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Short communication

Role of cerebral spermidine in the development of sensitization to convulsant activity of cocaine and lidocaine

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Abstract

We have previously shown that daily treatment with subconvulsant dose of cocaine resulted in the elevation of brain levels of polyamines such as putrescine and spermidine and the development of increased susceptibility to cocaine-induced seizures. The present study examined whether exogenously administered polyamines affect seizure activity caused by various doses of cocaine and lidocaine in mice. Thirty minutes after intracerebroventricular treatments with either saline, putrescine or spermidine (1–4 μmol), animals were injected intraperitoneally with cocaine or lidocaine (60–90 mg/kg); then the occurrence of clonic seizures was observed. Spermidine enhanced cocaine-induced seizure activity, while putrescine had no effect on it. Lidocaine-induced convulsions were also dose-dependently potentiated by spermidine. In addition, spermidine significantly enhanced seizure activity following an injection of *N*-methyl-DL-aspartate. The results suggest that spermidine plays an important role in the development of sensitization to convulsant activity by cocaine and lidocaine via modulation of *N*-methyl-D-aspartate receptors. © 1997 Elsevier Science B.V.

Keywords: Cocaine; Lidocaine; Convulsion; Spermidine; *N*-Methyl-D-aspartate receptor

Cocaine produces severe convulsions in animals [13] and humans [1] after injection of sufficient amounts. Even low doses of cocaine cause a progressive increase in susceptibility to seizures when administered repeatedly and intermittently [10,11,13]. We have shown that repeated administration of subconvulsant dose of cocaine to ddY mice results in the development of sensitization to seizure activity during initial few days. This sensitization, however, is followed by the development of tolerance to convulsions with further treatments [16,17]. In spite of many pharmacological and neurochemical studies, the mechanisms underlying the acute convulsant activity of cocaine and the development of sensitization and tolerance to seizures following repeated administration of cocaine remain yet unclear.

Spermidine, spermine and putrescine are the endogenous polyamines found in all brain regions of animals and humans, as well as in other organs. Polyamines are involved in cell proliferation and differentiation through activation of RNA and protein syntheses [6]. However, the exact role of polyamines in the brain has not been understood. Neurochemical investigation has revealed that the compounds act as a modulator of the *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptors [14,20]. Indeed, behavioral studies have indicated the involvement of polyamines in convulsant activity produced by agonists of the NMDA receptor [3–5,19] and electrical stimulation [8]. MK-801, a non-competitive antagonist of the NMDA receptor, has been shown to suppress the acute convulsant activity of cocaine and the development of increased susceptibility to convulsions induced by repeated administration of cocaine [9,16]. In addition, lidocaine-induced convulsions have been retarded by the NMDA receptor antagonists dextromethorphan [2] and MK-801 (unpublished observation). We recently found that daily treatment with subconvulsant dose of cocaine increased brain levels of spermidine and putrescine, whereas repeated treatment with lidocaine even at convulsant dose had no effect on cerebral polyamines [17,18]. Furthermore, injection of subconvulsant dose of lidocaine produced severe convulsions in mice that had received daily treatments with a subconvulsant dose of cocaine. However, the increased sensitivity to lidocaine-induced convulsions in the cocaine-pretreated animals disappeared with prolongation of the duration of

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cocaine pretreatments [18]. In light of these findings, it is conceivable that cerebral polyamines may modulate the convulsant activity of cocaine and play an important role in the one-way cross-sensitization and cross-tolerance to seizure activity from cocaine to lidocaine. In order to test this possibility, we examined the effects of intracerebroventricular (i.c.v.) treatments with putrescine or spermidine on convulsant activity produced by various doses of cocaine in mice, in comparison with the effects on convulsions by lidocaine and *N*-methyl-DL-aspartate (NMDLA).

Experiments were conducted on male Slc:ddY mice, weighing 30–35 g, that had been acclimated to the animal facilities at least for 10 days. All procedures were approved by the Animal Experiments and Ethics Committee of the Kawasaki Medical School. Putrescine and spermidine were dissolved in physiological saline, the pH being adjusted to 7.0 with HCl. According to the procedure described by Haley [7], either polyamine or saline was administered i.c.v. with 0–4 μmol doses in a volume of 10 μl, under slight anesthesia with ethyl ether. Among 81 subjects which were pretreated with the highest amount of spermidine, five animals that showed convulsions after the pretreatment alone were excluded from the experiment. Thirty minutes later, cocaine HCl (60–90 mg/kg, Shionogi and Co., Osaka, Japan), lidocaine HCl (60–90 mg/kg, Sigma Chemical Co., USA), and NMDLA (300 mg/kg, Sigma Chemical Co., USA) in physiological saline, were injected intraperitoneally (i.p.) in a volume of 0.01 ml/g b.wt. Immediately after injection, animals were placed individually in a transparent acrylic cage (40 × 30

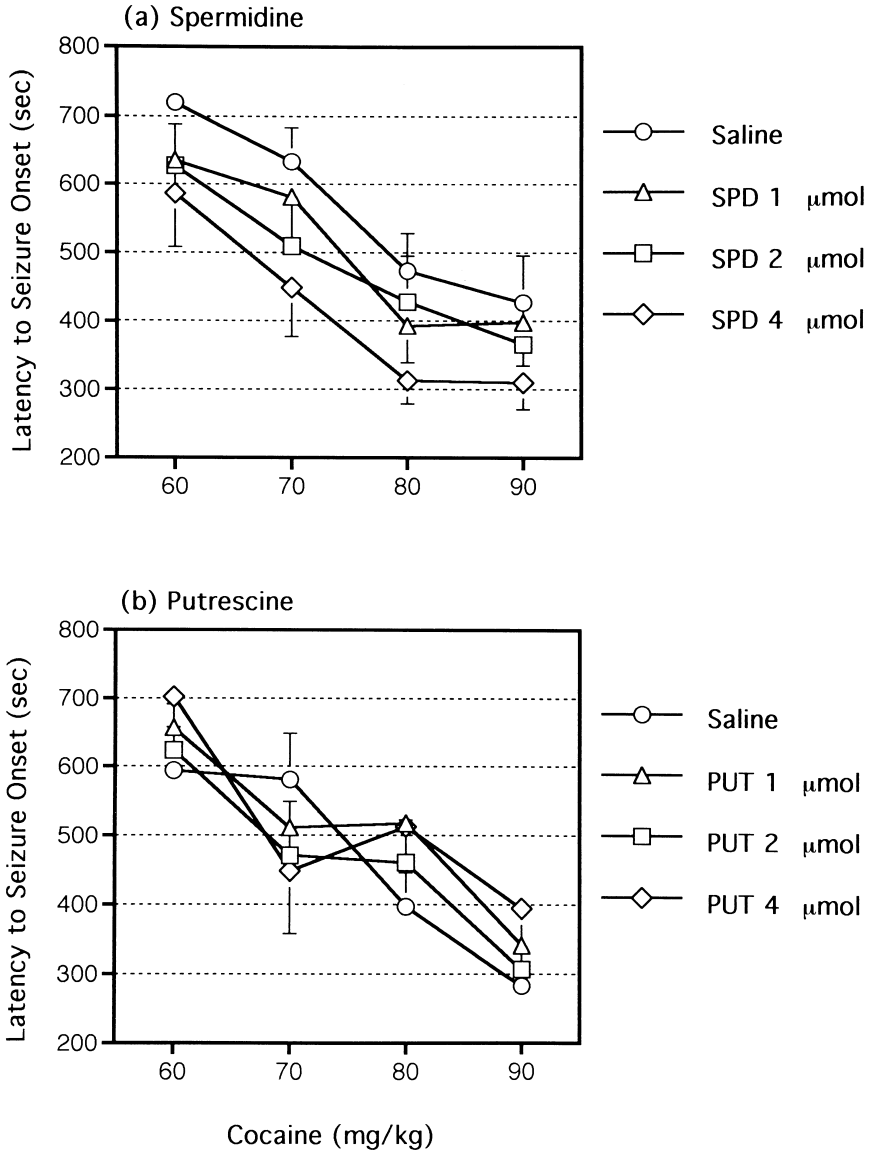

Fig. 1. Effects of spermidine (a) and putrescine (b) on seizure activity induced by an injection of various doses of cocaine in ddY mice. Animals were injected i.p. with 60–90 mg/kg cocaine, 30 min after the i.c.v. pretreatment with vehicle, 1, 2, or 4 μmol of spermidine (SPD) or putrescine (PUT). Each point and bar represent the mean value and the standard error, respectively, of the latency to seizure onset.

× 30 cm; w × d × h) for observation of seizure activity. Seizures were assessed either for 12 min after injection of cocaine and lidocaine or for 30 min after NMDLA; the latency to seizure onset was recorded for each animal. Clonic seizures observed after the treatment with drugs tested here were used as a marker of drug-induced convulsions [10,16]. Animals not convulsing during the observation period were given a maximum latency score of 720 or 1800 s dependent on the convulsants injected. Statistical analyses of data for the latency to seizure onset were conducted with analysis of variance (ANOVA) techniques followed by post-hoc comparisons using the Student–Newman–Keuls test. Each group consisted of 7–9 animals.

Injections of cocaine or lidocaine at doses of 60–90 mg/kg produced convulsant activities such as the loss of body posture with convulsive movements of all extremities and a bout of running/bouncing clonus. Cocaine dose-dependently reduced the latency to onset of seizure activity, reflecting an increase in convulsant activity (Fig. 1). I.c.v. treatments with spermidine significantly enhanced cocaine-induced seizures ($F_{3,107} = 4.32$, $P < 0.01$; 2-way ANOVA; Fig. 1a). Post-hoc analyses showed a significant difference in the latency to seizure onset between the groups of mice treated with 4 μmol spermidine and those with saline ($P < 0.05$). Treatments with putrescine, however, had no effects on seizure activity produced by cocaine (Fig. 1b). Lidocaine, another local anesthetic tested, also produced seizure activity in a dose-dependent manner (Fig. 2). While putrescine had no influence on lidocaine-

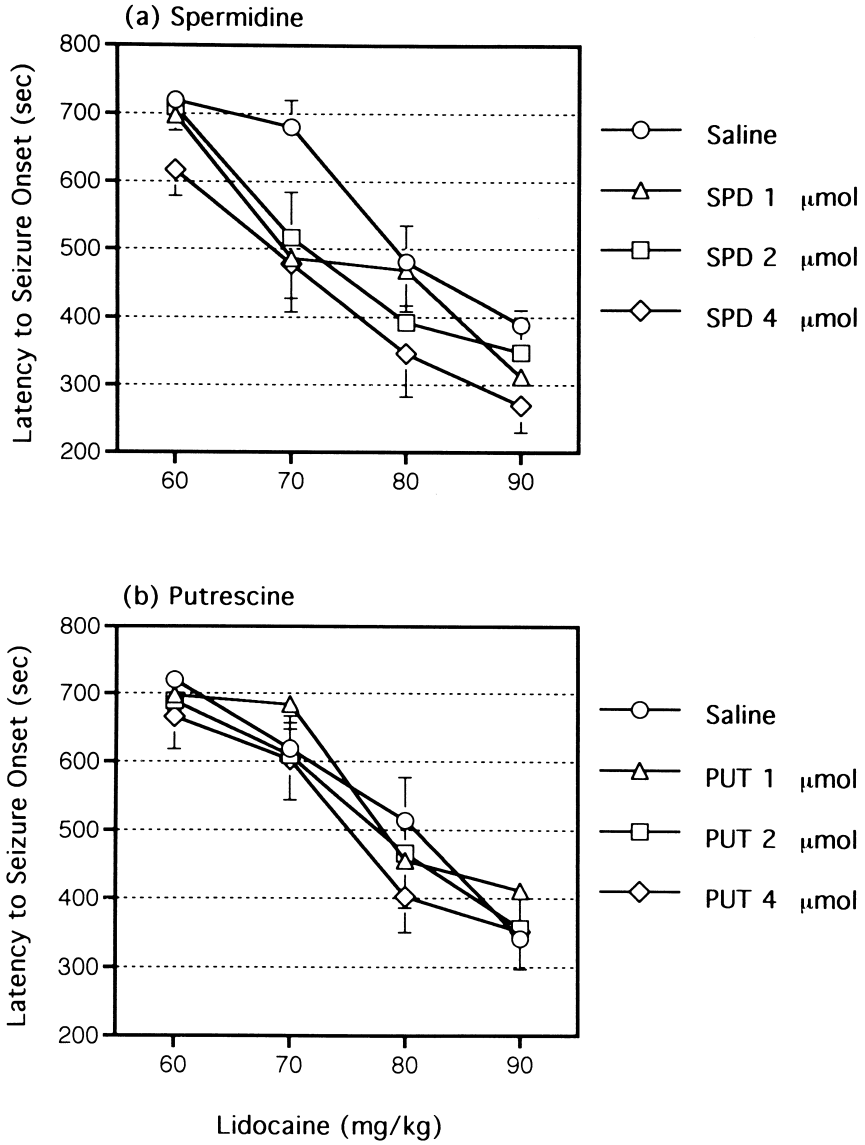

Fig. 2. Effects of spermidine (a) and putrescine (b) on seizure activity induced by an injection of various doses of lidocaine in ddY mice. Animals were injected i.p. with 60–90 mg/kg lidocaine, 30 min after the i.c.v. pretreatment with vehicle, 1, 2, or 4 μmol of spermidine (SPD) or putrescine (PUT). Each point and bar represent the mean value and the standard error, respectively, of the latency to seizure onset.

induced convulsions, spermidine apparently potentiated convulsant activity produced by lidocaine ($F_{3,128} = 5.77$, $P < 0.01$; 2-way ANOVA). Post-hoc comparison demonstrated significant differences in the latency to seizure onset between the groups of mice treated with 2 and 4 μmol spermidine and those of saline ($P < 0.05$).

Injection of NMDLA at a dose of 300 mg/kg elicited facial washing, tail-flicking and intensive scratching upper body with a hind limb, followed by a bout of running bouncing clonus and clonic–tonic convulsions. NMDLA-induced convulsions were significantly enhanced by spermidine, but not putrescine ($F_{3,33} = 3.12$, $P < 0.05$; 1-way ANOVA; Fig. 3). Post-hoc comparisons proved a significant difference in the latency of seizure onset between 4 μmol spermidine-treated mice and the saline-treated mice.

We previously measured endogenous levels of polyamines in the mouse brain. Spermidine and putrescine were estimated to be 290 ± 7 and 19 ± 1 pmol/mg of tissue weight, respectively in the forebrain obtained from mice 24 h after the daily treatment with saline for 2 days [18]. These levels are comparable to those reported by Shaskan et al. [15] and Paschen et al. [12]. Repeated daily treatment with subconvulsant dose of cocaine significantly elevated the forebrain levels of spermidine and putrescine by 15% and 100%, respectively [18]. Because polyamine levels were not determined after the i.c.v. treatment with spermidine and putrescine in this study, we cannot argue what extent exogenously administered polyamines were relevant to physiological conditions. However, the amounts of spermidine and putrescine used in this study were almost the same as those used in the previous study showing that spermidine, but not putrescine, potentiated NMDLA-induced convulsions [19].

Besides elevating the cerebral levels of putrescine and spermidine, repeated daily treatment with subconvulsant dose of cocaine caused the development of increased susceptibility to cocaine-induced seizures [17,18]. The findings suggest that such increased polyamines may be involved in the development of sensitization or tolerance to seizure activity following repeated administration of cocaine. Indeed, the present study revealed that i.c.v. treatment with spermidine potentiated the convulsant activity of cocaine in mice. Several lines of evidence, including the present study, show that treatment with spermidine and spermine enhanced seizure activity induced by NMDA receptor stimulation [3–5,19], while spermidine had no effect on convulsions produced by pentylenetetrazol, a blocker of GABA receptors [19] (unpublished observation). In addition, cocaine-induced seizures are mediated by NMDA receptors because MK-801 prevents seizure activity caused by cocaine [9,16]. Therefore, the present result, taken together with the previous findings, indicates that treatment with cocaine increases the cerebral levels of polyamine, especially spermidine, and that the increased polyamine in turn enhances seizure activity mediated by the NMDA receptor activation.

On the other hand, putrescine had no influence on the seizure activity of cocaine. This observation is inconsistent

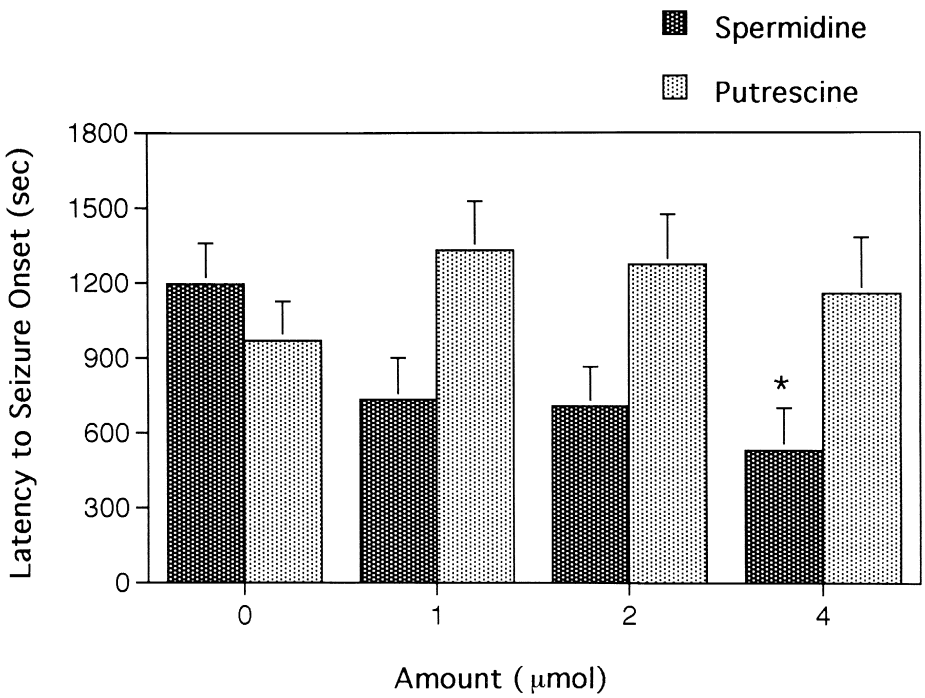

Fig. 3. Effects of spermidine and putrescine on seizure activity induced by an injection of *N*-methyl-DL-aspartate (NMDLA) in ddY mice. Animals were injected i.p. with 300 mg/kg NMDLA, 30 min after the i.c.v. pretreatment with vehicle, 1, 2, or 4 μmol of spermidine or putrescine. Each column and bar represent the mean value and the standard error, respectively, of the latency to seizure onset. The asterisk denotes the significance of difference in the latency when compared to that in the control group ($P < 0.05$; 1-way ANOVA with post-hoc comparison).

with a previous finding that treatment with putrescine retarded the development of amygdala-kindled seizures [8]. The difference between these two data described in the present study and in the previous report [8] suggests that mechanisms underlying amygdala-kindled seizures may be different from those of cocaine-induced convulsions. However, the exact reason for the discrepancy of the putrescine effects is not clear at present.

Repeated daily injections of subconvulsant dose of lidocaine caused rarely or very slowly the development of sensitization to convulsant activity in mice [11,13,18]. In addition, repeated daily treatments with lidocaine at even convulsant doses produced no changes in the brain levels of putrescine and spermidine [18]. Nonetheless, i.c.v. treatment with spermidine potentiated lidocaine-induced convulsions in the present study. The finding indicates that seizure activity produced by lidocaine may, at least in part, be mediated by NMDA receptors and/or polyamines. In fact, it has been revealed that lidocaine-induced convulsions were attenuated by the NMDA receptor antagonists dextromethorphan [2] and MK-801 (unpublished observation). Furthermore, we have previously shown that lidocaine at subconvulsant dose induced severe convulsions in animals that had received daily treatments with subconvulsant dose of cocaine, which elevated polyamine levels in the brain [18]. Therefore, these data strongly suggest that lidocaine even at low dose is likely to produce seizure activity under the condition that the levels of spermidine are increased after long-term abuse of cocaine.

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