CARDIOVASCULAR ACTION OF MESOIONIC COMPOUNDS,
3-SUBSTITUTED SYDNONIMINES*

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A number of the mesoionic compounds known as a new class of heterocycles have been examined in the search for potential therapeutic agents and their biological activities have been reviewed recently by Kier and Roche (1), and Ackermann (2). Oehme et al. (3) studied some mesoionic 3- or 3, 4-substituted sydnonimines and found that in urethane anesthetized rats the sydnonimines were hypotensive only in high doses (0.02 mM/kg i.v.), the effect not being pronounced, and reported that in the rat duodenum and the guinea pig ileum 3, 4-diphenylsyndnonimine had a spasmolytic effect equipotent to papaverine. However, no detailed cardiovascular actions of sydnonimines have so far been reported. Recently we found that the newly synthesized 3-substituted sydnonimines (4) have potential pharmacological actions such as vasodilating, vasoconstricting, hypotensive, hypertensive, sympathomimetic, or spasmolytic actions. This paper reports the chemical structure-activity relationship and mechanisms of the cardiovascular actions of 3-substituted sydnonimines, especially those of 3-morpholinosyndnonimine hydrochloride (CV-664, SIN-1), a potent hypotensive and spasmolytic compound.

METHODS

1. Anesthetics

Mongrel dogs of either sex, weighing from 6 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. Cats of either sex, weighing from 2 to 4.5 kg were anesthetized with chloralose (40 mg/kg i.v.) and urethane (250 mg/kg i.v. and 250 mg/kg i.p.), unless otherwise stated.

2. Blood pressure and heart rate in anesthetized animals

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) or a mercury manometer. The transducer was connected to an E & M or Nihon Kohden carrier preamplifier and blood pressure was recorded with an E & M Physiograph or Nihon Kohden Polygraph.

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Movement of the manometer was recorded on smoked paper. Heart rate was monitored continuously with an ECG-triggered Nihon Kohden or our own tachograph. Respiration was recorded by means of a tambour connected to the cannulated trachea or by an E & M impedance pneumograph. In a few experiments, central venous pressure was measured by inserting a polyethylene cannula filled with heparinized saline solution through the right external jugular vein to the superior vena cava. The cannula was connected to a saline manometer, the movement of which was recorded on smoked paper.

3. Coronary or inferior vena caval flow, and contractile force in anesthetized, open-chest dogs

Under artificial respiration with room air the chest was opened through either the fourth right or the fifth left intercostal space. The pericardium was incised and the edge pulled taut to bring the heart into an accessible position. Coronary flow was measured by two methods. In one, which was used when drugs were given intravenously into the cephalic vein, a flexible polyethylene coronary sinus cannula was inserted through an incision in the right atrium and its tip fixed into the sinus orifice. The sinus outflow was measured by diverting the blood through an extracorporeal electromagnetic flowmeter (Nihon Kohden MF-2) and into the right external jugular vein. Relative changes in oxyhemoglobin concentration of the sinus blood were recorded by interposing an E & M Flow-Thru oxymeter between the outflow tubing.

The second method was used when drugs were given intraarterially into the anterior descending branch of the left coronary artery. The branch was ligated and the distal segment immediately cannulated with a bent polyethylene arterial cannula and perfused with the blood flowing through the flowmeter from the left carotid artery. Inferior vena caval blood flow was measured by means of the flowmeter interposed between two cannulas inserted into the proximal and distal cut ends of the vessel. A Walton-Brodie strain gauge arch was fixed by biotissue adhesive (Aron Alpha, Sankyo Co.) to the surface of either the right or left ventricle for measurement of myocardial force of contraction (5). The blood was rendered non-coagulable by administration of an intravenous dose of 1,000 units/kg of heparin.

4. Limb circulation in dogs

Effects on the limb circulation were studied in three ways: simple measurement of arterial inflow, constant flow perfusion of the arterial side in intact, or in isolated hind limb. 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 intact or isolated hind limb consisted of similar cannulation of the femoral artery but instead of the flowmeter, a constant flowpump (Sigmamotor Model T-8) 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 intact limbs or around 100 mmHg in isolated limbs. Perfusion pressure was measured from a side arm in the tubing between the pump and the perfused artery. Measurement of the changes in the total volume of an isolated and fur-clipped hind limb was carried out as the method reported by Baum and Hosko (6) with minor modification.
for dogs. Briefly, the isolated limb was enclosed in a water-filled plethysmograph (a transparent vinyl-resin cylinder of 30 cm length and 8.5 cm diameter with 6 screws) which was connected to a venous pressure transducer (Nihon Kohden LPU-0.1). To maintain the temperature (38°C) of the water the plethysmograph was encased by a temperature regulated water box by circulating warm water. Outflow from the femoral vein was diverted into an open reservoir containing approximately 30 ml blood then backed into a centrally directed cannula of the femoral vein.

In constant flow perfusion preparations of intact hind limb, the lumbar sympathetic chain was exposed retroperitoneally through a flank incision and cut at L3 along with the rami at L3 and L4 and femoral nerve. The cut distal end of the sympathetic chain was stimulated by bipolar electrodes connected to an electronic stimulator (Nihon Kohden MSE-20).

5. Cross-circulation of the hind limb

Isolated but innervated hind limb of a recipient dog was perfused with blood from a donor dog. Blood from the donor was perfused through the femoral artery of the recipient limb and returned to the donor through the femoral vein with the Sigmamotor pump at a constant rate of blood flow which was adjusted so that limb pressure approximated systemic pressure. Systemic blood pressure of the donor and recipient dogs, and perfusion pressure of the recipient limb were recorded. The integrity of the preparation was checked by the intravenous injection of norepinephrine 5 to 10 µg/kg into the recipient body. The presence of a reflex vasodilatation in the recipient limb indicated that there was the intact innervation (sciatic nerve trunk) from the recipient body to the isolated limb.

6. Total aortic flow in anesthetized, open-chest cats

Cats of both sexes were anesthetized with sodium pentobarbital, 30 to 35 mg/kg i.v. Under artificial respiration with room air the chest was opened. The brachiocephalic and the left subclavian arteries were cleared from their points of origin for a distance of about 1.5 cm. The descending aorta was then cleared for a distance of about 2 cm below origin of the left subclavian artery. After heparinization (1000 units/kg i.v.) an extracorporeal circulation from the carotid to the femoral arteries was temporarily established in order to prevent interruption of blood supply to the body below the occluded aorta and overloading of the left ventricle during the introduction of cannulas into the proximal and distal part of the descending aorta. Blood flow through the descending aorta was measured by interpolating a flowmeter (E & M rotameter Type B) between the proximal and distal cut ends of the aorta after ligating the origins of the brachiocephalic and the left subclavian arteries which received blood traversed the flowmeter through cannulas inserted into the distal cut ends of the arteries. The flowmeter now recorded total aortic flow, being equal to the cardiac output minus coronary flow.

7. Heart-lung preparation of cats

This was performed according to the basic experimental technique for dogs described in detail by Hashimoto (7). Cardiac output was recorded by means of an automatic bubble flowmeter (Natume Seisakusho).
8. Nictitating membrane in anesthetized cats

Nictitating membrane responses from anesthetized cats were recorded with an isotonic lever system and smoked paper or 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 periods with square wave pulses of 2.5-5 V at a frequency of 20 per second and duration of 0.2 millisecond.

9. Spinal cats

Under ether anesthesia, the spinal cord was severed at the C$_{1-2}$ level. The carotid arteries were tied off and bilateral vagotomy performed.

10. Isolated, spontaneously beating guinea pig atria

The atria were dissected from the heart of guinea pig (300-450 g body weight) killed by a blow on the head. They were suspended in a 20-ml bath containing oxygenated Ringer solution maintained at 37°C. The amplitude of contractions was recorded on a smoked drum via a semiisometric lever counterweighted to exert 0.5 g tension on the strips. Pretreatment with reserpine (5 mg/kg i.p.) was carried out 24 hours prior to the experiment.

11. Guinea pig ileum

Distal sections of guinea pig ileum were 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 (µg/ml) of spasmogens were used: barium chloride, 20; acetylcholine chloride, 0.01; and histamine hydrochloride, 0.01.

12. 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.

13. Guinea pig tracheal chains

Guinea pig tracheal chain was prepared as described by Castilo and Beer (8). The chain, containing 4-5 tracheal rings, was mounted in 20-ml bath which contained aerated Ringer solution at 37°C. Histamine hydrochloride (1 µg/ml) was used to increase tone. Drug-induced effects were recorded on a smoked drum via an isotonic lever.

14. Drugs used

All drug doses in the text refer to the following salts: atropine sulphate (Merck), tripelemamine hydrochloride (Ciba), diphenhydramine hydrochloride (Vena, Tanabe), dibenamine hydrochloride, hexamethonium bromide (Methobromin, Yamanouchi), dichoroisoproterenol hydrochloride (Lilly), propranolol hydrochloride, phentolamine methansulfonate (Regitine, Ciba), papaverine hydrochloride (Fujisawa), hydralazine hydrochloride (Apresoline, Ciba), 2-aminoethyl nitrate-p-toluensulfonate (Cardisan), acetylcholine chloride (Merck), 1-epinephrine hydrochloride (Sankyo), dl-norepinephrine hydrochloride (Sankyo), tyramine hydrochloride (Wako Pure Chemical), histamine hydro-
### TABLE 1. Chemical structure, blood pressure response in anesthetized cats and inotropic action in isolated guinea pig atria of 3-substituted sydnonimines.

\[
R \cdot N \cdot C \cdot R' \\
\text{N} \quad \pm \text{C} = \text{NH} \cdot \text{HCl}
\]

| Series code | Code No. GV- | Chemical structure | \( R' \) | Blood pressure\(^1\) | Guinea pig atria\(^2\) |
|-------------|-------------|--------------------|--------|-----------------|------------------|
| 664 (SIN-1) |             | \( \text{O} \) \( \text{N} - \) | H      | ↓               | ↑                |
| 665        |             | \( \text{N} - \)   | H      | ↓               | not tested       |
| 655        |             | \( \text{N} - \)   | H      | ↓               | ↑↑↑              |
| 689        | SIN         | \( \text{CH}_3 \) | H      | ↓               | ↑                |
| 718        |             | \( \text{HCl} \cdot \text{NH} \cdot \) | H      | ↓               | ↑                |
| 667 (SIN-2) |             | \( \text{CH}_3 \) | H      | ↓               | ↑                |
| 695        |             | \( \text{CH}_3 \cdot \text{CH}_3 \cdot \) | H      | ↓               | ↑                |
| 719        |             | \( \text{CH}_3 \cdot \text{CH}_3 \cdot \) | H      | ↓               | ↑                |
| 692        |             | \( \text{N} - \)   | -\( \text{CH}_3 \) | ↑               | ↑↑↑              |
| 582        |             | \( \text{H} \)      | H      | ↑               | ↑↑↑              |
| 555 (SIC-11)|           | \( \text{H} \)      | H      | ↑               | ↑↑↑              |
| 654        |             | \( \text{H} \)      | H      | ↑               | ↑↑↑              |
| 553        | SIC         | \( \text{H} \)      | H      | ↑               | ↑↑↑              |
| 631 (SIC-12)|           | \( \text{CH}_3 \cdot \text{CH}_3 \cdot \) | H      | ↑               | ↑↑↑              |
| 693 (SIG-7) |             | \( \text{N} \cdot \text{CH}_3 \cdot \text{CH}_3 \cdot \) | H      | ↑               | ↑                |
| 694        |             | \( \text{CH}_3 \cdot \text{N} \cdot \text{CH}_3 \cdot \text{CH}_3 \cdot \) | H      | ↑               | ↑↑              |
| 565        |             | \( \text{H} \)      | -\( \text{CH}_3 \) | ↑               | not tested       |
| 630        |             | \( \text{HCl} \cdot \text{NH} \cdot \) | H      | ↑               | not tested       |

1) Hypotensive (↓) or hypertensive (↑) response in anesthetized cats, in the dose range of 0.02-5.0 mg/kg i.v.

2) Positive inotropic action in the spontaneously beating isolated guinea pig atria. 20-50% increase of amplitude in the concentration of 1-5 \( \times 10^{-3} \) g/ml (↑↑↑), 1 \( \times 10^{-2} \) g/ml (↑↑), and 5 \( \times 10^{-2} - 1 \times 10^{-1} \) g/ml (↑↑).
chloride, barium chloride. Other drugs used were reserpine (Serpasil, Ciba), sodium nitrite (Wako Pure Chemical) and synthetic angiotensin II.

RESULTS

1. Relationship of chemical structure to blood pressure responses of 3-substituted sydnonimines in anesthetized cats and dogs

Table 1 shows chemical structures of 3-substituted sydnonimines which are classified

| Dose mg/kg | Blood pressure | Heart rate |
|------------|----------------|------------|
|            | Control (mmHg) | % Change of | Time (min) to | Max. % |
|            | % Change of peak fall | Peak fall | 50% recovery | change |
| 0.05       | 118 | 30 | 16 | 40 | 214 | -9.4 |
|            | 135 | 7.5 | 6 | 25 | 200 | -3.0 |
|            | 146 | 11 | 15 | 60 | 207 | -3.5 |
| mean ± s.e. | 133 | 16.2 | 12.3 | 45.0 | 207.0 | -5.3 |
| 0.1        | 140 | 21 | 16 | 60 | 200 | 0 |
|            | 123 | 22 | 14 | 50 | 232 | 3 |
|            | 150 | 41 | 21 | 70 | 194 | 5 |
| 0.5        | 120 | 46 | 19 | 62 | 236 | 0 |
|            | 158 | 26 | 18 | 92 | 200 | 0 |
|            | 152 | 28 | 38 | 125 | 214 | -6.5 |
| mean ± s.e. | 140.6 | 32.6 | 22.0 | 79.8 | 215.2 | -1.7 |
| 1.0        | 145 | 38 | 23 | 123 | 188 | -18.5 |
|            | 176 | 34 | 35 | 120 | 204 | 3 |
|            | 126 | 30 | 16 | 70 | 167 | -5.4 |
|            | 126 | 30 | 13 | 60 | 171 | -18.7 |
|            | 138 | 54 | 13 | 135 | 169 | 5 |
| mean ± s.e. | 142.2 | 40.8 | 20.0 | 101.6 | 179.8 | -6.9 |
| 2.0        | 150 | 39 | 30 | 240 | 140 | -4 |
|            | 140 | 37 | 37 | 130 | |
|            | 143 | 22 | 10 | 90 | 168 | 8 |
|            | 155 | 68 | 20 | 120 | 221 | -13 |
|            | 140 | 57 | 13 | 85 | 230 | -33 |
| mean ± s.e. | 146.0 | 51.0 | 22.0 | 133.0 | 206.3 | -12.6 |
| 20         | 153 | 70 | 33 | 220 | 222 | -19 |

1) After bilateral cervical vagotomy.
2) After atropinization.
3) After unilateral cervical vagotomy.
4) Not determined.
into the two types by their side groups in the 3-position of the sydnonimine ring as well as their effects on blood pressure and isolated guinea pig atria. SIN-series compounds in the table which have morpholino-, piperidino-, pyrrolidino-, dialkylamino-, or piperazino-group in the 3-position of the ring with no substituent in the 4-position caused a marked, prolonged hypotension in the intravenous doses of less than 0.5 mg/kg, indicating that the direct attachment of the nitrogen of a 3-substituent to the nitrogen in the 3-position of the ring is required for the depressor activity. The dose of 0.02 mg/kg of 3-morpholinosydnino-

\[ \text{CV-655} \]

**FIG. 1.** Chemical structures and blood pressure responses in anesthetized cats. Note that CV-692, which has an additional methyl group in the 4-position of a depressor compound (CV-655), exerted a pressor response closely resembled to those of the SIC-series pressor agents (e.g. CV-654, see Table 1). BP, carotid blood pressure, mmHg; HR, heart rate, beats/min; Resp, respiration.
nimine hydrochloride (CV-664, SIN-1), for example, suppressed about 20 mmHg of mean blood pressure with duration of hypotension lasting over 30 minutes in pentobarbitalized dogs. Table 2 summarized the effects of SIN-1 on blood pressure and heart rate in 20 anesthetized cats. The hypotension induced by SIN-series compounds was dose-related and led to a decrease in pulse pressure. This decrease in pulse pressure was observed to be associated with a greater fall in systolic pressure. The dose of 1 mg/kg of 3-piperidinosydnominine hydrochloride (CV-655) produced sometimes initially a rapid, short-lived increase in blood pressure, followed by a lasting decrease of $37 \pm 27$ mmHg (mean±S.D.) in 5 anesthetized cats, as shown in Fig. 1. The onset of CV-655-induced hypotensive action was considerably slower than that of other depressor compounds listed in the Table 1. This initial pressor response is thought to be due to a cardiotonic action of this agent, which was demonstrated to be much stronger than that of CV-664 or other hypotensive derivatives on guinea pig atria (see Fig. 7 and Table 1). Heart rate and respiratory rate either slightly increased or decreased after a single injection of these compounds.

SIC-series compounds which have alkyl-, cycloalkyl-, or dialkyl-aminoalkyl-group in the 3-position, on the other hand, produced a prolonged rise in arterial pressure and increase in heart rate in the dose range of 0.2 to 5 mg/kg. 3-Cycloheptylsydnonimine hydrochloride (CV-654) produced a lasting mean increase of $28 \pm 12$ mmHg after the intravenous injection of 1 mg/kg (Fig. 1) in 3 anesthetized cats and 45 mmHg after 5 mg/kg in one cat. When the same dosage (1 mg/kg) of CV-654 was given repeatedly, spaced 30 to 50 minutes apart, the sustained pressor response became progressively smaller and it

![Fig. 2. Effect of repeated injections of CV-654 on blood pressure and heart rate in an anesthetized cat. Note that the sustained pressor response became progressively smaller and it was preceded by a transient depressor response. For legend to symbols, see Fig. 1.](image-url)
was preceded by a transient depressor response which became progressively larger, as shown in Fig. 2. The higher the dose, the more rapid was the development of tachyphylaxis. A high dose (5 mg/kg) of CV-654, for example, abolished the pressor response to a subsequent single injection and only produced transiently a depressor response. It is interesting to note that the addition of a methyl substituent in the 4-position of CV-655 provided a hypertensive agent (CV-692), the action of which was closely resembled to those of SIC-series compounds as shown in the Fig. 1.

2. Effects on blood pressure response to vasoactive drugs and procedures

The pressor responses of SIC-series compounds were not inhibited but augmented by pretreatment with hexamethonium and abolished by phentolamine (Fig. 1). The hypotensive effect of intravenously administered CV-664 or CV-655 (0.5–1.0 mg/kg) was not abolished by the acute pretreatment of cats or dogs with atropine (0.5–1.0 mg/kg i.v.), tripelennamine (15 mg/kg i.v.), diphenhydramine (5 mg/kg i.v.), dibenamine (15 mg/kg i.v.), hexamethonium (2.5–5 mg/kg i.v.), dichloroisoproterenol (3.0–5.0 mg/kg i.v.), propranolol (1.0 mg/kg i.v.), bilateral cervical vagotomy, spinal cord section with cervical vagotomy, or eversion.

In anesthetized dogs or cats, pressor responses induced by epinephrine (2.5–5 μg/kg i.v.), norepinephrine (2.5–5 μg/kg i.v.) and angiotensin II (0.2–0.5 μg/kg i.v.) were slightly inhibited (less than 30%) by CV-664 or CV-655 at intravenous doses of 0.5 to 1.0 mg/kg, but not influenced by CV-654 or CV-555. The pressor responses produced by electrical stimulation of peripheral stump of the cut splanchnic nerve (10 V, 0.2 millisecond, 40–60/sec for 10–15 seconds) and central stump of the cut sciatic nerve (15 V, 0.2 millisecond, 60/sec for 20 seconds) were slightly inhibited by the marked hypotensive doses of CV-664 for periods of time equivalent to the duration of the hypotensive effect as shown in Fig. 4. In one of 5 vagotomized cats, reflex pressor response to carotid occlusion was not influenced, but in the remaining animals the response was inhibited by more than 50 percent by intravenous dose less than 0.5 mg/kg of CV-664 during the hypotensive phase, and returned to pretreatment amplitude as the blood pressure approached control levels. The original
Fig. 4. Effects of CV-664 on pressor responses to carotid occlusion in a bilaterally cervical vagotomized cat (A) and to splanchnic nerve stimulation in an atropinized cat (B). HR, heart rate; BP, blood pressure; CVP, central venous pressure; Resp, respiration.

In three cats the vasodepressor effect of acetylcholine (0.1 μg/kg i.v.) and the bradycardic response to peripheral vagal stimulation (6 V, 0.2 millisecond, 20/sec for 10 seconds) were unchanged by CV-664 (0.5–2.0 mg/kg i.v.).

3. Cardiac contractile force
   a) Anesthetized, open-chest dogs

Intravenous injection of CV-664 (0.02–0.1 mg/kg) produced initially a slight but short-lived increase followed by a slight decrease of myocardial contractile force or did
not influence appreciably on contractions during the hypotensive response (Fig. 5). The SIC-series pressor substances produced an increase of contractile force and heart rate concomitant with the pressor response. This cardiotonic action, which was found to be more than 20 times less active than that of tyramine, was blocked by pretreatment with propranolol, being similar to that of tyramine as shown in the Fig. 5.

b) Spontaneously beating isolated guinea pig atria

In the isolated spontaneously beating atrial preparations, all of 3-substituted sydnonimines tested in a dose range of from 1 to 100 μg/ml produced more or less a positive inotropic action, the order of which potency was shown in the Table 1. After washing, con-
Effects of CV-664 on carotid blood pressure (BP, mmHg), inferior vena caval blood flow (IVCF), contractile force of right ventricle (CF), and heart rate (HR) in an anesthetized open-chest dog. The downward slight deflection on the recording of contractile force immediately after the injection is due to mechanical result of the pen. Tractions returned to control amplitude. The actions were inhibited by pretreatment with dichloroisoproterenol or propranolol, and in the reserpinized atrial preparations those were practically abolished. Typical experiments were illustrated in Fig. 7.

c) Heart-lung preparation of cats

In one of two preparations (total blood volume in each preparation was estimated about 150 ml), 5 mg of CV-664 injected into the venous inflow to the heart exerted only a slight increase of heart rate (from 156 to 174 beats/min) and cardiac output (from 66 to 69 ml/min). The second and the third doses of 10 and 20 mg, given about 10 minutes apart respectively, produced additional but slight increases in cardiac output and heart rate. In the second preparation, 20 mg of CV-664 caused a marked positive inotropic and chronotropic actions, as shown in Fig. 8, while the second injection of the same dose, given about 90 minutes apart, did not produce any appreciable changes.

4. Effects of CV-664 on systemic hemodynamics in anesthetized, open-chest cats

The effects of single intravenous injection of 0.5 mg/kg of CV-664 on systemic hemodynamics in 6 cats were compared with those of sodium nitrite (5.0 mg/kg), papaverine (2.0 mg/kg), and hexamethonium (0.5 mg/kg), as shown in Fig. 9. At these doses, CV-664 and sodium nitrite produced approximately equipotent peak hypotension and reduction of aortic blood flow. The duration of the CV-664-induced hypotension was considerably greater than that of sodium nitrite, despite approximately the same magnitude and duration in reduction of aortic blood flow, so that CV-664 reduced more remarkably total peripheral resistance than that of sodium nitrite. Hexamethonium at approximately equihypotensive
dose of sodium nitrite produced greater reduction of aortic blood flow and consequentially showed the least reduction of peripheral resistance among the above three drugs. In contrast with these three agents, papaverine increased aortic blood flow, so that the resistance decreased most significantly.

5. Effect of CV-664 on inferior vena caval blood flow in anesthetized, open-chest dogs

Intravenous injection of 0.05 mg/kg of CV-664 in 2 dogs decreased the inferior vena caval flow for the periods of time equivalent to the fall of systemic blood pressure. The decrease in the flow was observed to be associated with a greater fall in systolic pressure without concomitant decrease in heart rate and contractile force. Fig. 6 shows the original recording from a representative experiment.
6. Effects on femoral or coronary vascular bed of dogs

a) Blood flow

Intravenous injection of the hypotensive doses of CV-664 produced decrease of the blood flow concomitant with the reduction of systemic blood pressure. Direct injection of 0.5 to 5 μg/kg of CV-664 into the femoral or coronary artery caused transient increase in the rate of the blood flow in the autoperfused vascular beds. Fig. 10 illustrates the
Fig. 10. Effect of CV-664 on coronary blood flow in an anesthetized, open-chest dog following direct intracoronary artery injections (ic). CBF, coronary blood flow of the left descending branch; Contr., contractile force of left ventricle; BP, carotid blood pressure; PRU, peripheral resistance unit.

Characteristic effects of intracoronary artery injection of CV-664 on coronary blood flow, left ventricular contractile force and systemic blood pressure. As shown in this figure, when the drug reached the systemic circulation and produced hypotension, blood flow no longer increased and even decreased. Peripheral resistance of the coronary artery (femoral blood pressure in mmHg divided by the coronary flow in ml/min) was reduced.

b) Constant blood flow perfusion in intact hind limbs

Direct intraarterial injection of 0.05 to 1.0 mg of the hypertensive sydnonimines (CV-654, 555) into the femoral artery caused a prolonged increase in perfusion pressure which was sometimes preceded by an immediate and transient fall in perfusion pressure. When the same dosage was given repeatedly, the sustained rise in perfusion pressure became progressively smaller, while the initial decrease in the pressure was observed in all cases and became progressively larger, being similar to the blood pressure response after the repeated intravenous administrations to the anesthetized cat.
Fig. 11 shows effects of CV-664 on femoral vascular resistance in 5 constant blood flow perfusion preparations. When the drug was given intravenously, perfusion pressure decreased rapidly ($-29.3 \pm 6.9\%$, mean ±S.E.) as systemic blood pressure fell ($-24.7 \pm 8.7\%$). Thereafter, perfusion pressure increased to levels around the control ($+9.0 \pm 12.5\%$) within 5 minutes and again decreased to levels below the control. The period and magnitude of the second reduction of perfusion pressure ($-13.7 \pm 4.1\%$) were much less marked than those of the fall in systemic pressure ($-45.3 \pm 4.6\%$), suggesting an insufficient decrease in femoral vascular resistance to account for the CV-664-induced hypotension. Pretreatment of 2 dogs with dibenamine (15 mg/kg) did not affect significantly the systemic and perfusion pressure responses to CV-664, although the recovery of the responses was slightly slower than that in the non-treated animals. This slower recovery may be due to an interruption of the reflex vasoconstriction by dibenamine during CV-664-induced hypotension.

After the intravenous injection of CV-664 (1 mg/kg), constrictor responses in the perfused leg induced by the intraarterial injection of norepinephrine (0.5 μg) and by electrical stimulation of the lumbar sympathetic chain (5 V, 2 millisecond, 30/sec, for 20 seconds) were reduced by 36 to 61 percent and 36 to 59 percent, respectively, and a subsequent single injection of CV-664 caused but little additional reduction of the responses.

c) Constant blood flow perfusion in isolated hind limbs

In order to evaluate effects of the sydnonimines on the tone of the resistance and capacitance vessels, changes in perfusion pressure and hind limb volume after intraarterial injection of CV-664 or CV-555, and various vasoactive agents were observed in
FIG. 12. Constant blood flow perfusion experiments on isolated hind limb of dogs. Changes in perfusion pressure (PP) and hind limb volume (LV) after intrafemoral artery injection (ia) were observed in order to evaluate effects of CV-664 on the tone of the resistance and capacitance vessels, and compared with those of sodium nitrite, 2-aminooethyl-nitrate-p-toluensulfonate (AEN), papaverine (PAP), hydralazine (HYDRA-LAZ), acetylcholine (ACh), and histamine (HIS). Upper: the original recording from a typical experiment. Lower: the relation between increase in hind limb volume and decrease in perfusion pressure in 10 preparations after administrations of the various vasoactive drugs. VF, venous outflow; HbO₂, oxyhemoglobin concentration change in venous blood; BP, systemic blood pressure.

| Drug   | Dose (μg/ia) |
|--------|--------------|
| PAP    | 25 - 50      |
| NaN₂   | 100 - 500    |
| AEN    | 0.5 - 1      |
| PAP    | 25 - 50      |
| HYDRA-LAZ | 20   |
| ACh    | 0.05 - 0.1   |
| HIS    | 0.05 - 0.25  |

10 isolated hind limb preparations. Fig. 12 illustrates the original recording from a typical experiment and the relation between increase in hind limb volume and decrease in perfusion pressure. The relations indicated that CV-664 predominantly affects the
capacitance vessels, being similar to that of sodium nitrite or 2-aminoethyl nitrate-p-toluensulfonate. In sharp contrast to these drugs, hydralazine produced a long-lasting decrease of perfusion pressure without significantly affecting hind limb volume, as reported by Åblad and Mellander (9) in skeletal muscle in the cat. CV-555, 0.25-0.5 mg, produced a rise in perfusion pressure and a fall of leg volume.

7. Cross-circulation of the hind limb of dogs

In three successful cross-circulation experiments of the hind limb (the only connection between the limb and the body of the recipient dog was the intact sciatic nerve trunk), CV-655, 1 mg/kg, injected into the femoral vein of the body of the recipient dog did not decrease but increase slightly the recipient limb perfusion pressure indicating a lack of centrally mediated vasodilatation.

8. Nictitating membrane in anesthetized cats

In one out of five cats, 2 mg/kg i.v. of CV-664 slightly reduced (-15%) the nictitating membrane response to preganglionic sympathetic nerve stimulation, while in the other 4 cats did not alter the response. A second injection of 5 mg/kg i.v. also did not significantly affect the response. CV-555 or CV-582, 1 mg/kg i.v., produced an immediate and protracted increase in the tone of the nictitating membrane paralleled with rise in the blood pressure. The contraction of the membrane as well as the pressor response was abolished by prior treatment with phentolamine (3 mg/kg i.v.).

9. Spasmolytic action in guinea pig ileum and trachea, and rabbit intestine

The antagonistic effects of CV-664 and papaverine on contractions of guinea pig ileum induced by acetylcholine, histamine and barium chloride are summarized in Table 3.

| Acetylcholine | Histamine | Barium chloride |
|--------------|-----------|----------------|
| CV-664       | 4.2 × 10⁻⁶| 5.1 × 10⁻⁷      | 3.9 × 10⁻⁴ |
| Papaverine   | 4.4 × 10⁻⁴| 2.7 × 10⁻⁶      | 1.9 × 10⁻⁴ |

*Concentrations of 50% inhibition (g/ml)

Both agents were similar in potency and antagonized the contractions induced by all of the agonists in a relatively narrow range of concentrations, indicating that CV-664 is a nonspecific spasmolytic agent. Histamine-induced contraction of guinea pig tracheal muscle was inhibited by more than 50% by 10 μg/ml of CV-664. In the isolated rabbit intestine, 1 μg/ml of the compound inhibited slightly the spontaneous contractile activity and 10 μg/ml of the drug blocked almost completely the activity.

DISCUSSION

The most prominent feature of the 3-substituted sydnonimines studied on blood pressure response is that the derivatives have prolonged hypotensive or hypertensive properties,
depending on their 3-substituents. This dicotomy of action is thought to be due to either stability of the ring or of the ring-opened derivatives or special molecular structure of the sydnonimines. That is, the hypotensive and hypertensive agents in the Table 1 are stable to acid but suffer ring opening in alkali. And, a ring-opened derivative of 3-morpholinosydnonimine (CV-664, SIN-1), N-nitroso-N-morpholinoaminoacetonitrile (SIN-1A), is unstable, leading to the release of NO ion (4). The derivative is proved to be a rapid-acting and very potent vasodilator (10, 11), being similar to that of nitroglycerin, while a ring-opened derivative of 3-cyclohexylsydnonimine (CV-555, SIC-11), N-nitroso-N-cyclohexylaminoacetonitrile, is stable and proved to be inactive, as shown in Fig. 13. Similar reasoning may be applied to both compounds unsubstituted and substituted at position 4 (see)

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*Fig. 13. Chemical structures and blood pressure responses in anesthetized cats following intravenous administrations of two 3-substituted sydnonimines and their ring-opened derivatives. See text (under Discussion) for description of the responses.*
CV-655 vs. CV-692 in the Fig. 1). When comparing the onset of action and the potency of SIN-1A with those of SIN-1 or CV-655, it is also assumed that those are dependent upon the rate of the in vivo production of an active metabolite and the rate of the release of NO ion. But these considerations of structure and action are at present suggestive only.

Pressor or sympathomimetic responses of 3-substituted sydnonimines studied appear to be due mainly to activation of adrenergic receptors, probably through release of catecholamines. Supporting this view is the observation that hypertensive response and contraction of the nictitating membrane produced by the derivatives are abolished by pretreatment with phentolamine, and the positive inotropic action in the isolated guinea pig atria is inhibited in reserpinized preparations as well as suppressed by pretreatment with adrenergic beta-receptor blocking agents. In anesthetized, open-chest dogs, positive inotropic and chronotropic actions induced by CV-555 were also blocked by pretreatment with propranolol. In anesthetized cats or heart-lung preparations, tachyphylaxis to CV-654-induced pressor response or to CV-664-induced cardiotonic actions developed. These observations are similar to those of tyramine, which is known to produce its effect by releasing norepinephrine from endogenous catecholamine stores in the heart (12). Preliminary experiments showed that norepinephrine content of rat hearts determined 4 hours after an intraperitoneal dose of 25 mg/kg of either CV-664 or tyramine was depleted to about 80 and 70 percent, respectively, to the control levels (Hibino, unpublished data).

SIN-series compounds-induced hypotension probably results from a direct action on vascular beds, especially on capacitance vessels, being similar to that of the nitrites (9, 13). Evidence in support of this contention is derived from the following observations: a) the hypotension was not appreciably influenced by elimination of the central and autonomic nervous systems such as spinal transection, pharmacologic blockade of autonomic ganglia or of adrenergic, cholinergic or histamine receptors; b) in the cat nictitating membrane preparation, CV-664 did not consistently affect sympathetic nerve function; c) the hypotension led to a decrease in pulse pressure, primarily because of a decrease in systolic pressure; this is suggestive of a decrease in cardiac output induced by a decreased venous return, the mechanism of which is a pooling of blood in the capacitance vessels; in fact, intravenous injection of CV-664 reduced venous return and cardiac output; and, in constant blood flow perfusion experiments on isolated hind limb of dogs, intraarterial injection of CV-664 predominantly affected the capacitance vessels rather than the resistance vessels, being similar to those of the nitrites; d) in anesthetized, open-chest dogs, effective hypotensive doses of CV-664 exerted no appreciable effects on the myocardial contractile force or heart rate; in spontaneously beating guinea pig atria, CV-664 exerted only a slight positive inotropic action in a high concentration (1 × 10^{-4} g/ml), the action of which was reduced by pretreatment with propranolol; in the heart-lung preparation of a cat, CV-664 increased cardiac output and heart rate; e) additional evidence is that CV-664 has a non-specific musculotropic depressant activity.
SUMMARY

In anesthetized cats and dogs, intravenous injection (0.02–5.0 mg/kg) of 3-substituted sydnonimines caused a prolonged hypotension or hypertension, depending on their 3-substituents. Relationship between structure and action indicated that the direct attachment of the nitrogen of a 3-substituent such as morpholine, piperidine, dialkylamine to the 3-position of the sydnonimine ring was required for a marked, prolonged hypotension. The hypotension induced by 3-morpholinosydnonimine hydrochloride (CV-664, SIN-1) was not antagonized by autonomic blocking agents, spinal cord transection, or evisceration. Further investigations of the cardiovascular actions provided evidence that SIN-1-induced hypotension was assumed to be due mainly to a direct action on vascular beds, especially on capacitance vessels, being similar to that of the nitrites. The compounds which have alkyl-, cycloalkyl-, or dialkylaminoalkyl-group in the 3-position, on the other hand, produced a hypertension and other sympathomimetic actions which appear to be due mainly to activation of adrenergic receptors through release of catecholamines. Discussion was made on structure and action of the 3-substituted sydnonimines and their ring-opened derivatives.

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