PHARMACOLOGICAL STUDIES OF LYCORENINE, 
AN ALKALOID OF LYCORIS RADIATA HERB.: 
VASODEPRESSOR MECHANISM IN RATS

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Abstract—Vasodepressor mechanism of lycorenine (an alkaloid of Lycoris radiata Herb.) was investigated in anesthetized rats. Lycorenine (1–10 mg/kg i.v.) produced dose-related decreases in blood pressure and heart rate and tachyphylaxis developed with repeated injections. In the blood-perfused rat hindquarters, lycorenine (62.5–500 µg i.a.) produced dose-related decreases both in mean blood pressure and in perfusion pressure, and the lycorenine-induced decrease in perfusion pressure was abolished by phenoxybenzamine or hexamethonium. Lycorenine (more than 1 mg/kg i.v.) blocked the pressor response to sympathetic nerve stimulation, but failed to block the tachycardia induced by sympathetic nerve stimulation. Lycorenine (7.5 or 15 mg/kg i.v.) reduced the spontaneous splanchnic nerve activity. Lycorenine when given intracerebroventricularly produced decreases in blood pressure and heart rate only in large doses (over 500 µg). The maximal bradycardia induced by lycorenine was abolished by bilateral vagotomy. It is suggested that lycorenine may produce a decrease in blood pressure as the result of alpha-adrenergic blockade in conjunction with the reduction of the spontaneous sympathetic nerve activity, and produce bradycardia by modifying vagal activity.

Lycoris radiata Herb. is a poisonous plant which contains alkaloids such as lycorenine, lycorine, lycoramine, homolycorine, hippeastrine, galantamine, etc. We found a substance in the methanol-water extract of bulbs of Lycoris radiata Herb. which produced a decrease in blood pressure and with repeated injections tachyphylaxis developed to the vasodepressor effect in rats. This substance was identified as lycorenine. Although there are papers on the pharmacological effects of lycorine, lycoramine, and galanthamine among alkaloids of Lycoris radiata Herb., only one report on lycorenine (1) is available stating that this compound produced a decrease in blood pressure in dogs, cats, and rabbits, a transient increase of contractile force in the isolated toad heart, and an increase of motility of the isolated rabbit ileum. However, the mechanism of the vasodepressor action of lycorenine was not discussed. In the present study, the effects of lycorenine on the cardiovascular system and the autonomic nervous system were investigated in rats for elucidation of the vasodepressor mechanism.

MATERIALS AND METHODS

Animals: Male Donryu rats, weighing 250–350 g, were used for cardiovascular studies. Female Donryu rats (200–250 g), male ICR mice (30–35 g), and male Hartley guinea-pigs
(300–350 g) were also used for in vitro experiments.

**Measurements of blood pressure, perfusion pressure, cardiac output, and heart rate:** Rats were anesthetized with sodium pentobarbital (70 mg/kg, i.p.). Blood pressure was measured at the femoral artery by means of a pressure transducer (Nihon Kohden RP-3) and recorded on a polygraph (Nihon Kohden RM-150). In some experiments, blood pressure was measured at the carotid artery by means of a mercury manometer and recorded on a smoked drum. Heart rate was monitored with a tachometer (Nihon Kohden RT-2) triggered by blood pressure pulse. With the animals in a conscious state, blood pressure was directly measured using the procedure similar to that described by Weeks and Jones (2).

Cardiac output was measured by means of a square wave electromagnetic flowmeter (Nihon Kohden MF-5) and recorded on a polygraph (Nihon Kohden RM-150). The trachea was intubated and artificial ventilation was performed. The thorax was opened by a median sternotomy. A 3 mm probe (Nihon Kohden) was attached around the ascending aorta for measurement of the cardiac output. The intracerebroventricular (i.c.v.) injections were made into the lateral cerebral ventricle through a stainless steel cannula introduced according to the atlas of de Groot (3) for the rat whose head was fixed by a stereotaxic apparatus (Todai Noken type). A micrometer syringe was attached to the cannula via polyethylene tubing (PE 10). Lycorenine was given by adjusting the volume of its solution the concentration of which was 30.7 mg/ml (pH=7.0). Injection volume was less than 32.6 μl, because this volume of 0.9% NaCl solution had no influence on blood pressure and heart rate.

Perfusion of the hindquarters in rats was performed using the procedure similar to that described by Brody et al. (4). The trachea and the external jugular vein on either side were cannulated. As shown in Fig. 1, the aorta was approached by an abdominal midline incision and three silk ligatures were placed around the aorta, approximately 5 mm apart from each other. Following the i.v. administration of 1000 U/kg of heparin sodium, the aorta was ligated and connected bidirectionally to the external circuit shown in Fig. 1. The

![Fig. 1. Schematic diagram of the blood-perfused rat hindquarter.](image-url)
extra-corporeal circuit was constructed of silicone tubing with inside diameter of 1 mm and wall thickness of 1 mm and PE 100 tubing to be inserted into both ends of the silicone tubing for cannulation of the aorta. Two sections of glass T-tube were interposed in the external circuit in the positions shown in Fig. 1 and one portion of these tubes was connected to pressure transducers through PE tubing for the registration of systemic blood pressure and perfusion pressure. Following cannulation of the aorta, the silicone tubing was inserted into a Cum pump (Tokyo Rikakikai) and the flow rate was adjusted to such that perfusion pressure was essentially equivalent to systemic blood pressure. Intra-arterial injections to the hindquarters were made into the rubber tube interposed just before the distal cannula. The volume of intra-arterial injection was kept constant at 0.025 ml.

Stimulation of different segments of the sympathetic outflow from the spinal column: Stimulation of different segments of the sympathetic outflow from the spinal column was performed using the procedure similar to that described by Gillespie et al. (5). Rats were anesthetized with ether and tracheal intubation was performed. After d-tubocurarine chloride (1 mg/kg, i.v.), was given, the animal was ventilated artificially. In some experiments, adrenalectomies were performed. Rats were pithed by insertion of steel tubing (2 mm in diameter) through the orbit and the foramen magnum and down into the spinal column to the vertebral level of C6. After complete pithing with a pithing rod through this trocar, a monopolar stimulating electrode (0.5 mm in diameter) was inserted into the vertebral level T7–9 to obtain the pressor response or into the vertebral level C7-T1 to obtain the cardiac accelerator response. The stimulating electrode was insulated with a cashew coating except the tip which was 10 mm long for the pressor response or 3 mm long for the cardiac accelerator response. A steel needle (0.5 mm in diameter) inserted between the vertebral column and the back skin acted as an indifferent electrode. The spinal nerve roots were stimulated electrically by 1 msec pulses of supra-maximal voltage (approximately 60 V) for the pressor response or by 0.5 msec pulses of supra-maximal voltage (approximately 50 V) for the cardiac accelerator response. Progressively increasing stimulus frequencies were applied, each frequency being continued for 30 sec for the pressor response or for 2 min for the cardiac accelerator response.

Measurement of spontaneous discharges of the major splanchnic nerve: Spontaneous discharges of the major splanchnic nerve were measured using the procedure similar to that described by Okamoto et al. (6). Rats were anesthetized with urethane (1.25 g/kg, i.v.) or sodium pentobarbital (70 mg/kg, i.p.) and the skin of the animal’s back was incised and the muscles (a part of rhomboideus occipitalis and splenius) were removed to reach the left major splanchnic nerve. The major splanchnic nerve was isolated at its exit from the diaphragm, and sectioned at its entrance to the coeliac ganglion. In order to prevent drying, the nerve was kept in a liquid paraffin bath made of thin rubber. A bundle of fibers was placed on a pair of stainless steel electrodes and electrical activity of the nerve was displayed on a cathode ray oscilloscope (Nihon Kohden VC-7) and recorded on film by means of a continuous recording camera (Nihon Kohden PC-2B).

Measurement of postganglionic action potentials of the superior cervical ganglion: Rats
were anesthetized with urethane (1.25 g/kg, i.v.) and after cervical midline incision, the preganglionic and postganglionic fibers of the superior cervical ganglion were separated from the surrounding tissues and cut after ligations. The preparation was covered with liquid paraffin to prevent from drying. Pairs of stainless steel electrodes were used for stimulation and recording. Postganglionic action potentials in response to stimulation of preganglionic fibers were displayed on a cathode ray oscilloscope and recorded on film by means of a continuous recording camera.

In vitro experiments: Anti-cholinergic effect was examined on the isolated guinea-pig ileum and anti-noradrenergic and anti-serotonergic effects were examined on the isolated mouse seminal vesicle and the isolated rat uterus, respectively.

Drugs: Lycorenine was obtained from the bulbs of Lycoris radiata Herb. by the method of Ueo (7), mp. 202-204°C, [α]D 174.9° (C=1.65, CHCl3) [in the lit. (7), mp. 198-200°C, [α]D 180 (CHCl3)]. The NMR spectrum corresponded well to that described in the literature (8, 9). Lycorenine-related compounds (Fig. 2) were prepared in our laboratory. Other drugs used were atropine sulfate, dibenamine hydrochloride, ergotamine tartrate, phenoxybenzamine hydrochloride, serotonin creatinine sulfate, d-tubocurarine chloride, yohimbine hydrochloride (Tokyo Kasei), acetylcholine chloride (Ovisot, Daiichi), desipramine hydrochloride (Pertofran, Fujisawa), hexamethonium bromide (Methobromine, Yamanouchi), L-noradrenaline bitartrate (Wako), papaverine hydrochloride (Dainippon), phenolamine mesylate (Regitine, Takeda), propranolol hydrochloride (Inderal, Sumitomo), tripelemamine hydrochloride (Pyribenzamine, Takeda). Lycorenine and its related compounds were dissolved in dilute HCl solution and were neutralized with 1N-NaOH. Phenoxybenzamine was dissolved in a concentration of 10 mg/ml in propylene glycol containing 0.01N-HCl which was diluted with 0.9% NaCl solution to an appropriate concentration. Other compounds were dissolved in 0.9% NaCl solution. Drug doses refered to the salts except lycorenine and its-related compounds which were expressed in terms of the base. All drugs were given intravenously, unless otherwise mentioned. The significance of the difference of two means was evaluated with Student's t-test.

**Fig. 2.** Chemical structures of lycorenine and its related-compounds.
RESULTS

Decreases in blood pressure and heart rate induced by lycorenine and the development of tachyphylaxis to both the responses at repeated injections: Lycorenine produced dose-related decreases in blood pressure and heart rate in the dose range of 1–10 mg/kg in anesthetized rats (Fig. 3). Tachyphylaxis developed in both the responses with repeated injections of lycorenine (more than 2.5 mg/kg) at 30 min intervals in anesthetized rats and this tachyphylaxis was pronounced in doses over 7.5 mg/kg (Table 1). Tachyphylaxis to the vasodepressor action of lycorenine (7.5 mg/kg) also developed with repeated injections at 60 min intervals in anesthetized rats and with those at 30 min intervals in conscious rats (Table 1).

Lycorenine (7.5 mg/kg) produced a decrease in blood pressure which reached a maximum 2 min after the injection and bradycardia which occurred immediately after the injection and reached a maximum 5–10 min later in anesthetized rats (maximal bradycardia) (Fig. 4). Bilateral vagotomy resulted in a complete abolition of the maximal bradycardia induced 5–10 min after the injection of lycorenine (7.5 mg/kg), but it potentiated the degree and the duration of the decrease in blood pressure (Fig. 4).

Effects of various treatments on the decreases in blood pressure and heart rate induced by lycorenine in bilaterally vagotomized rats: Results are shown in Table 2. The decrease in blood pressure induced by lycorenine (5 mg/kg) was markedly reduced by hexamethonium
Table 1. Effects of the repeated injections of lycorenine on mean blood pressure and heart rate in pentobarbital anesthetized or conscious rats

1) Mean blood pressure

| Lycorenine Doses (mg/kg, i.v.) | n | A | B (min) | First injection | Mean blood pressure (mmHg) | Third injection |
|---------------------------------|---|---|---------|----------------|-----------------------------|-----------------|
|                                 |   |   |         | Control | Responses | Control | Responses | Control | Responses |
| 1.0                             | 5 | a | 30      | 135.5±7.7 | -19.0±2.8 | 138.0±7.3 | -14.9±2.8 | 135.6±7.0 | -12.5±2.7 |
| 2.5                             | 5 | a | 30      | 125.0±1.4 | -28.0±2.2 | 128.5±2.4 | -21.5±3.6 | 131.5±1.5 | -17.0±1.2** |
| 5.0                             | 6 | a | 30      | 130.4±3.7 | -33.3±2.9 | 138.3±3.8 | -18.3±1.9*** | 142.1±5.6 | -12.1±0.8*** |
| 7.5                             | 6 | a | 30      | 127.5±3.8 | -40.0±5.1 | 124.6±3.4 | -11.3±1.3** | 124.2±2.9 | -9.2±1.5** |
| 7.5                             | 5 | a | 60      | 128.5±3.7 | -41.5±3.4 | 129.5±4.4 | +6.5±2.7*** | 126.5±4.0 | +14.0±1.7*** |
| 7.5                             | 6 | b | 30      | 114.6±6.4 | -45.4±4.4 | 115.4±5.5 | -23.8±2.6** | 115.8±5.5 | -21.7±3.3** |
| 10.0                            | 5 | a | 30      | 127.5±2.6 | -42.5±7.5 | 115.0±5.0 | +19.0±4.6*** | 117.0±4.8 | +18.5±3.8*** |

2) Heart rate

| Lycorenine Doses (mg/kg, i.v.) | n | A | B (min) | First injection | Heart rate (beats/min) | Third injection |
|---------------------------------|---|---|---------|----------------|-------------------------|-----------------|
|                                 |   |   |         | Control | Responses | Control | Responses | Control | Responses |
| 1.0                             | 5 | a | 30      | 397.1±13.2 | -18.3±6.7 | 379.9±8.9 | +1.9±6.7 | 397.8±16.5 | +9.2±2.5* |
| 2.5                             | 5 | a | 30      | 375.3±11.6 | -30.4±4.6 | 380.6±17.9 | -7.9±3.8** | 394.8±25.4 | +2.0±2.9*** |
| 5.0                             | 6 | a | 30      | 372.2±6.6 | -41.3±9.0 | 372.5±13.1 | -13.0±1.4* | 400.0±8.6 | -12.0±1.0* |
| 7.5                             | 6 | a | 30      | 330.8±12.7 | -48.7±9.9 | 361.7±28.0 | -19.5±1.5* | 390.8±21.7 | -24.2±1.5 |
| 7.5                             | 5 | a | 60      | 402.6±14.5 | -65.3±8.4 | 359.8±26.2 | +22.6±4.8** | — | — |
| 7.5                             | 6 | b | 30      | 343.0±10.9 | +75.7±32.8 | 401.0±22.0 | +53.0±19.8 | 397.0±22.4 | +41.0±16.3 |
| 10.0                            | 5 | a | 30      | 375.8±17.8 | -56.5±11.8 | 332.2±28.9 | -23.4±4.3 | 339.0±29.2 | -28.3±5.1 |

Results are expressed as means±S.E.M.. Repeated injections were given at 30 or 60 min intervals, (column B). Column A indicates conditions under which measurements were made: (a) pentobarbital anesthesia (70 mg/kg, i.p.); (b) conscious state. Responses indicate maximal ones. *p<0.05; **p<0.01; ***p<0.001, Significantly different from first injection.
Table 2. Effects of various treatments on the decreases in blood pressure and heart rate induced by lycorenine (5 mg/kg, i.v.) in bilaterally vagotomized and pentobarbital anesthetized rats

| Treatments          | Doses (mg/kg, i.v.) | n  | Mean blood pressure (mmHg) | Heart rate (beats/min) |
|---------------------|---------------------|----|----------------------------|------------------------|
|                     |                     |    | Initial | After treatments | Maximal responses | Initial | After treatments | Maximal responses |
| Control             | —                   | 20 | 133.5±3.8 | — | — | 376.4±6.7 | — | — | 19.7±2.4 |
| Hexamethonium       | 15                  | 6  | 125.3±4.0 | 98.6±4.4 | — | 302.3±9.6 | 227.7±11.2 | — | 8.5±1.9 |
| Phenoxybenzamine    | 10                  | 8  | 127.5±2.6 | 125.6±5.1 | ±13.4±0.9*** | 370.1±8.2 | 402.8±11.2 | — | 14.8±4.1 |
| Spinal cut (Cl)     | —                   | 5  | — | 74.0±4.3 | ±6.1±1.1*** | — | 291.0±2.3 | — | 33.3±7.9* |
| Propranolol         | 2                   | 5  | 127.5±3.5 | 129.4±4.0 | — | 384.8±9.6 | 315.5±12.9 | — | 40.8±7.2** |
| Atropine            | 2                   | 5  | 137.0±6.8 | 141.5±7.1 | — | 398.0±7.3 | 386.4±6.4 | — | 13.6±1.5 |
| Tripelennamine      | 10                  | 6  | 124.2±3.1 | 116.3±3.2 | — | 338.2±9.8 | 355.0±10.3 | — | 8.7±0.7*** |
| Desipramine         | 1                   | 5  | 128.5±5.3 | 122.5±8.8 | — | 350.2±11.9 | 380.0±6.5 | — | 15.2±1.5*** |

Results are expressed as means±S.E.M.. *p<0.05; **p<0.01; ***p<0.001, Significantly different from control.
(15 mg/kg), phenoxybenzamine (10 mg/kg), and spinal cut (C1), but not by atropine (2 mg/kg) and propranolol (2 mg/kg). After treatment with desipramine (1 mg/kg) or tripelemamine (10 mg/kg), the duration of the vasodepressor action of lycorenine was shortened and the bradycardia was modified in such way that marked tachycardia followed transient slight bradycardia. The rise in heart rate appeared approximately 1 min after the injection and reached a maximum approximately 10 min later. In spinal rats treated with desipramine, lycorenine also produced marked tachycardia which was completely blocked by propranolol (2 mg/kg) (Table 3).

**Effect of lycorenine on cardiac output:** Lycorenine (7.5 mg/kg) produced an increase in cardiac output. The time course of the increase in cardiac output was roughly similar

| Pretreatment          | Mean blood pressure (mmHg) | Heart rate |
|-----------------------|-----------------------------|------------|
|                       | Control                   | Responses  |
|                       | 65.0±4.6                  | +7.5±0.8   |
|                       |                            | −5.0±1.8   |
|                       | 74.5±1.7                  | −9.0±1.0   |
|                       |                            | +3.1±0.3   |
|                       |                            | +14.2±2.0***|
| Control               | 291.0±2.3                 | −11.4±2.7  |
| Propranolol 2 mg/kg i.v. | 335.0±11.5                | +14.2±2.0***|
| Desipramine 1 mg/kg i.v. | 73.0±4.8                  | −11.0±2.2  |
|                       | 313.4±11.3                | −5.2±1.0   |

Results are expressed as means±S.E.M. obtained from 5 animals. ***p<0.001, Significantly different from control.

**Table 3. Cardiovascular responses to lycorenine (5 mg/kg, i.v.) in bilaterally vagotomized, spinal rats**

**Fig. 5.** Effect of lycorenine on blood pressure, cardiac output, and heart rate in pentobarbital anesthetized rats. Each point represents the mean obtained from 5 animals. Vertical bars indicate S.E.M.
to that of the decrease in blood pressure (Fig. 5).

**Effects of lycorenine and papaverine on blood pressure and perfusion pressure of the blood-perfused rat hindquarters:** Figure 6 illustrates responses obtained with several intra-arterial doses of lycorenine and papaverine. Lycorenine produced dose-related falls of mean blood pressure and of perfusion pressure in the dose range of 62.5–500 μg/animal,
and the fall of the former was greater than that of the latter. On the other hand, papaverine produced dose-related falls of perfusion pressure in the dose range of 6.25–125 μg/animal, but did not produce a definite fall of mean blood pressure even at the dose of 125 μg/animal. Pretreatment with hexamethonium (15 mg/kg) or phenoxybenzamine (15 mg/kg) blocked almost completely the lycorenine (500 μg, i.a.)-induced fall of perfusion pressure, but did not block the action of papaverine (25 μg, i.a.) (Fig. 7).

Effect of the i.c.v. injection of lycorenine on blood pressure and heart rate: Lycorenine produced decreases in mean blood pressure and heart rate only when doses over 500 μg/animal were injected into the lateral cerebral ventricle (Fig. 8). The time course of the vasodepressor action of lycorenine (i.c.v.) was similar to that of bradycardia and the maximal effects were obtained 10 min after the injection.

Effect of lycorenine on the pressor response and the tachycardia induced by stimulation of different segments of the sympathetic outflow from the spinal column in the pithed rats:

1) Effect on the pressor response

When the stimulating electrode was placed at the vertebral level of T7-9, stimulation produced a frequency (0.5–32 Hz)-related pressor response in the bilaterally adrenalectomized rats. Lycorenine at doses over 1 mg/kg significantly reduced this pressor response (Fig. 9). The duration of the blocking activity of lycorenine on the pressor response was investigated in the pithed, non-adrenalectomized rats. Lycorenine still reduced significantly the pressor response 2 hr after the i.v. injection of 7.5 mg/kg (Fig. 10).

2) Effect on the tachycardia

Stimulation at the vertebral level of C7-T1 produced a frequency (0.25–2 Hz)-related
tachycardia. Lycorenine at the dose of 15 mg/kg did not significantly reduce the tachycardia induced by stimulation even at low frequencies (0.25 and 0.5 Hz) (Fig. 11).

Effect of lycorenine on the spontaneous splanchnic nerve activity: Effect of lycorenine on the sympathetic nerve activity was investigated by measuring the spontaneous splanchnic nerve activity. It was confirmed that the activities recorded were exactly sympathetic, because they were augmented markedly by asphyxia brought about by stopping artificial respiration and reduced by the vasopressin (0.05 u, i.v.)-induced elevation of blood pressure. Lycorenine at the doses of 7.5 and 15 mg/kg produced a reduction in spontaneous discharges.

FIG. 10. Time course of the inhibitory effect of lycorenine on the rise in mean blood pressure caused by stimulation of the spinal column (vertebral level T7-9) in pithed rats. One msec pulses of 7.5 times the threshold voltage at the frequency of 10 Hz were applied for 30 sec. Each point represents the mean obtained from 5 animals. Vertical bars indicate S.E.M. -○--: saline i.v.; -□--: lycorenine 7.5 mg/kg, i.v. **p<0.01; ***p<0.001, Significantly different from control.

FIG. 11. Maximal tachycardia caused by supramaximal stimulation of the spinal column (vertebral level C7-T1) at various frequencies before (—○—) and 5 min after (—□—) i.v. injection of saline or lycorenine (15 mg/kg) in pithed rats. Each point represents the mean obtained from 5 animals. Vertical bars indicate S.E.M.
of the major splanchnic nerve in urethane anesthetized rats (Fig. 12) and pentobarbital anesthetized rats (Fig. 13). This reduction was recovered 10 min after the injection of lycorenine in urethane anesthetized rats, but not yet recovered 30 min after the injection in pentobarbital anesthetized rats. The reduction of spontaneous discharges of the splanchnic nerve was also observed at the second injection at 30 min intervals, although in some cases the reduction was less.

**Fig. 12.** Effect of lycorenine on the spontaneous discharges of the splanchnic nerve in urethane anesthetized rats.

**Fig. 13.** Effect of lycorenine on the spontaneous discharges of the splanchnic nerve in pentobarbital anesthetized rats.
Table 4. Effects of lycorenine and its related compounds (each 7.5 mg/kg, i.v.) on mean blood pressure and heart rate, and on the noradrenaline (NA, 0.2 g, i.v.)-induced pressor response in pentobarbital anesthetized rats

| Compounds               | n  | Mean blood pressure (mmHg) | Heart rate (beats/min) | Pressor response induced by NA |
|-------------------------|----|---------------------------|------------------------|-------------------------------|
|                         |    | Control Responses         | Control Responses     | n   | Inhibition (%) |
| Lycorenine              | 6  | 137.5±3.3 -43.3±4.7       | 394.5±12.9 -46.8±8.5   | 4   | 69.6±3.2       |
| Homolycorenine          | 5  | 136.0±5.6 -26.5±2.5*      | 321.4±9.0 -61.2±5.3    | 4   | 69.8±3.3       |
| Lycorenine-acetate      | 6  | 137.5±4.6 -47.9±2.4       | 405.0±4.1 -105.3±10.1* | 5   | 17.1±13.4*     |
| Compound 1              | 5  | 131.5±2.7 -14.0±2.6***    | 309.4±13.4 -36.8±1.5   | 5   | 67.3±3.4       |
| Compound 2              | 5  | 142.0±5.8 -48.0±8.2       | 362.0±10.2 -47.0±3.4   | 5   | 77.7±6.1       |
| Compound 3              | 5  | 129.0±5.9 +12.0±5.0***    | 376.0±11.2 -40.6±5.2   | 4   | 23.0±5.4***    |
| Compound 4              | 5  | 139.0±5.0 -73.5±6.6**     | 336.8±16.2 -58.2±11.7  | 5   | -16.5±4.1***   |

Results are expressed as means±S.E.M. Responses indicate maximal ones. Inhibitory effects of lycorenine and its related compounds on the NA-induced pressor response were examined 5 min after injection. *p<0.05; **p<0.01; ***p<0.001, Significantly different from lycorenine treated group.
Effect of lycorenine on the postganglionic action potentials of the superior cervical ganglion:
Lycorenine at 15 mg/kg did not block the postganglionic action potentials induced by stimulation of the preganglionic fibers.

Effects of lycorenine-related compounds on blood pressure and heart rate, and their alpha-adrenergic blocking activities: The chemical structures of lycorenine and its related compounds investigated in the present study are shown in Fig. 2. Effects of lycorenine and its related compounds (7.5 mg/kg) on blood pressure and heart rate are shown in Table 4. The order of vasodepressor potencies for lycorenine and its related compounds tested was the following: compound 4 > compound 2 = lycorenine acetate = lycorenine > homolycorine > compound 1. Compound 3 produced no depressor response. The vasodepressor actions of lycorenine, homolycorine, and compound 1 were similar in time course and maximal responses were attained 2 min after the injections. Lycorenine acetate produced a maximal vasodepressor action immediately after the injection and compound 4 produced a relatively short-lasting decrease in blood pressure, secondarily following temporal arrest of respiration.

Lycorenine produced maximal bradycardia 5-10 min after the injection, while other lycorenine-related compounds except compound 4 which produced maximal bradycardia 1 min after the injection, produced maximal bradycardia immediately after injections. The abilities of lycorenine and its related compounds to block the noradrenaline-induced pressor response are shown in Table 4. The order of alpha-adrenergic blocking potencies was the following: compound 2 = lycorenine = homolycorine = compound 1 > compound 3 = lycorenine acetate, and compound 4 had an opposite effect.

In vitro experiments: Lycorenine inhibited contractions of the isolated mouse seminal vesicle to noradrenaline and the isolated rat uterus to serotonin at relatively low concentrations, and it inhibited the contraction of the isolated guinea-pig ileum induced by acetylcholine at the higher concentration (Table 5). Lycorenine was 0.6, 0.36, 0.2, and 0.003 times as potent as yohimbine, dibenamine, ergotamine, and phenoxybenzamine respectively in terms of the anti-noradrenergic activity.

| Compounds       | Anti-NA a)  | ED50 (g/ml) | Anti-ACh c) |
|-----------------|-------------|-------------|-------------|
| Lycorenine      | 2.62×10⁻⁷   | 4.51×10⁻⁷   | 2.70×10⁻⁵   |
| Yohimbine       | 1.63×10⁻⁷   | 5.55×10⁻⁶   | 2.63×10⁻⁵   |
| Dibenamine      | 9.40×10⁻⁸   | —           | —           |
| Phenoxybenzamine| 6.96×10⁻¹⁰  | —           | —           |
| Ergotamine      | 5.52×10⁻⁸   | —           | —           |
| Papaverine      | —           | —           | 6.92×10⁻⁶   |

a) Concentration of NA: 8.0×10⁻⁷ g/ml.  b) Concentration of 5HT: 2.5×10⁻⁵ g/ml.  c) Concentration of ACh: 5.0×10⁻⁹ g/ml. Number of experiments was 5.
DISCUSSION

Lycorenine produced decreases in blood pressure and heart rate, and an increase in cardiac output in pentobarbital anesthetized rats. Therefore, the decrease in blood pressure by lycorenine can be ascribed to a decrease in peripheral resistance. In the present study we obtained three pieces of evidence showing that lycorenine decreases peripheral resistance mainly by the inhibition of the sympathetic vasoconstrictor tone, but not by the direct vasodilatation. The first evidence is that the vasodepressor action of lycorenine was markedly reduced in spinal rats and by prior treatment with hexamethonium or phenoxybenzamine. The second evidence is that in the blood-perfused rat hindquarters, the fall of perfusion pressure induced by lycorenine (i.a.) was almost completely blocked by treatment with hexamethonium or phenoxybenzamine, although that induced by papaverine (i.a.), a directly acting vasodilating agent, was not affected. It is clear that the abolition of the vasodepressor or vasodilator effect of lycorenine was not due to changes in blood or perfusion pressure level, since the blood pressure recovered almost to the initial level 5 min after treatment with phenoxybenzamine in the former experiments, and pretreatment with hexamethonium or phenoxybenzamine blocked the fall of perfusion pressure induced by lycorenine, but not by papaverine in the latter experiments. The third evidence is that in the blood-perfused rat hindquarters, the intra-arterial injection of lycorenine produced falls of both blood pressure and perfusion pressure, while papaverine produced a fall only of perfusion pressure, and not of blood pressure in the doses used here.

Lycorenine was found to possess alpha-adrenergic blocking activity since it produced inhibitory effects on the pressor responses induced by stimulation of the vertebral level T7-9 and by the i.v. administration of noradrenaline, and on the noradrenaline-induced contraction of the mouse seminal vesicles. These results indicate that the vasodepressor action of lycorenine is related in part to its alpha-adrenergic blocking activity on the vascular smooth muscle. However, there were no correlations between the potencies of the vasodepressor activities and the alpha-adrenergic blocking activities of lycorenine and its related compounds, that is, the order of vasodepressor potencies was : lycorenine > homolycorine > compound 1, although all three compounds had the same alpha-adrenergic blocking potencies. In addition, the vasodepressor action of lycorenine (7.5 mg/kg) appeared to be far shorter in duration than the alpha-adrenergic blocking action, since the former disappeared about 30 min after the injection in pentobarbital anesthetized rats, but the latter was still observed 2 hr after the injection in pithed rats. It is however, difficult to strictly compare both activities because of differences in the experimental conditions. Therefore, the effects of lycorenine-related compounds on blood pressure cannot be explained exclusively by their alpha-adrenergic blocking activities.

There are studies on effects and mechanisms of action of alpha-adrenergic blocking agents on the cardiovascular system. It has been reported (10, 11, 12) that not all alpha-adrenergic blocking agents produce pronounced and prolonged decreases in blood pressure, that there are alpha-adrenergic blocking agents which do not produce vasodepressor actions in spite of marked sympatholytic and adrenolytic action, and that there are alpha-adrenergic
blocking agents which produce a decrease in blood pressure by acting directly on the vascular smooth muscle or on the central nervous system with or without participation of the alpha-adrenergic blocking action. In other words, mechanisms of vasodepressor actions of alpha-adrenergic blocking agents cannot be exclusively ascribed to their alpha-adrenergic blocking activities. Therefore, it is possible that the vasodepressor action of lycorenine was produced by other mechanisms in addition to the alpha-adrenergic blockade.

As already mentioned, lycorenine has almost no direct vasodilatory action. In addition, lycorenine (15 mg/kg) has neither ganglionic blocking nor adrenergic neuron blocking activity. Thus, the vasodepressor action of lycorenine cannot be attributed to any of these actions. Lycorenine reduced the spontaneous discharges of the splanchnic nerve. Several investigators have proposed that clonidine produces hypotension by central inhibition of the sympathetic nerve activity (13, 14, 15, 16). Schmitt et al. (17, 18, 19) suggested that the inhibitory effects of clonidine and 1-dopa on the sympathetic nerve activity and their hypotensive actions are mediated by activation of certain alpha-adrenergic receptors in the central nervous system. Furthermore, they (18) suggested that alpha-adrenergic blocking agents such as yohimbine, 883F, dibozane, ethoxane, azapetine, and moxixylite may act on central alpha-adrenergic receptors as partial agonists. Lycorenine may have a similar action. In short, lycorenine may produce vasodilatation owing to the intrinsic alpha-adrenergic blockade in conjunction with the reduction of the spontaneous sympathetic nerve activity.

Lycorenine produced a decrease in blood pressure only in large doses when injected into the lateral ventricle. This is however, not necessarily incompatible with the above mentioned suggestion, because there are compounds which produce a decrease in blood pressure through central mechanisms, yet hypotension does not occur with i.c.v. administrations. For example, phentolamine produces a decrease in blood pressure when restricted to the head circulation in the cross-circulated isolated head preparations in dogs (20), but does not produce a decrease in blood pressure by the i.c.v. injection of even large dose in cats (21). Furthermore, there are species differences and the marked influence of anesthesia in cardiovascular effects induced by the i.v. and i.c.v. injection of yohimbine. (22). Therefore, the result of the i.c.v. administration of lycorenine should be carefully interpreted.

The development of tachyphylaxis to the vasodepressor action of lycorenine with repeated injections may be at least in part ascribed to maintenance of the alpha-adrenergic blocking action of the drug. That is, the alpha-adrenergic blockade induced by the injection of lycorenine was maintained up to the time of the next injection, although blood pressure levels did recover, presumably by recovery from the reduction of the sympathetic nerve activity and autoregulatory vasoconstrictor reaction (23). Tachyphylaxis to the depressor action which developed with repeated injections of lycorenine in anesthetized rats was independent of the depth of anesthesia as this phenomenon was observed in conscious rats.

The maximal bradycardia induced 5-10 min after the injection of lycorenine was probably mediated through the vagus, because it was abolished by bilateral vagotomy. However, the mechanism of the activation of the vagus induced by lycorenine remains to be investi-
gated. After treatment with desipramine or tripelennamine, lycorenine produced a marked tachycardia. It has been reported (24, 25, 26) that tripelennamine inhibits the uptake of catecholamines into the sympathetic nerve endings, and it seems likely that this uptake inhibition, rather than its anti-histaminic effect is responsible for the above mentioned effect of tripelennamine on the changes of heart rate induced by lycorenine. In the presence of desipramine, lycorenine produced a tachycardia also in the spinal rats and this tachycardia was completely blocked by propranolol therefore, the lycorenine-induced tachycardia may not be ascribed to the reflex increase of sympathetic nerve activity by the decrease in blood pressure or to mediation by a central mechanism. It seems that lycorenine releases catecholamines from the peripheral sympathetic nervous system by the desipramine resistant mechanism, and the tachycardia which is otherwise masked is produced by potentiation of the response to the released noradrenaline by desipramine which blocks the uptake of noradrenaline into the sympathetic nerve endings.

REFERENCES

1) MATSUI, M., KOIDA, M. AND HRAMATSU, Y.: Pharmacological studies on the alkaloids of Lycoris radiata Herb. (Report 1). Pharmacological action of lycorine and lycorenine. Yakugaku Kenkyu 34, 320–341 (1962) (Abs. in English)

2) WEEKS, J.R. AND JONES, J.A.: Routine direct measurement of arterial pressure in unanesthetized rats. Proc. Soc. exp. Biol. Med. 104, 646–648 (1960)

3) DE GROOT, J.: The Rat Forebrain in Stereotaxic Coordinates, N.V. Noord-Hollandsche Vitgevers Maatschappij, Amsterdam (1959)

4) BRODY, M.J., SHAFFER, R.A. AND DIXON, R.L.: A method for the study of peripheral vascular responses in the rat. J. appl. Physiol. 18, 645–647 (1963)

5) GILLESPIE, J.S., MACLAREN, A. AND POLLOCK, D.: A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat. Brit. J. Pharmacol. 40, 257–267 (1970)

6) OKAMOTO, K., NOSAKA, S., YAMORI, Y. AND MATSUMOTO, M.: Participation of neural factor in the pathogenesis of hypertension in the spontaneously hypertensive rat. Japan. Heart J. 8, 168–180 (1967)

7) UFO, S.: Alkaloids of Lycoris radiata Herb. Jikken Kagaku-Koza, Vol. 22, p. 449–468, Maruzen Company, Tokyo (1958) (in Japanese)

8) HAWKSWORTH, W.A., JEFFS, P.W., TIDD, B.K. AND TOUBE, T.P.: The alkaloids of the Amaryllidaceae. Part XII. The aromatic oxygenation patterns and stereochemistry of some trioxaryl alkaloids of the hemiacetal and lactone series. J. chem. Soc. 1965, 1991–2001 (1965)

9) OZEKI, S.: Studies on the alkaloids of Zephyranthes candida Herb. III. Structure of nerinine. Yakugaku Zasshi 85, 206–212 (1965) (Abs. in English)

10) NICKERSON, M.: The pharmacology of adrenergic blockade. Pharmacol. Rev. 1, 27–101 (1949)

11) GHOURI, M.S.K. AND HALEY, T.J.: Structure-activity relationships in the adrenergic-blocking agents. J. Pharm. Sci. 58, 511–538 (1969)

12) NICKERSON, M.: The Pharmacological Basis of Therapeutics, Edited by GOODMAN, L.S. AND GILMAN, A., Fourth Edition, p. 549–584, The MacMillan Company (1970)

13) KOBINGER, W.: Über den Wirkungsmechanismus einer neuen antihypertensiven Substanz mit Imidazolinstruktur. Arch. Pharmacol. 258, 48–58 (1967)

14) KOBINGER, W. AND WALLAND, A.: Kreislaufuntersuchungen mit 2-(2,6-Dichlorphenylamino)-2-imidazolinhydrochlorid. Arzneim.-Forsch. 17, 292–300 (1967)

15) SCHMITT, H., SCHMITT, M.H., BOISSIER, J.R. AND GIUDICELLI, J.F.: Centrally mediated decrease in sympathetic tone induced by 2-(2,6-dichlorophenylamino)-2-imidazoline
16) Schmitt, H., Schmitt, M.H., Boissier, J.R., Giudicelli, J.F. and Fichelle, J.: Cardiovascular effects of 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (ST 155) II. Central sympathetic structures. Europ. J. Pharmacol. 2, 340-346 (1968)

17) Schmitt, H., Schmitt, M.H. and Fenard, S.: Evidence for an α-sympathomimetic component in the effects of catapresan on vasomotor centres: antagonism by piperoxane. Europ. J. Pharmacol. 14, 98-100 (1971)

18) Schmitt, H., Schmitt, M.H. and Fenard, S.: Action of α-adrenergic blocking drugs on the sympathetic centres and their interactions with the central sympatho-inhibitory effect of clonidine. Arzneim.-Forsch. 23, 40-45 (1973)

19) Schmitt, H., Schmitt, M.H. and Fenard, S.: New evidence for an α-adrenergic component in the sympathetic centres: centrally mediated decrease in sympathetic tone by 1-dopa and its antagonism by piperoxane and yohimbine. Europ. J. Pharmacol. 17, 293-296 (1972)

20) Hilliard, C.C., Bagwell, E.E. and Daniell, H.B.: Effects of sympathetic and central nervous system alterations on the blood pressure responses to phentolamine. J. Pharmacol. exp. Ther. 180, 743-747 (1972)

21) Bergmann, F. and Ramu, A.: Effect of intraventricular phentolamine on hyper- and hypotensive vasomotor reflexes. Europ. J. Pharmacol. 4, 363-370 (1968)

22) Lang, W.J., Lambert, G.A. and Rush, M.L.: The role of the central nervous system in the cardiovascular responses to yohimbine. Archs int. Pharmacodyn. Thér. 217, 57-67 (1975)

23) Kisin, I.E. and Serебryakov, L.A.: Autoregulatory vasoconstriction and vasodilator drugs. Archs int. Pharmacodyn. Thér. 195, 24-32 (1972)

24) Isaac, L. and Goth, A.: Interaction of antihistamines with norepinephrine uptake: a cocaine-like effect. Life Sci. 4, 1899-1904 (1965)

25) Isaac, L. and Goth, A.: The mechanism of the potentiation of norepinephrine by antihistaminics. J. Pharmacol. exp. Ther. 156, 463-468 (1967)

26) Barnett, A., Taber, R.I. and Roth, F.E.: Activity of antihistamines in laboratory antidepressant tests. Int. J. Neuropharmacol. 8, 73-79 (1969)