Pharmacology Biochemistry & Behavior, Vol. 20, pp. 803–806, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

BRIEF COMMUNICATION

Phenytoin Potentiates Methamphetamine-Induced Behavior in Mice

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Received 30 June 1983

IZUMI, K., M. NOMOTO, T. KOJA, T. SHIMIZU, C. KISHITA AND T. FUKUDA. *Phenytoin potentiates methamphetamine-induced behavior in mice*. PHARMACOL BIOCHEM BEHAV **20**(5) 803–806, 1984.—We demonstrated that stereotyped behavior and tremor induced by methamphetamine (MA) were potentiated by pretreatment with phenytoin (PNT) in mice. Similar enhancing effects were obtained by pretreatment with carbamazepine. Gas chromatographic study demonstrated that pretreatment with PNT increased MA concentrations in the brain to approximately 2.5 times of control level. The increased MA concentrations were thought to be a major factor for the observed potentiation of MA-induced behavior by PNT. However, all MA-induced behavior were not equally potentiated; tremor was enhanced more than stereotypy. These results suggest that central neuronal mechanisms may also be involved in PNT-potentiated MA-induced behavior in mice.

Phenytoin Carbamazepine Methamphetamine Interaction Stereotyped behavior Tremor

METHAMPHETAMINE (MA), a derivative of phenylethylamine, is a central nervous system (CNS) stimulant sometimes associated with psychosis following chronic use. Administration of this drug increases locomotor activity and subsequently produces characteristic stereotyped behavior consisting of sniffing, licking or biting in rodents. An intraperitoneal (IP) injection of large doses (15–45 mg/kg) of dl-amphetamine in mice has been previously shown to induce tremors and convulsions in addition to increased locomotor activity and pronounced stereotyped behavior [3]. During experiments on epilepsy, we observed that MA-induced stereotyped behavior and tremor in mice are modified by pretreatment with phenytoin (PNT), an anticonvulsant most widely used for epileptic patients. The interaction between MA and PNT has not been previously demonstrated. A study investigating the interaction between MA and carbamazepine (CBZ) was also carried out, since the anticonvulsant effects of CBZ in animals resemble those of PNT.

METHOD

General

Male ddY mice (30–45 g) were used. All drugs used in the present study were injected IP in a volume of 0.1 ml per 10 g of body weight. MA HCl (Dainippon Seiyaku) and PNT-Na (Dainippon Seiyaku) were dissolved in distilled water. PNT (5–50 mg/kg) was administered 10 min prior to an injection of MA (10 mg/kg) in test mice. Control animals were injected with 0.85% saline solution, and 10 min later received the same dose of MA. In other experiments, the effects of pretreatment with PNT (50 mg/kg) on apomorphine (5 mg/kg; Sigma), ephedrine HCl (20 mg/kg; Iwaki), and L-DOPA (100–300 mg/kg; Nakarai) were examined. Apomorphine and ephedrine HCl were dissolved in 0.85% saline solution. L-DOPA was suspended in 0.5% carboxymethyl cellulose solution (sodium salt). This substance was administered 60 min following an injection of MK 486 (100 mg/kg), a peripheral aromatic amino acid decarboxylase inhibitor. In these experiments, mice were similarly pretreated with PNT 10 min before the administration of apomorphine, ephedrine or L-DOPA. In the final series of experiments, CBZ (50 mg/kg; CIBA GEIGY) emulsified in 0.85% saline solution was substituted for PNT 10 min prior to the injection of MA (10 mg/kg).

Behavioral Observation

Following administration of MA, apomorphine or other drugs, mice were placed individually in a plastic round box having a floor size of 44 cm diameter. Room temperature ranged from 23° to 25°C. Animals were continuously observed for 30 min and behavioral effects were recorded and scored at an interval of 2 min. The intensity of stereotyped behavior was assessed according to the scoring system of Naylor and Costall [4]: 0=animals same as saline-treated animals; 1=discontinuous sniffing, constant exploratory activity; 2=continuous sniffing and small head movements, periodic exploratory activity; 3=continuous sniffing and small head movements, discontinuous biting, gnawing and

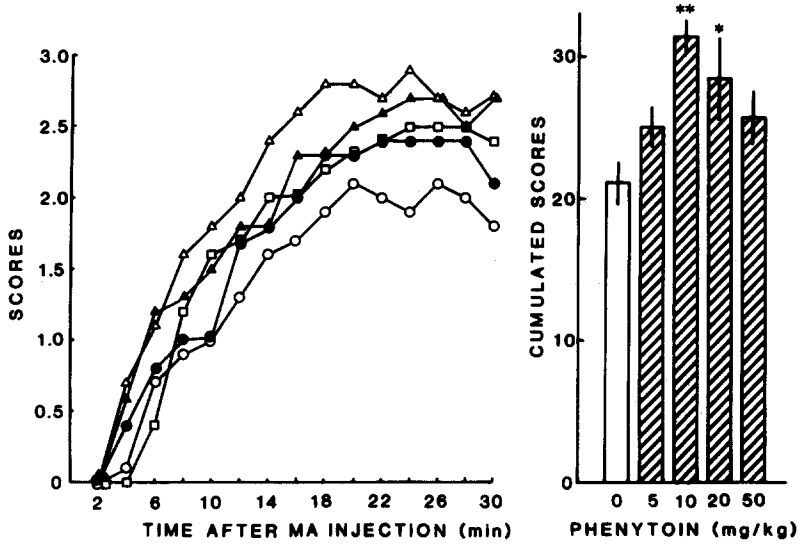

FIG. 1. Effects of phenytoin on stereotyped behavior induced by methamphetamine (MA) in mice. Animals were pretreated IP with various doses of phenytoin (●, 5 mg/kg; △, 10 mg/kg; ▲, 20 mg/kg; and □, 50 mg/kg) or saline (○) and 10 min later received MA (10 mg/kg, IP). Each point in the left panel showing the time-course effects represents the mean score of 10 animals. The cumulated scores were determined as the summation of each 2 min score for 30 min. Each bar in the right panel represents the mean±S.E. (n=10). *$p<0.05$; **$p<0.01$.

licking, brief periods of locomotor activity; and 4=continuous gnawing, biting and licking, no exploratory activity. The scoring system employed for tremor was as follows: 0=absent; 1= equivocal; 2=mild; 3=moderate; and 4=severe.

Methamphetamine Assay

To examine whether PNT is able to affect MA level, MA concentrations in the brain were determined by gas chromatographic method essentially as described by Yamanaka *et al.* [5] with a slight modification. Mice were injected with PNT (10 mg/kg, IP) or 0.85% saline solution 10 min prior to the administration of MA (10 mg/kg, IP). Animals were sacrificed by decapitation 20 min following the MA injection, and the whole brain was quickly removed on ice. Brain tissues were homogenized with a polytron (Kinematica; setting 7 for 15 sec) in 4 volumes of 0.4 N perchloric acid. Homogenization and subsequent centrifugation were carried out at 0–4°C. The homogenate was centrifuged at 14,000 × g for 20 min (Kubota KR-20000). One ml of the supernatant was mixed with 1.0 ml of 5 N NaOH saturated with NaCl and 6.0 ml of n-pentane. After shaking for 10 min, centrifugation was carried out at 1600 × g for 10 min and 5.0 ml of n-pentane phase was transferred to a 10 ml glass-stoppered test tube. After heating at 60°C for 30 min, the mixture was dried up under nitrogen. The residue was added by 0.1 ml of trifluoroacetic acid and mixed. One μl of this mixed solution was injected into the gas chromatograph (Shimadzu GC-4CM) equipped with a hydrogen flame ionization detector and a 3 mm i.d.×2.0 m glass column packed with 2% OV-17 on Uniport HP. The chromatographic conditions were as follows: column temperature, 110°C; injection temperature, 150°C; nitrogen gas flow rate, 40 ml/min; air pressure, 1 kg/cm^2; and hydrogen pressure, 0.5 kg/cm^2. Under these conditions, retention time for MA was found to be 4.80 min. The concentrations of MA were determined by interpolation on the standard curve for MA.

RESULTS

Behavioral

Administration of MA at a dose of 10 mg/kg to control mice initially increased locomotor activity. The increased locomotion was gradually replaced by sniffing and licking approximately 6 min after the MA injection. The scores for stereotyped behavior increased with time until 20 min. The maximum state of stereotypy was observed between 20 and 30 min following the injection (Fig. 1). With this dose of MA, all 10 mice showed weak fine whole body tremor with a latent period of 920±101 sec (mean±S.E., n=10) and piloerection.

Figures 1 and 2 demonstrate time-course and dose-dependent effects of PNT on the stereotyped behavior and tremor induced by MA. Animals which had received PNT alone at any dose used in the present study did not show abnormal behavior. Two-way analysis of variance revealed that the scores for stereotyped behavior in mice pretreated with PNT were significantly higher, $F(4,731)=36.16$, $p<0.01$, than those in control animals (Fig. 1). Furthermore, the scores increased with time, $F(14,731)=103.71$, $p<0.01$. In spite of these findings, the effects of PNT on MA-induced stereotypy were not dose-related. A peak effect was noted at a dose of 10 mg/kg but nonsignificant increases occurred after 5 and 50 mg/kg (Fig. 1).

On the other hand, the scores for tremor were found to be increased by pretreatment with PNT (5–50 mg/kg) in a dose-dependent fashion and as a function of time, $F(4,731)=73.40$, $p<0.01$, (Fig. 2). In fact, statistical analysis showed that there is a significant interaction between dose-dependent and time-course effects, $F(56,675)=2.35$, $p<0.01$, of the treatment with MA plus PNT on tremor. However, this enhanced

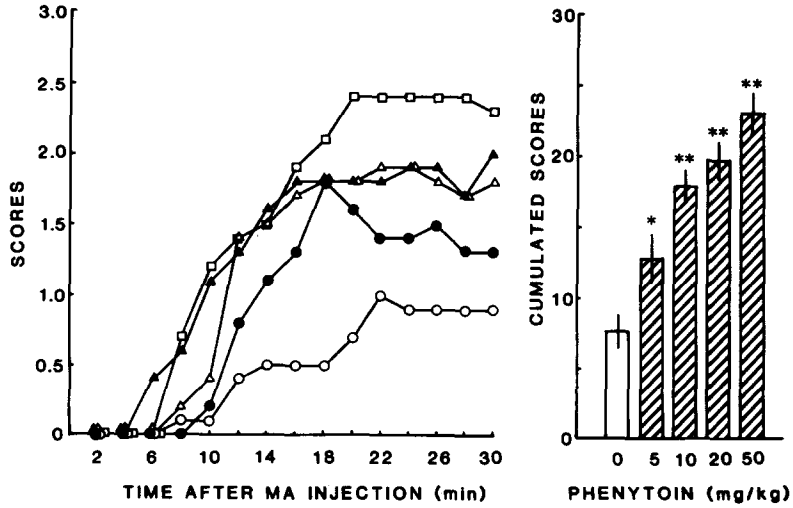

FIG. 2. Effects of phenytoin on tremor induced by methamphetamine (MA) in mice. Experimental conditions, number of animals tested and symbols on the figure are the same as those described in the legend for Fig. 1. *$p<0.05$; **$p<0.01$.

tremor per se did not seem to interrupt appearance of stereotyped behavior, since a simple regression analysis revealed a highly positive correlation ($r=0.965$, $F(1,8)=108.28$, $p<0.01$) between scores for tremor and stereotypy in mice injected with PNT (50 mg/kg) and MA (10 mg/kg).

In another series of experiments, we tested whether or not PNT has a capability to potentiate or provoke stereotypy and/or tremor in mice injected with other drugs affecting the dopaminergic neuronal activity or having structural similarity to MA. Those animals which had been injected with 0.85% saline and 10 min later received apomorphine (5 mg/kg) showed very weak tremor (cumulated scores of 1.5 ± 0.3 (n=6) in 30 min) and moderate degree of stereotyped behavior with scores of 22.7 ± 2.1 (n=6). Pretreatment with PNT significantly increased the scores for tremor (13.5 ± 1.7, n=6; $F(1,10)=48.54$, $p<0.01$) but did not increase those for stereotyped behavior (25.3 ± 2.2, n=6). Neither the administration of L-DOPA with MK 486 nor the additional pretreatment with PNT produced tremor or stereotyped behavior in mice during the 30-min observation period. Ephedrine did not produce either tremor or stereotypy in animals with or without PNT treatment.

Pretreatment with CBZ (50 mg/kg) enhanced the scores for tremor (21.2 ± 3.5, n=6; $F(1,18)=25.10$, $p<0.01$) and stereotyped behavior (27.3 ± 3.9, n=6; $F(1,18)=4.91$, $p<0.05$) induced by MA (10 mg/kg). Control scores for tremor and stereotypy were 8.29 ± 0.9 (n=14) and 21.0 ± 1.0 (n=14), respectively. Animals that had received CBZ alone at this dose did not show any abnormal behavior.

Methamphetamine Level

MA concentrations (14.68 ± 1.19 μg/g tissue; mean±S.E., n=5) in the brain in test mice which had received PNT (10 mg/kg) 10 min prior to the administration of MA (10 mg/kg) were found to be significantly higher than those (5.98 ± 0.93 μg/g tissue; n=5) in control animals which had been treated with 0.85% saline solution and 10 min later the same dose of MA, $F(1,8)=33.21$, $p<0.01$.

In a separate *in vitro* study, we examined whether or not PNT facilitates transfer of MA into the n-pentane phase during the extraction procedures. Added MA to the brain homogenates as an internal standard (5 μg) was extracted in the presence or absence of PNT (5 μg). Areas under the MA peak curve on gas chromatography were measured by a computer (Shimadzu Chromatopac C-R1A). The estimated areas in test samples extracted in the presence of PNT were 5514 and 4050, while those in control samples obtained in the absence of PNT were 7411, 7318 and 5342.

DISCUSSION

In the present study, we demonstrated that MA-induced behavior is potentiated by PNT or CBZ in mice. This potentiating action of PNT may be rather specific for MA, since among other drugs tested apomorphine was only the drug whose actions were enhanced by the anticonvulsant, albeit mildly. L-DOPA plus MK 486 or ephedrine did not provoke either tremor or stereotyped behavior in animals with or without PNT treatment.

Since PNT and CBZ are known to displace a number of drugs from protein binding sites, it was considered that serum levels of MA in unbound form might be increased in the presence of PNT or CBZ. Consequently, the MA levels in the brain could be raised when PNT- or CBZ-enhanced MA-induced behavior was present. In fact, the present gas chromatographic study demonstrated that the concentrations of MA in the brain in test mice which had been treated with PNT before the MA administration were approximately 2.5 times higher than those in control animals which had been injected with the same dose of MA without PNT pretreatment. It is unlikely that this increase in MA concentrations could be derived from an increase in transfer of MA into the n-pentane phase in the presence of PNT during the extraction procedures, since the estimated areas under the MA peak curve of test samples extracted in the presence of PNT did not significantly exceed the areas of control samples obtained in the absence of PNT. Thus, potentiation of MA-induced behavior by PNT or CBZ may be caused by drug interactions possibly due to protein binding.

However, behavioral effects were not equally potentiated; the potentiating effect of PNT on tremor induced by MA was obtained in a dose-dependent manner, whereas that

of PNT on stereotypy was not dose-related. A peak effect of PNT was noted at a dose of 10 mg/kg but a nonsignificant increase in score for stereotypy was observed at a dose of 50 mg/kg (Fig. 1). It was thought that the limited dose-dependency might be due to potentiated tremor by which stereotypy could be masked. However, the regression analysis did not support this possibility and implicated that the enhanced tremor per se did not interrupt appearance of stereotypy. These results suggest that potentiation of MA-induced behavior by PNT may not be explained only by drug interactions at protein binding sites. Therefore, the possibility may exist that other toxic effects of PNT or neuronal effects caused by PNT are coming in at higher doses and serving to suppress further increases in MA-induced stereotypy scores.

It has been shown that PNT possesses an ability to alter the neuronal activity in the CNS through stimulation of the sodium pump, blockade of sodium and calcium influx, enhancement of chloride-mediated inhibitory postsynaptic potentials (IPSP), interactions with membrane phospholipid components and inhibition of calcium-dependent protein phosphorylation and neurotransmitter release [1]. This last action has been believed to be shared with CBZ [2]. In conjunction with these findings, central neuronal mechanisms should be also considered to be involved in potentiation of MA-induced behavior by PNT or even CBZ.

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