Effects of Palmatine on Motor Activity and the Concentration of Central Monoamines and Its Metabolites in Rats

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ABSTRACT—We used behavioral and biochemical methods to investigate the sedative effect of palmatine on locomotor activity and the concentration of monoamine in rats. It was found that palmatine enhanced the hypomotility induced by $\alpha$-methyl-$p$-tyrosine, reserpine and 5-hydroxytryptophan, but reduced the hypermotility produced by L-dopa plus benserazide and $p$-chlorophenylalanine. Furthermore, palmatine significantly decreased the concentration of dopamine and homovanillic acid in the cortex and the concentration of serotonin in the brain stem, and it increased the concentration of 5-HT in the cortex and 5-hydroxyindole acetic acid in the brain stem. These results suggest that the sedative mechanism of palmatine may be related to the decrease in the concentration of catecholamine in the cortex and serotonin in brain stem and the increase in the concentration of 5-HT in the cortex.

Keywords: Palmatine, Locomotion, Monoamine concentration

Corydalis yanhusuo W.T.W. ANG (CY) is described in ancient Chinese medical books on analgesics and sedatives. One of the major constituents of CY is palmatine. In modern pharmacological studies, palmatine has been found to have antiarrhythmic and analgesic effects. However, because the sedative effect of palmatine has never been studied, the purpose of the present study was performed to investigate the sedative effect of palmatine and its mechanism by measuring locomotor activity and the concentrations of central monoamine and its metabolites.

MATERIALS AND METHODS

Male Sprague-Dawley rats (200–300 g) were injected intraperitoneally with palmatine (0.1, 10 mg/kg) dissolved in distilled water containing 10% phosphoric acid (vehicle, pH 4.5). The control animals received an equivalent volume of the vehicle.

Measurement of locomotor activity

The locomotor activity of individual rats was measured with the MK-ANIMEX activity meter (Model SE, Muromachi Kikai Co., Japan). The sensitivity and tuning of the instrument was adjusted to 35 $\mu$A to enable all kinds of motor behavior, including locomotion, rearing, grooming, sniffing and licking, to be jointly recorded. Activity was recorded for 2 hr, starting 5 min after inserting the animal into the test cage. Each rat was used only once, and a total of six rats were used for each treatment.

Palmatine (0.1, 1 mg/kg) was administered intraperitoneally. Thirty minutes later, locomotor activity was recorded. L-Dopa, benserazide, 5-HTP and PCPA were suspended in 5% CMC. $\alpha$-MT was dissolved in 0.9% saline. Reserpine was dissolved in the minimum amount of glacial acetic acid and diluted with water, the finally acetic acid concentration being less than 0.1%. L-Dopa, benserazide, 5-HTP and PCPA were prepared immediately before use.

The following compounds were given before administration of palmatine (10 mg/kg, i.p.): $\alpha$-MT (100 mg/kg, i.p.), 2 hr before; L-dopa (200 mg/kg, i.p.), 50 min before; benserazide (50 mg/kg, i.p.), 80 min before; reserpine (0.5 mg/kg, i.p.), 18 hr before; 5-HTP (50 mg/kg, i.p.), 30 min before; PCPA (200 mg/kg, i.p.), 24 hr before. Control mice received the vehicle only.
Quantification of monoamines and its metabolites by HPLC

Palmatine was given intraperitoneally at the dose of 10 mg/kg. Thirty minutes later, the rats were decapitated, and the whole brain of each animal was removed and separated into the cortex and brain stem. The concentrations of norepinephrine, dopamine, serotonin and their metabolites (VMA, HVA, 5-HIAA) were measured by high performance liquid chromatography (HPLC) with electrochemical detection (5–7). The HPLC Model M-45 (Waters Associates), Data Model M-730 (Waters Associates) and Electrochemical Detector Model LC-4B (Bioanalytical Systems) were used.

Statistical analyses

Results were analyzed statistically by the unpaired Student’s t-test or ANOVA followed by the Duncan multiple range test to compare the differences between groups; A P value < 0.05 was considered to indicate a significant difference.

RESULTS

Effects on the locomotor activity

As shown in Fig. 1, palmatine produced a significant decrease in locomotor activity, and this was dose-dependent response (P<0.05–0.01).

Rats treated with α-MT or reserpine alone appeared to be behaviorally depressed. The hypomotility produced by α-MT or reserpine was significantly augmented by palmatine (P<0.01) (Fig. 2).

As shown in Fig. 3, L-dopa plus benserazide produced significant hypermotility. The hypermotility produced by L-dopa plus benserazide was significantly reduced by palmatine (P<0.01).

Rats treated with 5-HTP also appeared to be behaviorally depressed. The hypomotility produced by 5-HTP was significantly augmented by palmatine (P<0.01) and
PCPA also produced a significant increase in locomotor activity. The increased locomotor activity could significantly be reduced by palmatine (P < 0.01) (Fig. 4).

**Fig. 3. Effects of palmatine on the locomotor activity produced by l-Dopa+benserazide in rats. VEH=vehicle controls. l-DOPA+BZ=l-Dopa, 200 mg/kg, 50 min prior to testing plus benserazide, 50 mg/kg, 80 min prior to testing. PAL. =palmatine 10 mg/kg, 30 min prior to testing. PAL./L-DOPA+BZ=palmatine plus l-dopa + benserazide. n = 6 (ANOVA for repeated measures followed by Duncan's multiple range test). * P <0.05, ** P <0.01.**

Effects on the concentration of monoamines and its metabolites in the brain stem and cortex

As shown in Table 1, palmatine significantly decreased the levels of DA (P < 0.05) and HVA (P < 0.01) in the cortex and the level of 5-HT in the brain stem (P < 0.01). It significantly increased the level of 5-HT in the cortex (P < 0.05) and the level of 5-HIAA in the brain stem (P < 0.05).

**Fig. 4. Effects of palmatine on the locomotor activity produced by 5-HTP, PCPA in rats. VEH=vehicle controls. 5-HTP=5-hydroxytryptophan, 50 mg/kg, 30 min prior to testing. PAL. =palmatine, 10 mg/kg, 30 min prior to testing. PAL./5-HTP=palmatine plus 5-hydroxytryptophan. PCPA=dL-p-chlorophenylalanine, 200 mg/kg, 24 hr prior to testing. PAL./PCPA=palmatine plus dl-p-chlorophenylalanine. n = 6 (ANOVA for repeated measures followed by Duncan's multiple range test). ** P < 0.01.**

DISCUSSION

As shown by the results in Fig. 1, palmatine significantly decreased the locomotor activity, showing that palmatine has a sedative effect. Furthermore, palmatine significantly augmented the hypomotility produced by α-MT or reserpine and reduced the locomotor stimulation produced by l-dopa plus benserazide. Generally, α-MT (an inhibitor of tyrosine hydroxylase) pretreatment inhibits the newly-synthesized pool of catecholamines (8), and reserpine depletes the stored-vesicular pool of CNS monoamines (9, 10). Therefore, the two compounds can reduce locomotor activity (11), whereas l-dopa (a precursor of dopamine) plus benserazide (a dopa decarboxylase inhibitor) (12) can produce hypermotility. These facts imply that the sedative effects of palmatine may be involved in the decrease of central catecholaminergic activity.

From the studies of monoamines and their metabolites in rat brains, we further found that palmatine could sig-
nificantly decrease the concentration of dopamine and its metabolite (HVA) in the cortex. DA is an excitatory neurotransmitter in the cortex (13). So, the sedative mechanism of palmatine may be related to its ability to decrease the dopamine concentration in the cortex.

Palmatine could augment the depressed locomotor activity induced by 5-HTP and depress the hypermotility induced by PCPA. Generally, 5-HTP (a precursor of serotonin) can increase the central 5-HT level in the whole brain with a subsequent decrease in locomotor activity (14). In contrast, PCPA (a serotonin synthesis inhibitor) can cause catecholamine arousal with a subsequent increase in locomotor activity (15). These facts imply that the sedative effect of palmatine may be involved in the increase of the serotonergic activity.

From the studies of monoamines and their metabolites in rat brains, we found that palmatine significantly increased the concentration of 5-HT in the cortex, but decreased the concentration of serotonin in the brain stem. Palmatine decreased the ratio of 5-HIAA/5-HT in the cortex and increased the ratio of 5-HIAA/5-HT in the brain stem. These results indicate that palmatine decreased the 5-HT turnover rate in the cortex and increased the 5-HT turnover rate in the brain stem. Generally, because 5-HT facilitates the inhibition of motor neurons in the cortex (16), the 5-HT levels of the cortex were increased. Because 5-HT excites the motor neurons in the brain stem (16), the 5-HT levels of the brain stem were decreased. The present results demonstrate that the inhibitory effect of palmatine on motor activity in rats was related to the increase of 5-HT levels in the cortex and the decrease of 5-HT levels in the brain stem.

In conclusion, the sedative mechanism of palmatine may be related to the decrease of the catecholaminergic activity in the cortex and serotonergic activity in the brain stem and the increase of the serotonergic activity in the cortex.

### Table 1. Effects of palmatine on monoamines and metabolites in the cortex and brain stem in rats

|         | Cortex (ng/g) | Brain stem (ng/g) |
|---------|--------------|------------------|
|         | control      | palmatine        | control     | palmatine     |
| DA      | 2155 ± 379   | 1688 ± 158       | 4574 ± 416  | 3514 ± 457   |
| 5-HT    | 1903 ± 180   | 1305 ± 42*       | 2322 ± 214  | 1398 ± 260   |
| VMA     | 703 ± 76     | 969 ± 77*        | 1410 ± 204  | 499 ± 715**  |
| HVA     | 286 ± 47     | 196 ± 16         | 315 ± 31    | 297 ± 49     |
| 5-HIAA  | 429 ± 60     | 220 ± 25**       | 519 ± 100   | 416 ± 46     |
| 5-HIAA  | 410 ± 112    | 308 ± 70         | 290 ± 41    | 421 ± 26*    |

Mean ± S.E. (n=6). *P < 0.05, **P < 0.01. P value is compared with the control group by Student's t-test.

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