

Down-regulation of norepinephrine transporter function induced by chronic administration of desipramine linking to the alteration of sensitivity of local anesthetics-induced convulsions and the counteraction by co-administration with local anesthetics.

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Abstract

Alterations of norepinephrine transporter (NET) function by chronic inhibition of NET in relation to sensitization to seizures induced by cocaine and local anesthetics were studied. [³H]norepinephrine uptake into hippocampus region but not cortex, striatum or amygdalae isolated from mice treated daily for 5 days with desipramine, an inhibitor of NET was significantly decreased. Daily treatment of cocaine increased [³H]norepinephrine uptake into hippocampus. The decrease in norepinephrine uptake induced by chronic desipramine treatment was reversed by co-administration of lidocaine, bupivacaine or tricaine with desipramine. Daily administration of desipramine increased the incidence of appearance of lidocaine-induced convulsions and decreased that of cocaine-induced convulsions. The changes in the convulsive sensitivity to lidocaine and cocaine induced by repeated administration of desipramine was reversed by co-administration of lidocaine with desipramine.

These results suggest that down-regulation of hippocampal NET induced by chronic administration of desipramine may be relevant to desipramine-induced sensitization of lidocaine convulsions. Inhibition of Na⁺ channels by local anesthetics may regulate desipramine-induced down-regulation of NET function. While repeated administration of cocaine caused up-regulation of hippocampal NET function. Cocaine kindling developed by repeated administration of cocaine may be due to the different mechanisms from those for desipramine-induced sensitization of lidocaine

seizures.

1. Introduction

Although cocaine and synthetic local anesthetics block the Na⁺ channel, thereby inhibiting nerve conductance, they produce seizures in their toxic doses. These compounds bind with high affinity to open state of Na⁺ channel, thus represent the property of frequency-dependent inhibition of the channel. This property of the action may result in selective depression of GABAergic neurons, thereby allowing exciting neuronal activity to lead convulsions (Dohi et al., 2002). It is known that repeated administration of subconvulsive dose of cocaine to rodent easily sensitizes seizure activity of cocaine, called as 'cocaine kindling' (Post and Weiss, 1988). Repeated administration of subconvulsive dose of lidocaine also increased the susceptibility to lidocaine-induced seizures (Post and Weiss, 1988, Doriss and Lotzf, 1992). However, the time course of the development of lidocaine kindling which requires daily injection for several weeks is much slower than cocaine-kindling which develops during several days. In addition, the difference between genetic factors of mouse strain in susceptibility of the development of lidocaine kindling did not parallel those for cocaine kindling (Marley et al., 1991). Therefore, although the blockade of Na⁺ channel appear to be responsible in part for the seizure kindling properties, the additional actions of cocaine appear to be involved in cocaine kindling.

There is convincing evidence suggesting that cocaine inhibits the monoamine uptake systems to exert psychostimulant action, while synthetic local anesthetics have been believed to devoid of this effect. However, some

synthetic local anesthetics including procaine and meprylcaine, inhibit monoamine transporter (MAT) with less potent than cocaine, while lidocaine lacks the effect on MAT (Woodward et al.,1995; Sato et a., 2000; Joyce et al., 2001). Repeated intermittent administration for 5 days of cocaine, procaine and meprylcaine induced sensitization their convulsive activity dependently on their MAT inhibition potency and cross sensitization to lidocaine convulsions, whereas repeated treatment of lidocaine for this period did not produce any sensitization (Shimosato et al., 1996; Sato et al., 2000; Arai et al., 2003). Thus, MAT is suggested to do important role on seizure progression. In fact, after chronic administration of desipramine or maprotiline, inhibitors of norepinephrine transporter (NET), convulsive activity of lidocaine was markedly increased, while chronic administration of inhibitors for serotonin transporter or dopamine transporter had minor effect on the convulsive activity of lidocaine (Arai et al., 2003).

It has been reported that norepinephrine might act as an endogenous modulator of convulsive seizures (Weinshenker and Szot, 2002). Recent studies using genetically engineered mice suggest an anticonvulsive role of endogenous norepinephrine against various seizure-inducing stimuli. For instances, depletion of NE due to the genetic deletion of dopamine β -hydroxylase (Dbh^{-/-}) showed an increased susceptibility to seizure-inducing stimuli, such as pentylenetetrazol (PTZ) (Szot et al. 1999), while an increase in synaptic norepinephrine level by genetic deletion of NET decreased vulnerability to seizures induced by PTZ and cocaine

(Kaminski et al. 2005). However, local anesthetics-induced convulsions have different feature against pharmacological manipulation of monoaminergic neuronal activity (Dohi et al.,2005). For instances, the treatments which decreased norepinephrine content in brain decreased susceptibility of lidocaine-induced seizures, while these enhanced susceptibility of PTZ-induced seizures (Yoshimura et al. 1991). Acute and chronic treatment NET inhibitor antidepressants have both pro- and anti-convulsant properties against PTZ convulsions (Ahern 2005) but produced sensitization of lidocaine-induced convulsions (Arai et al., 2003; Yoshimura et al., 1991). Thus, noradrenergic system may exert proconvulsant control against lidocaine-induced seizures. Taken together, it may be predicted that changes in neurotransmission of noradrenergic system produced by chronic inhibition of NET is responsive to sensitization of lidocaine seizures. Chronic inactivation of NET displays numerous alterations in noradrenergic neuronal activities at presynaptic and postsynaptic sites. NET is thought to be regulated by persistent alterations in extracellular norepinephrine concentrations. However, there have been mixed results regarding NET regulation, up- or down-regulation or no change in the expression or function following chronic exposure to NET inhibitors including cocaine and desipramine.

In this study, the following experiments were designed to elucidate the role of alteration of NET function by chronic inhibition of NET in the development of sensitization of lidocaine- and cocaine-induced seizures; (1)

whether chronic administration of desipramine, a NET inhibitor induces alteration of NET function was studied by measuring the [3H]norepinephrine uptake in brain regions from desipramine-treated mice in relation to development of sensitization to lidocaine- and cocaine- induced convulsion; (2) whether chronic administration of cocaine produces the similar events to desipramine. Actually it was found that chronic NET inhibition by desipramine caused down-regulation of NET function in hippocampus in relation to sensitization of lidocaine convulsions. However, chronic administration of cocaine caused up-regulation of NET function unexpectedly. Therefore, (3) whether chronic inhibition of Na⁺ channel has some influence on the alteration of NET function induced by chronic inhibition of NET was examined, because chronic administration of cocaine bring both inhibition of NET and Na⁺ channel.

2. Result

2.1. Effects of repeated treatments with desipramine, cocaine or desipramine in combination with local anesthetics on [3H]norepinephrine uptake in brain regions

In order to demonstrate the alteration of NET functions by chronic administration of desipramine or cocaine on [3H]norepinephrine uptake was measured in P2 fraction from brain regions of mice treated with these drugs. Specific uptake of [3H]norepinephrine in hippocampus was significantly decreased in mice treated with desipramine for 5 days (Fig.1A). There was no significant difference in [3H]norepinephrine uptake in cortex, striatum and amygdalae. Therefore, it is suggested that chronic treatment of desipramine caused down-regulation of NET function in the restricted brain area. On the other hand, the chronic administration of cocaine did not decrease, but rather increased [3H]norepinephrine uptake in hippocampus with no significant difference in other area (Fig. 1B). When cocaine was administered *in vivo*, Na⁺ channel beside MAT is an another target molecule for cocaine regulation of neuronal activity. We assumed one possibility that chronic inhibition of Na⁺ channel by cocaine simultaneous inhibition of NET may cause some influence on MAT function. Thus, we examined whether the combined treatment of lidocaine and desipramine would affect on the reduction of [3H]norepinephrine uptake induced by chronic treatment of desipramine (Fig.2). When desipramine was treated daily for 5 days in combination with lidocaine, the decrease of uptake induced by desipramine

alone disappeared. Lidocaine treatment alone had no influence on [3H]norepinephrine uptake activity. These results showed that the co-administration of lidocaine with desipramine counteracted to the down-regulation induced by desipramine alone. To test this effect of lidocaine is common to local anesthetics, effects of co-administered of bupivacaine and tricaine which display no inhibitory activity on MAT (Sato et al., 2000) on desipramine-induced down-regulation of NET function in hippocampus (Fig.3). Reduction of [3H]norepinephrine uptake induced by chronic administration of desipramine was counteracted with co-administration with bupivacaine and tricaine. The treatment with these local anesthetics by themselves did not affect on NET function. These results may suggest that the chronic blockade of Na⁺ channel counteracted the down-regulation of NET induced by chronic NET inhibition.

2.2. Effects of repeated treatments with desipramine with or without lidocaine on lidocaine- and cocaine-induced convulsions

We have previously reported that daily treatment of NET inhibitors, desipramine and maprotiline markedly enhanced seizure activity of lidocaine. As it is suggested that chronic treatment of desipramine actually produced down-regulation of NET and the co-administration of lidocaine with desipramine counteracted to the down-regulation induced by desipramine alone, the effect of daily treatment of desipramine in combination with or without lidocaine on seizure activity of lidocaine was

examined (Fig. 4). Percent incidence of lidocaine-induced convulsions (n=10) increased in mice with chronic injection of desipramine (n=15) and this increase was disappeared in mice with chronic injection of desipramine in combination with lidocaine (n=16). On the other hand, the % incidence of cocaine-induced convulsion (n=10) almost disappeared in mice with chronic injection of desipramine (n=10) and this decrement was recovered in mice with chronic injection of desipramine with lidocaine (n=10). These results suggest that chronic treatment of lidocaine protect induction of neuronal changes from chronic administration of desipramine. Taken together, the enhancement of lidocaine convulsions or inhibition of cocaine convulsions by chronic treatment of desipramine may reflect the down-regulation of NET by desipramine. Recovery from the alteration of lidocaine- and cocaine-induced convulsions by chronic co-treatment of lidocaine with desipramine may reflect the blockade of the down-regulation of NET by chronic co-treatment of lidocaine.

3. Discussion

It is thought that adaptive changes that follow chronic reuptake blockade of monoamines by antidepressant drugs are responsible for their clinical efficacy. Down-regulation of MAT is thought as one such molecular change. Chronic treatment with desipramine has been reported to decrease density of binding site for nisoxetine, a high affinity ligand for NET (Bauer and Tejani-Butt, 1992; Weinshenker et al., 2002; Benmansour et al., 2004) or

norepinephrine uptake (Benmansour et al., 2004), although some reports suggested the increase or no change in NET density by chronic desipramine treatment (Biegon 1986; Shores et al, 1994; Racagni et al., 1983; Cheetham et al., 1996). These differences may arise from various experimental conditions including doses, period during or after withdrawal of desipramine. The present study showed that [³H]norepinephrine uptake decreased in P2 fractions of hippocampus from desipramine-treated mice in agree with the observation by Benmansour et al. (2004). One of the target regions for seizures of local anesthetics is hippocampus where an increase in metabolic activity (Invar and Shapiro, 1981) and in the level of c-fos mRNA and protein were produced by stimulation of CNS with local anesthetics (Chae et al., 1999). Down-regulation of NET increases norepinephrine in the synapse and this mechanism could be one of the causes for the sensitization of lidocaine-convulsions by chronic treatment with NET inhibitors, as norepinephrine is proconvulsive against lidocaine-induced convulsions. It is reported that the systemic administration of excitatory amino acids markedly increased c-Fos, c-Jun protein and nuclear transcription factor activator protein-1 only in granule cell layer of the dentate gyrus in hippocampus (Yoneda et al 1999; Kitayama et al., 2003). Although the observed reduction of [³H]norepinephrine uptake by chronic desipramine in hippocampus in the present study was significant but small, the above observation reported allow us to speculate that the reduction of NET large enough to control seizure activity would be produced in the restricted area in

hippocampus but this alteration was masked by non-responded other area of hippocampus, because P2 fraction contains large margin of hippocampus.

Convulsive activity of cocaine received little affect with desipramine which markedly enhanced lidocaine-induced convulsions (Jackson et al., 1990; Yoshimura et al. 1991) and was diminished by α -receptor antagonist, prazosin (Jackson et al., 1990). These results suggest that the inhibition of NET by cocaine itself could be involved in the activation of cocaine-induced convulsions. The decrease of convulsive activity of cocaine in NET deleted mice (Kaminski et al., 2005) could be due to the lack of the target NET for cocaine to argue its convulsive activity. Thus, decrement of cocaine-induced convulsions in chronic desipramine treated mice also could be due to down-regulation of NET.

Although whether the altered NET function by desipramine is due to changes in the expression of NET protein is remained to be elucidated, it is the intrigued findings at the present study that the enhancement of lidocaine-induced convulsions, decrease of cocaine-induced convulsions and decrease of [3 H]norepinephrine uptake in P2 fractions of hippocampus produced by chronic desipramine treatment were all counteracted with co-administration of lidocaine with desipramine. These may provide the evidence that chronic co-administered lidocaine protected NET function from down-regulation induced by desipramine. This protective effect is not specific to lidocaine, because other amido- and esteric-type anesthetics, bupivacaine and tricaine, both produced the similar antagonistic effect on

desipramine-induced reduction of [³H]norepinephrine uptake in hippocampus. Therefore, the reverse effect of the local anesthetics may relate to the inhibition of Na⁺ channels. Chronic treatment with desipramine reduced NET levels in both wild type and Dbh^{-/-} mice, demonstrating that norepinephrine is not required for the down-regulation of NET (Weinshenker et al., 2002), but interaction of NET with desipramine per se produced altered expression of NET. Although the mechanisms for regulation of NET by local anesthetics are not known, the changes in the neuronal activity by inhibiting voltage-gated Na⁺ channels may relate to altered expression of NET function bypassing the effect of released norepinephrine. NET is a family of Na⁺/Cl⁻-dependent neurotransmitter transporter and thus its activity is controlled by cellular Na⁺ gradient. Local anesthetics by blocking Na⁺ channel could alter transport activity of NET and/or affinity for substrate or inhibitors to NET. 5'-Flanking region of NET gene contains nerve growth factor (NGF) responsive element and cause down-regulation of NET expression through interaction with NGF (Ikeda et al., 2001). Whether chronic inhibition of Na⁺ channel may produce plastic changes in NET function by affecting ionic circumstance or altered expression of NET by altering release of neurotrophic factors such as NGF are remained to be elucidated.

Although activation of the brain dopaminergic system is thought to be the primary mechanism by which cocaine exerts its reinforcing and behaviorally activating effects, the role of noradrenergic system has began to

be appreciated in relation of the alterations of NET by chronic cocaine-intake with behavioral changes. Recent studies have shown that cocaine self-administration in monkey up-regulated nisoxetine binding site in the bed nucleus of the stria terminalis (Macey et al., 2003), the A1 noradrenergic cell group, the forebrain projections of both the ventral and dorsal noradrenergic bundles (Thomas et al., 2005), and in the insular cortex from brains of cocaine addicts (Mash et al., 2005), although previous studies reported unresponsive to cocaine exposure in rodent (Bemansour et al., 1992; Belej et al., 1996). In the present study, [³H]norepinephrine uptake increased in P2 fractions of hippocampus from mice treated daily with cocaine for 5 days. Taken together that local anesthetics probably by blocking Na⁺ channels reversed the down-regulation of NET induced by chronic inhibition of NET by desipramine, another possibility could be speculative that the chronic inhibition of Na⁺ channel by cocaine itself may influence on the regulation of NET expression by cocaine. Although up-regulation of NET function by chronic cocaine relate to which behavioral effects of cocaine is not known, cocaine kindling due to the different mechanisms from down-regulation of NET as seen in desipramine-induced sensitization of lidocaine seizures.

In summary, the present findings suggest that 1) chronic occupation of NET with desipramine caused NET down-regulation which is a cause of sensitization to seizures induced by lidocaine, 2) the simultaneous blockade of Na⁺ channel by local anesthetics with chronic occupation of NET with

desipramine produced the reverse regulation against the down-regulation of NET by desipramine, 3) cocaine kindling may be due to the different mechanisms, because chronic cocaine produced up-regulation of NET function.

4. Experimental procedures

4.1. Animals

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

4.2. Treatment with drugs

For daily pretreatment, subconvulsive dose of cocaine (35 mg / kg) and lidocaine (35 mg /kg) or desipramine (20 mg / kg) and desipramine (20 mg / kg) together with lidocaine (35 mg / kg) or saline were injected intraperitoneally once a day for 5 days. Desipramine with this regimen produced the maximum enhancement of lidocaine-induced convulsions (Arai et al., 2003). After 48 hrs after the last pretreatments, minimum convulsive dose of lidocaine (40 mg /kg) or about 50 % convulsive dose of cocaine (40 mg / kg) was administrated intraperitoneally to see the behavioral alteration. To avoid the possibility of the influence of the residual drugs of the last dose in

brain tissue, especially desipramine, 48 hrs washout period after the last dose was chosen to perform the behavioral and [³H]norepinephrine uptake experiments as described previously (Arai et al, 2003).

4.3. Measurement of [³H]norepinephrine uptake

Mice were sacrificed 48 h after last dose of pretreatment and then brains were removed from animals and dissected into cortex, hippocampus, striatum and amygdalae. These regions were homogenized with Teflon homogenizer in 0.32 M sucrose. The homogenates were centrifuged at 800 g for 10 min, and the supernatant was centrifuged at 20,000 g for 20 min. The pellet (P2; crude synaptosomal fraction) was washed with 0.32 M sucrose at 3 times. This P2 were suspended with Krebs-Ringer HEPES-buffered solution (KRH; 125 mM NaCl, 5.2 mM KCl, 1.2 mM CaCl₂, 1.4 mM MgSO₄, 1.2 mM KH₂PO₄, 5 mM glucose, 20 mM HEPES, pH7.3). The P2 suspension was incubated for 10 min at 37°C with 20 nM [³H]norepinephrine containing monoamine oxidase inhibitor pargyline 50 μM, antioxidant ascorbic acid 100 μM, serotonin uptake inhibitor zimelidine 100 nM and dopamine uptake inhibitor GBR12935 100 nM. The incubation was terminated by vacuum filtration through Whatman glass fiber filter of type C and washing three times with KRH at 4°C. The filter radioactivity was detected by liquid scintillation counting. Nonspecific uptake was determined in the presence of 100 μM cocaine.

4.4. Estimation of behavior

The seizures 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 group was determined as described previously (Arai et al., 2003).

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Regent

Fig. 1. Effects of repeat treatment with Desipramine or cocaine on [³H] norepinephrine uptake.

Animals were injected with desipramine (20 mg / kg; A) or cocaine (35 mg / kg; B) once a day for 5 days, followed by preparation of P2 fraction after 48 h from murine cortex (CX), hippocampus (HC), striatum (ST) and amygdalae (AM). The P2 suspension was incubated for 10 min at 37°C with [³H]norepinephrine containing 50 μ M pargyline, 100 μ M ascorbic acid, 100 nM zimelidine and 100 nM GBR12935. The data obtained from six separate experiments. *Represent P<0.05 and **Represent P<0.01 compared to control.

Fig. 2. Effects of repeat treatment with lidocaine, desipramine or desipramine co-administration of lidocaine on [³H] norepinephrine uptake.

Mice were injected with desipramine (20 mg/kg), lidocaine (35 mg/kg) or simultaneously with desipramine (20 mg/kg) and lidocaine (35 mg/kg) once a day for 5 days. P2 fractions were prepared 48 h later from the following brain regions: cortex (CX), hippocampus (HC), striatum (ST) and amygdala (AM). The P2 suspension was incubated for 10 min at 37°C with [³H]norepinephrine containing 50 μ M pargyline, 100 μ M ascorbic acid, 100 nM zimelidine and 100 nM GBR12935. The data were obtained from six separate experiments. Values represent mean \pm S.D. percent of specific

uptake of [³H]norepinephrine. The specific uptake of saline-injected control in cerebral cortex (CX), hippocampus (HC), striatum (ST) and amygdala (AM) were 1.09 ± 0.18 , 1.38 ± 0.08 , 0.83 ± 0.16 and 0.54 ± 0.09 fmol/ μ g protein, respectively. *Represent $P < 0.05$ compared to control.

Fig. 3. Effects of repeat treatment with local anesthetics, desipramine or desipramine co-administration of local anesthetics on [³H] norepinephrine uptake.

Mice were injected with desipramine (20 mg/kg), lidocaine (35 mg/kg), Bupivacaine(35 mg/kg), Tricaine (80 mg/kg) or simultaneously with desipramine (20 mg/kg) and the local anesthetics once a day for 5 days. The P2 fraction was prepared 48 h later from hippocampus. The P2 suspension was incubated for 10 min at 37°C with [³H]norepinephrine containing 50 μ M pargyline, 100 μ M ascorbic acid, 100 nM zimelidine and 100 nM GBR12935. The data obtained from at least four separate experiments. Values represent mean \pm S.D. percent of specific uptake of [³H]norepinephrine. The specific uptake in saline-injected control is 1.41 ± 0.08 fmol/ μ g protein. *Represent $P < 0.05$ compared to control.

Fig. 4. Effects of repeat treatment with desipramine or desipramine co-administration of lidocaine on lidocaine or cocaine convulsion.

Animals were injected with desipramine (20 mg/kg) or simultaneously with desipramine (20 mg/kg) and lidocaine (35 mg/kg) once a day for 5 days,

followed by administration of lidocaine or cocaine after 48 h. Values represent % incidence of convulsion. n=number of mice used. One-side Fisher's exact probability test was used for seizure susceptibility. **Represent $P < 0.01$ compared to saline-injected animals. ††Represent $P < 0.01$ compared to desipramine-injected animals.

Fig. 1

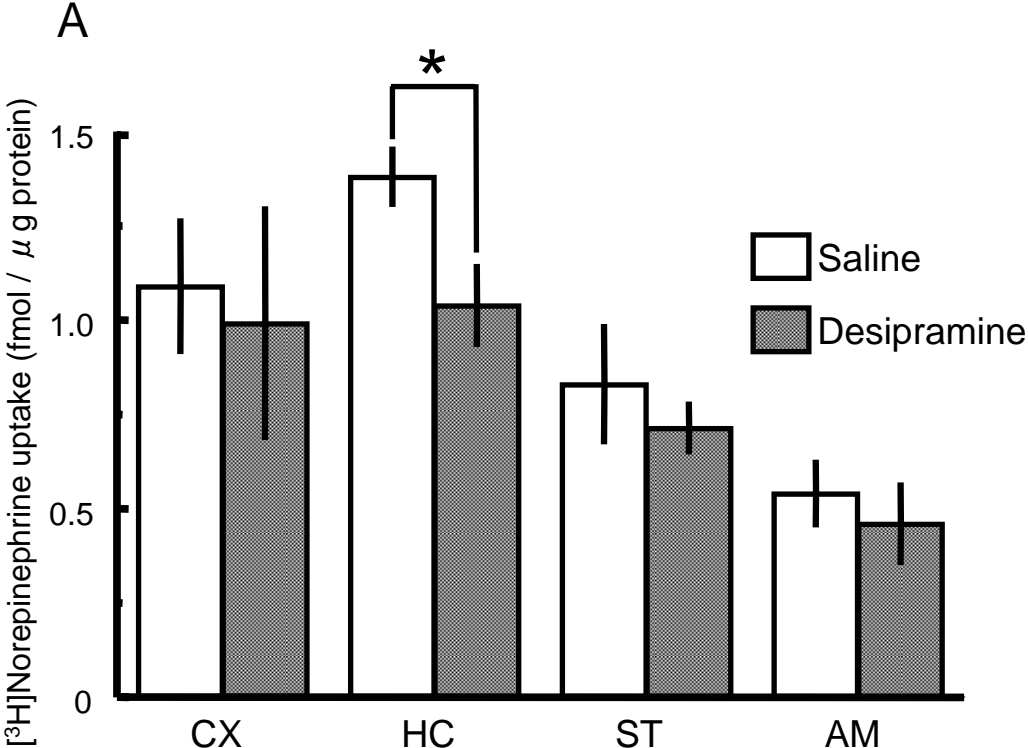


Fig. 1

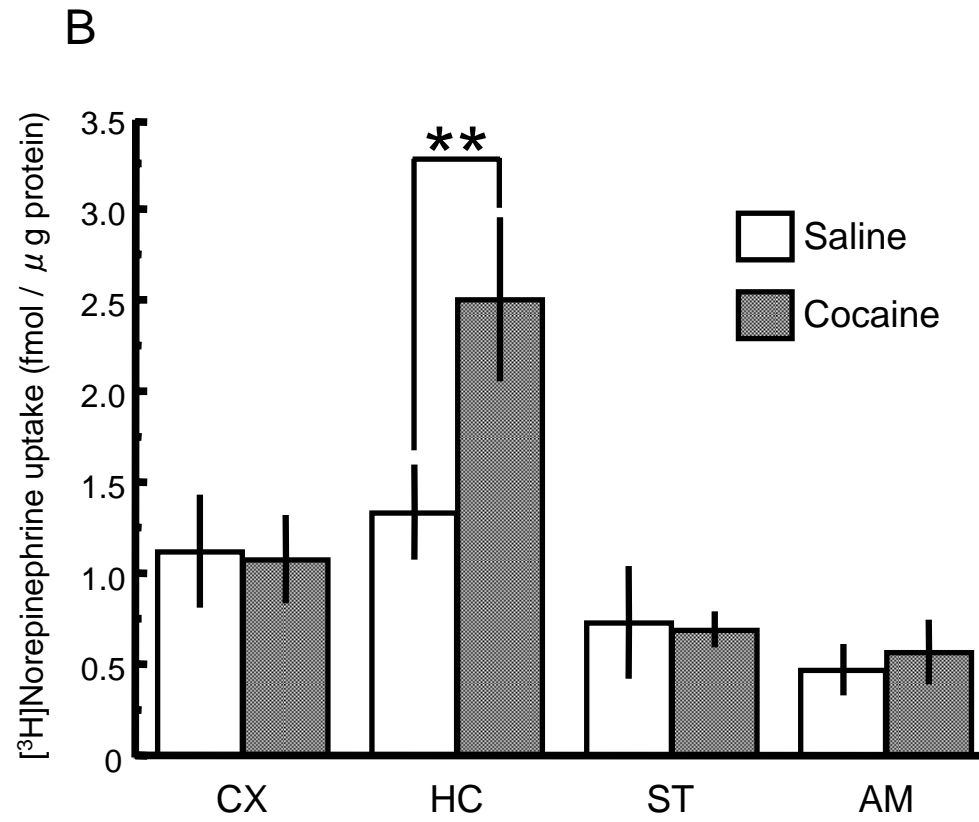


Fig. 2

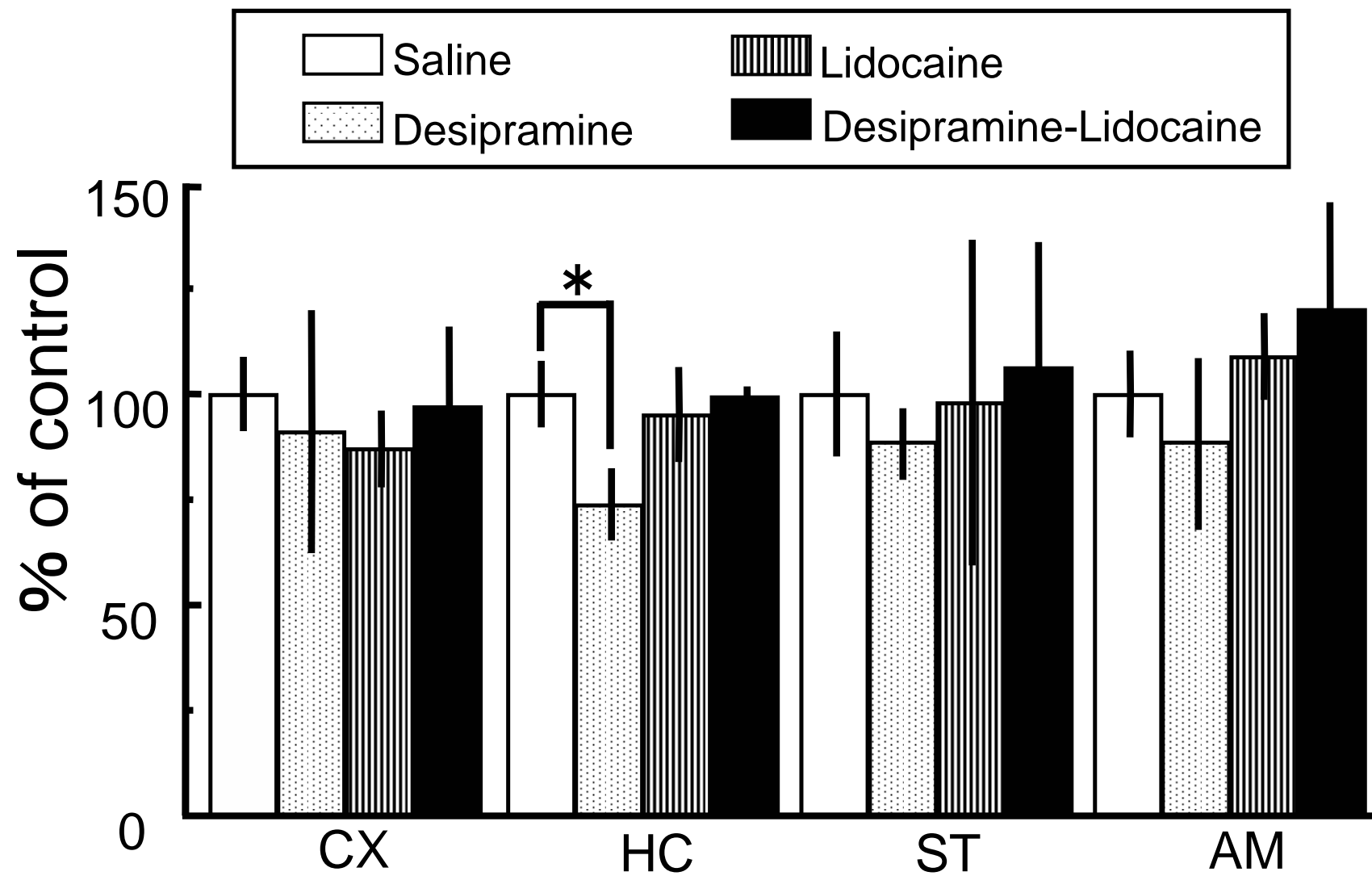


Fig. 3

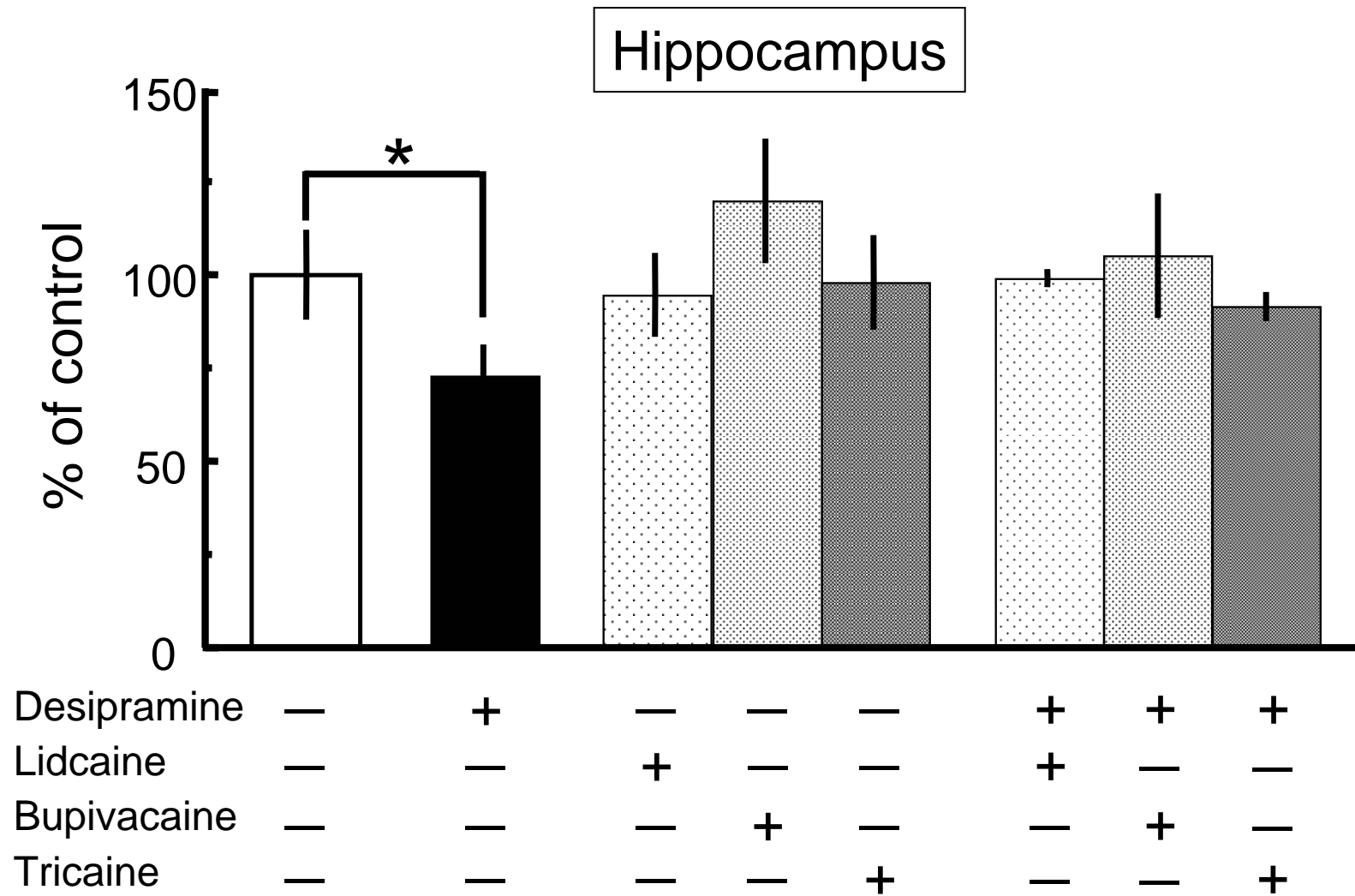


Fig. 4

