EFFECTS OF N-ETHOXYCARBONYL-3-MORPHOLINOSYDNIMINE (SIN-10) ON THE CARDIOVASCULAR SYSTEM

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Sydnonimine derivatives, a series of mesoionic compounds, have a peculiar feature in their molecular structure and biological activities. The smooth muscle relaxing action of the compounds has been reported on the rat duodenum and the guinea-pig ileum by Oehme et al. (1) and hypotensive effect by Daeniker and Druey (2).

Recently, 3-substituted sydnonimines were newly synthetized (3) and were found to have potent vasodepressor activities (4, 5). Of these compounds, a derivative, whose imino radical at 5 position was acylated with ethoxycarbonyl group to make N-ethoxy-carbonyl-3-morpholinosydnonimine (SIN-10), became more stable and was reported to produce a potent and persistent hypotension in anesthetized dogs and rabbits (4, 5).

The present study deals with the effects of SIN-10 on cardiovascular system and the analysis of mechanism of action involved in hypotensive effect of the drug in anesthetized dogs and cats.

METHODS

Experiments were carried out using adult mongrel dogs weighing 5.9–11.5 kg and cats weighing 2.0–4.3 kg of either sex anesthetized with pentobarbital sodium (30 mg/kg, i.v.).

Respiration was recorded with a pressure transducer connected with tracheal cannula. Blood pressure was measured from the femoral or common carotid artery by means of an electronic manometer. An intracorporeal probe was attached to each of the common carotid, femoral, renal and pulmonary artery, and blood flow was measured with a square wave electromagnetic flowmeter. (Nihon Kohden MF-5).

Coronary circulation and myocardial metabolism were investigated using isolated perfused dog heart. The heart of a dog was isolated and a glass cannula was inserted into each of the aorta and pulmonary artery. With another dog as a donor, its common carotid artery was connected with the aortic cannula of the isolated heart. The coronary venous blood was allowed to flow out through the pulmonary artery cannula into a blood reservoir and returned to the femoral vein of the donor dog. Heparin (10 mg/kg) was used to prevent blood coagulation. Drugs were injected into coronary artery.
Coronary blood flow was continuously recorded by means of an automatic bubble flowmeter and an electromagnetic flowmeter which were interposed between the carotid artery of the donor and the aortic cannula of the isolated heart. Perfusion pressure was measured with an electronic manometer from the side tube of the aortic cannula. Cardiac contractions were recorded with a strain gauge transducer. Blood samples were collected from the arterial and venous side of the flow tubing. The determination of blood oxygen, lactic and pyruvic acid was carried out by the methods of Van Slyke-Saito (6), Barker-Summerson (7), and Friedmann-Haugen (8), respectively. Redox potentials (Eh) were calculated from the blood concentrations of lactic (L) and pyruvic acid (P) according to the following equation (9). \( \text{Eh} = -204 - 30.7 \log (L/P) \).

Eleven dogs were used to study on the heart preparation in situ. The heart was exposed by the midsternal and intercostal thoracotomy. The left circumflex coronary artery was cannulated with a glass cannula via the left subclavian artery and perfused with the blood led from the right common carotid artery. Coronary blood inflow and perfusion pressure were recorded with an electromagnetic flowmeter and an electronic manometer, respectively, set in the perfusion circuit. In some experiments, the myocardial contractile force (MCF) and the rate of contraction (dF/dt) or the left intraventricular pressure (LVP) and its rate of rise (dP/dt) were measured with a strain gauge sutured on the left ventricle and by direct needle puncture of the left ventricle, respectively. All recordings were made on an ink-writing polygraph. Drugs were administered intravenously or orally.

For testing the ganglionic action the splanchnic ganglion (S.G.) of the dog and the superior cervical ganglion (U.C.G.) of the cat were prepared. Each of the peripheral cut end of S.G. and the pre- and postganglionic nerve fibers of U.C.G. dissected free from surrounding tissues was mounted on a bipolar non-polarizable electrode for stimulation (1 msec, 20–50/sec, 2–5 V). Thus induced rise in blood pressure and contractions of the nictitating membrane were used as an indicator for demonstrating ganglionic blockade.

RESULTS

1. Effects on respiration and blood pressure

Intravenous injection of 1 mg/kg of SIN-10 produced hypotension in which the fall
in systolic pressure was more marked than that in diastolic, resulting in a decrease in the pulse pressure. Maximal hypotensive effects were observed 10 to 20 minutes after the injection, and thereafter mean arterial pressure gradually returned to its original level, though the pulse pressure remained diminished even after 80 to 100 minutes. Respiratory rate and depth were increased as systemic blood pressure fell. With 2 to 5 mg/kg, the hypotensive and respiratory stimulating effects of the drug became more pronounced. The duration of the hypotension was so prolonged that mean blood pressure and pulse pressure were not recovered 4 to 5 hours after drug administration. Respiration was remarkably accelerated. Fig. 1 shows the hypotensive and respiratory stimulating effects of SIN-10 (5 mg/kg) given orally.

2. Effects on arterial blood flow

a. Common carotid artery: With 1 mg/kg of SIN-10 given intravenously, there occurred a gradual decrease in blood flow in the common carotid artery associated with a fall in systemic blood pressure. In some cases, the flow continued to decrease even after the recovery of mean blood pressure. Fig. 2 illustrates the effect of 5 mg/kg of SIN-10 and 0.2 mg/kg of nitroglycerin. Both of the drugs produced a similar pattern of decreased blood pressure. The common carotid blood flow was decreased by SIN-10, but transiently increased by nitroglycerin.

b. Femoral artery: With 5 mg/kg of SIN-10, blood flow in the femoral artery was slightly decreased concomitantly with a gradual fall in systemic blood pressure. Nitroglycerin (0.2 mg/kg) caused an initial transient decrease in blood pressure, while blood flow remained unchanged (Fig. 3).

c. Renal artery: Blood flow in the renal artery was almost unchanged by treatment
Fig. 4. Effects of nitroglycerin and SIN-10 on renal blood flow (RBF) and blood pressure (BP) in an anesthetized dog.

with 2 mg/kg of SIN-10 given intravenously. Nitroglycerin in a dose of 0.2 mg/kg also produced little change in the blood flow with a slight fall in blood pressure (Fig. 4).

d. Pulmonary artery: Intravenous injection of SIN-10 (2 mg/kg) resulted in a de-
Figs. 5. Effects of SIN-10 on femoral blood pressure (FBP) and pulmonary blood flow (PBF) in an anesthetized open-chest dog.

Table 1. Effects of SIN-10 (1 mg) given intracoronarily on perfusion pressure (PP), coronary blood flow (CBF), myocardial oxygen consumption (Qo2), myocardial redox potentials (ΔEh), heart rate (HR) and myocardial contractile force (MCF) in isolated perfused dog hearts.

| Exploit. No. | Dose (mg) | PP (mmHg) | CBF (ml/min/100 g) | Qo2 (ml/min/100 g) | ΔEh (mV) | HR (beats/min) | MCF (mm) |
|--------------|-----------|-----------|---------------------|---------------------|----------|----------------|---------|
|              |           | Bef   | Aft. | Bef   | Aft. | Bef. | Aft. | Bef. | Aft. | Bef. | Aft. | Bef. | Aft. | Bef. | Aft. | Bef. | Aft. | Bef. | Aft. |
| BC-1         | 1         | 55    | 62   | 90.1  | 90.1 | 1.4  | 2.3  | 8.75 | 7.75 | 92   | 94   | 11   | 25   |
| BC-2         | 1         | 75    | 70   | 166.6 | 183.3 | 4.2  | 4.6  | 6.59 | 5.12 | 106  | 107  | 13   | 15   |
| BC-3         | 1         | 55    | 50   | 116.6 | 83.3  | 1.4  | 0.5  | 5.71 | 5.86 | 78   | 76   | 13   | 12   |
| BC-6         | 1         | 55    | 55   | 69.3  | 69.3  | 2.8  | 2.8  | 5.81 | 5.25 | 88   | 88   | 14   | 16   |
| BC-9         | 1         | 100   | 100  | 68.1  | 68.1  | 1.7  | 2.4  | 8.63 | 11.11| 74   | 75   | 13   | 13   |
| BC-10        | 1         | 65    | 60   | 53.9  | 33.9  | 6.2  | 6.5  | 5.35 | 3.71 | 140  | 146  | 11   | 7    |
| Mean         |           | 67.5  | 66.2 | 94.1  | 91.3  | 3.0  | 3.2  | 6.81 | 6.47 | 96   | 98   | 13   | 15   |
| % change     |           | 1.9   | -3.1 | -0.6  | 6.5   | 1.0  | -18.8 |

crease in the pulmonary blood flow with a concomitant fall in systemic blood pressure (Fig. 5).

3. Effects on coronary circulation and myocardial metabolism

a. Isolated perfused dog heart: Intracoronary injection of 1 mg of SIN-10 did not produce any appreciable changes in perfusion pressure (PP), coronary blood flow (CBF), heart rate, myocardial contractile force (MCF), myocardial oxygen consumption and myocardial redox potentials in five of six cases. Results obtained were summarized in Table 1, and a typical recording is shown in Fig. 6. Only in experiment numbered BC-1, perfusion pressure markedly increased, and a total increase of 18.8% in MCF was noted. With the same dose, SIN-1A caused an immediate fall in PP and an increase in CBF.

b. In situ heart preparation: SIN-10 in an intravenous dose of 1 mg/kg produced a gradual decrease in blood pressure in which the systolic pressure predominantly fell and the phasic pattern of coronary blood flow was so changed that the diastolic flow was considerably increased, while the systolic flow remained unchanged. However, a back flow was evident when perfusion pressure fell to a lower level than 60 mmHg. As shown in
Fig. 6. Effects of SIN-10 (1 mg) given intracoronarily on perfusion pressure (PP), myocardial contractile force (MCF) and coronary blood flow (CBF) measured with electromagnetic flowmeter and with automatic bubble flowmeter in an isolated perfused dog heart.

Fig. 7. Effects of SIN-10 on femoral blood pressure (FBP), coronary blood flow (CBF), left intraventricular pressure (LVP) and its maximal rate of rise (dP/dt) in an anesthetized-open-chest dog.
A: immediately, B: 10 minutes, C: 30 minutes after SIN-10 injection.
Fig. 7, with 1 mg/kg of SIN-10 given intravenously there occurred a gradual decrease in coronary mean blood flow accompanied by a persistent decline in femoral blood pressure, left intraventricular pressure (LVP) and dP/dt. When 5 mg/kg of the drug was administered orally, coronary blood flow decreased with a concomitant and long-lasting fall in perfusion pressure.

4. Analysis of mechanism of hypotensive action

a. Cholinergic action Intravenous injection of 1 mg/kg of SIN-10 resulted in a gradual and persistent reduction in the pulse pressure accompanying a decrease in coronary blood flow. These effects were similarly demonstrated after the animal was treated with atropine 0.5 to 1 mg/kg (Fig. 8).

b. Ganglionic blockade: Electrical stimulation of the major splanchnic nerve resulted in a rise of systemic blood pressure with two peaks in all seven dogs used. This rise was found to be considerably decreased but the response pattern remained unchanged after treatment with 5 mg/kg of SIN-10 in four dogs tested (Fig. 9), while the rise in blood pressure was almost completely abolished after 2 mg/kg of hexamethonium given to three dogs intravenously. In four anesthetized cats, effect of SIN-10 on the contractions of the nictitating membrane produced by stimulation of the pre- and postganglionic nerves of the superior cervical ganglion was compared with that of nitroglycerin and hexamethonium.

Fig. 8. Effects of atropine treatment on the hypotensive action of SIN-10 in an anesthetized open-chest dog. Atr.: atropine sulphate, FBP: blood pressure, CBF: coronary blood flow.

A: a few minutes, B: 10 minutes after SIN-10 administration.

Fig. 9. Effects of SIN-10 on pressor response to stimulation of the major splanchnic nerve.

A: control, B: 30 minutes after SIN-10 (5 mg/kg) injection.
A typical experiment is shown in Fig. 10. The intravenous injection of nitroglycerin (0.5 mg/kg) resulted in a slight reduction of the contractile response of the membrane to sympathetic pre- and postganglionic stimulation, and complete recovery of the response followed within 30 minutes. With 0.5 mg/kg of SIN-10 there was no appreciable change of the contractile response to nerve stimulation. SIN-10 in a dose of 1 mg/kg produced a moderate fall in blood pressure and slightly reduced the contractile response to preganglionic stimulation as comparable to the effect of nitroglycerin. In two cases receiving 2 mg/kg of SIN-10, the membrane responses to both pre- and postganglionic stimulation were depressed as well. The intravenous administration of hexamethonium (0.5 mg/kg) and atropine (0.5 mg/kg) or the topical application of hexamethonium to the superior cervical ganglion produced a marked reduction or elimination of the contractile response to preganglionic stimulation, while postganglionic one remained unchanged. The intravenous injection of 2 mg/kg of hexamethonium completely abolished the preganglionic response with a moderate enhancement of the contraction produced by postganglionic stimulation.

c. Adreno- and sympatholytic actions: Intravenous injection of adrenaline (2-5 μg/kg) in five dogs resulted in a rise of systemic blood pressure with an increased blood flow in the common carotid and pulmonary artery. The pressor response to adrenaline was
compared before and after intravenous administration of SIN-10 and nitroglycerin. As shown in Fig. 11, the adrenaline-induced rise in blood pressure was reduced from 60 mmHg to 20 mmHg and the increased blood flow in the pulmonary artery almost disappeared during the hypotension resulting from SIN-10 (2 mg/kg). Nitroglycerin also reduced the adrenaline-induced rise in blood pressure from 130 mmHg to 40 mmHg. In four cases, the average increase in blood pressure due to adrenaline was 96% during control period but reduced to 22% after SIN-10 (2 mg/kg) and 40% after nitroglycerin (0.5 mg/kg).

DISCUSSION

The administration of SIN-10 in anesthetized dogs resulted in a gradual and persistent hypotension consisting of more marked decrease in the systolic pressure than that in the diastolic. The onset, magnitude, and duration of hypotension depended on the dose and route of administration of the drug. The hypotensive effect occurred slowly and gradually more than a few minutes after the intravenous injection of SIN-10, and much longer time was needed when it was given orally.

The study on the structure-activity relationship between 3-morpholinosydnonimine (SIN-1) and N-nitroso-N-morpholino-aminonitrile (SIN-1A), a ring-opened product of SIN-1, has shown that SIN-1A has a more potent hypotensive property with rapid onset and shorter duration than SIN-1 (4, 5). In addition, it was described that the sydnonimines are stable in acid (2) but suffer ring-opening in alkali to give nitrosoamide (10), and that SIN-1A is unstable and readily releases nitroso ions (3). It is conceivable that the delayed onset of the hypotensive action of the sydnonimines, SIN-1 and SIN-10, resulted from their increased molecular structural stability due to the ring-closing of SIN-A and the introduction of ethoxycarbonyl group at 5 position in SIN-1. The hypotensive activity of these compounds is probably due to some degradation products such as nitrosoamide derivatives (10), nitroso ions (3) or other unknown metabolites.

Although a comparable decline in pulse pressure was noted after treatment with SIN-10 and nitroglycerin, there were some qualitative differences in their effects on arterial blood flow. SIN-10 caused a prevalent decrease in the blood flow in common carotid, coronary, femoral and renal artery, whereas nitroglycerin (0.2–0.5 mg/kg) produced a
Transient initial increase in common carotid and coronary but little changes in femoral and renal blood flow.

These observations suggest that the hypotensive effect of SIN-10 is brought about through a similar mechanism to that of nitroglycerin, but that SIN-10 has a weaker vasodilating action on the resistance vessels than nitroglycerin (11). The decrease in the pulmonary blood flow by SIN-10 reflects the decreased venous return and cardiac output resulted from pooling of the blood in the capacitance vessels dilated by the drug (4, 12). Thus, the diminished circulating blood volume in arterial system reduces left intraventricular pressure and its maximal rate of rise.

The fact that the hypotensive property of SIN-10 was not modified by the pretreatment with atropine ruled out any cholinergic mechanism involved. The diminished pressor responses to adrenaline and sympathetic nerve stimulation were comparably observed after nitroglycerin was used. From these results it was considered that such a reduction in adrenergic response is based on the nonspecific relaxing action of these drugs on vascular smooth muscle itself. Both SIN-10 and nitroglycerin diminished the contractile amplitude of the nictitating membrane elicited by preganglionic stimulation, and they also depressed the contraction produced by postganglionic stimulation as well. Such effect of these drugs is quite different from that of hexamethonium which abolished the response to preganglionic stimulation but slightly potentiated the membrane contraction during postganglionic stimulation. The reduced response by SIN-10 to ganglionic stimulation seems not to be due to ganglionic blockade but its smooth muscle relaxing action.

Kikuchi et al. (4) have demonstrated that SIN-1, a prototype of mesoionic compounds, produces a slight and transient positive inotropic effect blocked by treatment with propranolol. They concluded that SIN-1 has a tyramine-like catecholamine releasing action in rat heart. In the present study, SIN-10 did not show any sign of sympathomimetic action like SIN-1.

In perfused dog heart preparations, a hypotensive dose of SIN-10 produced no appreciable changes in perfusion pressure and coronary blood flow, while SIN-1A caused an increase in coronary blood flow. SIN-10 seems to be a less active vasodilator than SIN-1A.

SIN-10 was found to increase respiratory rate and depth. This occurred with the development of systemic hypotension and lasted even after the recovery of blood pressure to almost control level. Oehme et al. (1) have described that the synnonimine compounds cause death after convulsions. Whether or not synnonimine compounds have any action on central nervous system has not been explored but a possible explanation for the respiratory effect of the drug can be drawn from its central origin.

**SUMMARY**

Effects of SIN-10 on cardiovascular system were investigated using dogs and cats anesthetized with pentobarbital sodium. The results obtained are as follows:

1. In anesthetized dogs, intravenous or oral administration of SIN-10 in doses of
1 to 5 mg/kg produced a persistent decrease in blood flow of the common carotid, femoral, renal, pulmonary and coronary artery with a gradual and prolonged decline in systemic blood pressure consisting of more marked decrease in the systolic pressure than in the diastolic. The rate and depth of respiration was increased. Nitroglycerin produced a transient increase of blood flow in carotid and femoral artery with a slight fall in systemic blood pressure.

2. In isolated, perfused dog hearts, 1 mg of SIN-10 given intracoronarily did not produce any appreciable changes in perfusion pressure, coronary blood flow, myocardial contractile force, heart rate, myocardial oxygen consumption and redox potentials.

3. Pretreatment of experimental animals with atropine (0.5–1.0 mg/kg) did not modify the hypotensive effects of SIN-10.

4. The vasopressor response to adrenaline (2 μg/kg) given intravenously was considerably decreased by SIN-10 and by nitroglycerin.

5. Pressor response to electrical stimulation of the major splanchnic nerve was little affected by 0.5 mg/kg of SIN-10 but attenuated after 2–5 mg/kg were employed. Hexamethonium (2 mg/kg) almost completely abolished the response to the sympathetic pre-ganglionic stimulation.

6. In anesthetized cats, SIN-10 (1–2 mg/kg) and nitroglycerin (0.5 mg/kg) very slightly decreased contractions of the nictitating membrane elicited by electrical stimulation of not only pre- but also postganglionic fibers of the superior cervical ganglion, while hexamethonium (0.5 mg/kg) suppressed selectively the membrane contraction produced by preganglionic stimulation and slightly potentiated that elicited by postganglionic stimulation.

7. The hypotensive effect of SIN-10 is likely due to the smooth muscle relaxation of capacitance vessels which resulted in a pooling of the blood in venous beds and reduction of cardiac output. The mode of action of SIN-10 is similar to that of nitroglycerin.

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