Inhibition of serotonin transporters by cocaine and meprylcaine through 5-HT_{2C} receptor stimulation facilitates their seizure activities

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Abstract

The present study examined whether the inhibition of serotonin transporters (SERT) contributes to cocaine- and other local anesthetics-induced convulsions, and which subtypes of 5-HT receptor are involved in the convulsions. For this purpose, cocaine, meprylcaine and lidocaine, all of which have different effects on SERT, were used as convulsants and the effects of serotonin reuptake inhibitors (SSRIs), specific agonists and antagonists for 5-HT receptor subtypes were evaluated in mice.

Administration of SSRI, zimelidine, citalopram and fluoxetine, 5-$HT_{2A,2C}$ receptor agonist, R(-)-DOI and the 5-HT_{2C} receptor agonists, mCPP and MK212 resulted in a marked increase in incidence of convulsions and a reduction in the threshold of lidocaine-induced convulsions, while the 5-HT_{2B} receptor agonist, BW723C86, had little influence. On the other hand, SSRI did not affect the measured parameters in meprylcaine- and cocaine-induced convulsions. R(-)-DOI, mCPP and MK212 reduced the threshold of meprylcaine or cocaine with less extent than the reduction of lidocaine threshold. Incidence of cocaine- and meprylcaine-induced convulsions were significantly reduced by 5-$HT_{2A,2B,2C}$ antagonist, LY-53857 and 5-HT_{2C} antagonist, RS 102221. The threshold of cocaine and meprylcaine was significantly increased by both antagonists. 5-HT_{2A} antagonists MDL-11,939 and ketanserin, and 5-HT_{2B} antagonist SB-204741 except at high doses had little effect on cocaine- and meprylcaine-induced convulsions. None of these antagonists altered the parameters of lidocaine-induced convulsions. Pretreatment with fluoxetine but not citalopram increased the plasma concentration of lidocaine. These results suggest that the increase of serotonergic neuronal activity through 5-HT_{2C} receptor

stimulation was responsible for increased activity of local anesthetics-induced convulsions and support the involvement of this mechanism in cocaine- and meprylcaine- but not in lidocaine-induced convulsions through their direct inhibitory action on central SERT.

Theme: Neurotransmitters, modulators, transporters, and receptors

Topic: Uptake and transporters

Keywords: Serotonin transporter; Convulsion; SSRI; Cocaine; Local anesthetics.

1. Introduction

Cocaine and synthetic local anesthetics produce stimulation of central nervous systems at toxic doses as observed in seizures both in rodents and humans. It has been generally accepted that seizures induced by cocaine and local anesthetics are related to the inhibition of central inhibitory nervous systems such as GABAergic and other inhibitory neurons due to their blockade of Na^+ current , thereby stimulating excitatory neuronal activity [6].

Although changes in susceptibility to seizures induced by cocaine and local anesthetics have been related to altered brain monoaminergic functions, they are not always uniform [6]. The norepinephrine (NE) system does not appear to modulate the proconvulsant effect of cocaine [14, 19] or is anticonvulsant to cocaine [17] but it dose enhance synthetic local anesthetic-induced convulsions [37]. Cocaine-induced convulsions are controlled positively and negatively by dopamine (DA) D_1 and D_2 receptor stimulation, respectively [30], while the involvement of dopaminergic activity is minor in lidocaine-induced convulsions [2, 37].

It has been suggested that the manipulation of serotonergic activity significantly modifies cocaine-induced seizures. Co-administration of cocaine with fenfluramine racemer, which increases synaptic serotonin (5-HT) levels, increased the occurrence and decreased the time of onset in status epileptics, without affecting lethality [26]. In addition, the occurrence and severity of cocaine-induced convulsions were increased by the selective serotonin reuptake inhibitor (SSRI) fluoxetine [20, 24]. Other SSRIs such as citalopram, paroxetine and tricyclic antidepressant imipramine also facilitated cocaine-induced convulsions [20]. On the contrary, 5-HT_2 receptor antagonists antagonized cocaine-induced convulsions [21, 24, 26]. An increase in brain content of 5-HT also intensified convulsions by local anesthetics, and a decrease in

brain 5-HT content increased the threshold [1, 4, 5]. These results are consistent with the idea that an increase in serotonergic neuronal activity facilitates convulsive activity of both cocaine and local anesthetics.

Cocaine is known to inhibit monoamine neurotransmitter transporters, including 5-HT transporters (SERT). Therefore, the accumulation of 5-HT in the synapse due to the inhibition of SERT by cocaine could be involved in the increase in serotonergic neuronal activity. However, it could be also possible that cocaine indirectly activates the neurons through cocaine-induced inhibition of Na^+ current of inhibitory neurons innervating to 5-HT neurons.

While synthetic local anesthetics are generally believed to lack the effect on monoamine transporters, certain local anesthetics have been shown to have cocaine-like reinforcing effects in animals [9, 15, 35, 36]. We and others have recently demonstrated that some synthetic local anesthetics such as procaine and meprylcaine do inhibit monoamine transporters (MAT) in rat brain synaptosomes [36], in COS cells transfected with MAT cDNAs [25] and in SH-SY5Y human neuroblastoma cells [16], while lidocaine displays no inhibitory action [25, 34].

Using local anesthetics as convulsants, all of which have different effects on MAT, including cocaine which is a potent inhibitor of MAT, meprylcaine with a relatively potent inhibitory effect on MAT and lidocaine, and a typical local anesthetic that does not inhibit MAT [25], the present study further examined the role of 5-HT on seizure development; 1) the intrinsic role of the inhibition of SERT in the development of seizures was examined by comparing the effects of SSRIs which may substitute some pharmacological properties of cocaine and the agonists and antagonists for 5-HT receptors on convulsions induced by cocaine, meprylcaine and lidocaine, 2) receptor subtypes of 5-HT involved in the development of

convulsions induced by these local anesthetics were analyzed using specific agonists and antagonists for 5-HT receptor subtypes.

2. Materials and methods

2.1. Animals

Male ICR mice 6 ~ 7 weeks old (25 ~ 35 g) were used. All procedures and handling of animals were performed according to the guideline "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*

The full chemical names and the supplier were as follows: BW723C86 [1-[5-(2-thienylmethoxy)-1*H*-3-indolyl]propan-2-amine hydrochloride; Tocris Cookson, Bristol, UK], cocaine hydrochloride (Takeda Pharmaceutical Co. Ltd., Osaka Japan), R(-)-DOI [(R)-(-)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride; Sigma-Aldrich, St. Louis, MO], MK212 [6-chloro-2-(1-piperazinyl)pyrazine hydrochloride; Tocris Cookson], SB 204741 [*N*-(1-methyl-5-indolyl)-*N'*-(3-methyl-5-isothiazolyl) urea; Tocris Cookson], ketanserin tartrate [3-[2-[4-(4-fluorobenzyl)-1-piperidinyl]-2,4[1*H*,3*H*]-quinazolinedione tartrate; Tocris Cookson], mCPP [1-(3-chlorophenyl)piperazine hydrochloride; Tocris Cookson], LY-53,857

[6-methyl-1-(1-methylethyl)-ergoline-8β-carboxylic acid 2-hydroxy-1-methylpropyl ester maleate; Sigma-RBI], MDL 11,939 [α-phenyl-1-(2-phenylethyl)-4-piperidinemethanol; Tocris Cookson], RS 102221 [8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulphonamido)phenyl-5-oxopentyl)-1,3,8-triazaspiro[4,5]decane-2,4-dione hydrochloride; Tocris Cookson].

SB 204741 was dissolved in DMSO and diluted as required in saline (final concentration of DMSO was less than 1 %). Other drugs were dissolved in saline. The doses of drugs were chosen based upon their functional selectivity at a particular 5-HT_2 receptor the affinity profile for each of the 5-HT_2 receptor ligands [18].

2.3. Seizures

Mice were intraperitoneally injected with convulsants and placed individually in plastic cages for observation of seizure activity. Seizures induced by drugs were characterized by ataxia, short loss of the righting reflex and clonic and tonic convulsions. The percent age of animals that exhibited convulsions in each treatment group was determined in the 15 min following the injection of convulsants. The convulsions were scored as, 1= weak, intermittent chronic convulsion; 2= typical clonic convulsion lasting more than 1 min; 3= clonic and tonic convulsion with increased locomotor activity or jumping. Thirty minutes after the injection of SSIR, 5-HT_2 receptor agonists, antagonists or an equal volume of saline, the mice were challenged with lidocaine, meprylcaine or cocaine. Percent incidence of the appearance of convulsions was determined from the proportion of the number of mice having intermittent chronic convulsions (score 1) to the number of mice used. To determine the

threshold for lidocaine-, meprylcaine-, and cocaine-induced convulsions, the occurrence of clonic convulsion (score 1) was measured by infusing lidocaine solution (4.0 mg/ml), meprylcaine solution (2.0 mg/ml), or cocaine solution (2.0 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 and the dosage administered was calculated per body weight. Preparation of drug solution, intraperitoneal injection or intravenous infusion of the drug into mice and the observation of behavior were performed by different persons with blind.

2.3. Statistical analysis

One-side Fisher's exact probability test was applied for the frequency of the seizures. Dunnett's multiple range test and Welch test were used to calculate the differences in seizure severity and seizure threshold, respectively.

2.5. Measurement of plasma lidocaine concentrations

Blood was obtained by cardiopuncture, mixed with 6 mM EGTA and centrifuged at 2500 rpm for 10 min, and 0.5 ml of plasma was used for solid phase extraction. C18 cartridges (360 mg/cartridge) were preconditioned by 6 ml of acetonitrile, followed by 6 ml of water. 25 μl of working standard solution containing 500 ng of phenacemide, an internal standard (I.S.), was added to 0.5 ml of plasma, which was then diluted to 2 ml with HPLC water. The sample was transferred onto the preconditioned C18 cartridge. A low vacuum was used to maintain the

extraction speed at ~ 0.5 ml/min. After loading the samples, the cartridge was washed four times, each time with 1.5 ml of washing solution consisting of acetonitrile-pH 9.0 of phosphate buffer (10:90 v/v). The washing speed was also adjusted to ~ 0.5 ml/min. At the end of washing, a high vacuum was applied so as to remove the remaining solvent. The washed cartridge was eluted with 2.5 ml of acetonitrile-pH 4.0 phosphate buffer (40:60 v/v) at ~ 0.5 ml/min. At the end of elution, high vacuum was again applied to recover all the eluting solution. After the vortex, 50 µl of the effluent was injected onto the column for quantitation.

Chromatographic conditions

The mobile phase was acetonitrile-pH 5.9 phosphate buffer (0.05 M) (20:80 v/v), which was degassed prior to use and delivered at a flow rate of 1.0 ml/min. The mobile phase was prepared from LC-grade solvents. The UV detection was carried out at 210 nm.

Chromatography was carried out using a conventional HPLC system consisting of a PU-980 intelligent HPLC pump (Jasco, Tokyo Japan), an autosampler fitted with 100-µl sample loop (AS-950-10 intelligent sampler, Jasco), a reversed phase analytical column (Silica rod column, C18, Chromolith perfomance RP-18, 100 x 4.6 mm, MERCK), UV-970 intelligent UV/VIS detector (Jasco), an integrator (Jasco/JMBS HSS-900, Jasco), and a data processor (BORWIN, Jasco).

3. RESULTS

3.1 Effect of SSRIs on convulsions induced by lidocaine and cocaine

Pretreatments of zimelidine, citalopram, and fluoxetine at 3 ~ 20 mg/kg dose-dependently

increased the incidence of convulsions induced by 40 mg/kg of lidocaine and decreased the threshold of the convulsions (Table 1). The severity of lidocaine convulsions as evaluated by an increase in score of the convulsions was increased by SSRI (scores of convulsions induced by lidocaine, lidocaine + zimelidine 20 mg/kg and lidocaine + citalopram 10 mg/kg were 1.0, 2.8 ± 0.3 and 2.4 ± 0.3, respectively). Zimelidine and citalopram at 20 mg/kg did not affect on the incidence of meprylcaine- and cocaine-induced convulsions and did not reduce their threshold at 10 and 20 mg/kg. Score of cocaine convulsions was not affect by citalopram (scores of convulsions induced by cocaine and cocaine + citalopram 10 mg/kg were 2.4 ± 0.3 and 2.9 ± 0.1, respectively).

3.2. *Effect of 5-HT_2 receptor agonists on convulsions induced by lidocaine and cocaine*

5-$HT_{2A, 2C}$ receptor agonists, R(-)-DOI at 0.1 mg/kg significantly increased the incidence of convulsions induced by lidocaine and reduced the threshold of the convulsions at 0.03, 0.1 and 1 mg/kg (Table 2). 5-HT_{2B} receptor agonist, BW723C86 had no influence on the incidence and the threshold of lidocaine convulsions at 2 and 5 mg/kg and significantly reduced the threshold at 5 mg/kg. 5-HT_{2C} receptor agonist, mCPP and MK212 at 1 and 10 mg/kg increased the incidence. mCPP at 1 ~ 10 mg/kg and MK212 at 0.1 ~ 10 mg/kg produced a profound reduction of the threshold of lidocaine convulsions. The effects were dose-dependent and the threshold was reduced to 66.4 % and 35.2 % of the control by mCPP and MK212 at 10 mg/kg, respectively. Score of lidocaine convulsions was increased by mCPP and MK212 (scores of convulsions induced by lidocaine, lidocaine + mCPP 10 mg/kg and lidocaine + MK212 10

mg/kg were 1.0, 3.0 and 3.0, respectively). Threshold of meprylcaine-induced convulsions was reduced significantly by R(-)-DOI at 0.1 mg/kg, mCPP at 10 mg/kg and MK212 at 10 mg/kg. mCPP at 1 and 10 mg/kg and MK212 at 10 mg/kg significantly reduced threshold of cocaine-induced convulsions. The extent of the reduction by these agonists in threshold of convulsions induced by meprylcaine and cocaine was much less than that of lidocaine. None of the agonists had influence on incidence of meprylcaine- and cocaine-induced convulsions.

3.3 Effect of 5-HT_2 receptor antagonists on convulsions induced by cocaine, meprylcaine, and lidocaine

Incidence of cocaine-induced convulsions was significantly reduced by 5-$HT_{2A,2B,2C}$ antagonist, LY-53857 at 5 mg/kg and 5-HT_{2C} antagonist, RS 102221 at 2 mg/kg (Table 3). The threshold of convulsions induced by cocaine was dose-dependently increased by LY-53857 and RS 102221 at 1 ~ 5 mg/kg. 5-HT_{2A} antagonists MDL 11,939 and ketanserin except at 3 mg/kg and 5-HT_{2B} antagonist SB204741 had no influence on the parameters of cocaine convulsions. Incidence of meprylcaine-induced convulsions was also significantly reduced by LY-53857 at 5 mg/kg and RS 102221 at 2 mg/kg and the threshold was increased by both agents. Ketanserin, except at 3 mg/kg, MDL 11,939 and SB204741 had no influence on the parameters of meprylcaine convulsions. None of LY-53857, RS 102221, MDL 11,939, ketanserin and SB204741 at any dose examined altered the incidence or threshold of lidocaine-induced convulsions, while enhancement by citalopram of parameters of convulsion activity of lidocaine was antagonized by RS 102221.

3.4. *Effect of SSRI treatment on blood levels of lidocaine*

Effects of the treatment with citaroplam and fluoxetine on plasma concentration of lidocaine was investigated (Fig. 1). The onset time of convulsions after injection of 40 mg/kg was 4.02 ± 0.35 min in the absence of citalopram. Times were 3.53 ± 0.30 min and 1.82 ± 0.24 min in the presence of 10 and 20 mg/kg of citalopram, respectively. A similar onset time was observed with fluoxetine. Thus, the plasma concentration immediately before or during convulsions (3 min or 5 min after injection of lidocaine) was determined. Pretreatment with 10 and 20 mg/kg of citalopram had no effect on the concentration of plasma lidocaine after injection of lidocaine at 3 min. With pre-treatment of fluoxetine at 10 and 20 mg/kg, plasma concentration of lidocaine was 189 % and 360 % higher than the control at 3 and 5 min, respectively, after the injection of lidocaine.

4. Discussion

Local anesthetics activate limbic discharge, which is most pronounced in the amygdaloid nuclear complex [28, 31, 32], though they inhibit nerve conductance primarily by blocking Na^+ channels. It is recognized that these seizures are due to selective suppression of inhibitory neurons such as γ-aminobutyric acid (GABA) *in vivo* because the firing rate of GABAergic neurons is high and local anesthetics-induced inhibition of nerve conductance tend to be frequency-dependent [3, 12, 13, 29]. Therefore, GABA mimetic drugs such as benzodiazepins,

barbiturates, $GABA_A$ receptor agonists and inhibitors of GABA transaminase [7] effectively block local-anesthetic-induced seizures. However, cocaine-induced seizures tend to be resistant to standard anticonvulsant therapies, including benzodiazepines, barbiturates, and phenytoin [7, 10, 33]. Convulsions induced by cocaine and synthetic local anesthetics also display different features. Certain symptoms of cocaine-induced seizures, including high locomotor activity, jumping and tonic-convulsion were not observed in lidocaine-induced seizures. Some pharmacological effects of cocaine are due to the inhibition of monoamine re-uptake. Convulsions induced by lidocaine together with SSRIs or 5-HT_2 receptor agonists displayed these three symptoms, as reflected in the increased score of lidocaine convulsions. Therefore, the increase of serotonergic activity not only increases the incidence of local anesthetics-induced convulsions, but also intensifies the symptoms. However, fluoxetine has an inhibitory action on various cytochrome p450 isoenzymes [11]. Therefore, fluoxetine could affects the metabolism of lidocaine. This is shown by an increase in plasma concentration of lidocaine in combination with fluoxetine in the present study. Fluoxetine-treated rats had higher brain levels of cocaine [8]. Citalopram has marginal inhibitory effect on CYP2D6 [27], but did not affect on brain cocaine level [8] or plasma lidocaine level at the present study. There is no evidence of drug interaction of zimelidine. Thus, the results of fluoxetine should be undervalued. The threshold of convulsions induced by cocaine and meprylcaine which have inhibitory action on SERT was reduced only slightly by 5-HT_2 agonists and was hardly affected by SSRI while these agents produced a marked increase in incidence and a profound reduction of the threshold of convulsions induced by lidocaine which has no inhibitory action on SERT. The evidence suggests that the serotonergic neuronal activity was stimulated by cocaine or meprylcaine themselves and

thus 5-HT_2 agonists and SSRI had a weak effect on cocaine- and meprylcaine-induced convulsions or rare at all. The increase in serotonergic activity could be resulted from the inhibition of SERT by cocaine and meprylcaine and/or from the inhibition of inhibitory interneuronal input to serotonergic neurons by their blocking action of Na^+ channels. However, the latter possibility seems not probable, because convulsions induced by lidocaine were not blocked by 5-HT_2 antagonists while convulsions induced by cocaine and meprylcaine were antagonized by 5-HT_2 antagonists nevertheless all of three compounds have common feature of potent inhibitor of Na^+ channels. Therefore, it is suggested that the activation of serotonergic neurons by cocaine or meprylcaine is due to direct inhibition of SERT on the serotonergic neurons.

Moreover, the density of 5-HT receptors mediates sensitivity to the convulsant effect of cocaine across different strains of mice: cinanserin, a 5-HT_2 antagonist, more potently attenuates cocaine-induced convulsions in C57BL/6J (6J) mice, exhibiting a lower density of 5-HT_2 receptors in the amygdaloid ridge, hypothalamus and midbrain relative to C57BL/6ByJ(6ByJ) mice [22]. 5-HT_{2c} agonists, mCPP, MK212, and the SSRI fluoxetine more strongly potentiated cocaine-induced convulsions in 6ByJ mice than in 6J mice [23]. The present results show that mCPP and MK212 increased the convulsive activity of lidocaine, and at lesser extent. of meprylcaine and cocaine. 5-$HT_{2A,2C}$ agonist, R(-)-DOI also increased the convulsive activity of lidocaine and little affected on convulsive activity of meprylcaine and cocaine. Futhermore, relatively specific antagonists for 5-HT_2 receptor subtypes, 5-$HT_{2A,2B,2c}$ antagonist, LY-53,857, and 5-HT_{2c} antagonist, RS 102221, reduced the incidence of cocaine- and meprylcaine-induced convulsions and increased the threshold. 5-HT_{2B} agonist, BW723C86 and 5-HT_{2A} antagonists, ketanserin and MDL 11,939, or 5-HT_{2B} antagonist, SB204741 had no or little influence on the

convulsive activity of all of convulsants. These results are consistent with the conclusion that 5-HT_{2C} sites, appear to be sites involved in cocaine- and meprylcaine-induced convulsions [24]. Effectiveness of 5-HT receptor antagonists in blocking cocaine- and meprylcaine-convulsions is also suggested.

In summary, the present study suggested that the increase in serotonergic neuronal activity had profound potentiation of local anesthetics-induced convulsion as shown with marked facilitation of lidocaine-induced convulsions by SSRI and 5-HT_{2C} agonists. Serotonergic neuronal activity was stimulated by cocaine and meprylcaine but not by lidocaine through the inhibition of SERT and this mechanism is involved in the convulsion activity of cocaine and meprylcaine. Comparing the effects of specific agonists and antagonists for 5-HT receptors on convulsions induced by lidocaine, meprylcaine and cocaine, stimulation of post synaptic 5-HT_{2C} contributes to intense convulsions of cocaine and meprylcaine.

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Figure legend

Fig.1. Effects of citalopram and fluoxatine on plasma concentration of lidocaine in mice. Lidocaine at 40 mg/kg was injected intraperitoneally 30 min after the injection of citalopram, fluoxetine or saline. Blood sample was taken by cardiopuncture in vials containing EGTA at 3 and 5 min after the injection of lidocaine, and was serum separated and kept at –80°C for lidocaine assay. *Significantly different from control, $p<0.001$, n=4-16.

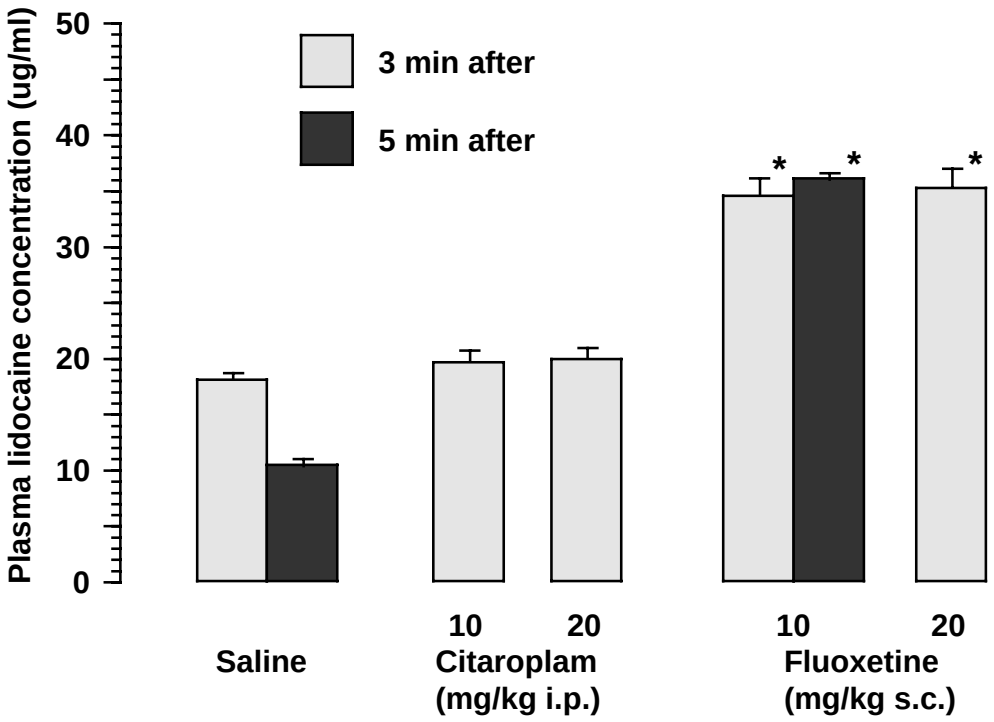
Plasma lidocaine concentration (ug/ml)
50
40
30
20
10
0
3 min after
5 min after
*
*
*
10
20
10
20
Saline
Citaroplam
(mg/kg i.p.)
Fluoxetine
(mg/kg s.c.)

Table 1. Effects of selective serotonin uptake inhibitors on seizure activity induced by lidocaine, meprylcaine and cocaine in mice

Addition	mg/kg	% Incidence	N_1	Threshold[a)] (mg/kg i.v.)	N_2
Lidocaine (40 mg/kg i.p.)-induced convulsion					
Saline		6.7	15	25.12 ± 0.33	13
Zimelidine	3 (i.p.)			22.65 ± 0.74**	10
	10 (i.p.)	50.0*	10	19.90 ± 0.71***	10
	20 (i.p.)	60.0**	10	18.75 ± 0.56***	10
Saline		10.0	20	25.06 ± 0.69	16
Citalopram	3 (i.p.)	33.3	12	18.64 ± 0.61***	10
	10 (i.p.)	66.7***	15	13.92 ± 0.59***	11
	20 (i.p.)	93.3***	15	12.61 ± 0.77***	10
Saline		10.5	19	25.61 ± 0.47	10
Fluoxetine	5 (s.c.)	30.0	10	19.63 ± 0.52***	10
	10 (s.c.)	80.0***	10	15.96 ± 0.53***	10
	20 (s.c.)	88.9***	9		
Meprylcaine (85 mg/kg i.p.)-induced convulsion					
Saline		40.0	10	12.05 ± 0.78	7
Zimelidine	10 (i.p.)			12.64 ± 0.54	7
	20 (i.p.)	50.0	10	11.78 ± 0.59	7
Citalopram	10 (i.p.)			11.64 ± 0.70	7
	20 (i.p.)	70.0	10	11.48 ± 0.55	7
Cocaine (50 mg/kg i.p.)-induced convulsion					
Saline		53.3	15	12.55 ± 0.27	15
Zimelidine	10 (i.p.)			12.88 ± 0.23	7
	20 (i.p.)	50.0		12.79 ± 0.74	7
Citalopram	10 (i.p.)	70.0	10	12.94 ± 0.28	10
	20 (i.p.)	75.0	12	12.60 ± 0.25	10

One-sided Fisher's exact probability test was used for seizure susceptibility: *$p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. saline. [a)]Data are expressed as the mean ± S.E.M. of the threshold for lidocaine- or cocaine-induced convulsions in mice. The Welch test was used for the convulsion threshold, ** $p<0.01$, *** $p<0.001$ vs. saline. N_1, number of mice used; N_2, number of mice used.

Table 2. Effects of 5-HT_2 receptor agonists on seizure activities of lidocaine, meprylcaine and cocaine in mice

Pretreatments (mg/kg i.p.)		% Incidence	N_1	Convulsive threshold [a)] (mg/kg i.v.)	N_2
Lidocaine (40 mg/kg i.p.)-induced convulsion					
Saline		13.3	15	25.13 ± 0.37	14
R(-)-DOI	(0.01)			23.91 ± 1.21	8
	(0.03)			20.51 ± 0.83***	8
	(0.1)	80.0**	10	15.07 ± 0.93***	8
Saline		13.3	15	25.12 + 0.33	13
BW723C86	(2)	14.3	7	24.00 ± 0.55	10
	(5)	16.7	12	23.89 ± 0.24*	7
Saline		6.7	15	24.61 ± 0.35	17
mCPP	(1)	50.0*	12	19.66 ± 0.64***	10
	(3)			17.41 ± 0.77***	10
	(10)	100.0***	25	16.33 ± 0.27***	15
Saline		6.7	15	23.92 ± 0.41	15
MK212	(0.1)			19.44 ± 0.49***	10
	(1)	58.3**	12	14.66 ± 0.46***	10
	(10)	100.0***	10	8.66 ± 0.49***	8
Meprylcaine (85 mg/kg i.p.)-induced convulsion					
Saline		40.0	10	13.18 ± 0.54	10
R(-)-DOI	(0.03)			11.84 ± 0.62	7
	(0.1)	60.0	10	9.68 ± 0.21***	7
BW723C86	(5)	30.0	10	14.14 ± 0.31	7
mCPP	(1)			13.50 ± 0.87	7
	(10)	50.0	10	11.76 ± 0.34*	7
MK212	(1)			13.31 ± 0.61	7
	(10)	60.0	10	11.27 ± 0.25**	7
Cocaine (50 mg/kg i.p.)-induced convulsion					
Saline		60.0	10	12.12 ± 0.39	10
R-(-)-DOI	(0.03)			13.32 ± 0.58	7
	(0.1)	60.0	10	12.16 ± 0.20	8
BW723C86	(5)	50.0	10	11.49 ± 0.14	7
Saline		53.3	15	12.71 ± 0.19	20
mCPP	(1)			10.40 ± 0.41***	10
	(10)	50.0	12	11.64 ± 0.23**	10
MK212	(1)			12.79 ± 0.22	10
	(10)	66.7	12	11.42 ± 0.23***	10

One-sided Fisher's exact probability test was used for seizure susceptibility: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. saline. [a)]Data are expressed as the mean ± S.E.M. of the threshold for lidocaine- or cocaine-induced convulsions in mice. The Welch test was used for the convulsion threshold, * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. saline. N_1, number of mice used; N_2, number of mice used.

Table 3. Effects of 5-HT_2 receptor antagonists on seizure activities of cocaine, meprylcaine and lidocaine in mice

Convulsants (mg/kg i.p.)	Pretreatments (mg/kg i.p.)		% Incidence	N_1	Threshold[a)] (mg/kg i.v.)	N_2
Cocaine (50)						
	Saline		80.0	15	12.37 ± 0.30	14
	LY-53,857	(0.1)			12.15 ± 0.77	7
		(1)			14.68 ± 0.33***	7
		(5)	25.0**	16	19.53 ± 0.38***	13
	Saline		73.3	15	12.41 ± 0.25	14
	RS 102221	(0.3)			13.56 ± 0.65	7
		(1)			14.90 ± 0.60**	7
		(2)	23.5**	17	19.63 ± 0.60***	13
		(5)			16.98 ± 0.26***	8
	Saline		53.3	15	12.50 ± 0.21	15
	MDL 11,939	(0.5)			13.54 ± 0.63	7
		(2)	50.0	12	12.42 ± 0.42	10
		(5)			12.96 ± 0.40	7
		(10)			13.27 ± 0.43	7
	Ketanserin	(0.3)			13.43 ± 0.58	7
		(1)	66.7	12	12.38 ± 0.37	10
		(3)	50.0	10	15.03 ± 0.44***	8
	SB204741	(0.3)			12.53 ± 0.41	7
		(2)	58.3	12	12.58 ± 0.27	10
		(5)			12.21 ± 0.67	7
Meprylcaine (90)						
	Saline		73.3	15	13.15 ± 0.34	13
	LY-53,857	(5)	10.0**	10	20.82 ± 0.41***	13
	RS 102221	(2)	20.0*	10	19.43 ± 0.40***	13
	Saline		70.0	10	12.16 ± 0.37	10
	MDL 11,939	(2)	50.0	10	12.50 ± 0.37	7
	Ketanserin	(3)	60.0	10	13.90 ± 0.56*	7
	SB204741	(2)	70.0	10	12.38 ± 0.77	7
Lidocaine (60)						
	Saline		86.7	15	23.00 ± 0.36	18
	LY-53,857	(5)	100.0	10	23.36 ± 0.52	13
	RS 102221	(2)	90.0	10	22.37 ± 0.58	13
	Saline		80.0	10	23.51 ± 0.48	18
	Ketanserin	(3)	80.0	10	23.46 ± 0.61	10
	SB204741	(2)	80.0	10	22.75 ± 0.52	10
	Saline		80.0	10	24.63 ± 0.69	10
	MDL 11,939	(2)	90.0	10	24.88 ± 0.71	8
Lidocaine (40)						
	Saline		6.7	15	25.06 ± 0.69	16
	Citalopram	(10)	66.7***	15	13.92 ± 0.59***	11
	Citalopram + RS 102221	(10) (2)	10.0##	10	21.01 ± 0.38###	13

One-sided Fisher's exact probability test was used for seizure susceptibility: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. saline; ## $p<0.01$ vs citalopram. [a)]Data are expressed as

the mean ± S.E.M. of the threshold for cocaine-, meprylcaine- or lidocaine- induced convulsions in mice. The Welch test was used for the convulsion threshold, * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. saline; ### $p<0.001$ vs. citalopram. N_1, number of mice used; N_2, number of mice used.