EXCITATION BY LYONIOL-A OF VAGALafferent NERVES AND THE REFLEX AUTONOMIC AND SOMATIC ACTIONS IN RATS

HIDEKI ONO, HIDEOMI FUKUDA* AND YOSHIHISA KUDO**

Department of Toxicology and Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, * Hongo, Bunkyo-ku, Tokyo 113, Japan and Department of Pharmacology, Mitsubishi-Kasei Institute of Life Sciences,** Minamiooya 11, Machida, Tokyo 194, Japan

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Lyoniol-A (2 mg/kg, i.v.), a toxic component isolated from the Ericaceous tree, markedly caused the increase in rate of discharges from vagal afferent nerves in the rat. This agent also caused respiratory depression, hypotension and the relief of decerebrate rigidity. These effects of lyoniol-A on the respiration and the rigidity were absent under conditions of bilateral cervical vagotomy, and the hypotension was abolished by sectioning of both vagal and carotid sinus nerves. These results suggest that the excitation by lyoniol-A of the afferent activities reflexly produces respiratory depression, hypotension and the relief of decerebrate rigidity.

Keywords—lyoniol-A; lyoniol-B; veratridine; vagal afferent; respiration; blood pressure; decerebrate rigidity

INTRODUCTION

Lyoniol-A (Fig. 1), a component isolated from a poisonous tree Lyonia ovalifolia var. elliptica, causes abnormal posture in mammals which is characterized by torsion, retrocollis and locomotor ataxia.1) We investigated the pharmacological actions of the compound on the central and peripheral nervous systems, and found that the potent muscle spindle excitant action might be largely responsible for the so-induced abnormal behaviours.2−5)

Lyoniol-A also induces symptoms related to the autonomic nervous system including retching, salivation, hypotension and respiratory depression. Moran et al.6,7) found that andromedotoxin, a compound which structurally resembles lyoniol-A, produced a hypotension and respiratory depression which were induced reflexly via vagal and carotid sinus afferent nerves. Thus, the possibility that lyoniol-A may also cause an increase in the visceral afferent discharges is worthy of investigation.

In the present study, the effects of lyoniol-A on the vagal afferent discharges were studied in the rat in situ, and the role of afferent impulses in the depressant effect of lyoniol-A on the respiration, the blood pressure and the decerebrate rigidity was examined.

FIG. 1. Structures of Lyoniol-A and Lyoniol-B

lyoniol-A \( R = \text{COCH}_3 \)
lyoniol-B \( R = \text{H} \)
MATERIALS AND METHODS

Afferent Discharges from the Vagal Nerve — Twenty-one male Wistar rats were anesthetized with urethane (1 g/kg, i.p.) and α-chloralose (25 mg/kg, i.p.). The right and left vagal nerves were cut centrally at the cervical region, and either the right or left one was placed on bipolar silver-silver chloride wire electrodes for recording afferent impulses (Fig. 2). Action potentials were displayed on an oscilloscope (Nihonkohden VC-6) and photographed on a moving film (Nihonkohden PC-II). Spikes higher than an arbitrary threshold were then transformed into square wave pulses and fed into an integrator (time constant 0.1 s), the output of which was recorded by an ink-writing recorder (San-Ei Instrument Rectigraph 8S). In a few preparations, the vagal efferent discharges from the contralateral side were simultaneously recorded. To eliminate spontaneous respirations, the rats were immobilized with d-tubocurarine chloride (2 mg/kg, i.p.) and artificially respired. Rectal temperature was maintained at 37–38°C using a heating pad.

Respiration — Fifteen rats were anesthetized with urethane and α-chloralose. Spontaneous respiration was recorded with a tambour connected to a tracheal cannula in the vagotomized and non-vagotomized rats. The vagotomy was performed acutely at the cervical region.

Blood Pressure — Rats were anesthetized with urethane and α-chloralose. Carotid arterial blood pressure was recorded with a mercury manometer, under conditions of artificial respiration. Bilateral vagotomy was performed in 14 rats. Both vagotomy and crushing of the carotid sinus nerves were performed bilaterally in 9 rats. Spinal rats were prepared by transecting the spinal cord at the C1 level and crushing the medulla oblongata. Pithed rats were obtained by destroying the spinal cord using a steel wire.

Decerebrate Rigidity — Under ether anesthesia, the mid-brain was sectioned between the inferior and superior colliculi.5 The rats showing sustained rigidity were selected (n = 15). Electromyogram (EMG) was recorded by a coaxial needle electrode inserted into the gastrocnemius muscle. EMG activities were amplified (Nihonkohden VC-6), transformed into square wave pulses and fed into an integrator (reset time 3 s), the output of which was recorded by an ink-writing recorder. The rats were artificially respirated to prevent respiratory depression.

Materials — Drugs used were lyoniol-A, lyoniol-B (deacetyl-lyoniol-A), veratridine (Aldrich Chemicals) and chlorpromazine · HCl (Wintamin®, Shionogi). All were dissolved in 0.9% saline solution and injected into the cannulated femoral vein.

RESULTS
Effects on the Afferent Discharges from the Vagal Nerve
The afferent discharges from the vagal nerve bundle increased during the inspiratory phase of the artificial respiration and decreased during the expiratory phase (Fig. 2). Lyoniol-A (1 and 2
FIG. 3. Effect of Lyoniol-A (2 mg/kg, i.v.) on the Vagal Afferent Activities
Upper and lower traces: photographed and integrated recordings of afferent discharges, respectively. Lyoniol-A produced prolonged increase in the afferent discharges.

FIG. 4. Effect of Lyoniol-B and Veratridine (0.1 mg/kg, i.v.) on the Vagal Afferent Activities
Lyoniol-B and veratridine produced transient increase in the afferent discharges.
mg/kg) caused an immediate increase in the afferent discharges and this effect lasted for more than 1 hour. The increased frequency of discharges in the expiratory phase of the respiratory cycle after lyoniol-A was higher than that in the pre-drug inspiratory phase (Fig. 3).

Lyoniol-B (deacetyl-lyoniol-A) (Fig. 1) and veratridine (0.05, 0.1 and 0.2 mg/kg) facilitated the afferent discharges immediately after the administration, although the durations of action were shorter than that of lyoniol-A (Fig. 4).

The possibility that lyoniol-A acts on the respiratory center and changes the vagal efferent activity can be excluded, since no significant effect of lyoniol-A was observed on the vagal respiratory efferent discharges.

Effects on Respiration

Lyoniol-A (2 mg/kg) markedly reduced the rate of spontaneous respiration in normal rats with intact vagal nerves (Fig. 5A). The reduction of the respiratory rate was obviously due to the prolongation of the expiratory phase. The time course coincided with the time course of the drug effect on the afferent activities; the inhibitory effect of lyoniol-A on respiration continued for more than 1 hour.

Sectioning of both right and left vagal nerves reduced the respiratory rate by eliminating the Hering-Breuer inflation reflex which accelerates the respiratory rate. In these preparations, lyoniol-A (2 mg/kg) did not induce respiratory depression (Fig. 5B). In addition, bilateral vagotomy done after lyoniol-A administration eliminated this depressant effect of the drug (Fig. 5C).

Lyoniol-B and veratridine also caused similar inhibitory actions on the spontaneous respiration, but the durations of effects were shorter than those seen with lyoniol-A.

Effects on Blood Pressure

Lyoniol-A (1 and 2 mg/kg) lowered the blood pressure for over 1 hour after the administration to anesthetized intact rats. To assess the involve-

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![Diagram of respiratory effects](image)

**FIG. 5. Effect of Lyoniol-A (2 mg/kg, i.v.) on the Respiration in the Rat**

Spontaneous respiration were recorded with a tambour connected to a tracheal cannula. A: intact vagal nerve preparation. B: vagotomy was performed before the drug administration. C: vagotomy was performed after the drug administration. At squares ( □ ), right (R) of left (L) vagal nerves were sectioned. At dots ( ● ), drugs were given into the femoral vein. The inhibitory effect of lyoniol-A was absent under condition of cervical vagotomy.
ment of afferent activity in the effect of lyoniol-A, vagal nerves or both vagal and carotid sinus nerves were sectioned bilaterally (Table I). In 5 of the vagotomized rats, lyoniol-A continued to lower blood pressure, although in 4 rats, lyoniol-A elevated the blood pressure. Consequently, lyoniol-A increased the standard error of the mean (SEM) of the blood pressure changes, without affecting the mean.

In rats in which both the vagal and carotid...

TABLE I. **Effect of Lyoniol-A on Blood Pressure**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Saline 2 ml/kg P (before)</th>
<th>Δ P (5 min) (n)</th>
<th>Saline 4 ml/kg P (before)</th>
<th>Δ P (5 min) (n)</th>
<th>Lyoniol-A 1 mg/kg P (before)</th>
<th>Δ P (5 min) (n)</th>
<th>Lyoniol-A 2 mg/kg P (before)</th>
<th>Δ P (5 min) (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>106.6±10.3</td>
<td>+11.2±6.2 (5)</td>
<td>104.5±8.6</td>
<td>−32.0±11.4a1 (4)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Vagotomized</td>
<td>86.4± 8.5</td>
<td>+3.0±2.6 (5)</td>
<td>92.6±9.0</td>
<td>− 2.7± 8.2 (9)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Vagotomized and carotid sinus crushed</td>
<td>81.2± 9.2</td>
<td>+2.6±2.5 (5)</td>
<td>71.5±8.2</td>
<td>+21.0±7.2a1 (4)</td>
<td></td>
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</tbody>
</table>

P (before): blood pressure before drug administration. Δ P (5 min): blood pressure changes 5 min after administration. +: hypertension. −: hypotension. Each value represents mean ± SEM (mmHg).

a) Statistical significance (p < 0.05) to saline-treated control (student’s t-test, two-tailed).

FIG. 6. **Effects of Lyoniol-A (2 mg/kg, i.v.) and Chlorpromazine (CPZ)·HCl (0.2 mg/kg, i.v.) on Decerebrate Rigidity in the Rat**

EMG activities were recorded from the gastrocnemius muscle by a coaxial needle electrode under conditions of artificial respiration. A: intact vagal nerve preparations. B: vagotomized preparations. At dots (●), drugs were given into the femoral vein. The inhibitory effect of lyoniol-A was abolished by vagotony.
sinus nerves had been sectioned, lyoniol-A significantly elevated the blood pressure. In spinal rats, lyoniol-A significantly elevated the blood pressure, and the hypertension was abolished by the destruction of the spinal cord (pithed rat).

**Effects on Decerebrate Rigidity**

Lyoniol-A inhibited decerebrate rigidity, as already shown in our earlier report. The inhibitory action of lyoniol-A on decerebrate rigidity was abolished by bilateral vagotomy (Fig. 6). In contrast, chlorpromazine which preferentially reduces γ-efferent discharges caused the same degree of inhibitory actions on the intact and vagotomized preparations.

**DISCUSSION**

Since the method we used preferentially recorded the higher amplitude impulses, the recorded afferent impulses may be regarded as those from the pulmonary stretch receptors which send larger fibers to the vagal nerve. The function of the pulmonary stretch receptor is to send information on inflation of the lung and to terminate inspiration (Hering-Breuer reflex). Since the vagotomy which blocked the afferent input to the respiratory center abolished the depressant action of lyoniol-A on the respiration, it was concluded that lyoniol-A reduced the respiratory rate by increasing the afferent activity.

It was reported that andromedotoxin causes hypotension via vagal and carotid sinus afferent nerves and that the drug activates central sympathetic activities. In the present study, blood pressure in the intact anesthetized rat was lowered by lyoniol-A. In rats in which both vagal and carotid sinus nerves were sectioned, lyoniol-A significantly elevated blood pressure. These results suggest that the depressor effect of lyoniol-A is induced via afferent nerves while the pressor effect is due to a central action or induced by afferent signals which do not pass the vagal or carotid sinus nerves. In the spinal rat, lyoniol-A significantly elevated the blood pressure, although the drug did not show any pressor effects on the pithed rat. These results suggest that excitation of spinal or supraspinal vasomotor neurones is involved in the elevation of the blood pressure and that the increased afferent impulses indirectly lowered the blood pressure. The variation of effect of lyoniol-A as seen in vagotomized preparations may be due to the balance between the central action and the indirect action via carotid sinus afferent impulses (Table I). These results suggest that lyoniol-A increases the afferent impulses from the carotid sinus baroreceptor and such is supported by the finding that lyoniol-B increased the afferent impulses from the aortic arch baroreceptor in the rabbit.

It has been shown that visceral afferent impulses reflexly affect the somatic central nervous system. For example, afferent discharges from the heart and the lung depress the spinal cord α-activities and type J pulmonary endings depress monosynaptic reflex in the spinal cord. Ginzel et al. reported that nicotine, veratridine, phenylbiguanide and 5-hydroxytryptamine reflexly relieve cat decerebrate rigidity (γ-type) via vagal or carotid sinus nerves and inhibit γ-efferent activities of the spinal cord. In the present study, the inhibitory effect of lyoniol-A on γ-type decerebrate rigidity was also abolished by bilateral vagotomy. Thus, the inhibitory effect of lyoniol-A on the rigidity is probably due to the increased vagal afferent activity.

In summary, it was concluded that the excitatory action of lyoniol-A on the vagal afferent activities induced respiratory depression, hypotension and the relief of decerebrate rigidity. These results support the view that the viscerosomatic reflex action of some drugs relieves the rigidity. Effects of lyoniol-A on visceral afferent nerves may contribute to some extent to the toxic autonomic and somatic nervous actions when the drug is given to the whole animal.

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REFERENCES


