EFFECT OF LYONIOL-A ON DECEREBRATE RIGIDITY AND ELECTROENCEPHALOGRAM IN RATS

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Lyoniol-A is a toxic component isolated from a poisonous tree in Japan (Lyonia ovalifolia var. elliptica). In our previous studies, it was shown in experimental animals that injection of lyoniol-A caused postures characterized by torsion, retrocollis, spasm and locomotive ataxia (1), and moreover it was suggested in the evoked electromyographic (EMG) study (2) that the sites of action involved the supraspinal and spinal structures.

One of the interesting properties of lyoniol-A is that it causes remarkable locomotive ataxia. It was found in rabbits that administration of lyoniol-A for about 15 days caused a histological degeneration in certain nuclei of the extrapyramidal system (3). Since extrapyramidal nuclei are closely related to gamma-system, the effect of lyoniol-A on the intercollicular decerebrate rigidity mainly due to hyperactivity of gamma-efferent outflow from the brain (4) was examined. For comparison with lyoniol-A effects of chlorpromazine which markedly abolished the rigidity of this type (5), mephenesin (6, 7) and strychnine were studied.

While analyzing the action of lyoniol-A in electroencephalographic (EEG) study, the compound was found to accelerate the arousal pattern in the motor cortex of curarized rats. For this reason, the effects of lyoniol-A on the cortical and subcortical EEG patterns in the encéphale isole and cerveau isolé rats were examined. The stereochemical structure of lyoniol-A has been established (8) (Fig. 1).

Fig. 1. Chemical structure of lyoniol-A.

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MATERIALS AND METHODS

Male rats of Wistar strain (Nippon Rat Co.) weighing from 200 to 450 g were used.

1. Decerebrate rigidity

Under ether anesthesia, the mid-brain was sectioned by a spatula between the inferior and superior colliculi (9). In the course of recovery from anesthesia, rigidity appeared in the forelegs, or hindlegs, or both, and sometimes in the neck. Rats showing sustained rigidity were selected.

The rat was placed on its back and forelegs fixed. EMG was recorded by a coaxial needle electrode inserted into the gastrocnemius muscle. The tension of 10 g was loaded, parallel to the tibial bone, to the ipsilateral hindleg toe in order to obtain a stable frequency of EMG. EMG activities obtained from the gastrocnemius muscle were amplified with a Nihonkohden RB-2 amplifier, transformed into square waves and fed into an integrator, the output of which was amplified and recorded by an inkwriting recorder (Nihonkohden WI-180).

Exposed neural structures were covered with warm mineral oil, and body temperature was maintained constant using an infrared lamp. Location of lesions was confirmed histologically. In some experiments, arterial blood pressure was recorded from the carotid artery by means of a transducer (Nihonkohden MP-4T).

2. Muscle contractions by single and paired stimuli

Experiments were performed on rats anesthetized with chloralose (50 mg/kg, i.p.) and urethane (0.5 g/kg, i.p.). The arrangement was approx. the same as described in the study using cats (10). The twitch tension of the gastrocnemius muscle in response to nerve stimulation was recorded by means of a transducer secured by a string to the distal tendon. The distal stump of the cut tibial nerve was stimulated by supramaximal square wave pulses, 0.1 Hz, 0.1 msec in duration from a Nihonkohden MSE-3 stimulator with an isolator.

3. EEG

The rat was fixed on the stereotaxic apparatus (Todai Noken type) under ether anesthesia, immobilized by d-tubocurarine chloride and artificially ventilated. The concentric needle electrodes (external diameter 0.5 mm) insulated except tips were implanted deeply in the head of the caudate nucleus, hippocampus, amygdala and hypothalamus (9). EEG recordings were done by Nihonkohden WE-92C electroencephalograph. Coordinates of nuclei in millimeters are as follows: caudate nucleus, anterior 7.4, lateral 3.5, vertical +1.5; hippocampus, anterior 4.2, lateral 2.0, vertical +2.0; amygdala, anterior 5.0, lateral 5.0, vertical −3.0; hypothalamus, anterior 5.8, lateral 1.0, vertical −3.0. Silver ball monopolar electrodes were used to record EEG from the exposed motor (anterior) and limbic (posterior) cortex. The indifferent electrode was fixed to the neck muscle.

Encéphale isolé and cerveau isolé preparations were prepared as follows. The brain stem was sectioned under ether anesthesia with a steel spatula at C1 or C2 level (encéphale isolé) or at intercollicular level (cerveau isolé). Exposed neural structures were covered with warm mineral oil, and body temperature maintained constant using infrared lamp. Following experiments, location of lesions or electrodes was confirmed histologically.
Several control experiments were included in each series.

4. Chemicals

Drugs used were lyoniol-A, chlorpromazine-HCl (Wintamin, Shionogi), mephenesin (Myanol, Chugai), strychnine nitrate (Hoei-yakko), pentylenetetrazol (Cardiazol, Sankyo), picrotoxin (Tokyokasei) and pentobarbital-Na (Nembutal, Dainippon). All were dissolved in 0.9% saline and injected into the femoral vein through a cannula.

RESULTS

1. Effects on the decerebrate rigidity

In the decerebrate rigid rats, the frequency of tonic spontaneous discharge in the gastrocnemius muscle was 120–250 Hz. With the injection of 2.0 ml/kg of saline, decrease of the rate of discharge was a maximum of 10%. Lyoniol-A (0.5 mg/kg) gradually depressed
the rate of discharge: 50% and 75% decrease in 5 and 10 min respectively (Fig. 2A). A higher dose of 2.0 mg/kg of lyoniol-A caused a transient increase of rate followed by a complete depression (Fig. 2B); in such a state of the EMG activity, the behaviour of retching was sometimes observed. Approx. 10 min after the injection, the EMG activity reappeared intermittently (Fig. 2B), and in this period abnormal postures characterized by torsion, spasm and retrocollis were sometimes observed.

For comparison with lyoniol-A effects of strychnine, chlorpromazine and mephenesin on the EMG activity of decerebrate rigid rats were studied (Figs. 2 and 3). Strychnine nitrate (125 mg/kg) increased the rate of discharge: 20% and 15% in 1 and 5 min respectively. Chlorpromazine-HCl (125 mg/kg) markedly reduced the rate and 10 min later depressed it completely. Mephenesin (12.5 mg/kg) decreased the rate to 60%.

Effect of the drugs on blood pressure was examined. Mean blood pressure of the decerebrate rats was 100-140 mmHg. Lyoniol-A (0.5 mg/kg) did not change the blood pressure; a dose of 2.0 mg/kg produced a temporary rise of blood pressure (35 mmHg), which returned to control level within a few min. Depression of discharges induced by lyoniol-A were observed even after the return of blood pressure to the control level. Thus, a direct relationship between the depressant effect of lyoniol-A and the changes of blood pressure could not be determined. Strychnine nitrate (125 mg/kg) or mephenesin (12.5 mg/kg) produced little or no change in blood pressure. Chlorpromazine-HCl (250 mg/kg) produced a fall of blood pressure (70 mmHg). A dose of 1.25 mg/kg of acetylcholine chloride, sufficient to cause a fall of 50 mmHg or more, produced no significant effects on EMG activity.

2. Effects on the refractory period of muscle

When the pulse interval of the paired stimuli was less than 1.0 msec, the twitch tension was the same as that produced when stimulated with single pulses. Thus 1.0 msec was considered to be the absolute refractory period of indirectly stimulated skeletal muscle. As the pulse interval increased, some of the fibers within the muscle responded to both the first and the second stimulus with a consequent summation of the twitch tension.

Neither the twitch tensions obtained with single pulses nor those with paired pulses were affected by the dose of lyoniol-A (0.5-1.0 mg/kg) as well as mephenesin (12.5-25.0 mg/kg) and chlorpromazine-HCl (0.25-1.0 mg/kg). Thus it was confirmed that the drugs, in doses used in the experiment, had no effect on the refractory period.

3. Effect on EEG

a. Control EEG patterns

Spontaneous EEG patterns of d-tubocurarine-immobilized rats with intact brain were almost the same as those originally reported by Oshima et al. (11). EEG arousal (desynchronized) pattern was characterized by a low amplitude fast wave in the motor cortex and regular wave (θ wave) in the hippocampus.

There are no published reports on the EEG experiment using rat encéphale isolé or cerveau isolé preparation. For this reason EEG patterns in these preparations of rats were carefully compared with those of cats (12, 13) and it was found that the EEG patterns in
Fig. 4. Electroencephalographic response induced by lyoniol-A in curarized rats with intact brain.
A: control EEG patterns. B: 5 min after the injection of lyoniol-A (2.0 mg/kg, i.v.). A marked decrease of amplitudes and an increase of fast waves were observed in the motor cortex after lyoniol-A.
Abbreviations: M.C. motor cortex, C.N. caudate nucleus, HYP hypothalamus, AMG amygdala, HIP hippocampus, L.C. limbic cortex, A.L. anterior lobe (cerebellum), ECG electrocardiogram.

Fig. 5. Electroencephalographic response induced by lyoniol-A in rat encepha le isolé preparations. A: with lyoniol-A (1.0 mg/kg); further reduction of amplitude and increase of mean frequency in the motor cortex were seen.
B: with lyoniol-A (2.0 mg/kg). arousal pattern was not observed in the motor cortex. Abbreviations: see Fig. 4.
the encephale isolé and cerveau isolé rats were similar. In the present study, the spontaneous EEG patterns in encephale isolé rats were not significantly different from those in intact rats (Figs. 4 and 5A).

In the cerveau isolé preparations, the continuous sleep patterns characterized by frequent spindle bursts were observed in the motor cortex as well as in the caudate nucleus, although in the hippocampus and amygdala the arousal patterns similar to those in intact animals were observed (Fig. 5B).

b. Lyoniol-A

In rats with intact brain, lyoniol-A (2.0 mg/kg) accelerated the EEG arousal patterns: further reduction of amplitude and increase of mean frequency in the motor cortex and reduction of amplitude in the limbic cortex. No clear modification of electrical activity

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**Fig. 6. Electroencephalographic response induced by drugs in rat encephale isolé and cerveau isolé preparations. Abbreviations: see Fig. 4.**
was found in the hippocampus, amygdala or other nuclei examined. Marked arousal patterns induced by lyoniol-A, which were also observed in smaller doses of 0.5-1.0 mg/kg, lasted for more than 1 hr.

In the encephale isolé preparation also, lyoniol-A (1.0 mg/kg) accelerated arousal patterns. There was additional reduction of amplitude and increase of mean frequency in the motor cortex as was seen in the intact rat and prolongation of the time of each hippocampal arousal pattern (Fig. 5A). With 0.5 mg/kg of lyoniol-A, acceleration of the arousal response was also observed, although it was weaker and sometimes indefinite.

In the cerveau isolé preparation, only a slight decrease in amplitude of hippocampal arousal patterns appeared following the injection of 2.0 mg/kg of lyoniol-A, while patterns in the motor cortex and other areas were not affected (Fig. 5B).

c. Other drugs

The mode of action of strychnine, pentobarbital, pentylenetetrazol and picrotoxin on the EEG was studied in comparison with that of lyoniol-A (Fig. 6).

Strychnine nitrate (250 µg/kg) caused the reduction of amplitude and the increase of mean frequency in the motor cortex but little affected hippocampal arousal patterns in encephale isolé preparation. In the cerveau isolé preparation, the same dose caused prolongation of the time of hippocampal arousal pattern as well as an increase of frequency in the motor cortex. Pentobarbital-Na (10 mg/kg) caused sleep patterns with slow waves and high amplitudes in the motor cortex and the disappearance of arousal patterns in the hippocampus in the encephale isolé preparation. In the motor cortex of the cerveau isolé preparation, the same dose changed the spindle wave to the slow wave.

Pentylenetetrazol (10 mg/kg) caused an increase of frequency and reduction of amplitude in the motor cortex of the encephale isolé preparation; in the cerveau isolé preparation even a higher dosage of 20 mg/kg revealed no remarkable change in either the motor cortex or hippocampus. Picrotoxin (0.75 mg/kg) caused an increase of frequency and reduction of amplitude in the motor cortex and the prolongation of the time of hippocampal arousal pattern in the encephale isolé preparation. In the cerveau isolé preparation, no change in the motor cortex and a short time prolongation of hippocampal arousal pattern were observed in a dose of 1.5 mg/kg.

DISCUSSION

The effect of lyoniol-A was similar to that of chlorpromazine or mephenesin in reducing the rigidity of intercollicular decerebrate rats (Fig. 2), although our previous study involving the evoked EMG showed that the compound excited supraspinal and spinal structures (2). Possible sites of drug action reducing the decerebrate rigidity should include the following: i) neuromuscular junction and muscle, ii) muscle spindle, iii) spinal interneurones, iv) motoneurone, and v) lower brain stem.

Previous studies (1, 2) showed that lyoniol-A had no effect on neuromuscular junction or muscle, only slightly enhanced the crossed extensor reflexes, and induced in the evoked EMG a late response of supraspinal origin. It appears that the effect of lyoniol-A on
neuromuscular junction, muscle or spinal structures is not the important factor in the reduction of decerebrate rigidity. It could be possible that drugs show a depressant effect on the rigidity by affecting the refractory period of the muscle contraction (10, 14). This however can be ruled out in the present study since the indirectly stimulated muscle contractions were not influenced by the dosage of lyoniol-A (0.5-1.0 mg/kg), mephenesin (12.5-25.0 mg/kg) or chlorpromazine-HCl (0.25-1.0 mg/kg), which reduced the decerebrate rigidity of the rat.

Lyoniol-A markedly increased the rate of afferent discharge of the muscle spindle (15). It is unlikely that reduction of decerebrate rigidity with lyoniol-A is the result of an increase of afferent discharge of muscle spindle induced by the compound, since only depression of the afferent discharge can reduce the decerebrate rigidity. In addition, the effect of lyoniol-A on the blood pressure did not affect the decerebrate rigidity. Conclusively, it appears that lyoniol-A reduces the intercollicular decerebrate rigidity by affecting the lower brain stem.

The EEG patterns observed in the control records of the encephale isolé and cerveau isolé rats (Fig. 5) were similar to those of cats (12, 13). The spinal and peripheral effects of lyoniol-A do not seem to be essential for the activation of the motor cortex induced by the compound, since in the encephale isolé preparation the EEG arousal pattern of the motor cortex was accelerated by the compound as was seen in the animals with intact brain (Figs. 4 and 5). The lower brain stem would be necessary for the acceleration by lyoniol-A in the arousal pattern of motor cortex, since the compound failed to change the sleep patterns in the cerveau isolé preparation in which the mid-brain was transected at the intercollicular level. The effect of lyoniol-A on the EEG arousal patterns seems to be similar to that of pentylenetetrazol or picrotoxin in so far as these effects were abolished by transection at the intercollicular level; on the other hand, the effects of strychnine or pentobarbital did not disappear with intercollicular transection.

The mode of action of lyoniol-A is complicated especially regarding excitation (EEG) and depression (decerebrate rigidity). From the results of these and previous studies (1), where lyoniol-A caused postures complicated by excitation and depression, it appears that action sites stimulating peculiar postures are multivocal.

SUMMARY

1. Effects of lyoniol-A on the rat decerebrate rigidity and the EEG patterns in intact, encephale isolé and cerveau isolé rats were examined.

2. Intravenous injection of lyoniol-A (0.5-2.0 mg/kg) reduced the tonic EMG activity of the decerebrate rats. The reduction of rigidity caused by lyoniol-A seems to be due to the effect on the lower brain stem.

3. Lyoniol-A (2.0 mg/kg) accelerated the EEG arousal patterns of the motor cortex in intact and encephale isolé rats; in the cerveau isolé preparation, however, it showed no change of the EEG sleep pattern in the motor cortex. From the results, it is suggested that the lower brain stem is involved in the accelerated EEG arousal patterns induced by
lyoniol-A.

4. It would appear that action sites of lyoniol-A stimulating peculiar postures are multivocal.

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REFERENCES

1) Fukuda, H., Watanabe, K. and Ito, T.: Yakugaku Zasshi 89, 382 (1969) (in Japanese)
2) Fukuda, H., Watanabe, K. and Ito, T.: Jap. J. Pharmac. 19, 394 (1969)
3) Kishida, T., Ota, H., Tsuimura, K., Yasue, M. and Kato, Y.: Yokohama Medical Bulletin 14, 107 (1963)
4) Granit, R., Holmgren, B. and Merton, P.A.: J. Physiol. 130, 213 (1955)
5) Henatsch, H.D. and Ingvar, D.H.: Arch. Psychiat. Nervkr. 195, 77 (1956)
6) Matsushita, A. and Smith, C.M.: Brain Research 19, 411 (1970)
7) Klarv, E.M. and Maxwell, D.R.: Br. J. Pharmac. Chemother. 29, 400 (1967)
8) Yasue, M., Sakakibara, J. and Kato, T.: Chem. Pharm. Bull., Tokyo 18, 854 (1970)
9) DeGroot, J.: Roy. Neth. Acad. Sci. 52, 1 (1959)
10) Crankshaw, D.P. and Raper, C.: Br. J. Pharmac. Chemother. 34, 579 (1968)
11) Oshima, K., Miyama, T. and Kawamura, H.: Jap. J. Physiol. 12, 601 (1962)
12) Bremer, T.: Bull. Acad. Roy. Med., Belgique 2, 68 (1937)
13) Kawamura, H., Nakamura, Y. and Tokizane, T.: Jap. J. Physiol. 11, 564 (1961)
14) Rosenberg, F.J. and Cooke, W.J.: J. Pharmac. exp. Ther. 155, 145 (1967)
15) Kudo, Y., Watanabe, K. and Fukuda, H.: Folia pharmac. jap. 65, 186 § (1969) (in Japanese)