THE CENTRAL EFFECTS OF XYLOPININE IN MICE

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The depression of the spontaneous movement of mice by xylopinine, 2,3,10,11-tetramethoxy-5,6-13a-tetrahydro-8-dibenzo(a-g)quinolizine, has been reported by Nakanishi (1). In the unanesthetized rabbit, the intravenous injection of xylopinine produced high voltage slow waves and increased spindle waves in EEG. The threshold voltage for eliciting the reticular arousal response by stimulation of the ascending reticular formation was also elevated.

Recently, Hsu and Kin (2) have studied the pharmacological effects of tetrahydropalmatine and its analogues. They showed that tetrahydropalmatine exerted sedative-tranquilizing effects in mice.

The present experiments were designed to study sedative-tranquilizing effects of xylopinine in mice.

METHODS

The experiments were performed in the mice, weighing 14 to 18 g, of both sexes. The drugs used in this experiment were all injected intraperitoneally in 0.2 ml of aqueous solution per 10 g. The drugs used were xylopinine citrate, tetrabenazine hydrochloride, chlorpromazine hydrochloride, methamphetamine hydrochloride, *trans*-dl-2-phenylcyclopropylamine maleate (SKF-385), caffeine and amobarbital sodium. Because of less solubility in water SKF-385 was suspended in the physiological saline solution. The locomotor movement of mice was measured as a number of rotations of the rotation apparatus devised by Takagi *et al.* (3). The sleeping time of mice was determined by measuring the period of time of the disappearance of the righting reflex over 20 seconds and was expressed in minutes. The antagonistic effects of xylopinine were assayed on the lethality in mice due to seizures produced by the injection of the convulsive drugs and by the electroshock. For the induction of the electroshock seizure the apparatus of Woodbury and Davenport (4) was used. Further details of the experimental methods will be described under the RESULTS.

RESULTS

I. Effects of xylopinine on the motor movement of the unanesthetized mice

When the mice were transferred from the feeding cage to the rotation apparatus in the daytime, the animals showed the exploratory movements for a while, and some

of the spontaneous movements involving exploratory movements could be recorded as a rotation. However, these movements decreased gradually 40 to 50 minutes after the transfer. Accordingly, further study was carried out on driving movements in response to an auditory stimulus or a clamping of the tail by the forceps for testing the central effect of xylopinine. The number of rotations thus caused by the driving movements was counted at a time interval of 5 minutes for 30 minutes before and 60 to 120 minutes after the administration of drug. The mean rotation number in a group of 5 mice after the administration of drug was expressed as a percentage of that prior to drug. The time course of the driving rotation after the injection of 0.2 ml/10 g of the physiological saline solution was regarded as a control. As is illustrated in Fig. 1, the injection of the physiological saline solution increased the driving rotation at first and thereafter decreased it. However, the percent change in the driving rotation during 60 minutes after the injection was within the ranges of 38 to -34% and the mean decrease during 60 minutes was 11%. The spontaneous movement of mice was increased immediately but transient-

FIG. 1. Effects of xylopinine, chlorpromazine and tetrabenazine on the driving movement in mice.

- a. 0.2 ml/10 g of physiological saline solution,
- b. 10.0 mg/kg of xylopinine,
- c. 10.0 mg/kg of tetrabenazine,
- d. 10.0 mg/kg of chlorpromazine,

Ordinate: percent change of the driving movement in mice. Abscissa: minutes after the injection of the drugs.

FIG. 2. Effects of xylopinine, chlorpromazine and tetrabenazine on the driving movement in mice.

Ordinate: percent decrease of the mean of the driving movement for each 5 minutes during 60 minutes after the injection of the drugs. Abscissa: doses in mg/kg in logarithmic scale.

Abbreviation: chl.; chlorpromazine, tet.; tetrabenazine, xyl.; xylopinine.

ly after the injection of xylopinine, chlorpromazine or tetrabenazine. But, thereafter, the animals showed only a decrease in the spontaneous and locomotor activities. On the other hand, the driving movement of the mice in response to the auditory or clamping stimuli was progressively depressed by the injection of the drugs and at last the mice failed to respond to the stimuli. The duration of the depression varied with the drugs and also with the dose employed. The time of the decrease in the driving movement over 80% after the injection of 10 mg/kg was 25 minutes for xylopinine and 35 minutes for tetrabenazine, while chlorpromazine showed only the sign of the recovery of the driving movement about 140 minutes after the injection.

In order further to obtain the dose-effect relationship, the effect on the driving movement of the injection of xylopinine, chlorpromazine and tetrabenazine in the doses of 5, 10 and 20 mg/kg was tested, respectively. The percent decrease in the mean values of the driving rotation for each 5 minutes during 60 minutes after each dose was plotted against the logarithmic dose of the drugs (Fig. 2). The mean decrease of the movement caused by the injection of the physiological saline solution alone was 11%. Figs. 1 and 2 show that xylopinine, tetrabenazine and chlorpromazine all decrease the driving movement of mice, and the decreasing effect of xylopinine is weakest and somewhat different from those of chlorpromazine and tetrabenazine, the dose response curves of which are relatively parallel. For example, the mean decrease of the movement produced by the injection of 10 mg/kg was 66% for xylopinine, 83% for tetrabenazine and 90% for chlorpromazine.

II. Effects of xylopinine on the sleeping time of mice induced by amobarbital sodium

Each group of nine mice was injected 5, 10 and 20 mg/kg of xylopinine, chlorpromazine and tetrabenazine, respectively, immediately after an intraperitoneal injection

TABLE 1. Effects of xylopinine, chlorpromazine and tetrabenazine on amobarbital sleeping time in mice.

	Mean sleeping time ±S.E. (minutes)	P
Barb. 70 mg/kg	56 ± 12	
Barb. 70 mg/kg & xyl. 20 mg/kg	112 ± 23	0.05 > p > 0.02
Barb. 70 mg/kg & xyl. 10 mg/kg	79 ± 11	0.20 > p > 0.10
Barb. 70 mg/kg & xyl. 5 mg/kg	79 ± 23	0.50 > p > 0.30
Barb. 70 mg/kg	57 ± 8	
Barb. 70 mg/kg & chl. 20 mg/kg	277 ± 45	0.001 > p
Barb. 70 mg/kg & chl. 10 mg/kg	173 ± 24	0.001 > p
Barb. 70 mg/kg & chl. 5 mg/kg	96 ± 15	0.05 > p > 0.02
Barb. 70 mg/kg	44 ± 6	
Barb. 70 mg/kg & tet. 20 mg/kg	210 ± 14	0.001 > p
Barb. 70 mg/kg & tet. 10 mg/kg	119 ± 7	0.001 > p
Barb. 70 mg/kg & tet. 5 mg/kg	90 ± 9	0.01 > p > 0.001

S.E. ; standard error of mean.

Barb. ; amobarbital sodium, xyl. ; xylopinine, chl. ; chlorpromazine, tet. ; tetrabenazine.

of 70 mg/kg of amobarbital sodium. The period of time of disappearance of the righting reflex was used as a measure of the sleeping time. The experiment was carried out on mice separated each other at the room temperature of 22° to 24°C. The measured figures of large deviation were rejected by the formula of Grubbs (5) at the confidence of 99%, and thereafter the mean sleeping time and the standard error of mean were calculated (Table 1). Fig. 3 shows the relation between the logarithmic dose of the drugs and the multiples of a control sleeping time of 70 mg/kg of amobarbital sodium alone. The combination of 70 mg/kg of amobarbital sodium with 10 mg/kg of each drug prolonged the sleeping time, being by 1.7 times for xylopinine, 3.0 times for tetrabenazine and 3.3 times for chlorpromazine. These results also show that chlorpromazine is most effective and xylopinine is less effective in prolonging the amobarbital sleeping time in mice.

III. Effects of xylopinine on the seizures produced by some convulsive agents

The effects of xylopinine on the lethality of mice after the seizures induced by some central convulsive agents as well as on the pattern of the seizures were observed.

FIG. 3. Effects of xylopinine, chlorpromazine and tetrabenazine on amobarbital sleeping time in mice.

Ordinate : multiples of control sleeping time due to intraperitoneal injection of 70 mg/kg of amobarbital sodium in mice. Abscissa : doses in mg/kg in logarithmic scale.

(Xylopinine, chlorpromazine and tetrabenazine were injected intraperitoneally immediately after the injection of amobarbital sodium.)

Abbreviation : chl. ; chlorpromazine, tet. ; tetrabenazine, xyl. ; xylopinine.

FIG. 4. Effects of xylopinine on the lethality of caffeine.

a. 30 mg/kg of xylopinine was injected 10 to 15 minutes before the injection of caffeine,

b. caffeine alone.

Plotted on the logarithmic probability paper.

Ordinate : percent lethality, Abscissa : doses in mg/kg.

1) *Effects on the lethality caused by caffeine*

The LD 50 of caffeine was 275 mg/kg (292 to 259 mg/kg at $p=0.05$). The injection of xylopinine in the dose of 30 mg/kg 10 to 15 minutes before the injection of caffeine decreased the LD 50 of caffeine to 220 mg/kg (233 to 207 mg/kg at $p=0.05$). The results can be seen in Fig. 4. From the figure it is shown that the pretreatment of mice with xylopinine intensifies the lethal effect of caffeine. However, toxic symptoms of caffeine by itself, restlessness, hypersensitivity to the external stimuli, decreased locomotion and clonic or tonic convulsions, were not significantly modified by the pretreatment with xylopinine. The pretreatment of mice with 30 mg/kg of chlorpromazine or tetrabenazine affected the lethality of caffeine similarly. The pretreated mice were all killed by even LD 10 of caffeine alone. However, the pretreatment of the mice with chlorpromazine and tetrabenazine also did not modify the manifestation and duration of the toxic symptoms of caffeine by itself.

2) *Effects on the lethality caused by SKF-385*

The effective dose of SKF-385 induced the elevated flexion of the tail, restlessness, increase of the spontaneous movement, motor excitement accompanied with hopping movement and tremor, hypersensitivity to the external stimuli and clonic convulsion. Some of the animals died immediately after the manifestation of such symptoms and the others survived repeating a series of such seizures. The LD 50 of SKF-385 in mice was 37 mg/kg (50 to 27 mg/kg at $p=0.05$). Because of slow onset of monoamine oxidase inhibitory action of SKF-385, doses of SKF-385 were injected 40 to 50 minutes prior to xylopinine. The LD 50 of SKF-385 combined with 30 mg/kg of xylopinine was 38 mg/kg (53 to 28 mg/kg at $p=0.05$). The results are shown in Fig. 5. The lethal effect of SKF-385 was not statistically significantly decreased by xylopinine. The toxic symptoms of mice produced by the injection of SKF-385 were also not modified by xylopinine. Any reversal of the behavior of mice in response to xylopinine was not observed by the pretreatment with a wide range of the dose of SKF-385.

3) *Effects on the lethality caused by methamphetamine*

The lethal effects of methamphetamine in mice were studied at the room temperature of

FIG. 5. Effects of xylopinine on the lethality of SKF-385.

a. SKF-385 alone,

b. 30 mg/kg of xylopinine was injected 40 to 50 minutes after the injection of SKF-385.

Plotted on the logarithmic probability paper.

Ordinate : percent lethality,
Abscissa : doses in mg/kg.

25° to 27°C. The administration of methamphetamine induced behavioral excitements closely similar to those induced by SKF-385. The LD 50 of methamphetamine in the mice placed in each cage individually was 60 mg/kg (108 to 33 mg/kg at $p=0.05$). The pretreatment of such mice with 30 mg/kg of xylopinine resulted in a statistically significant increase of the LD 50 of methamphetamine. The result shows that the pretreatment with xylopinine is protective against the lethal effect of methamphetamine in mice. When each group of four mice was allowed to keep in one cage, the LD 50 of methamphetamine was 11 mg/kg (17.8 to 7.4 mg/kg at $p=0.05$). The size of the cage was 16×13×20 cm. These results clearly show that the grouping of the mice in one cage intensifies the lethal effect of methamphetamine. The LD 50 of methamphetamine in the mice grouped in one cage and treated with 10 mg/kg of xylopinine 10 to

FIG. 6. Effects of xylopinine on the lethality of methamphetamine in mice.

A. Mice were housed individually after the injection of methamphetamine.

B. Mice were grouped together in one cage after the injection of methamphetamine.

Plotted on the logarithmic probability paper. Ordinate : percent lethality, Abscissa : doses in mg/kg.

Abbreviation : meth. ; methamphetamine, xyl ; xylopinine.

15 minutes before was 18 mg/kg (27 to 12 mg/kg at $p=0.05$), and that in the mice pretreated with 30 mg/kg of xylopinine was 17.5 mg/kg (29.5 to 10.3 mg/kg at $p=0.05$) (Fig. 6). These results clearly show that the protective effect of xylopinine against the lethal effect of methamphetamine is also effective in the mice grouped in one cage. However, the fact that the protective effect of xylopinine did not increase with a rise of the dose suggests that this effect did not result from the sedation but the other pharmacological effect of xylopinine.

In another series of experiments, the effects of chlorpromazine and tetrabenazine on the lethal effects of methamphetamine were studied in mice, grouped four mice in one cage. For one dose of methamphetamine eight mice were used at the room temperature of 18 to 20°C. At this range of room temperature, there was no increase in the toxicity of methamphetamine in grouped mice and the LD 50 of methamphetamine was 73 mg/kg (103 to 51 mg/kg at $p=0.05$). The pretreatment of the mice with 10 mg/kg of chlorpromazine 10 to 15 minutes before the administration of methamphetamine sedated the animals and raised the LD 50 of methamphetamine to 97 mg/kg (129 to 73 mg/kg at $p=0.05$), whereas the pretreatment with 10 mg/kg of tetrabenazine did not significantly affect the LD 50 of methamphetamine.

When the mice were injected 8 mg/kg of methamphetamine at the room tempera-

TABLE 2. Depressant effects on xylopinine on the excitement due to methamphetamine in mice.

Xylopinine (mg/kg)	40	30	10
% depression	93	53	3
Mean time $\pm$ S.E. of depression*	27 $\pm$ 2.9	18 $\pm$ 1.9	13

S.E. ; standard error of mean.

* Mean time of depression was calculated on animals depressed by xylopinine.

ture of 25 to 27°C the animals were excited and showed a continuously running movements about 10 minutes after the injection and this lasted above 60 minutes. The injection of xylopinine in the doses of 10, 30 and 40 mg/kg 10 minutes after the injection of methamphetamine reduced markedly the excitatory movement of mice and sometimes abolished it. The cessation of this locomotion over 30 seconds was used as a measure of the depressive effect of xylopinine on the motor excitement. Table 2 shows the percentage of the depressed animals and the time (minutes) of the depression. The experiment was carried out on 30 mice for each dose, grouping 2 mice in one cage.

4) Effects on the electroshock seizure

The electric current from the Woodbury-Davenport's apparatus was delivered on the both eye balls wetted with the physiological saline solution. Fixing the stimulation time at 0.15 sec, the effects of xylopinine on the threshold current for eliciting the maximal extensor seizure were studied in 20 mice for each dose. The stimulus was delivered to the mice after they showed a sedation in response to the dose of the drugs. The threshold current for eliciting the maximal extensor seizure in 50% of animals was 5.5 mA. This was decreased to 3.9 and 3.0 mA following the dose of 10 and 40 mg/kg of xylopinine, respectively (Fig. 7). These results show that the pretreatment of the animals with xylopinine intensify the manifestation of the seizure. On the other hand, the pretreatment of the

FIG. 7. Effects of xylopinine, chlorpromazine and tetra-benzazine on the threshold current for maximal extensor seizure in mice.

a. Each drug was given in 10 mg/kg,

b. Each drug was given in 40 mg/kg.

Plotted on the logarithmic probability paper.

Ordinate : percent of mice which show the maximal extensor seizure, Abscissa : mA.

Abbreviation : xyl. ; xylopinine, tet. ; tetra-benzazine, chl. ; chlorpromazine, n. : non-treated.

animals with chlorpromazine or tetrabenazine did not affect the threshold current to evoke the maximal extensor seizure. However, all the drugs tested did not modify the pattern of the seizure in response to the electroshock.

DISCUSSION

The central effects of xylopinine on the behavior of mice were studied in this report. As was reported by Nakanishi (1), the injection of xylopinine in doses of 10 to 100 mg/kg depressed the spontaneous movement of mice for a relatively short period. The driving movements of the intact mice in response to external stimuli were reduced profoundly or even abolished by the sedative doses of xylopinine as well as chlorpromazine and tetrabenazine. Though the degree of the depression varied with the dose injected, the duration of the depression was longest after chlorpromazine and shortest after xylopinine. Comparing the mean decrease of the driving movements for 60 minutes produced by the same doses of the drugs, the effect of xylopinine was found to be weakest. Deviation of the dose response curve of xylopinine from the almost parallel curves of chlorpromazine and tetrabenazine may be due to the central excitatory effects of the smaller doses of xylopinine as pointed out by Nakanishi (1).

The administration of xylopinine as well as chlorpromazine and tetrabenazine prolonged the sleeping time produced by the intraperitoneal injection of 70 mg/kg of amobarbital sodium in mice. Chlorpromazine was most active, and xylopinine was less effective. However, from the results cited above it is concluded that xylopinine exerts a weak but definite sedative effect on the spontaneous movement in mice.

Further, the effects of xylopinine on the seizure and lethality produced by the convulsive agents and the electroshock were studied in mice. The pretreatment of mice with the sedative doses of xylopinine significantly decreased the LD 50 of caffeine but did not modify the toxic symptoms of caffeine. The similar results were also observed in mice pretreated with chlorpromazine and tetrabenazine. The threshold current to produce the maximal extensor seizure in 50% of mice was similarly reduced by the pretreatment with the sedative doses of xylopinine. The lack of antagonism between xylopinine and caffeine might be derived from the biphasic, i.e. convulsive and paralytic effects of caffeine, as was the weakness of antagonism between caffeine and anesthetics (6, 7).

Pletcher *et al.* (8) have shown that the pretreatment of rabbit with iproniazid prevents the reduction of brain noradrenaline and serotonin produced by reserpine. Chessin *et al.* (9) have reported that mice, rats, guinea-pigs and rabbits pretreated with iproniazid respond to the administration of reserpine with marked motor excitation instead of the usual depression and that the blood pressure of the anesthetized cats pretreated with iproniazid is elevated by the administration of reserpine. The similar reversal of the action of reserpine has been shown in the isolated atrium of rabbit pretreated with iproniazid (10), and in the blood pressure of dog pretreated with SKF-385 (11).

However, Nakanishi (1) has reported that the depressor responses of the anesthetized rabbit, cat and dog to xylopinine were rather somewhat potentiated by SKF-385. He (12) has shown that the pretreatment of unanesthetized rabbit with SKF-385 does not modify the EEG resting pattern produced by xylopinine. In the present experiments, too, there was not observed reversal of the sedative effect of xylopinine by the pretreatment with SKF-385 in mice. On the other hand, the sedative dose of xylopinine did not modify toxic symptoms and LD 50 of SKF-385. The lack of protection of xylopinine against the toxic symptoms of SKF-385 and no reversal by SKF-385 of sedative effect of xylopinine may relate with a little liberation of brain catecholamine by xylopinine, as shown by Higuchi *et al.* (13).

The pretreatment with methamphetamine did not affect the EEG resting pattern produced by xylopinine in unanesthetized rabbits. But the pretreatment with xylopinine significantly reduced the increase in threshold voltage for the recruiting response by methamphetamine (12). The pretreatment of mice with the sedative dose of xylopinine significantly increased the LD 50 of methamphetamine in mice placed individually and in grouped mice. However, this protective effect of xylopinine on the lethality of mice produced by methamphetamine did not increase along with the increase of the dose. The similar protective effect was also observed by the pretreatment of mice with chlorpromazine or tetrabenazine. The increase of the spontaneous, locomotor movement of mice produced by lower doses of methamphetamine was depressed and even abolished for a while by the sedative dose of xylopinine. It remained to be settled if the behavioral antagonism between xylopinine and methamphetamine is related with the antagonism of the drugs in the threshold voltage for eliciting the EEG recruiting response in the rabbit.

SUMMARY

Sedative-tranquilizing effects of xylopinine in mice were studied. The effects obtained are summarized as follows:

1. The driving movements of mice in response to external stimuli is decreased by xylopinine as well as chlorpromazine and tetrabenazine. The suppressive effect on driving movement is weakest in xylopinine and most active in chlorpromazine.
2. Xylopinine prolongs the amobarbital sleeping time of mice in a much lesser extent than chlorpromazine and tetrabenazine.
3. The pretreatment of mice with xylopinine decreases the LD 50 of caffeine. Chlorpromazine and tetrabenazine also affect the lethality of caffeine similarly. However, the pretreatment with the drugs does not modify the manifestation and duration of toxic symptoms caused by caffeine.
4. Xylopinine does not affect the lethality and toxic symptoms of SKF-385. Moreover when xylopinine is administered following pretreatment with SKF-385, the mice only show the characteristic depressant effects of xylopinine and do not exhibit any behavioral excitement.

5. Xylopinine increases the LD 50 of methamphetamine in mice housed individually and in grouped mice. Chlorpromazine and tetrabenazine also affect the LD 50 of methamphetamine similarly.

6. Xylopinine depresses the excitatory running movement due to methamphetamine.

7. The threshold current for maximal extensor seizure of mice evoked by electroshock is reduced by the pretreatment with xylopinine but not modified by chlorpromazine and tetrabenazine. However, all the drugs do not modify the pattern of convulsion due to electroshock.

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