MSE-3) into stainless-steel stimulating electrodes, to which the end of the strip was attached. The strips were stimulated by rectangular-wave pulses of 1-msec duration and 5 mA strength at a frequency of 1 to 2/sec at a constant resting tension of 750 mg. The contractile tension was measured by means of the Nihon Kohden force-displacement transducer and was recorded on the polygraph.

8. Rabbit ileum

Isolated rabbit ileum was suspended in a 50-ml bath containing aerated Tyrode solution maintained at 37°C and the amplitude of isotonic contractions was recorded on a smoked drum via a lever.

9. Guinea pig ileum

A distal section of guinea pig ileum was suspended in a 20-ml bath containing aerated Tyrode solution maintained at 32°C and contractile activity was recorded on a smoked drum via an isotonic lever. The following concentrations (ug/ml) of spasmogens were used: barium chloride, 20; acetylcholine chloride, 0.01; and histamine hydrochloride, 0.01.

10. Drugs used and methods of administering drugs

Drugs used were: atropine sulfate (Merck), 1-epinephrine hydrochloride (Sankyo), dl-norepinephrine hydrochloride (Sankyo), 1-isoproterenol hydrochloride (Nikken Kagaku), acetylcholine chloride (Merck), histamine hydrochloride, barium chloride, synthetic angiotensin II, glyceryl trinitrate (Nitroglycerin, Nihon Kayaku), isosorbide dinitrate (Nitrol, Ehsai), 2-aminoethyl nitrate-p-toluensulfonate (Cardisan, Takeda), and pentaerythritol tetranitrate (Pentritol, Tokyo Tanabe; Hasethrol, Shionogi).

For sublingual administration of SIN-10 and the nitrates to anesthetized dogs, the crushed tablets or granules were placed on the buccal mucosa and were moistened with a few drops of water. The mixture was rubbed into the mucosa with a spatula. The crystals of SIN-10 and 2-aminoethyl nitrate-p-toluensulfonate were diluted with mannitol and were administered as described above. For oral administration of the drugs, animals
were starved for one day before experiments. The crushed tablets, granules or the crystals diluted with mannitol were administered to anesthetized dogs with 10 ml of water by a stomach tube.

SIN-10 was dissolved in saline for intravenous or intraarterial injection. Other drug doses are expressed in terms of the salt.

RESULTS

1. Blood pressure and heart rate in anesthetized animals

   a) Dogs: When SIN-10 was given in a single rapid intravenous injection (0.01 to 2.0 mg/kg) systolic blood pressure gradually decreased in one or two minutes after the administration and then diastolic pressure was depressed. Doses as low as 0.0125 to 0.025 mg/kg produced slight hypotensive responses, as shown in Table 1. The hypotension induced by the drug in a dose range from 0.025 to 2.0 mg/kg was usually greatest 20 to 30 minutes after administration of the drug and persisted for 1 to 6 hours depending on the dose given, although the duration of action varied from animal to animal depending on depth of anesthesia. In any dosage, pulse pressure usually decreased in association with a greater fall in systolic pressure. Heart rate generally tended to increase but sometimes decreased after injection of the drug. The respiratory change observed was a slight hyperpnea during the hypotension.

   FIG. 1. Effect of SIN-10 on carotid blood pressure (BP), respiration (RESP), heart rate (HR) and ECG in an anesthetized cat.

| Dog No. | Dose, i.v. (mg/kg) | % Change of blood pressure | Time to peak fall (min) | Duration of action $T^{1/2}$ (min) |
|---------|-------------------|--------------------------|------------------------|----------------------------------|
| 67021   | 0.0125            | -10.9/-5.3*              | 13                     | 25                               |
| 67023   | 0.025             | -11.2/-1.8               | 25                     | 100                              |
| 67025   | 0.025             | -11.8/-5.5              | 20                     | 120                              |
| 67022** | 0.025             | -9.9/-18.4              | 27                     | 35                               |

* Systolic and diastolic blood pressure.
** Artificially ventilated and open-chest dog.
Fig. 2. Effect of SIN-10 on femoral blood pressure (BP), heart rate (HR), coronary blood flow (Cor. F.), left ventricular dp/dt (LV dp/dt) and left ventricular pressure (LP) in an anesthetized, open-chest dog.

Table 2. Effect of SIN-10 0.1 and 0.5 mg/kg i.v. on blood pressure, peak LV dp/dt, coronary blood flow or coronary perfusion pressure and heart rate in 7 anesthetized, open-chest dogs.

|                  | Control       | 5 min          | 15 min         | 30 min         | 120 min        |
|------------------|---------------|----------------|----------------|----------------|----------------|
|                  | 0.1 mg/kg i.v.|                |                |                |                |
| Blood pressure   | (systolic/diastolic, mmHg) | 167.8/114.3 ± 6.6/3.9 | -14.1/-11.1 ± 3.6/3.4 | -21.8/-17.0 ± 5.1/4.3 | -21.6/-16.1 ± 6.1/6.5 | -10.9/-10.0 ± 5.3/5.9 |
| Coronary flow    | (ml/min)      | 30.5 ± 5.8     | -14.5 ± 9.3    | -36.0 ± 10.9   | -25.8 ± 11.1   | -27.3 ± 9.7       |
| Peak LV dp/dt    | (mmHg/sec)    | 3318 ± 627     | 0.4 ± 5.8      | -16.8 ± 11.2   | -9.8 ± 11.2    | 8.3 ± 10          |
| Heart rate       | (beats/min)   | 177.8 ± 14.0   | 4.8 ± 3.4      | 0.6 ± 2.2      | 0.9 ± 2.2      | 3.3 ± 4.9         |
|                  | 0.5 mg/kg i.v.|                |                |                |                |
| Blood pressure   | (systolic/diastolic, mmHg) | 132.6/99.6 ± 3.9/5.9 | -23.2/-22.9 ± 8.1/6.8 | -37.2/-38.9 ± 7.1/6.5 | -33.2/-36.2 ± 6.4/6.5 | -15.5/-16.5 ± 5.8/7.6 |
| Coronary perfusion pressure (mmHg) | 151.3 ± 10.6 | -3.2 ± 3.4 | -9.2 ± 1.6 | -9.8 ± 4.2 | -0.3 ± 1.5 |
| Peak LV dp/dt    | (mmHg/sec)    | 3367 ± 88      | -9.3 ± 18.2    | -16.7 ± 11.9   | -24 ± 9.5      | -9.6 ± 15.7       |
| Heart rate       | (beats/min)   | 196.2 ± 13.8   | 1.3 ± 3.1      | -8.0 ± 5.0     | -10.0 ± 5.7    | -2.8 ± 3.6        |
b) Cats: Effect of SIN-10 on blood pressure in anesthetized cats was similar in nature to that in anesthetized dogs. Fig. 1 shows the effect of 2 mg/kg of the drug. The hypotension induced by SIN-10 was not significantly affected by the acute pretreatment of cats with atropine (1.0 mg/kg i.v.), bilateral cervical vagotomy, spinal cord section (C-2) with cervical vagotomy, or evisceration.

2. Coronary circulation and peak LV dp/dt in anesthetized, open-chest dogs

Effects of SIN-10 (0.1 and 0.5 mg/kg i.v.) on coronary circulation and peak LV dp/dt

| TABLE 3. Effect of nitroglycerin 0.6 mg/dog sublingual on blood pressure, peak LV dp/dt, coronary perfusion pressure and heart rate in anesthetized dogs. |
|---|---|---|---|
| Control | 1-2 min | 3 min | 10 min |
| Blood pressure (syst/diast, mmHg) | 114.3/76.0* | -22.0/-28.4 | -20.2/-18.9 | -6.4/-10.8 |
| Coronary perfusion pressure (mmHg) | 156.0 | -17.7 | -15.2 | +0.2 |
| Peak LV dp/dt (mmHg/sec) | 3120, 3620 | -13.5, +56 | -31.0, +11 | -4.9, -13.5 |
| Heart rate | 187.8 | +3.3 | +1.0 | 0 |

*Average ± standard error in 4 dogs.
**Values in two dogs.

![TIME (10 sec) ECG]

![BP (mmHg) 150 100]

![SIN-10 50µg ic]

![Cor. F (ml/min) 12 0]

![HR (beats/min) 180 120]

![LP (mmHg) 150 100]

![LVdp/dt (mmHg/sec) 4000 0]

**Fig. 3. Effect of intracoronary injections of SIN-10 on the electrocardiogram (lead II), femoral blood pressure (BP), coronary blood flow of the left descending coronary artery (Cor. F), heart rate (HR), left ventricular pressure (LP) and its maximal rate of rise (LV dp/dt) in an anesthetized, open-chest dog.**
were shown in Fig. 2 and Table 2. The drug produced decrease in the coronary blood flow concomitant with the reduction in systemic blood pressure. In 5 constant blood flow perfusion experiments of the left circumflex coronary artery, the drug slightly decreased the perfusion pressure in parallel with the falls in systemic blood pressure and left intraventricular pressure, except one animal in which the perfusion pressure was not influenced despite a hypotensive response. In 5 of 7 dogs, peak LV dp/dt was lowered concomitant with the reduction in systemic blood pressure. In other 2, peak LV dp/dt increased despite the falls in blood pressure the degrees of which were less marked than those in the cases of the decreased peak LV dp/dt.

Table 3 shows the effects of nitroglycerin administered sublingually in 4 dogs. Fall in blood pressure was observed within 1 minute and greatest 1 to 3 minutes after the administration and then gradually wore off within 15 to 30 minutes. Perfusion pressure in the coronary artery decreased in parallel with the hypotension. However, recovery of the former was faster than that of the latter. Peak LV dp/dt decreased in one dog but increased in another after the administration of nitroglycerin. Increase in the dp/dt may result from reflexly mediated compensatory responses (8, 9).

Direct injection into the coronary artery of SIN-10 did not affect the coronary blood flow even in the high doses which lower systemic blood pressure (Fig. 3).

3. **Femoral circulation in anesthetized dogs**

Direct injection into the femoral artery of SIN-10 did not affect blood flow of the artery, as observed in the case of the coronary artery. Constant blood flow perfusion experiments of the femoral artery were carried out in 6 dogs, in 4 of which the perfused leg was denervated by cutting the sciatic and femoral nerves. In 5 of the 6 animals, intravenous injection of 0.5 mg/kg of SIN-10 caused a slight reduction in the perfusion pressure

| Dog No. | Blood pressure (mmHg)* | Perfusion pressure (mmHg) | Perfusion rate (ml/min) | Heart rate (beats/min) |
|---------|-------------------------|--------------------------|------------------------|-----------------------|
|         | Control | 4-5 min | 30 min | Change | Control | 4-5 min | 30 min | Change | Control | 4-5 min | 30 min |
| 68071   | 151/112 | -26/-22 | -45/-24 | 110 | -16 | +2 | 23 | 191 | 198 | 192 |
| 68072   | 187/152 | -29/-18 | -53/-38 | 162 | -20 | -7 | 30 | 175 | 180 | 178 |
| **Average** | 169/132 | -28/-20 | -49/-31 | 136 | -18 | -2.5 | 26.5 | 183 | 189 | 185 |
| 68073** | 190/143 | -28/-20 | -58/-29 | 132 | +21 | +26 | 80 | 167 | 175 | 191 |
| 69010** | 176/123 | -25/-14 | -44/-13 | 113 | -9 | +1 | 64 | 194 | 206 | 217 |
| 69011** | 162/110 | -46/-33 | -60/-38 | 135 | -21 | -21 | 43 | 203 | 196 | 176 |
| 69009** | 182/133 | -27/-12 | -39/-15 | 106 | -5 | -2 | 77 | 166 | 206 | 223 |
| **Average** | 178/127 | -32/-17 | -50/-24 | 122.0 | -3.5 | +1.0 | 66.0 | 182.5 | 195.8 | 201.8 |
| **± S.E.** | 5.9/7.1 | 4.9/6.2 | 5.2/3.9 | 6.7 | 8.8 | 9.7 | 8.4 | 9.4 | 7.3 | 11.0 |

*Systolic and diastolic blood pressure.

**Sciatic and femoral nerves were cut just before the perfusion experiments.
in parallel with gradual fall in systemic blood pressure. However, in contrast to decrease in systemic pressure which was greatest about 30 minutes after the injection, the perfusion pressure returned or approached to the pre-drug level, as shown in Table 4. In one denervated leg, perfusion pressure of the artery increased exceptionally from the onset of hypotensive action induced by the drug.

4. Effects on blood pressure responses to carotid occlusion and to vasoactive drugs in anesthetized animals

In 5 bilaterally vagotomized cats, reflex pressor response to carotid occlusion was

![Graph](image)

**Fig. 4.** Effect of SIN-10 in denervated, perfused leg of an anesthetized dog. Test drugs were injected into the perfusion circuit. 1: epinephrine 0.5 μg; 2: angiotensin 0.5 μg; 3: isoproterenol 0.05 μg; 4: norepinephrine 0.5 μg; BP: femoral blood pressure, mmHg; PP: perfusion pressure in the femoral artery, mmHg; PF: rate of blood flow in the perfused artery, ml/min; HR: heart rate, beats/min.

**Table 5.** Sublingual and oral doses of SIN-10 and four different nitrates to anesthetized dogs.

| Drugs                | Sublingual | Dose | Oral       |
|----------------------|------------|------|------------|
|                      | mg/dog     | mg/kg| mg/dog    |
| SIN-10               | 2(2)*      | 0.22 | 4(4)       |
|                      | 0.24       |      | 0.47–0.56  |
| Glyceryl trinitrate  | 0.3(3)     | 0.029–0.033 | 3(3) | 0.33–0.38 |
| (GTN)                | 0.6(2)     | 0.053 |           |
|                      | 0.067      |      |            |
| Isosorbide dinitrate | 5(1)       | 0.48 | 20(3)      |
| (ISDN)               | 10(3)      | 1.05–1.22 | 2.33–2.94 |
| 2-Aminoethyl nitrate | 2(1)       | 0.21 | 20(1)      |
| p-toluenesulfonate   | 4(2)       | 0.47, 0.67 | 2.31      |
| Pentacythritol tetranitrate | 20(2) | 2.1  | 60(2)      |
| (PETN)               |            |      | 4.5, 7.0   |

*Number of trials.
not affected by intravenous doses of 0.5 and 2.0 mg/kg of SIN-10. In 3 cats, pressor responses to epinephrine and norepinephrine (5 μg/kg i.v.) were slightly inhibited by the drug at intravenous doses of 0.5 and 2.0 mg/kg. In 5 dogs, pressor responses to these amines were not consistently affected by the drug (2.0 mg/kg i.v. at the time of peak fall of blood pressure produced changes of from 58±6 to 60±19 by epinephrine and from 45±2 to 43±14 mmHg by norepinephrine, mean±S.E.).

In constant flow perfusion experiments of the femoral artery of dogs, constrictor responses in the perfused leg induced by the intraarterial injection of epinephrine and norepinephrine (0.5 to 1 μg) were reduced by about 50 percent, while that induced by angiotensin II (0.5 μg) was slightly increased after the intravenous injection of SIN-10 (0.5 mg/kg). Vasodilator response induced by isoproterenol (0.05 μg) was slightly inhibited.

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![Graph](image)

**Fig. 5.** Hypotensive responses to SIN-10 and nitrates after sublingual administration in anesthetized dogs. Ps: systolic blood pressure; Pd: diastolic blood pressure. For further details, see Table 7 and text.
The original recording from a representative experiment was shown in Fig. 4.

In 3 cats the vasodepressor effects of acetylcholine (0.5 μg/kg i.v.) and histamine (0.5 μg/kg i.v.) were slightly inhibited by SIN-10 (0.5 and 2.0 mg/kg).

5. The hypotensive responses to SIN-10 and nitrates after sublingual and oral administration in anesthetized dogs

The hypotensive potencies of SIN-10 after sublingual and oral administration in anesthetized dogs were compared with those of known representative nitrates. Table 5 shows the drugs tested (with the abbreviations) and doses used which were chosen from the recommended doses for sublingual or oral use in man (10). Fig. 5 shows the hypotensive responses to each drug after sublingual administration. The results showed that GTN acted within 0.5 to 1 minute, and the action wore off within 10 to 30 minutes. AEN and ISDN also acted within 1 to 2 minutes, but the action of AEN was longer lasting than that of ISDN, lasted more than 2 hours. SIN-10 led to a more slowly developing action than the 3 nitrates and the effect occurred in 10 to 15 minutes and the action lasted for more than 4 hours after the sublingual dose of 0.4 mg/kg of the drug. The effect of PETN was not apparently observed in the dose used.

\[
\text{ANESTHETIZED DOGS}
\]

\[
\begin{align*}
\text{TIME IN MIN} & \\
\text{Ps : systolic blood pressure ; Pd : diastolic blood pressure.}
\end{align*}
\]

Fig. 5. Hypotensive responses to each drug after sublingual administration. The results showed that GTN acted within 0.5 to 1 minute, and the action wore off within 10 to 30 minutes. AEN and ISDN also acted within 1 to 2 minutes, but the action of AEN was longer lasting than that of ISDN, lasted more than 2 hours. SIN-10 led to a more slowly developing action than the 3 nitrates and the effect occurred in 10 to 15 minutes and the action lasted for more than 4 hours after the sublingual dose of 0.4 mg/kg of the drug. The effect of PETN was not apparently observed in the dose used.

In the experiments of oral administration, SIN-10 was as active orally as sublingually, as shown in Fig. 6. GTN, ISDN and AEN, on the other hand, were all much less effective orally than when given sublingually. To be effective when given orally, the doses of the nitrates had to be more than several times larger than the sublingual ones.

6. Blood pressure and heart rate in unanesthetized normotensive and hypertensive rats

The comparative vasodepressor responses to SIN-10, AEN and PETN after oral ad-
TABLE 6. Effects of SIN-10, 2-aminoethylnitrate p-toluenesulfonate (AEN) and pentaerythritol tetranitrate (PETN) on systolic blood pressure in spontaneously hypertensive and normotensive rats.

| Drug    | Dose mg/kg p.o. | mmHg change in systolic blood pressure |
|---------|-----------------|----------------------------------------|
|         |                 | 0         | 1         | 3         | 5(hr)     |
|         |                 | Spontaneously hypertensive rats | Normotensive rats |
| SIN-10  | 5               | 181.2*    | -25.6 ± 3.9** | -9.2 ± 5.4 | -1.2 ± 3.6 |
| SIN-10  | 10              | 168.0     | -38.0 ± 9.6 | -17.6 ± 9.3 | -8.6 ± 6.6 |
| AEN     | 50              | 181.4     | -32.0 ± 4.1 | -7.2 ± 10.7 | -2.6 ± 6.6 |
| PETN    | 100             | 171.5     | -7.6 ± 7.1  | -2.6 ± 4.6  | -1.4 ± 3.9  |

*Pretreatment blood pressure.
**Average ± standard deviation in 5 different rats.

ministration in normotensive and spontaneously hypertensive rats are presented in Table 6. In both groups of rats, SIN-10 (10 mg/kg) and AEN (50 mg/kg) produced a similar hypotension, but the former showed longer duration of action than the latter. Heart rate tended to increase during the hypotension. On the other hand, PETN (100 mg/kg) did not significantly affect the blood pressure in normotensive and hypertensive rats.

7. Other pharmacodynamic activities

a) Nictitating membrane in anesthetized cats: Nictitating membrane responses to pre-ganglionic sympathetic nerve stimulation and to epinephrine (5 μg/kg i.v.) were unaffected by intravenous injection of SIN-10 (0.5 and 2.0 mg/kg).

b) Spontaneously beating isolated guinea pig atria: In the isolated spontaneously beating atrial preparations, SIN-10 in a dose range of from 1 to 100 μg/ml did not produce positive or negative inotropic and chronotropic actions (Fig. 7). In a concentration of 200 μg/ml, the drug showed slightly negative chronotropic and inotropic actions (depression of less than 5 percent).

c) Guinea pig ventricular strip: In the isolated, electrically stimulated ventricular strip, SIN-10 in a dose range of from 1 to 100 μg/ml did not affect the isometric con-

![Fig. 7. Effects of SIN-10 on the contractile force (ATR. CONTR.) and rate (ATR. RATE) of spontaneously beating guinea pig atria and on the contractile force (VENTR. CONTR.) of electrically driven guinea pig ventricular strip.](image-url)
tractile force (Fig. 7).

d) Isolated rabbit ileum: SIN-10 in a concentration of 10 μg/ml was without effect. A high concentration (100 μg/ml) of the drug also did not influence the spontaneous contractile activity, although it sometimes depressed the tone slightly.

e) Isolated guinea pig ileum: The contractions induced by acetylcholine, histamine and barium were not antagonized by 50 μg/ml of SIN-10 and were slightly antagonized by more than 100 μg/ml of the compound.

DISCUSSION

In anesthetized animals, SIN-10 produced a gradually developing and prolonged hypotensive action which was characterized by decrease in pulse pressure, primarily as a result of a reduction in systolic pressure. The hypotension was not antagonized by atropine, bilateral cervical vagotomy, spinal transection. During the hypotension induced by the agent, the pressor response to bilateral carotid occlusion and the response of the nictitating membrane to preganglionic sympathetic stimulation were not affected, and epinephrine and norepinephrine blood pressure responses were not consistently affected, although SIN-10 caused reduction in responses of the perfused vascular bed to epinephrine and norepinephrine but not to angiotensin given directly into the perfusion circuit. These data suggest that SIN-10 did not produce its hypotensive effect through the central, sympathetic and parasympathetic nervous systems or a specific blockade of the alpha-adrenergic receptive system.

In blood flow experiments, the rates of blood flow in the femoral and coronary arteries did not increase by intraarterial injection of SIN-10 and they decreased in parallel with the hypotension induced by intravenous injection of the drug. In constant flow perfusion studies, a slight reduction in perfusion pressure of innervated or denervated perfused limbs was not associated with full development of the hypotension. Although a slight but parallel fall in perfusion pressure in the coronary artery was observed in association with a reduction in systemic and left intraventricular pressure, it can be assumed that the reduction in the perfusion pressure may be due mainly to a depression in extravascular pressure induced by a fall in left intraventricular pressure. We can conclude from the experiments described above that SIN-10-induced hypotension results not from a dilator action of the peripheral resistance vessels but rather from a decreased cardiac output by either a decreased venous return, the mechanism of which is a pooling of blood in the capacitance vessels, or by a depressed cardiac function. As reported in our previous paper (1), investigations of the cardiovascular action of 3-morpholinosydnonimine hydrochloride, SIN-1, provided evidence that SIN-1 exerted a more rapid fall of blood pressure than SIN-10 and that SIN-1-induced hypotension was assumed to be due mainly to a direct action on vascular beds, especially on capacitance vessels. In the present study a fall in peak LV dp/dt was usually observed during a SIN-10-induced hypotension. The measurement of a direct drug action on myocardial contractility in the whole animal preparation is a most difficult problem because LV dp/dt is influenced by various factors such as end-diastolic pressure,
stroke volume, aortic pressure, heart rate, and extrinsic inotropic influences (11, 12). In spontaneously beating guinea pig atria or electrically stimulated ventricular strips, SIN-10 did not produce a marked negative inotropic or chronotropic action in high concentrations. In isolated, perfused dog hearts, Takenaka et al. (13) observed that intracoronary SIN-10 1 mg did not exert any appreciable changes in perfusion pressure, coronary blood flow, myocardial contractile force, heart rate, myocardial oxygen consumption and redox potentials. It can be assumed, therefore, that the fall in peak LV dp/dt during the hypotension by SIN-10 might be caused from a fall in aortic pressure and stroke volume.

SIN-10 was as active orally as sublingually, in contrast to the nitrates which are known to be relatively inactive orally but highly effective sublingually (10). The results suggest that SIN-10 is equally absorbed through both mucous membranes and is not inactivated in the gastrointestinal tract and hepatic circulation. This finding coincides with the results that no remarkable differences in the LD_{50} values of SIN-10 in mice and rats were found whether the drug was given enterally or parenterally (Yokotani et al., unpublished observations).

From the results in this and previous (1, 2) reports, it is presumed that SIN-10 per se might not be a hypotensive agent but that it might be converted in the body to active metabolite(s). But these considerations of structure and action are at present suggestive only.

**SUMMARY**

N-Ethoxycarbonyl-3-morpholinosydnonimine SIN-10, produced a gradually developing and prolonged hypotensive action which was characterized by decrease in pulse pressure, because of a greater fall in systolic than diastolic pressure. The hypotension was not antagonized by atropine, cervical vagotomy or spinal transection. In the cat nictitating membrane preparation, sympathetic nerve function and adrenergic smooth muscle receptor site were unaffected by this agent. Intraarterial injections of SIN-10 did not increase the rate of blood flow in femoral and coronary vascular beds. In constant flow perfusion experiments, the perfusion pressure of the femoral artery did not reduce in parallel with the SIN-10-induced hypotension. The drug did not produce significant effect on isolated atria, ventricular strip and ileum in a concentration of from 1 to 100 μg/ml. It is suggested that SIN-10 exerts its characteristic hypotensive effect not by affecting peripheral resistance vessels but by mainly affecting capacitance vessels.

It was observed that SIN-10 was as active orally as sublingually in anesthetized dogs.

**Acknowledgement:** We are grateful to Mr. T. Jimpu for skillful technical assistance.

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