Chronic inhibition of the norepinephrine transporter in the brain participates in the seizure sensitization to cocaine and local anesthetics

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Abstract

Involvement of chronic inhibition of monoamine transporters (MAT) in the brain concerning the sensitization of cocaine- and local anesthetic-induced seizures was studied in mice. Repeated administration of subconvulsive doses of meprylcaine as well as cocaine, both of which inhibit MAT, but not lidocaine, which does not inhibit MAT, increased seizure activity and produced sensitization to other local anesthetics. Effects of 5 daily treatments of monoamine transporter inhibitors on lidocaine-induced convulsions were examined 2 or 3 days after the last dose of the inhibitors. The daily treatments of GBR 12935, a specific inhibitor of dopamine uptake, significantly increased the incidence and the intensity of lidocaine-induced convulsions at 20 mg/kg and decreased the threshold of the convulsions. The daily treatments of desipramine and maprotiline, selective norepinephrine uptake inhibitors, markedly increased the incidence and intensity of lidocaine-induced convulsions, and decreased the threshold with dose-dependent manner between 5 and 20 mg/kg. The daily treatments of citaloplam, a selective serotonin uptake inhibitor, 10 and 20 mg/kg, produced no significant increase in the incidence or intensity of lidocaine-induced convulsions but decreased the threshold of the convulsions. These results suggest that the chronic intermittent inhibition of monoamine uptake increases susceptibility to cocaine- and local anesthetic-induced seizures, and a norepinephrine transporter is an integral component of this sensitization.

Theme: Neurotransmitters, modulators, transporters, and receptors

Topic: Uptake and transporters

Keywords: Inhibition of monoamine transporters; Seizure; Kindling; Local anesthetics; Cocaine

1. Introduction

A seizure is a well-known complication of synthetic local anesthetics and of cocaine abuse. Local anesthetics activate a limbic discharge, which is most pronounced in the amygdaloid nuclear complex [43, 45, 46], though local anesthetics primarily block the Na^+ channel, thereby inhibiting nerve conductance. It is believed that local anesthetics induce a seizure by depressing GABAergic and other inhibitory neurons, thereby allowing the potentiation of excitatory neuronal activity [4, 5, 10, 11, 44].

The repeated administration of cocaine increases its central stimulating activity, displayed as an increase in locomotor activity [28]. It has also been demonstrated that intermittent repeated administration of relatively high doses of cocaine to rodents increase cocaine-induced seizures, called “cocaine kindling” [5, 28]. Cocaine has two prominent pharmacological actions, a local anesthetic action and an inhibition of the monoamine transporter (MAT) that, in turn, blocks the reuptake of monoamines released from the nerve endings. The latter action, especially the blockade of catecholamine reuptake, is closely related to the increase in locomotor activity caused by repeated cocaine administration [28]. However, it is not evident that the blockade of monoamine reuptake accounts for seizure kindling, because the dose required for cocaine kindling is higher than that required to induce locomotor sensitization [47], and that development of cocaine-kindled seizures is attenuated by chronic treatment with anticonvulsant carbamazepine, though carbamazepine has little effect on locomotor sensitization to cocaine [47].

It has been generally believed that synthetic local anesthetics do not inhibit MAT. However, we and others have recently demonstrated that some synthetic local anesthetics such as procaine and meprylcaine do inhibit MAT in rat brain synaptosomes [50], in COS cells transfected with MAT cDNAs [35] and in SH-SY5Y human neuroblastoma cells [19], while lidocaine displays no inhibitory action [35, 48]. Shimosato et al. [41] demonstrated that the repeated administration of cocaine sensitizes to lidocaine-induced convulsions, but chronic treatment of lidocaine did not affect the cocaine-induced convulsions. In light of our previous observation

that a daily treatment for 2 days with nomifensine, which has an inhibitory effect on norepinephrine and dopamine uptake, increased the incidence of lidocaine-induced convulsions [36], we hypothesized that the inhibition of MAT results in a greater susceptibility to local anesthetic-induced convulsions.

In the present study, the following experiments were performed to elucidate the role of chronic inhibition of MAT in the development of drug-induced seizure kindling, 1) whether the repeated administration of local anesthetics other than cocaine inhibit MAT increase seizure activity, 2) whether the chronic administration of uptake inhibitors for noreinephrine, dopamine or serotonin, which may substitute some pharmacological properties of cocaine, produce a susceptibility to seizures induced by lidocaine, a typical local anesthetic that does not inhibit MAT.

2. Materials and methods

2.1. Animals

Male ICR mice 6 ~ 7 weeks of age (25 ~ 35 g) were used. All procedures and handling of the animals were performed according to the guideline by "Guiding Principles for the Care and Use of Laboratory Animals" approved by The Japanese Pharmacological Society as well as the guideline of Hiroshima University.

2.2. *Treatments with drugs*

Different subconvulsive dose of cocaine (30, 35, 40 mg/kg) and meprylcaine (80, 85 mg/kg) and minimal convulsive doses of lidocaine (45, 45 mg/kg) were injected intraperitoneally once daily for 4 days. On the 5 th day, lidocaine (50 mg/kg, i.p.) and cocaine (40 mg/kg, i.p.) were challenged in cocaine (35, 40 mg/kg) and in meprylcaine (80, 85 mg/kg) treated mice, respectively. Monoamine uptake inhibitors, GBR 12935 (i.p.), desipramine (i.p.),

maprotiline (i.p.) and citalopram (i.p.), were injected once daily for 5 days. For the timing of the drug challenge after the chronic treatment of monoamine uptake inhibitors, Sacchetti et al. [34] have reported that the administration of 10 mg/kg i.p. desipramine once daily for 14 days significantly raised the basal extracellular norepinephrine in the dorsal hippocampus at 24 hrs but not 48 hrs after the last drug injection, and the increase in extracellular norepinephrine by the challenge dose of desipramine in rats treated chronically with desipramine was observed in rats 48 hrs but not 24 hrs after the last injection. From these results, the authors discussed that residual brain concentrations of desipramine were presumably high enough to markedly block the norepinephrine uptake at 24 hrs, but not 48 hrs, after the last dose of the chronic desipramine [34]. To avoid the possibility of the influence of the residual drugs of the last dose of monoamine uptake inhibitors, lidocaine was challenged 2 days and 3 days after the last dose to seek the effect on the incidence and the intensity of lidocaine-induced convulsions and the threshold of the convulsions, respectively. In some experiments, lidocaine was challenged 5 and 9 days after the last injection of desipramine.

2.3. Seizures

Mice were intraperitoneally injected with convulsants and placed individually in plastic cages for observation of seizure activity. The seizures induced by the drugs were characterized by ataxia, a short loss of the righting reflex, and clonic and tonic convulsions. The percentage of animals that exhibited convulsions in each treatment group was determined 15 min after the injection of the convulsants. The convulsions were scored as, 1= weak, intermittent clonic convulsion; 2= typical clonic convulsion lasting more than 1 min; 3= clonic and tonic convulsion with increased locomotor activity or jumping. To determine the threshold for lidocaine-induced convulsions, the occurrence of clonic convulsion was measured by infusing lidocaine solution (4.0 mg/ml) into a tail vein at a rate of 0.21 ml/min via a 24 gauge butterfly needle attached to a syringe pump, while the dosage administered was calculated per body weight. Other experimental procedures were described previously [36].

2.4. Statistical analysis

One-side Fisher's exact probability test was applied for the frequency of the seizures. Student's *t* test and Welch test were used to calculate the differences in seizure severity and seizure threshold, respectively.

3. Results

3.1. Effects of repeated treatment with cocaine, meprylcaine and lidocaine on local anethetic-induced seizures.

Meprylcaine, which has a relatively potent inhibitory action on MAT, was compared to cocaine which is a potent inhibitor of MAT and lidocaine which lacks this property in terms of convulsive activity during chronic treatment. Treatment once daily with subconvulsive doses of cocaine, 30 ~ 40 mg/kg, for 4 days produced convulsions from the 2 nd day, and the incidence of convulsions markedly increased dose- and time-dependently. At 40 mg/kg, all mice developed convulsions on the 3 rd and 4 th day. In mice treated once daily with subconvulsive doses of meprylcaine, 80 and 85 mg/kg, convulsions appeared from the 2 nd day and % incidence of convulsions on the 4 th day reached to 50 and 70, respectively. Lidocaine daily treatment for 4 days at even minimal convulsive doses did not affect the convulsive activity during the period of the treatment (Fig. 1). In cocaine-treated mice on the 5 th day, lidocaine at a dose of 50 mg/kg in which the incidence was 20 % in control mice produced convulsions in all animals. In meprylcaine-treated mice on the 5 th day, cocaine with a subconvulsive dose in normal mice produced convulsions. These results shows that meprylcaine as well as cocaine but not lidocaine produced sensitization to its own convulsive activity and a cross sensitization to other local anesthetics although the sensitizing effect of meprylcaine was less potent than cocaine.

3.2. *Effects of repeated administration with inhibitors of monoamine transporters on lidocaine-induced seizures.*

Cocaine and meprylcaine have a selective inhibitory action on MAT but inhibit all of the uptake of dopamine, norepinephrine and serotonin. Effects of a chronic administration of more selective inhibitors of dopamine uptake, GBR 12935, of norepinephrine uptake, desipramine and maplotiline, and of serotonin, citalopram, on lidocaine-induced convulsions were examined under various conditions to clarify whether either the inhibition of dopamine, norepinephrine or serotonin uptake produce a susceptibility to convulsions.

2 days after the last injection of the daily treatment with GBR 12935, 20 mg/kg once daily for 5 days, the incidence of lidocaine-induced convulsions and the intensity of symptoms of convulsions as shown in convulsion score significantly increased (Table 1). Threshold of lidocaine-induced convulsions tested at 3 days after the last injection of GBR 12935, 20 mg/kg, significantly decreased (Table 2). In mice treated daily for 5 days with desipramine, the incidence of lidocaine-induced convulsions markedly increased 2 days after the last dose of desipramine, and the effect of desipramine was dose-dependent between 5 and 20 mg/kg (Table 1). The sensitization produced by daily dose of 20 mg/kg lasted until the 5 th day after the last dose of desipramine, with a gradual decline. Daily treatment of maprotiline with the similar dosage for 5 days also significantly increased the incidence of lidocaine-induced convulsions. Daily treatment with desipramine, 10 and 20 mg/kg and maprotiline, 10 and 20 mg/kg remarkably intensified the intensity of convulsions induced by lidocaine as shown in an increase of lidocaine's convulsive score (Table 1). 3 days after the last dose of 5 daily treatments of 10 and 20 mg/kg of desipramine and maprotiline, the convulsive threshold for lidocaine was profoundly lower (Table 2).

In the present study, we observed that acute treatment with citalopram, which is an SSRI without action on cytochrome P-450 and thereby considered to avoid the influence on lidocaine metabolism, significantly increased the incidence of lidocaine-induced convulsions; %

incidence of convulsions induced by lidocaine (40 mg/kg) in the control and citalopram (10 mg/kg of citalopram was injected 30 min before the injection of lidocaine)-treated group were 9.4 and 66.7, respectively ($p<0.05$). Therefore, we next examined the chronic effect of citalopram on seizure activity related to lidocaine. The 5 daily treatments of citalopram, 20 mg/kg but not 10 mg/kg produced slight, but not significant increase in the incidence of convulsions induced by lidocaine, and did not change the seizure score of lidocaine (Table 1). Seizure threshold of lidocaine was significantly reduced by citalopram at 10 and 20 mg/kg but to a lesser degree than with desipramine and maprotiline (Table 2).

4. Discussion

Cocaine is known to inhibit MAT including dopamine transporter (DAT), norepinephrine transporter (NET) and serotonin transporter (SERT). The mood-enhancing and psychomotor stimulation effects of cocaine are thought to be related primarily to the inhibition of MAT, especially DAT [32]. While synthetic local anesthetics are generally believed to lack this effect, certain local anesthetics such as procaine have cocaine-like reinforcing effects in animals [7, 18, 29, 49, 50]. Recently, it has been demonstrated that certain local anesthetics including procaine do inhibit MAT [19, 35, 48], and this effect, especially inhibition of DAT, would explain their cocaine-like effect [5, 48]. Among local anesthetics tested in our former study, meprylcaine had relatively potent inhibitory action on MAT although less potent than cocaine, while lidocaine had not [35]. Thus the effect of a repeated administration of cocaine, meprylcaine and lidocaine in the development of seizures were compared to elucidate the relation of the inhibition of MAT in the development of kindling. It has been reported that sensitization to cocaine-induced convulsions by a daily treatment with a minimal convulsive dose of cocaine reached a peak response after 4 to 5 days and by a daily treatment with a sub-convulsive dose of cocaine appeared on the 5 th day, and the treatment for a longer period produced tolerance [23], although the sensitivity to cocaine kindling differ with strain of mice [21]. In consistent with these results, daily treatments of subconvulsive doses of cocaine produced the susceptibility to

lidocaine-induced convulsions during 4 days treatment with dose- and time-dependent manner in the present study. Daily treatments of meprylcaine also increased the susceptibility to convulsions with less potency than cocaine in relate with their potency of MAT inhibition [35]. In cocaine- and meprylcaine-treated mice, a cross sensitization to other local anesthetics was displayed, although a repeated administration of lidocaine did not produce the sensitization of convulsions. These results showed that the chronic administration of local anesthetics, cocaine and meprylcaine, which inhibit monoamine uptake, can facilitate convulsive activity but lidocaine, which lacks this property, did not. Thus, the local anesthetic action alone was either unrelated or insufficient to develop seizure kindling, although lidocaine has been reported to induce seizure kindling by repeated administration for longer periods than used here [27]. Therefore, it is suggested that the chronic inhibition of MAT is closely related with development of local anesthetic-induced kindling.

It has been shown that cocaine and meprylcaine inhibit the uptake of norepinephrine, dopamine and serotonin with almost equal potency [35]. The administration of cocaine and meprylcaine could inhibit the uptake of these amines *in vivo*. We have previously observed that injection for 2 days of nomifensine, which inhibits both norepinephrine and dopamine, facilitated lidocaine-induced convulsions [36]. Daily administration of more selective inhibitors of norepinephrine uptake, desipramine, but not selective inhibitor of dopamine, GBR 12935, for 2 days actually potentiated lidocaine-induced convulsions 1 day after the last injection of monoamine uptake inhibitors (data not shown). However, it has been reported that the residual brain concentrations of desipramine could block norepinephrine uptake at 24 hrs, but not 48 hrs, after the last dose of the chronic desipramine (10 mg/kg i.p. desipramine once daily for 14 days) [34]. To avoid the possible influence of the residual drugs of the last dose of monoamine uptake inhibitors, lidocaine was challenged at 2 days and 3 days after the last dose of monoamine uptake inhibitors to seek their effects on the incidence and severity of lidocaine-convulsion and threshold of the convulsions, respectively. Desipramine and maplotiline at 5 ~ 20 mg/kg dose-dependently produced a remarkable increase in the incidence of lidocaine-induced convulsions, lidocaine's convulsive score, and reduction in the convulsive threshold for

lidocaine. The sensitization by desipramine to lidocaine convulsions lasted for 5 days after withdrawal of the inhibitors. It has been reported that extracellular norepinephrine concentration was increased in *locus coeruleus* and cingulated cortex following the acute administration of desipramine as low as 3 mg/kg, i.p. in rats [22]. It could be assumed that the reuptake of norepinephrine is effectively blocked by the systemic administration of these doses of norepinephrine uptake inhibitors used in the present study. Thus, repeated previous inhibition of norepinephrine uptake produced plastic changes that lead to increased susceptibility to local anesthetic-induced convulsions. Previously, Jackson et al. [17] reported that acute treatments with a norepinephrine uptake inhibitor and α_2 adrenoceptor–antagonists, both of which increase noradrenergic synaptic activity, neither increased nor decreased cocaine-induced convulsions, concluding that no noradrenergic mechanism is involved in the cocaine-induced convulsion. However, Russell and Stripling [33] showed that cocaine's noradrenargic action modulated a kindling expression via a daily electrical stimulation of the left prepyriform cortex. Further, we observed that concurrent, acute pharmacological manipulations to stimulate the central noradrenergic nervous system enhanced the convulsions induced by lidocaine, while those for inhibition decreased the convulsions [5, 51]. Therefore, the present study indicates that previously increased noradrenergic activity results in potentiation of convulsive activity of local anesthetics.

Although the lack of acute effects of a dopamine uptake inhibitor on lidocaine-induced convulsions do not support the involvement of dopaminergic functions in local anesthetic convulsions [36], Ciarlone [3] reported that depletion of brain dopamine without changing the norepinephrine levels decreased the convulsant thresholds for lidocaine and procaine. In the present study, a daily treatment with GBR 12935 at 20 mg/kg for 5 days increased the incidence of lidocaine convulsions and intensified the symptoms of convulsions as shown in an increase of score and decreased the threshold, although the effects of GBR 12935 were less potent than desipramine and maprotiline. It has been reported that GBR 12935 at 15 mg/kg produced not only an increase in dopamine level in dialysate of nucleus accumbens but profound increase in norepinephrine level in ventral tegmental erea in rats [29], suggesting the cross talk between

monoaminergic systems occurring upon systemic administration of the uptake inhibitor. Therefore, the sensitization to lidocaine convulsions by chronic GBR 12935 at a high dose of 20 mg/kg may be due to either the activation of dopaminergic neronal system or via the indirect interaction with noradrenergic nervous system.

It has been suggested that cocaine-induced convulsions are modified by an acute manipulation of serotonergic activity rather than noradrenergic and dopaminergic activity. For example, a cocaine-induced seizure is markedly enhanced by acute treatment with the selective serotonin reuptake inhibitors (SSRI) [24, 30, 31], while the 5-HT_2 receptor antagonists antagonize cocaine seizure [25, 31, 37]. Thus, the SERT is thought to be a primary site of action mediating the modification of seizures induced by acute injections of cocaine. Intraperitoneal administration of citalopram, a potent and selective inhibitor of serotonin uptake has shown to increase significantly dialysate serotonin in the dorsal raphe of rats at 1 mg/kg, a greater effect at 10 mg/kg [12]. In the present study, we observed that acute treatment with 10 mg/kg of citalopram actually increased the incidence of lidocaine-induced convulsions. However, the chronic treatment with citaloplam, 10 mg/kg and even higher dosage of 20 mg/kg had no effect on the incidence or severity of symptoms of lidocaine-induced convulsions with a slight decrease in threshold. Thus, chronic inhibition of SERT may play a minor role in the facilitation of a seizure induced by local anesthetics, though acute inhibition plays an important role.

The direct blockade of monoamine uptake can account for the elevation of extracellular level of monoamine neurotransmitters, which would alter the pre- and post-synaptic neuronal functions. For examples, down-regulation of α_2-adrenoceptor-mediated responses in the CNS are common responses observed to the chronic treatment with antidepressant drugs that increase synaptic norepinephrine concentration [2, 8, 9]. Although the obvious changes of MAT would be expected with the blockade of monoamine uptake, there have been mixed results regarding MAT regulation following a chronic exposure to cocaine and antidepressants [52]. Experimental differences in the dose and period of drug administration, drug withdrawal times, brain regions studied, etc., may contribute to the variable results [52]. Behavioral studies in rodents showed that motor stimulant effects of dopamine agonists were potentiated by the repeated, but not

acute, administration of various antidepressant or by repeated convulsive shock, accompanied with concomitant activation of dopamine D_2-like receptors [1, 42]. Several studies of postsynaptic mechanism have indicated that an excitatory amino acid transmission is involved in cocaine seizure kindling. Increased susceptibility to a cocaine-induced seizure is associated with the up-regulation of cortical NMDA receptors [16], with a marked increase in NMDA receptor density in the sriatum, amygdala and hippocampus and an increase in the affinity of NMDA receptors in the hippocampus [15]. Moreover, cocaine kindling is blocked by co-administration with an antagonist of N-methyl-D-aspartate (NMDA) glutamate receptors, dizocilpine (MK-801) [16, 20, 38]. Pretreatment with nitric oxide synthase (NOS) inhibitors prior to cocaine administration abolished the cocaine-induced up-regulation of NMDA receptors and the induction and expression of cocaine kindling without affecting the acute cocaine-seizures [13, 14, 26]. These findings suggest that iNOS induction followed by an increase in the NMDA receptor function after repeated exposure to cocaine is associated with fundamental cellular processes required for the cascade leading to cocaine sensitization. Shimosato et al reported an increase in polyamine levels associated with changes in the sensitivity to convulsions during chronic treatment with cocaine, and that the intracerebroventricular administration of spermidine enhanced seizures induced by cocaine, lidocaine and NMDA [39, 40]. Spermidine may play an important role in the development of susceptibility to seizure activity by cocaine via the modulation of NMDA receptors.

Further study is required concerning the mechanism of the development of the susceptibility to a local anesthetic-induced seizure by the chronic administration of MAT inhibitors; e.g., whether the chronic inhibition of MAT alters such pre-synaptic receptor functions, transporter functions and/or postsynaptic functions as the molecular changes in the NO/NMDA signaling pathway.

In conclusion, the present results suggest that intermittent chronic inhibition of MAT, especially NET in the brain, induces plastic changes that facilitate seizure activity induced by local anesthetics. Such changes may be involved in cocaine kindling.

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Figure legend.

Fig.1 Effects of daily treatment with cocaine (A), meprylcaine (B) and lidocaine (C) on seizure activity in mice. Drugs were injected intraperitoneally once a day. After 4 days of treatment with cocaine (A) or meprylcaine (B), lidocaine 50 mg/kg, a minimal convulsive dose on day 1 as shown in (C), or cocaine 40 mg/kg, a subconvulsive dose on day 1 as shown in (A), were administered to mice treated with cocaine (A) or meprylcaine (B), respectively. Number of animals used were 15-20 mice each group.

Table 1
Effects of chronic treatment with specific inhibitors of dopamine-, norepinephrine- and serotonin-transporter on seizure activity induced by lidocaine in mice.

Pretreatment (mg/kg i.p.)		Term of pretreatment (days)	Term of rest (days)	Lidocaine (40 mg/kg i.p.) % incidence	N_1	Score [a)]	N_2
Saline		5	2	12.0	50	1.2 ± 0.2	6
GBR12935	(5)	5	2	20.0	25	2.0 ± 0.4	5
	(20)	5	2	45.0**	20	1.7 ± 0.2†	9
Saline		5	2	7.2	83	1.2 ± 0.2	6
Saline		5	5	6.7	15	1.0	1
Saline		5	9	6.7	15	1.0	1
Desipramine	(2)	5	2	6.7	15	2.0	1
	(5)	5	2	40.0**	15	1.5 ± 0.2	6
	(10)	5	2	73.3**	15	1.9 ± 0.2†	11
	(20)	5	2	88.6**	35	2.4 ± 0.1††	31
	(20)	5	5	40.0*	15	2.5 ± 0.4	6
	(20)	5	9	26.7	15	1.5 ± 0.3	4
Saline		5	2	10.3	68	1.1 ± 0.1	7
Maprotiline	(2)	5	2	13.3	15	2.0 ± 1.4	2
	(5)	5	2	40.0*	15	2.2 ± 0.3††	6
	(10)	5	2	52.0**	25	2.2 ± 0.2††	10
	(20)	5	2	65.0**	20	2.2 ± 0.2††	13
Saline		5	2	9.4	53	1.2 ± 0.2	5
Citalopram	(10)	5	2	3.3	30	1.0	1
	(20)	5	2	20.0	30	1.2 ± 0.2	6

GBR12935, desipramine, maprotiline, citalopram, or an equal volume of saline were injected once every day at the indicated dose for 5 days, and then 2, 5 or 9 days after the last injection the mice were challenged with lidocaine.

[a)] Seizure score is seizure severity quantified according to the following rating scale: 1= weak, intermittent clonic convulsion, 2= typical clonic convulsion lasting more than 1 min, 3= clonic and tonic convulsion with increased locomotor activity or jumping. These scores were counted for each seizure-generalized mouse. Data are expressed as mean ± S.E.M. of the seizure score in mice.

One-side Fisher's exact probability test was used for seizure susceptibility; *$p<0.05$; **$p<0.01$ vs. saline. Student't *t* test was used to calculate differences in seizure severity; $^{\dagger}p<0.05$; $^{\dagger\dagger}p<0.01$ vs. saline.

N_1; number of mice used. N_2; number of mice with convulsions.

Table 2
Effects of chronic treatment with specific inhibitors of dopamine-, norepinephrine- and serotonin- transporter on seizure threshold for lidocaine in mice.

Pretreatment (mg/kg i.p.)		Term of pretreatment (days)	Term of rest (days)	Lidocaine convulsive threshold (mg/kg i.v.)	*N*
Saline		5	3	24.7 ± 0.1	10
GBR12935	(20)	5	3	22.3 ± 0.7*	10
Saline		5	3	24.8 ± 0.2	40
Desipramine	(2)	5	3	24.1 ± 0.5	10
	(5)	5	3	21.4 ± 0.9*	8
	(10)	5	3	18.8 ± 0.6**	8
	(20)	5	3	14.9 ± 0.6**	13
Saline		5	3	25.1 ± 0.5	23
Maprotiline	(2)	5	3	23.7 ± 0.6	10
	(5)	5	3	19.3 ± 0.4**	10
	(10)	5	3	15.9 ± 0.5**	10
	(20)	5	3	14.3 ± 0.9**	8
Saline		5	3	24.8 ± 0.2	40
Citalopram	(10)	5	3	21.0 ± 0.5**	35
	(20)	5	3	22.3 ± 0.5**	20

Drugs were injected once every day at the indicated dose and days. Three days after the last injection the mice were challenged with lidocaine. Lidocaine (4.0 mg/ml) was infused into a tail vain at a rate of 0.21 ml/min until the onset of seizure. Data are expressed as mean ± S.E.M. of the threshold for lidocaine-induced seizure activity in mice. The Welch test was used for seizure threshold; *$p<0.01$; **$p<0.001$ vs. saline. *N*; number of mice used.

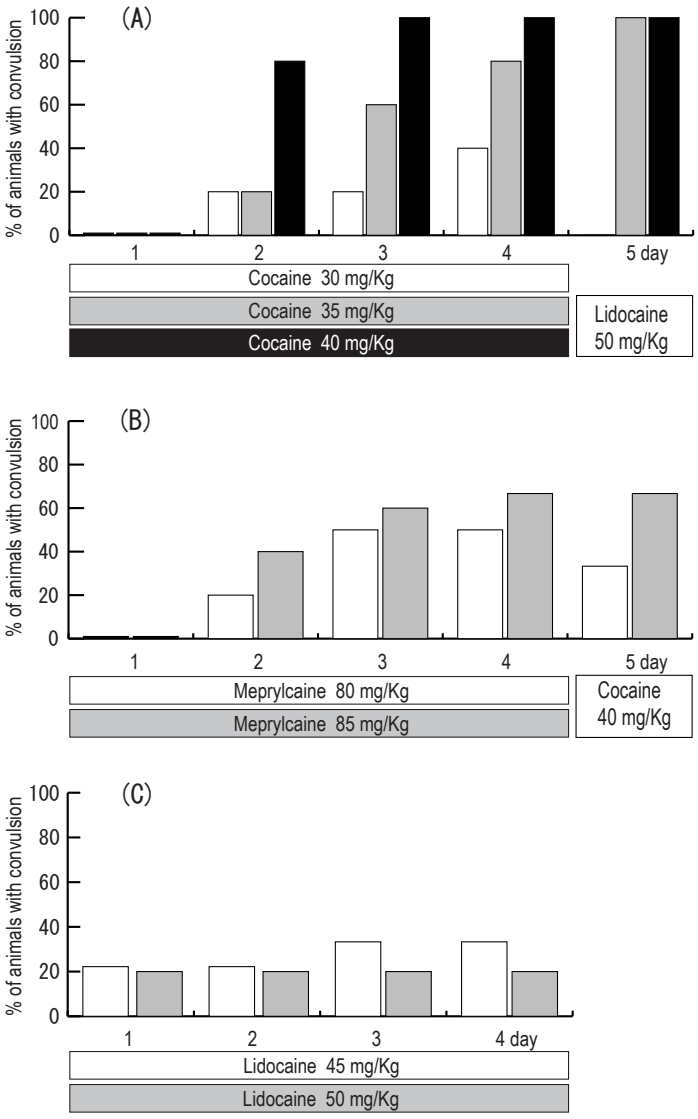
(A)
% of animals with convulsion
100
80
60
40
20
0
1
2
3
4
5 day
Cocaine 30 mg/Kg
Cocaine 35 mg/Kg
Cocaine 40 mg/Kg
Lidocaine
50 mg/Kg
(B)
% of animals with convulsion
100
80
60
40
20
0
1
2
3
4
5 day
Meprylcaine 80 mg/Kg
Meprylcaine 85 mg/Kg
Cocaine
40 mg/Kg
(C)
% of animals with convulsion
100
80
60
40
20
0
1
2
3
4 day
Lidocaine 45 mg/Kg
Lidocaine 50 mg/Kg

Fig. 1