PAP 9704, a Korean Herbal Medicine Attenuates Methamphetamine-Induced Hyperlocomotion via Adenosine A2A Receptor Stimulation in Mice

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The effect of PAP 9704, a traditional prescription in Korea consisting of Polygala tenuifolia, Acorus gramineus, and Poria cocos at a ratio of 1:1:1 (dry weight), on methamphetamine (MA)-induced hyperlocomotion was examined in mice. The increased locomotor activity induced by MA (1 mg/kg/d, i.p. × 7) was significantly attenuated by co-administration with PAP 9704 (100 or 200 mg/kg/d, p.o. × 7) in a dose dependent manner. Consistently, it was found that the hyperlocomotor activity occurred in parallel with the expression of striatal fos-related antigen immunoreactivity. The adenosine A2A receptor antagonist, 1,3,7-trimethyl-8-(3-chlorostyryl)-xanthine (0.5 or 1.0 mg/kg, i.p.), significantly reversed the pharmacological action of PAP 9704 in a dose related manner, but the adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dimethylxanthine (0.5 or 1.0 mg/kg, i.p.) and the A2B receptor antagonist alloxazine (1.5 or 3.0 mg/kg, i.p.) did not significantly affect this pharmacological action. Our results suggest that PAP 9704 prevents MA-induced hyperlocomotion, at least in part, via the stimulation of the adenosine A2A receptor.

Key words PAP 9704; methamphetamine; locomotor activity; adenosine A2A receptor; striatum; fos-related antigen immunoreactivity

PAP 9704, a traditional prescription of Korea (Polygala tenuifolia : Acorus gramineus : Poria cocos = 1 : 1 : 1) has been used in the clinical treatment for the neurodegenerative conditions, such as amnesia and dementia.1) We have demonstrated that PAP 9704 possesses a protective effect in response to neurotoxicity induced by kainate.2) It has been demonstrated that Korean Panax ginseng shows preventive effects on the methamphetamine (MA)-induced neurotoxic3) and behavioral side effects.4) In our preliminary study, we have observed that PAP 9704 exhibits Panax ginseng-like sedative- and anti-psychotropic effects.5) Therefore, we attempted to investigate whether PAP 9704 could prevent MA-induced hyperlocomotion in this study.

The purine nucleotide adenosine might act as a neuromodulator of the CNS and an endogenous neuroprotectant.6) Adenosine exerts its effects on the neuronal activity via four G protein-coupled receptors, A1, A2A, A2B and A3.7) While adenosine A1 and A2B receptors are widely distributed in the brain, the A2A receptor distribution is restricted to the dopamine-innervated regions.8) A1 receptors are expressed at low levels in the brain. Stimulation of either A1 or A2A receptors induces a psychomotor depression and counteracts the motor activating effects of dopamine receptor agonists.9) Adenosine analogs have also been found to decrease striatal dopamine turnover and dopamine release.10) This antagonistic adenosine–dopamine interaction in the striatum seems to be mediated by either presynaptic A1 receptors or postsynaptic A2A receptors, co-localized with D2 dopamine receptors in striatopallidal neurons.11) Transcription factors encoded by the Fos and Jun families of immediate early genes have been studied as potential mediators of drug-induced neural plasticity.12—15) The acute administration of psycho-stimulants produces a rapid but transient induction of several Fos- and Jun-like proteins in the striatal complex. In contrast, chronic drug exposure desensitizes the ability of these proteins to be induced and results instead in the gradual accumulation of a novel Fos-like protein termed chronic Fos-related antigen (FRA) protein.13,14) Recent work has definitively identified the chronic FRA as an isoform of Δ-Fos B.13) Therefore, it is thought that FRA induction is, in part, relevant to various behavioral features of prolonged cocaine exposure.12—15)

We evaluated the impact of PAP 9704 on the MA-induced behavioral effects and expression of the striatal fos-related antigen-immunoreactivity (FRA-IR) to learn whether adenosine receptors play a role in the pharmacological effects of PAP 9704, since adenosine A1 and A2A receptors regulates dopaminergic neuronal activity as described above.

MATERIALS AND METHODS

Animals and Treatments All animals were handled in accordance with the NIH guidelines for the humane care of laboratory animals. Male BALB/C AnNcrj mice (Bio Genomics, Inc., Charles River Technology, Gapyung-Gun, Gyeonggi-Do, Korea) weighing about 25 g were maintained on a 12:12 h light:dark cycle and fed ad libitum. They were adapted to these conditions for 2 weeks before the experiment. All the rodents were drug and seizure naive before testing. MA hydrochloride (NIDA/NIH, Rockville, MD,
U.S.A.) was dissolved in sterilized saline (1 mg/kg, i.p.). PAP 9704 is a mixture of Polygala tenuifolia, Acorus gramineus, and Poria cocos at a ratio of 1:1:1 (dry weight). Polygala tenuifolia contains onjisaponins and sugars.16) Acorus gramineus consists mainly of essential oils such as asarone, eusarone and thymol.17) Poria cocos consists of the polysaccharide pachymann, and the triterpenoids pachymic acid, eburicoic acid, and tumulosic acid.18) However, a careful review of the literature indicates that the pharmacokinetic properties of this prescription remain to be fully determined. PAP 9704 was supplied by Boryung Pharmaceutical Company, Ansan, Gyeonggi-Do, Korea.21) Previously we demonstrated the neuroprotective properties of PAP 9704 in vivo.2,23) PAP 9704 was dissolved in sterilized water (100 or 200 mg/kg, p.o.), and was administered daily for 7 consecutive days. MA injections were performed 2 h after every PAP 9704 treatment. Adenosine receptor antagonists [A1 receptor antagonist 8-cyclopentyl-1,3-dimethylxanthine (CPT; 0.5 or 1.0 mg/kg, i.p., RBI, Natick, MA, U.S.A.), adenosine A2a receptor antagonist 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine (CSC; 0.5 or 1.0 mg/kg, i.p., Sigma, St. Louis, MO, U.S.A.) and A2B receptor antagonist alloxazine (ALX; 1.5 or 3.0 mg/kg, i.p., RBI, Natick, MA, U.S.A.)] were administered 30 min prior to the last MA injection.

Locomotor Activity Ten minutes after the last treatment with each drug, locomotor activity was measured for 30 min using an automated video-tracking system (Noldus Information Technology, Wagenin, The Netherlands). Eight test boxes (40×40×30 cm high) were operated simultaneously by an IBM computer. Animals were studied individually during locomotion measurement in each test box, where they were adapted for 10 min before starting the experiment. A printout for each session showed the pattern of the ambulatory movements of the test box. The distance traveled in cm by the animals in horizontal locomotor activity was analyzed. Data were collected and analyzed between 0900 and 1700 h.14,15)

Fos-Related Antigen Immunoreactivity (FRA-IR) Brains were taken 12 h after the final MA treatment and used for immunocytochemical analysis. The coronal sections containing dorsomedial striatum were processed for FRA immunocytochemistry. Prior to overnight incubation with the primary antibody, sections were prewashed in 0.2% Triton X-100 for 15 min, followed by 4% normal goat serum for 20 min. After the primary antibody, sections were prewashed in 0.2% Triton X-100 for 1 h. Then incubated with a secondary biotinylated antiserum for 90 min followed by 4% normal goat serum for 20 min. After the chromogen was used to visualize immunoreactive cells.

RESULTS AND DISCUSSION

The body weight (B.W.) of the animals treated with saline + saline [25.2±0.5 g (1 d) vs. 27.9±0.4 g (7 d), p<0.05] or with saline + PAP 9704 (200 mg/kg) [25.0±0.5 g (1 d) vs. 27.7±0.3 g (7 d), p<0.05] significantly increased over time. The B.W. of animals receiving MA did not change within the 7 d [25.3±0.3 g (1 d); 24.9±0.4 g (7 d)]; however, B.W. did significantly increase when MA was co-administered with PAP 9704 (100 mg/kg) [PAP 9704 (100 mg/kg) + MA: 25.0±0.4 g (1 d) vs. 27.0±0.4 g (7 d); MA vs. PAP 9704 (100 mg/kg) + MA, p<0.05] or PAP 9704 (200 mg/kg) [PAP 9704 (200 mg/kg) + MA: 25.4±0.5 g (1 d) vs. 27.4±0.4 g (7 d), p<0.05; MA vs. PAP 9704 (200 mg/kg) + MA, p<0.05].

MA-induced locomotor activity significantly increased over time [Saline + MA, 1 d vs. 7 d; F (1,28)=4.19, p<0.05]. Treatment with PAP 9704 attenuated MA-induced hyperlocomotion [at 7 d, Saline + MA vs. PAP 9704 (100 mg/kg) + MA; F (1,28)=4.92, p<0.05: at 1, 3, and 7 d, Saline + MA vs. PAP 9704 (200 mg/kg) + MA; at 1 d, F (1,28)=4.72, p<0.05: at 3 d, F (1,28)=9.94, p<0.005: at 7 d, F (1,28)=21.18, p<0.0001] (Fig. 1A). Neither adenosine receptor antagonists nor PAP 9704 significantly altered locomotor activity in mice. Adenosine antagonists at the doses used in this study did not significantly affect MA induced locomotor activity at 7 d. Although neither the adenosine A1 antagonist, CPT, nor A2B antagonist, ALX, affected PAP 9704-mediated pharmacological action, A2a antagonist, CSC, significantly counteracted PAP 9704-mediated pharmacological action in a dose-dependent manner [Saline + PAP 9704 (200 mg/kg) + MA vs. “CSC 0.5 mg/kg” or “CSC 1.0 mg/kg” + PAP 9704 (200 mg/kg) + MA; F (1,28)=5.43, p<0.05 or F (1,28)=34.61, p<0.0001]. Striatal FRA-IR was significantly increased 12 h after the last injection of MA [F (1,28)=34.61, p<0.0005]. PAP 9704 (200 mg/kg) significantly decreased MA induced increases in the striatal FRA-IR [F (1,28)=18.94, p<0.0003]. CSC (1.0 mg/kg) significantly reversed the PAP 9704-evoked reduction in FRA-IR [F (1,18)=16.56, p<0.005]. Consistently, FRA induction was positively correlated with adenosine A2a antagonism by CSC (p<0.05). However, CPT and ALX failed to alter the effects of PAP 9704 (Fig. 2).

In this study, we demonstrated that MA-induced increases in locomotor activity are related to corresponding FRA-IR in the mouse striatum, and that PAP 9704 attenuates these effects of MA, at least in part, via adenosine A2a receptor stimulation. Our data provide interesting functional clues to the relationship between striatal FRA-IR and behavioral features. It has been recognized that the striatal complex is the primary site of the behavioral effect of chronic psychostimulant administration.12,13) For example, chronic psychostimulant administration induces expression of chronic FRA-IR in the striatal complex, while FRA-IR is not induced significantly by acute administration.23,24) Therefore, FRA appeared to be the transcriptional mediator underlying some of the long-lasting biochemical adaptations of MA. Although the nucleus accumbens is more sensitive to some of the effects of
neurostimulants than is the striatum during a course of chronic exposure.[1,11,14] PAP 9704-induced reduction in FRA-IR increases was more pronounced in the striatum than in the nucleus accumbens (data not shown) in this study.

In our preliminary study, we found that each constituent (Polygona tenuifolia, Acorus gramineus or Poria cocos) in the PAP 9704 fails to significantly attenuate locomotor facilitation induced by repeated treatment with MA (data not shown), although polygalasaponins from Polygona tenuifolia and water extract of Acorus gramineus show central inhibitory effects on response induced by a single injection of apomorphine, pentylenetetrazole, cocaine or MK-801.[25,26] Therefore, it remains to be further characterized what components are responsible for the mechanism of actions of PAP 9704.

Fig. 1. Effects of PAP 9704 on the Locomotor Stimulations Following Chronic Methamphetamine (MA) Administration

1 d, 3 d or 7 d = a single dose of MA (1 mg/kg, i.p./d), three doses of MA (1 mg/kg/d, i.p.×3) or seven doses of MA (1 mg/kg/d, i.p.×7). (A) Effects of adenosine receptor antagonists on the PAP 9704-mediated pharmacological action following final MA administration (1 mg/kg/d, i.p.×7). (B) Details are in Animals and Treatments, Materials and Methods. PAP 100 or 200 = PAP 9704 (100 or 200 mg/kg, p.o.). CPT = 8-cyclopentyl-1,3-dimethylxanthine (A1 receptor antagonist), CSC = 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine (A2A receptor antagonist), ALX = 8-alloxazine (A2B receptor antagonist). Each value is the mean±SEM of 15 animals. *p<0.01 vs. corresponding Saline+MA, †p<0.01 vs. corresponding Saline+MA, ‡p<0.01 vs. corresponding Saline+PAP 200+MA, §p<0.01 vs. corresponding Saline+PAP 200+MA (ANOVA with DMR test).

It is suggested that both adenosine A1 and A2A receptor agonists inhibit MA-induced behavioral effects via different mechanisms.[27] In this study, we found that PAP 9704 shows A1A receptor agonist-like effects, because PAP 9704-mediated pharmacological action was significantly reversed by the specific A1 receptor antagonist CSC. Most A2A receptors are located on striatopallidal GABAergic neurons. Stimulation of these receptors increases the release of GABA in the striatum and globus pallidus and counteracts the D2-mediated effects.[11] Similarly, A2A receptors of cholinergic terminals are activated by A2A agonist CGS 21680, and then GABAergic neurons might be activated. Further, dopaminergic neurons might be inhibited by activated GABAergic neurons.[28] Therefore, we cannot rule out the possibility that significant reductions in the behavioral effects and FRA-IR induced by PAP 9704 may occur through complex pharmacological interactions between A2A receptor and DA/GABA receptor complex, although direct evidences should be gathered.

In summary, our results suggest that PAP 9704 attenuates MA-induced hyperactivity, in part, through adenosine A2A receptor stimulation, although the precise pharmacological mechanisms mediated by PAP 9704 remain to be fully determined.

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