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Effect of 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline on Methamphetamine- and Cocaine-Induced Behavioral Sensitization

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AKIYAMA, K., H. UJIKE, K. SAKAI, Y. SHIMIZU, M. KODAMA AND S. KURODA. *Effect of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline on methamphetamine- and cocaine-induced behavioral sensitization.* PHAMACOL BIOCHEM BEHAV **61**(4) 419–426, 1998.—The present study investigated the effect of pretreatment with 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX), an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist, on behavioral sensitization induced by methamphetamine (METH) and cocaine at doses that are transitional relative to the induction of an acute response of locomotion and interrupting episodes of sniffing and head movement. Male Sprague–Dawley rats were randomly assigned to four groups that received daily either 20 mg/kg NBQX + 3 mg/kg METH, 40 mg/kg NBQX + 3 mg/kg METH, vehicle + 3 mg/kg METH, or vehicle + saline daily for 10 days. In another experiment, rats of four groups received daily either 20 mg/kg NBQX + 15 mg/kg cocaine, 40 mg/kg NBQX + 15 mg/kg cocaine, vehicle +15 mg/kg cocaine, or vehicle + saline daily for 10 days. NBQX did not attenuate activity/stereotypy induced by acute administration of either psychostimulant. Pretreatment with NBQX did not affect augmentation of activity/stereotypy scores by repeated administration of METH or cocaine. There was no significant difference in the intensity of activity/stereotypy between the NBQX + METH and vehicle + METH groups and between the NBQX + cocaine and vehicle + cocaine groups following a challenge injection with 2 mg/kg METH alone or 15 mg/kg cocaine alone, respectively, given 7 days after the last dose of repeated treatment session. Pretreatment with NBQX alone for 10 days (20 mg/kg for 5 days and 40 mg/kg for subsequent 5 days) did not affect the intensity of activity/stereotypy induced by a challenge injection with 2 mg/kg METH given 7 days after the last dose of repeated injection session. NBQX at 40 mg/kg had no apparent effect on acute METH (3 mg/kg)-induced dopamine release in the striatum. These results suggest tht AMPA receptors are unlikely to be involved in induction of behavioral sensitization that is manifested as augmented activity/stereotypy following repeated administration of METH or cocaine. © 1998 Elsevier Science Inc.

Methamphetamine Cocaine Behavioral sensitization AMPA receptors NBQX

IT has been reported that excitatory amino acid (EAA) receptors are implicated in a variety of neural plasticity. Although the role of *N*-methyl-D-aspartate (NMDA) receptors in long-term potentiation and kindling has been well documented, several lines of evidence have indicated that α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors may also be involved (13).

Repeated administration of an intermediate dose of psychostimulants, such as amphetamine (AMPH) and methamphetamine (METH), to rodents leads to progressive augmentation of stereotyped behavior, a process known as behavioral sensitization (1,5,18,20). Animals receiving previously repeated injections of psychostimulants still exhibit an augmented response induced by a challenge injection even after long-term abstinence, indicating that behavioral sensitization has long-lasting properties. Several studies have reported a putative role of NMDA receptors in the induction of behavioral sensitization, because MK-801, a selective antagonist of NMDA receptors, can prevent AMPH- (8) and cocaine (6)-induced behavioral sensitization. On the other hand, little is

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known about the role of AMPA receptors in behavioral sensitization, although there have been so far a few studies on this aspect (7,11). 6,7-Dinitroquinoxaline-2,3-dione (DNQX), a nonselective antagonist of AMPA/kainate (KA) receptors, has been reported to suppress the induction and expression of AMPH-induced behavioral sensitization in mice (7). Recently, a number of selective antagonists of AMPA receptors have been developed, and tested for their ability to affect dopamine-mediated behaviors (25).

2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX) is one of such selective AMPA receptor antagonists (21), and it has recently been investigated as to whether this compound would inhibit AMPH-induced behavioral sensitization (11). Whereas other selective antagonists of AMPA receptors have been shown to bear antipsychotic-like activity, the suppressive effect of NBQX on dopamine-mediated behavior may be ascribed to general motor impairment (25). The present study further examined this question, by investigating the effect of NBQX pretreatment with each METH or cocaine injection on the behavioral sensitization of stereotypy induced by each psychostimulant, and also by examining the effect of NBQX pretreatment on acute METH-induced striatal dopamine release.

METHOD

Animals

Male Sprague–Dawley rats (Charles River, Yokohama, Japan) weighing 220–240 g at purchase were used. They were housed under a 12 L:12 D cycle (lights on 0700, off 1900 h) with constant temperature (25°C) and humidity and allowed free access to food and water. All animals used were handled gently for 3 min once daily for 1 week and then subjected to the following drug treatment. All animals used in the procedures were treated in strict accordance with the Guidelines for Animal Experiments at Okayama University Medical School.

Drugs and Chemicals

NBQX was generously donated by Novo Nordisk (Malov, Denmark), and purchased from Tocris Cookson (Langford, UK) in part. It was dissolved in 0.1 N NaOH and thereafter diluted with 0.01 N NaOH and H_2O, and subjected to pH adjustment at pH 7.5 using 0.1 N HCl, as described previously (19). The same vehicle as used in preparation of the NBQX solution was referred to as vehicle control for NBQX. METH and cocaine were purchased from Dainippon Pharmaceutical Co. (Osaka, Japan) and Takeda Pharmaceutical Co. (Osaka, Japan), respetively, and dissolved in saline.

Experiment 1: Effect of NBQX on Acute Motor Changes Induced by a Single Dose of METH and METH-Induced Behavioral Sensitization

Four groups, each consisting of six rats, were used. Three groups received once daily for 10 days an intraperitoneal (IP) injection of either the vehicle, 20, or 40 mg/kg NBQX, and 15 min later, an injection of 3 mg/kg METH. Development of activity/stereotypy after repeated administration of METH alone or in combination with NBQX was evaluated in these three groups. Rats in the control were injected once daily for 10 days with vehicle and saline at an interval of 15 min. METH was withdrawn from all the animals for 7 days, and then a challenge of 2 mg/kg of METH was given. On days 1, 5, and 10 of the repeated injection session and the day of challenge, the rats were placed individually in an observation cage made of transparent plastic with demensions 310 × 360 × 175 mm, allowed 1 h acclimation prior to each injection and were videotaped thereafter for 30 s every 6–12 min. Another two groups, each of which consisted of six rats, were prepared, and they received once daily for 10 days an IP injection of either the vehicle or NBQX (20 mg/kg for the first 5 days and 40 mg/kg for the subsequent 5 days) and 15 min later, an injection of saline. Seven days after the last injection of this repeated administration, rats of the two groups were subjected to a challenge of 2 mg/kg of METH. Activity/stereotypy-involving sniffing and repetitive head movement was rated according to the rating systems of Ujike et al. (23,24) using a 0–5 score: 0: asleep or still; 1: locomotion with normal exploration and normal pattern of sniffing and head movement; 2: increased rate of sniffing and head movement associated with hyperlocomotion and rearing; 3: discontinuous stereotyped sniffing and stereotyped up–down head movement with periodic locomotion activity; 4: almost continuous stereotyped sniffing and head movement, but sometimes interrupted by brief locomotion; 5: continuous and intense stereotyped sniffing and repetitive head movement at one location only.

Experiment 2: Effect of NBQX on Acute Motor Changes Induced by a Single Dose of Cocaine and Cocaine-Induced Behavioral Sensitization

Four groups each consisting of six rats were used. Three groups received once daily for 10 days an IP injection of either the vehicle, 20, or 40 mg/kg NBQX, and 15 min later, an injection of 15 mg/kg cocaine. Development of activity/stereotypy after repeated administration of cocaine alone or in combination with NBQX was evaluated in these three groups. Rats in the control group of six rats were injected once daily for 10 days with the vehicle and saline at an interval of 15 min. Cocaine was withdrawn from all the animals for 7 days, and then a challenge of 15 mg/kg of cocaine was given. Behavior assessment was conducted in the same manner as in the Experiment 1.

Experiment 3: Effect of NBQX on Striatal Dopamine Release Induced by a Single Dose of METH

To examine the effect, if any, of NBQX on METH-induced dopamine release in the striatum, in vivo microdialysis was conducted. Eight animals were placed on a stereotaxic frame under pentobarbital sodium (50 mg/kg, IP) anesthesia, and a U-shaped dialysis probe, made as described previously (19), was implanted into the left anterior dorsal striatum [coordinates: rostral +2.4 mm, lateral +3.0 mm from the bregma, ventral −5.9 mm from the dural surface according to the atlas of Pellegrino et al. (16)]. Forty-eight hours after implantation of the probe, the animals were subjected to in vivo microdialysis. For this, the dialysis probe was perfused continuously at a flow rate of 2 μl/min with artificial cerebrospinal fluid (aCSF; Na, 140 mM; K, 3.35 mM; Ca, 1.26 mM; Mg, 1.15 mM; Cl, 151 mM, pH 6.5). Starting 2 h after the commencement of perfusion, perfusates began to be collected every 20 min, and the initial three fractions of the perfusates were used to determine the basal levels. At 60 min after start of perfusate collection, the rats were injected IP either the vehicle ($n = 4$) or 40 mg/kg NBQX ($n = 4$). Twenty minutes later, all the rats were injected IP with 3 mg/kg METH, and a sample collection was continued for 160 min thereafter. The perfusates were injected directly into a revesed-phase high-performance liquid chromatography column connected to a coulometric electro-

chemical detection system (ESA Co., USA; guard electrode = +0.4 V, oxidation electrode = +0.05 V, reduction electrode = −0.35 V) to measure the dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations. Dopamine and DOPAC were separated at 35°C by ion-pair reversed-phase chromatography using Cosmosil ODS-C18 5-μm mresin (Nakalai Co., Japan), with a mobile phase that comrised 0.05 M sodium dihydrogen phosphate buffer (pH 4.3), containing EDTA (0.05 mM), octanesulfonic acid (1 mM), 5% methanol, and 5% acetone. The mean concentrations of dopamine and DOPAC in the three fractions collected before either vehicle or NBQX injection were regarded as baseline levels, and the levels of dopamine and DOPAC shown in Fig. 7 were expressed as percentages relative to the respective baseline values.

Statistical Analysis

Scores of the activity/stereotypy (Experiments 1 and 2) were nonparametrically analyzed as reported previously (4,14). Briefly, the scores were analyzed by Kruskal–Wallis test at each postinjection time point, and when there was a statistically significant difference, Mann–Whitney *U*-test was used to determine differences between specific groups at each time. Repeated two-way ANOVA measures were used to evaluate whether postinjection changes in dopamine and DOPAC levels (Experiment 3) differed between groups. Differences at *p*-values of less than 0.05 were considered to be statistically significant.

RESULTS

Experiment 1: Effect of NBQX on Acute Motor Changes by a Single Dose of METH and METH-Induced Behavioral Sensitization

Figure 1 shows the result of acute effect of NBQX on motor changes induced by a single dose of METH in Experiment 1. Kruskal–Wallis tests revealed a significant difference from 6–60 min. A single injection of METH at dose of 3 mg/kg in combination with either vehicle or NBQX induced hyperactivity. Mann–Whitney *U*-tests revealed that neither 20 nor 40 mg/kg of NBQX affected acute METH-induced activity/stereotypy scores except for the inhibitory effect of 20 mg/kg of NBQX at 60 min. Figure 2 shows the result of developmet of activity/stereotypy at days 1, 5, and 10 during repeated administration of METH in combination with either vehicle or NBQX in Experiment 1. Kruskal–Wallis tests revealed a significant difference from 6–60 min, from 12–60 min, and at 12 min, and from 24–60 min for vehicle + METH, 20 mg/kg NBQX + METH and 40 mg/kg NBQX + METH groups, respectively. Mann–Whitney *U*-tests revealed that repeated administration of 3 mg/kg METH for 10 days induced progressive augmentation of activity/stereotypy scores, and that neither 20 nor 40 mg/kg of NBQX affected augmentation of activity/stereotypy scores. Figure 3A shows the result of challenge test with 2 mg/kg METH after repeated administration of METH in combination with either vehicle or NBQX. Kruskal–Wallis tests revealed a significant difference from 12–18 min and from 36–60 min. Mann–Whitney *U*-tests revealed that the activity/stereotypy of the groups previously exposed to repeated doses of vehicle + 3 mg/kg METH and NBQX + 3 mg/kg METH was significantly more intense than that of the group exposed to repeated doses of saline. The intensities of the activity/stereotypy in the groups pretreated with repeated doses of NBQX combined with 3 mg/kg METH did not differ significantly from that of the group pretreated with repeated doses of the vehicle + METH. An acute dose of NBQX at 40 mg/kg alone caused slight muscular hypotonia and ataxia. Figure 3B shows the result of challenge test with 2 mg/kg METH after repeated administraion of saline in combination with either NBQX or vehicle. Mann–Whitney *U*-tests revealed no significant difference in intensities of activity/stereotypy between these two groups.

Experiment 2: Effect of NBQX on Acute Motor Changes Induced by a Single Dose of Cocaine and Cocaine-Induced Behavioral Sensitization

Figure 4 shows the result of acute effect of NBQX on motor changes induced by a single dose of cocaine in Experiment 2. Kruskal–Wallis tests revealed a significant difference from 6–60 min. A single injection of cocaine at dose of 15 mg/kg in combination with either vehicle or NBQX induced hyperactivity. Mann–Whitney *U*-tests revealed that neither 20 nor 40 mg/kg of NBQX affected acute cocaine-induced activity/stereotypy scores. Figure 5 shows the result of developmet of activity/stereotypy at days 1, 5, and 10 during repeated administration of cocaine in combination with either vehicle or NBQX in Experiment 2. Kruskal–Wallis tests revealed a significant difference from 6–48 min and at 60 min, from 12–24 min, and from 6–24 min and at 36 min for vehicle + cocaine, 20 mg/kg NBQX + cocaine and 40 mg/kg NBQX + cocaine groups, respectively. Mann–Whitney *U*-tests revealed that repeated administration of 15 mg/kg cocaine for 10 days induced progressive augmentation of activity/stereotypy scores, and that neither 20 nor 40 mg/kg of NBQX affected augmen-

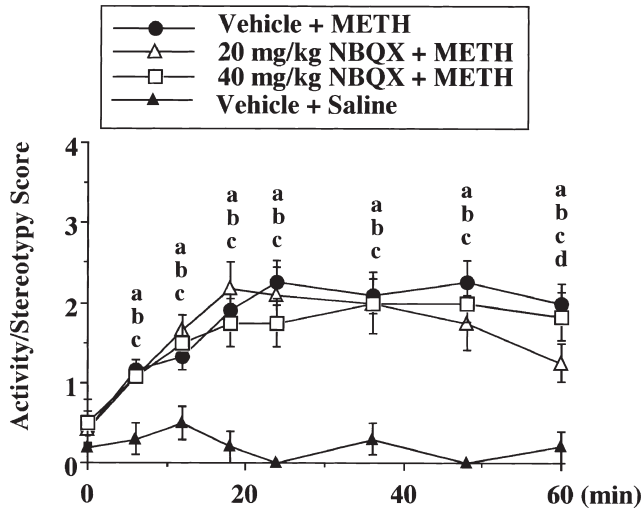


FIG. 1. Acute effect of NBQX on motor changes induced by a single dose of METH. Four groups each consisting of six rats were used. Three groups received an intraperitoneal (IP) injection of either vehicle, 20, or 40 mg/kg NBQX; then 15 min later, all three groups were injected IP with 3 mg/kg METH. The control group of six rats were injected with vehicle + saline. Each point represents the mean ± SEM for six rats per group. (a) The vehicle + METH group vs. The vehicle + saline group, $p < 0.02$ at 12 min, $p < 0.01$ at the rest of time points; (b) the 20 mg/kg NBQX + METH group vs. the vehicle + saline group, $p < 0.01$; (c) the 40 mg/kg NBQX +METH group vs. the vehicle + saline group, $p < 0.02$ at 12 min, $p < 0.01$ at the rest of the time points; (d) the vehicle + METH group vs. The 20 mg/kg NBQX + METH group, $p < 0.05$ (Kruskal–Wallis test and Mann–Whitney *U*-test).

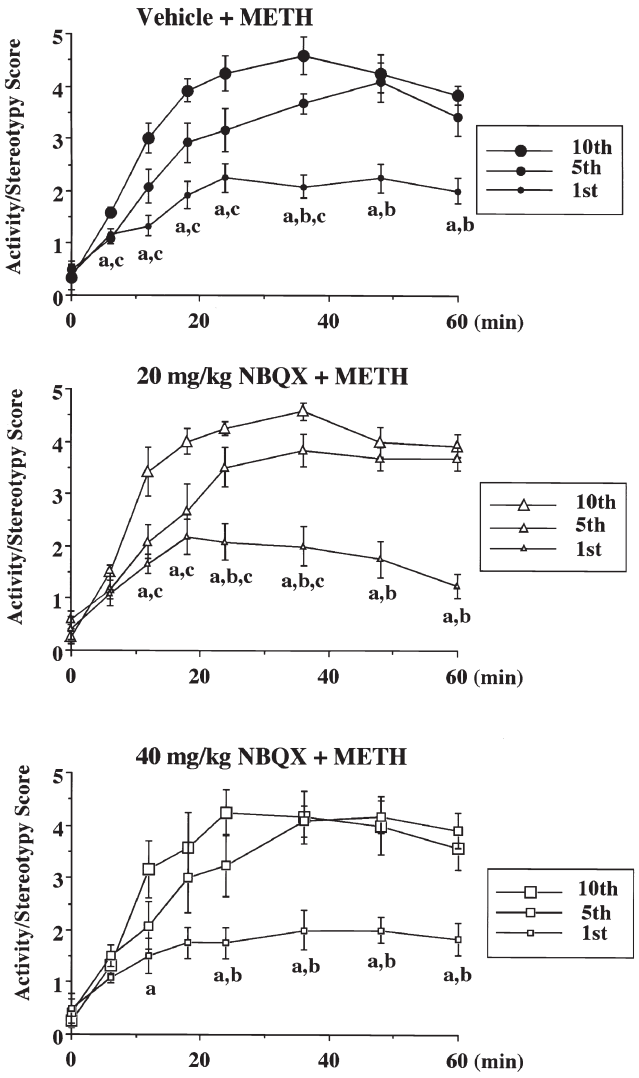


FIG. 2. Development of activity/stereotypy after repeated administration of METH in combination with either vehicle or NBQX. Three groups, each consisting of six rats, received once daily for 10 days an IP injection of either vehicle, 20, or 40 mg/kg NBQX, 15 min before an injection of 3 mg/kg METH. The activity/stereotypy was evaluated on days 1, 5, and 10 of the repeated injection session. Each point represents the mean ± SEM for six rats per group. (a) First vs. 10th injection, $p < 0.02$ at 6 min, $p < 0.01$ at the rest of the time points in the vehicle + METH group, $p < 0.05$ at 12 min, $p < 0.01$ at the rest of the time points in the 20 mg/kg NBQX + METH group, $p < 0.05$ at 12 min, $p < 0.02$ at 36–60 min, $p < 0.01$ at 24 min in the 40 mg/kg NBQX + METH group; (b) first vs. fifth injection, $p < 0.01$ at 36 and 48 min, $p < 0.02$ at 60 min in the vehicle + METH group, $p < 0.05$ at 24 min, $p < 0.01$ at 36–60 min in the 20 mg/kg NBQX +METH group, $p < 0.05$ at 24 min, $p < 0.01$ at 36–60 min in the 40 mg/kg NBQX + METH group; (c) 5th vs. 10th injection, $p < 0.01$ at 6 min, $p < 0.05$ at 12–36 min in the vehicle + METH group, $p < 0.05$ at 12–36 min in 20 mg/kg NBQX + METH group (Kruskal–Wallis test and Mann–Whitney *U*-test).

tation of activity/stereotypy scores, which were peaked between 12 and 24 min. Figure 6 shows the result of challenge test with 15 mg/kg cocaine after repeated administration of cocaine in combination with either vehicle or NBQX. Kruskal–Wallis tests revealed a significant difference from 6–54 min. Mann–Whitney *U*-tests revealed that the activity/stereotypy of the groups previously exposed to repeated doses of vehicle + 15 mg/kg cocaine and NBQX + 15 mg/kg cocaine was significantly more intense than that of the group exposed to repeated doses of saline. The intensities of the activity/stereotypy in the groups pretreated with repeated doses of NBQX combined with cocaine did not differ significantly from that of the group pretreated with repeated doses of the vehicle + cocaine at most of the time points.

Experiment 3: Effect of NBQX on Striatal Dopamine Release Induced by a Single Dose of METH

METH (3 mg/kg) induced a robust increase in the striatal extracellular dopamine and a decrease in the striatal extracellular DOPAC. Coadministration of NBQX did not have any effect on METH-induced increase in dopamine or decrease in DOPAC (repeated-measures of two-way ANOVA without group vs. time interaction, Fig. 7).

DISCUSSION

The present study investigated the effect of coadministration of NBQX, an AMPA receptor antagonist, on METH- and cocaine-induced behavioral sensitization. NBQX is a potent and selective antagonist of AMPA receptors with no apparent affinity for the glycine sites on the NMDA receptor complex (27), and is active in vivo when administered systemically (19,21). The dose range of 20–40 mg/kg (IP) NBQX that we chose according to our previous in vivo microdialysis study (19) is the same as was effective in suppressing ischemic excitotoxicity (3) and kindled seizures (13). In the latter study (13), the ability of NBQX to block central AMPA receptors was considered to reach its peak 30–60 min after injection. Thus, the interval between NBQX and METH or cocaine injections that was adopted in the present study may be justified by the conceivable temporal profile of AMPA receptor blockade deduced from the antiepileptic effect of NBQX (13).

A variety doses of psychostimulants affect differentially the number of locomotion versus intensity of stereotypy, because it is considered that mechanisms underlying locomotion induced by a small dose differ from those underlying stereotypy induced by an intermediate dose (20). Thus, repeated administration of the intermediate dose of AMPH (2.5–7.5 mg/kg/day) results in diminished locomotion during the early time course due to concomitant intensified stereotypy, whereas repeated administration of the smaller doses (0.5–1.5 mg/kg/day) of AMPH leads to predominant augmentation of locomotion (20). The doses of METH (3 mg/kg/day) and cocaine (15 mg/kg/day) used during the repeated administration session in the present study are apparently larger than a small dose that would result in simple augmentatation of locomotor response after repeated administration (20). Accordingly, the present study focusses on the quantitative changes in the intensity of stereotypy.

The major findings of the present study are that NBQX did not inhibit activity/stereotypy induced by acute administration of METH or cocaine, nor did its coadministration prevent the induction of behavioral sensitization induced by repeated administration of these psychostimulants. In addition, repeated administration of NBQX + saline had no effect on the intensity of activity/stereotypy induced by a challenge of METH, indicating lack of cross-sensitization between NBQX and METH. Vanover et al. (25) recently reported that NBQX at doses up to 40 mg/kg suppressed spontaneous locomotor

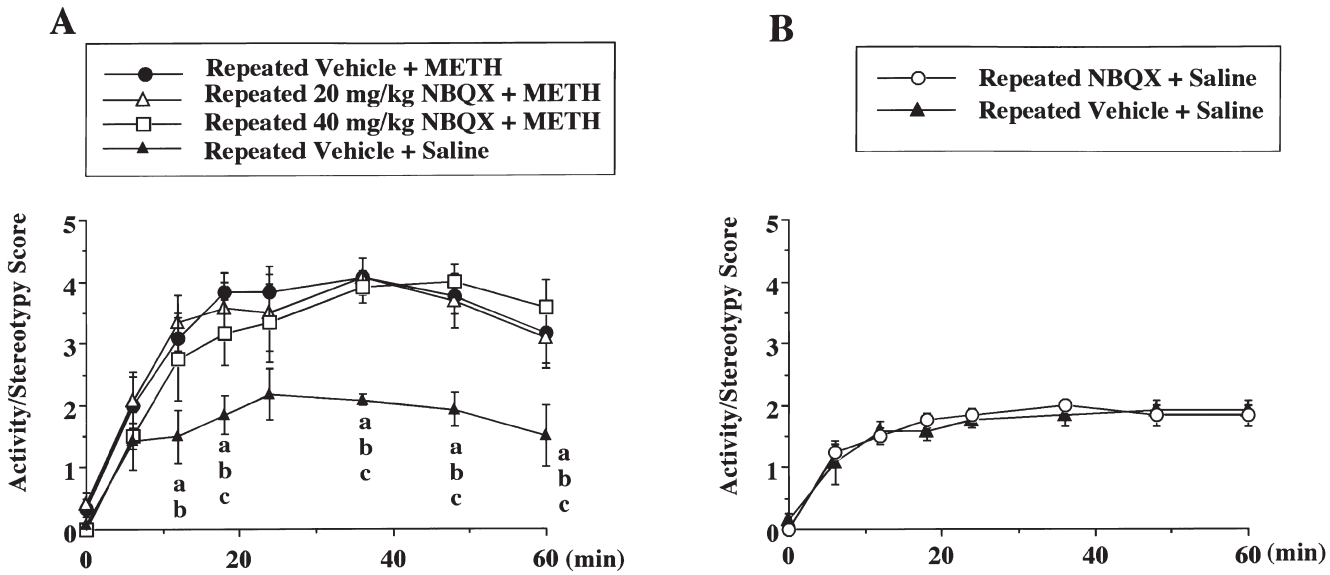


FIG. 3. (A) Challenge test with 2 mg/kg METH after repeated administration of METH in combination with either vehicle or NBQX. Rats randomly assigned to four groups each consisting of six rats received daily either vehicle + 3 mg/kg METH, 20 mg/kg NBQX + 3 mg/kg METH, 40 mg/kg NBQX + 3 mg/kg METH, or vehicle + saline during the 10-day repeated treatment session. Treatment was then withdrawn from all the animals for 7 days, followed by a challenge with 2 mg/kg of METH. Each point represents the mean ± SEM for six rats per group. (a) repeated vehicle + METH group vs. repeated vehicle + saline group, $p < 0.02$ at 12 min, $p < 0.01$ at 18, 36, and 48 min, $p < 0.05$ at 60 min; (b) repeated 20 mg/kg NBQX + METH group vs. repeated vehicle + saline group, $p < 0.01$ at 12, 18, and 36 min, $p < 0.02$ at 48 min, $p < 0.05$ at 60 min; (c) repeated 40 mg/kg NBQX +METH group vs. repeated vehicle + saline group, $p < 0.05$ at 18 min, $p < 0.01$ at 36–60 min (Kruskal–Wallis test and Mann–Whitney *U*-test). (B) Challenge test with 2 mg/kg METH after repeated administration of saline in combination with either NBQX or vehicle. Rats randomly assigned to two groups each consisting of six rats received daily either NBQX (20 mg/kg for the first 5 days and 40 mg/kg for the subsequent 5 days) + saline or vehicle + saline during the 10-day repeated treatment session. Treatment was then withdrawn from all the animals for 7 days, followed by a challenge with 2 mg/kg of METH. Each point represents the mean ± SEM for six rats per group.

activity, but not AMPH-induced locomotion or stereotypy. Lack of inhibitory effect of NBQX on acute AMPH-induced stereotypy is consistent with the results depicted in Figs. 1 and 4 in the present study. As reported in the previous studies (11,13), we observed that NBQX induced slight mucular hypotonia and ataxia. It cannot be ruled out that this motor-incapacitating effects may contribute to suppression of spontaneous locomotion caused by NBQX.

Using 6,7-dinitroquinoxaline-2,3-dione (DNQX), which is able to bind to glycine sites on the NMDA receptor complex as well as AMPA/KA receptors (9,15,27), Karler et al. (7) reported that its coadministration with each dose of repeated AMPH decreased the percentages of mice exhibiting stereotypy induced by a challenge of AMPH alone, and suggested that non-NMDA ionotropic EAA receptors might be involved in the induction of behavioral sensitization. However, Li et al. (11) recently reported that, following a challenge dose of AMPH, neither a proportion of rats exhibiting stereotypy nor temporal profile of locomotion counts was affected by a coadministration of NBQX with each dose of AMPH. The METH results of the present study are mostly in agreement with those of AMPH in a report by Li et al. (11), although the latter investigators noted that the double NBQX pretreatments before and after a each dose of AMPH prevented the development of poststereotypy ambulatory hyperactivity. In the present study, there appears to be less numbers of time points corresponding to significant differences among 1st, 5th and 10th days during repeated doses of NBQX + cocaine than during repeared doeses of vehicle + cocaine (Fig. 5). It is noted that cocaine-induced behavior was peaked around 20 min after injection, and tapered thereafter. Significant differences observed around this time point in the NBQX + cocaine groups in Fig. 5, in conjunction with the result of a cocaine challenge (Fig. 6), suggest that NBQX did not prevent the induction of cocaine-induced behavioral sensitization. This conclusion differs from the study by Li et al. (11). This discrepancy may be ascribed to diffrences between the two studies in doses of cocaine used [7.5 mg/kg in the study by Li et al. (11), 15 mg/kg in the present study], the method of behavioral assessment [locomotion count in the study by Li et al. (11), activity/stereotypy score in the present study], and length of the abstinece period duration, which was set 7 days to exclude the effect of prior administration of NBQX in the present study.

The striatum receives extensive glutamatergic inputs derived from the cerebral cortex, and different populations of neurons in the striatum differentially express AMPA receptor subunits, which appear to mediate many of the effects of glutamate in the striatum (2). In addition, a number of studies have suggested that AMPA receptors participate in corticostriatal glutamate afferent-mediated tonic modulation of extracellular dopamine levels in the striatum (19,26). On the contrary, it has been recently reported that agents ("ampakine") that selectively enhance currents mediated by AMPA receptors attenuate the stereotypic rearing in rats induced by acute administration of METH (10). In this context, the microdialysis result of the present study that NBQX does not modify acute METH-induced dopamine efflux in the striatum

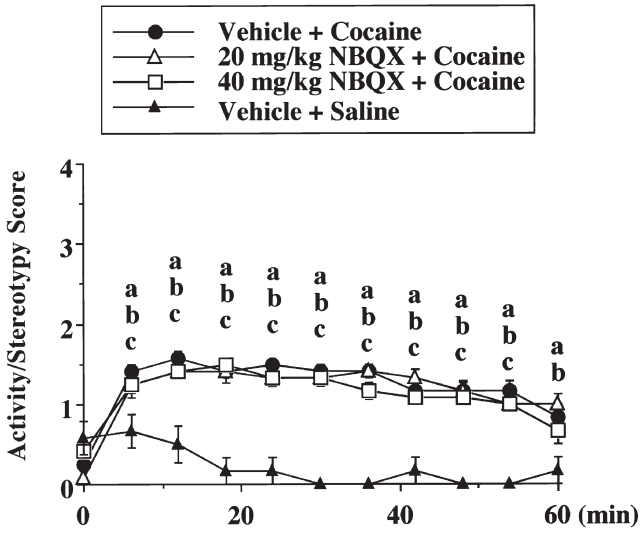


FIG. 4. Acute effect of NBQX on motor changes induced by a single dose of cocaine. Four groups each consisting of six rats were used. Three groups received an IP injection of either vehicle, 20, or 40 mg/kg NBQX; 15 min later, all the three groups were injected with IP 15 mg/kg cocaine. The control group of six rats were injected with vehicle + saline. Each point represents the mean ± SEM for six rats per group. (a) The vehicle + cocaine group vs. vehicle + saline group, $p < 0.05$ at 60 min, $p < 0.01$ at the rest of the time points; (b) the 20 mg/kg NBQX + cocaine group vs. vehicle + saline group, $p < 0.05$ at 6 min, $p < 0.01$ at the rest of the time points; (c) the 40 mg/kg NBQX + cocaine group vs. vehicle + saline group, $p < 0.05$ at 6 min, $p < 0.01$ at the rest of the time points (Kruskal–Wallis test and Mann–Whitney *U*-test).

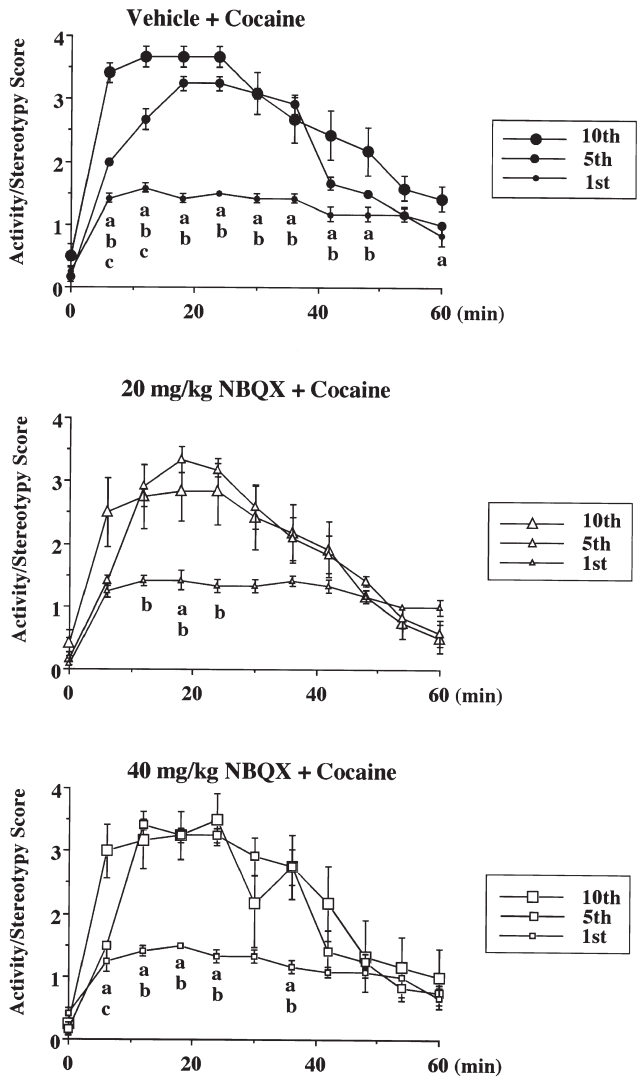


FIG. 5. Development of activity/stereotypy after repeated administration of cocaine in combination with either vehicle or NBQX. Three groups received once daily for 10 days an IP injection of either vehicle, or 20 or 40 mg/kg NBQX, 15 min before an injection of 15 mg/kg cocaine. The activity/stereotypy was evaluated on days 1, 5, and 10 of the repeated injection session. Each point represents the mean ± SEM for six rats per group. (a) First vs. 10th injection, $p < 0.01$ at 6–30 min, $p < 0.05$ at 36–48 and 60 min in the vehicle + cocaine group, $p < 0.05$ at 18 min in the 20 mg/kg NBQX + cocaine group, $p < 0.05$ at 6,12, and 36 min, $p < 0.01$ at 18 and 24 min in the 40 mg/kg NBQX + cocaine group; (b) first vs. fifth injection, $p < 0.01$ at 6–36 min, $p < 0.02$ at 42 and 48 min in the vehicle + cocaine group, $p < 0.01$ at 12–24 min in the 20 mg/kg NBQX + cocaine group, $p < 0.01$ at 12–24 min and 36 min in the 40 mg/kg NBQX + cocaine group; (c) 5th vs. 10th injection, $p < 0.01$ at 6 and 12 min in the vehicle + cocaine group, $p < 0.05$ at 6 min in the 40 mg/kg NBQX + cocaine group (Kruskal–Wallis test and Mann–Whitney *U*-test).

(Fig. 7) further substantiates that AMPA receptor blockade after IP administration of NBQX plays a minor or nonspecific role in affecting AMPH/METH-mediated behaviors. It is likely that AMPA receptors exert tonic facilitative modulation of basal dopamine in the striatum (19), but that this portion of extracellular dopamine apparently differs from METH-induced dopamine release.

In spite of the previous (11) and present studies, the effect of glutamate acting on AMPA receptors on dopamine-mediated behaviors remains to be explored in further studies using other selective AMPA receptor antagonists and AMPA receptor modulators. Accumulating evidence suggests that assemblies of glutamate receptor subunits are differentially distributed among subpopulations of striatal neurons (2). Different pharmacological properties of selective AMPA receptor antagonists in dopmaine-mediated behavior paradigms (25) may be ascribed to preferential affinity for a particular subunit assembly.

Several lines of evidence have indicated that the expression of behavioral sensitization to psychostimulants is associated with alterations of EAA neurotransmission. Stephans et al. (22) reported that high potassium-induced glutamate efflux in the prefrontal cortex was greater in METH-pretreated rats than controls. Pierce et al. (17) recently reported that microinjection of AMPA into the nucleus accumbens core induced greater motor activity in rats exhibiting sensitization following chronic cocaine administration than in nonsensitized rats. These investigators also found that a cocaine challenge increased the extracellular level of glutamate in the core in rats sensitized to cocaine, and that the sensitized motor response to cocaine challenge was prevented by pretreatment with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), an AMPA/KA receptor antagonist. Notably, it has also recently been reported that repeated AMPH administration alters the expres-

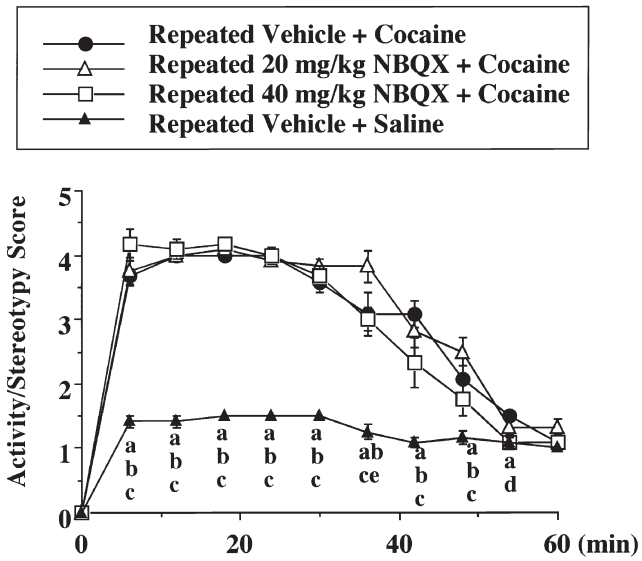
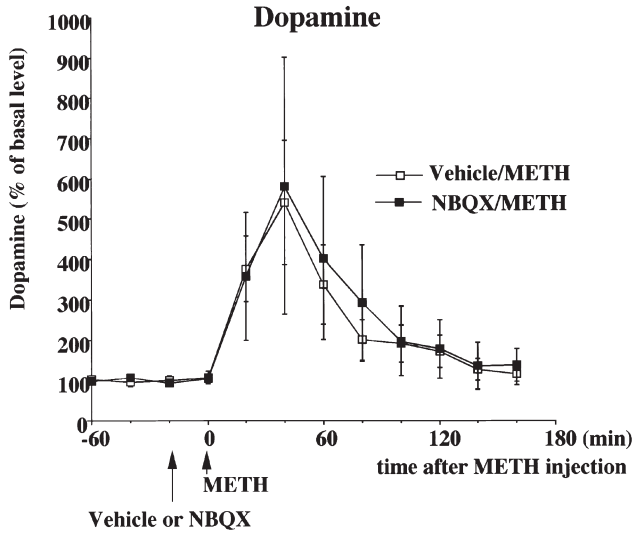


FIG. 6. Challenge test with 15 mg/kg cocaine after repeated administration, in combination with either vehicle or NBQX. Rats randomly assigned to four groups, each consisting of six rats, received daily either vehicle + 15 mg/kg cocaine, 20 mg/kg NBQX + 15 mg/kg cocaine, 40 mg/kg NBQX + 15 mg/kg cocaine, or vehicle + saline during the 10-day repeated treatment session. Treatment was withdrawn from all animals for 7 days, followed by a challenge with 15 mg/kg of cocaine. Each point represents the mean ± SEM for six rats per group. (a) Repeated vehicle + cocaine group vs. repeated vehicle + saline group, $p < 0.01$ at 6–42 and 54 min, $p < 0.05$ at 48 min; (b) repeated 20 mg/kg NBQX + cocaine group vs. repeated vehicle + saline group, $p < 0.01$ at 6–48 min; (c) repeated 40 mg/kg NBQX + cocaine group vs. repeated vehicle + saline group, $p < 0.01$ at 6–36 min, $p < 0.02$ at 42–48 min; (d) repeated vehicle + cocaine group vs. repeated 40 mg/kg NBQX + cocaine group, $p < 0.01$ at 54 min; (e) repeated 20 mg/kg NBQX + cocaine group vs. repeated 40 mg/kg NBQX + cocaine group, $p < 0.05$ at 36 min (Kruskal–Wallis test and Mann–Whitney *U*-test).



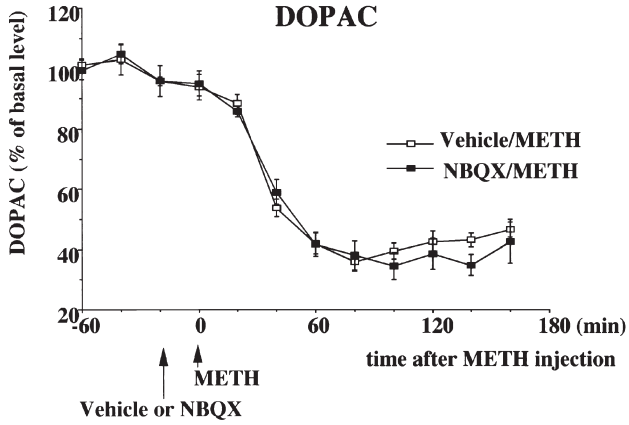


FIG. 7. Effect of NBQX on striatal dopamine release and DOPAC reduction by a single dose of METH. Rats were injected IP with either vehicle ($n = 4$) or 40 mg/kg NBQX ($n = 4$) 20 min before an IP injection with 3 mg/kg METH, and striatal perfusates were analyzed for dopamine and DOPAC. Each point represents the mean ± SEM for four rats per group. No significant differences between the two groups in METH-induced increase in dopamine or decrease in DOPAC (repeated measures of two-way ANOVA without group vs. time interaction).

sion of mRNA for AMPA receptor subunits in rat nucleus accumbens and prefrontal cortex (12). These findings suggest that enhanced EAA neurotransmission in the ventral striatum and prefrontal cortex may be involved in the expression of behavioral sensitization.

In conclusion, the present study demonstrates that NBQX, a selective antagonist of AMPA receptors, does not inhibit the acute activity/stereotypy effect of METH (3 mg/kg) or cocaine (15 mg/kg), nor does it prevent the induction of behavioral sensitization of activity/stereotypy induced by chronic administration of these psychostimulants. These results, however, does not exclude the possibility that AMPA receptors are somehow involved in long-term neuronal plasticity involved in the behavioral sensitization.

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