Modulation of Cocaine- and Methamphetamine-Induced Behavioral Sensitization by Inhibition of Brain Nitric Oxide Synthase

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ABSTRACT
Evidence suggests the existence of multiple interactions between dopamine, glutamate and nitric oxide (NO) in brain structures associated with psychomotor stimulation. The present study was undertaken to investigate the effect of the relatively selective inhibitor of the neuronal nitric oxide synthase (NOS) isoform, 7-nitroindazole (7-NI), on the development of sensitization to the locomotor stimulating effect of cocaine and methamphetamine (METH). Male Swiss Webster mice that received 15 mg/kg cocaine once a day for 5 days developed a marked locomotor sensitization to a challenge cocaine (15 mg/kg) or cross-sensitization to a challenge METH (0.5 mg/kg) injection given after a 10-day drug-free period. This treatment also produced a context-dependent sensitization as evident by the sensitized response to a challenge saline injection. Pretreatment with 7-NI (25 mg/kg) 30 min before cocaine administration (5 days) completely blocked the induction of sensitization to cocaine, the cross-sensitization to METH and the conditioned locomotion induced by cocaine. 7-NI when given alone, either acutely or for 5 days, had no significant effect on the locomotor activity of animals. Animals treated with METH (1.0 mg/kg) for 5 days developed marked sensitization to challenge METH (0.5 mg/kg), cross-sensitization to challenge cocaine (15 mg/kg) and context-dependent locomotion. Pretreatment with 7-NI (25 mg/kg) attenuated the sensitized response to METH and the cross-sensitization to cocaine as revealed after a 10-day drug-free period. However, the METH-induced conditioned locomotion was unaffected by the pretreatment with 7-NI. The present study supports the role of brain NO in the development of sensitization to both psychostimulants, cocaine and METH. However, it appears that the inability of 7-NI to completely abolish the sensitized responses induced after METH administration is the result of the resistible conditioned locomotion caused by METH, but not by cocaine.

Repeated administration of psychostimulants such as cocaine and amphetamine to rodents causes the development of “reverse tolerance” known as sensitization. The increased sensitivity to the locomotor stimulating effect of these drugs (behavioral sensitization) is believed to be relevant to the psychopathology, neurotoxicity (Post et al., 1988) and drug addiction and craving (Robinson and Berridge, 1993) that develop in humans abusing psychostimulants.

Although evidence suggests that enhanced dopamine transmission in the nucleus accumbens and striatum is associated with behavioral sensitization to cocaine (Kalivas and Stewart, 1991; Weiss et al., 1992) and amphetamine (Robinson and Becker, 1986; Robinson et al., 1988), the role of glutamatergic transmission is also apparent. Several studies indicate that blockade of the NMDA type of glutamate receptors attenuates the development of behavioral sensitization to cocaine and amphetamine (Karler et al., 1989; 1990; 1994; Wolf and Jeziorski, 1993; Wolf et al., 1994). Increase in excitatory amino acid transmission in the nucleus accumbens (Pierce et al., 1996) and up-regulation of the NMDA receptors (Itzhak and Stein, 1992) may underlie some of the processes in the development of cocaine sensitization. Also, the neurotoxicity produced by repeated METH administration has been associated with an increase in glutamate release in the striatum (Nash and Yamamoto, 1992).

The relationship between activation of the NMDA receptor and stimulation of the neuronal isoform of NOS (Garthwaite, 1991; Snyder, 1992) prompted us to investigate the effect of NOS inhibitors on the development of sensitization to the convulsive effect of cocaine (cocaine kindling). We reported that pretreatment with L-NAME or Nω-nitro-L-arginine (NO-Arg) completely blocked the development of cocaine kindling and protected the animals against cocaine-induced death (Itzhak, 1993, 1994). Similarly, it has been reported that L-NAME attenuated the development of sensitization to the convulsive effect of cocaine (Itzhak et al., 1994). The present study supports the role of brain NO in the development of sensitization to both psychostimulants, cocaine and METH. However, it appears that the inability of 7-NI to completely abolish the sensitized responses induced after METH administration is the result of the resistible conditioned locomotion caused by METH, but not by cocaine.
locomotor stimulating effect of cocaine (Pudiak and Bozarth, 1993). However, conflicting results were reported on the effect of l-NAME on the induction of behavioral sensitization to amphetamine (Stewart et al., 1994) and METH (Ohno and Watanabe, 1995; Abekawa et al., 1995; Inoue et al., 1996).

The NOS inhibitors used in the studies above (e.g., l-NAME and NO-Arg) are not selective for the neuronal NOS isoform, and the inhibition of the endothelial NOS isoform may alter cocaine and amphetamine pharmacokinetics. Recently, however, we reported that the relatively selective inhibitor of the neuronal NOS isoform, 7-NI (Moore et al., 1993) blocked the process of cocaine-induced kindling (Itzhak, 1996). In addition, we found that 7-NI blocked METH-induced neurotoxicity in the striatum (Itzhak and Ali, 1996). The development of cocaine kindling and METH-induced neurotoxicity usually requires the administration of relatively high doses of cocaine and METH, respectively. The present study was undertaken to investigate whether blockade of brain NOS by 7-NI attenuates the induction of behavioral sensitization to cocaine and METH, a process which requires the administration of relatively low doses of the psychostimulants. Results indicate that 7-NI blocks the induction of sensitization to cocaine, the cross-sensitization with METH and cocaine-induced conditioned locomotion. However, 7-NI only partially blocked METH-induced sensitization; the 7-NI-resistant conditioned locomotion produced by METH, but not by cocaine, may explain the differential effect of 7-NI on cocaine- and METH-induced sensitization.

**Material and Methods**

**Drugs.** Cocaine-HCl and METH-HCl were purchased from Sigma Chemical Co. (St. Louis MO) and prepared in saline solution (0.9% NaCl). 7-NI was purchased from Research Biochemicals International (Natick, MA) and dissolved in a solution containing dimethyl sulfoxide/propylene glycol/distilled water (1:3:6) (considered as "vehicle").

**Animals and schedule of drug administration.** Male Swiss Webster mice (28–31 g; Charles River, Wilmington, MA) were maintained on a 12-h light/dark lighting schedule and housed in groups of 4 with free access to food and water. The principles of laboratory animal care (NIH publication No. 85–23, revised 1985) were followed. Animals’ weights were monitored daily before drug administration. Drug solutions were freshly prepared daily and administered by intraperitoneal (i.p.) injection in a volume of 0.1 ml/10 g of body weight. All drug administrations were performed once a day between 11:00 A.M. and 5:00 P.M. At least 10 animals were used for each drug treatment. Animals were divided into six groups, and during the first 5 days of the experiment each group received two injections which were separated by a 30-min period: (1) vehicle/saline, (2) 7-NI (25 mg/kg)/saline, (3) vehicle/cocaine (15 mg/kg), (4) 7-NI (25 mg/kg/cocaine (15 mg/kg), (5) vehicle/METH (1.0 mg/kg), (6) 7-NI(25 mg/kg)/METH (1.0 mg/kg). Because locomotor activity on day 1 was compared with that on day 5, the drug treatments on days 1 and 5 were delivered in the animal’s test cage (see below for details). On days 2, 3, and 4 the drug treatment was delivered in the animal’s home cage. On days 6 and 7, animals remained drug free in their home cage. On day 8, animals were transferred to the test cage and received a single injection of saline (test for conditioned locomotion). On days 9 through 14, animals remained drug free in their home cage. On day 15, 10 days after the drug treatment was stopped, each group of animals received a challenge injection of either cocaine (15 mg/kg) or METH (0.5 mg/kg). Accordingly, on day 15, separate groups of cocaine-experienced animals were tested for sensitization to cocaine or for cross-sensitization to METH. Similarly, on day 15, METH-experienced animals were tested for sensitization to METH or for cross-sensitization to cocaine.

**Measurement of locomotor activity.** To compare the acute behavioral effects of cocaine (or METH) with the repeated drug administrations, each animal’s locomotor activity was measured on days 1 and 5. The test for conditioned locomotion was done on day 8, and the test for sensitization on day 15, i.e., 10 days after the drug treatments were stopped. On the test day, 2 h before the experiment began, animals were transferred to a room separate from the animal colony room, where the test cages were kept. Animals were taken from the home cage, given the first injection, and immediately placed in individual test cages; this was a standard transparent rectangular redent cage (42 × 24 × 20 cm high). Routinely, animals were allowed a 30-min habituation period to the new environment before the second injection was given, a time point at which animals’ activities began to be recorded. Based on several experiments in which animals’ locomotor activities were monitored during the first 30 min in the test cage, it appears that animals usually return to their normal activity within this time period. After spending 20 to 30 min in the test cage, total and ambulatory counts were stabilized at a relatively low pace compared with the first 10 to 15 min. Animals’ activity was monitored by activity meter, Opto-Varimex Mini (Columbus Instruments, Columbus, OH), which consists of an array of 15 infrared emitter/detector pairs, spaced at 2.65-cm intervals, measuring activity along a single axis of motion. Each emitter and detector were mounted alongside the length of the cage (42 cm long). Both the total counts and the ambulatory counts were recorded and transferred by a computer counter interface to an IBM computer. Ambulatory counts correspond to horizontal activity, whereas the difference between the total and ambulatory counts corresponds to vertical activity. Counts were usually registered every 10 min for a period of 30 to 120 min.

**Statistical analysis.** The results of the time course of acute drug administration and the comparisons between the effect of the drugs on day 1 vs. day 5 were analyzed by a two-way ANOVA (drug treatment × time) with time as the repeated measure. Bonferroni multiple comparison adjustment was performed to determine differences between specific groups. When the effect of the various drugs on the behavior of animals was analyzed at a fixed time point, one-way ANOVA followed by post hoc Neuman-Keuls test was performed.

**Results**

**Effect of 7-NI on response of animals to acute and repeated cocaine administration.** The administration of a single injection of cocaine (15 mg/kg) to Swiss Webster mice resulted in a marked increase in ambulatory counts, compared with the administration of vehicle/saline. Cocaine’s peak effect was 10 min after i.p. administration; within 30 to 40 min ambulatory counts reached a plateau which was similar to the activity of control animals (fig. 1). A two-way ANOVA (drug treatment × time) revealed a significant drug effect (P = .0075), time effect (P = .0089) and interaction (P = .0115). The effects of two different doses of 7-NI on locomotor activity produced by acute cocaine administration were tested (fig. 1). A relatively low dose of 7-NI (15 mg/kg) resulted in approximately 50% reduction in ambulatory counts compared with the vehicle/cocaine group (7-NI effect, P = .0361; time effect, P = .0019; interaction, P = .08). However, a dose of 25 mg/kg 7-NI, which we previously found to reduce brain NOS activity by about 75% (Itzhak, 1996) and to protect mice against METH-induced neurotoxicity (Itzhak and Ali, 1996), completely abolished the locomotor stimulation caused by acute cocaine administration (fig. 1). Comparison between vehicle/cocaine and 7-NI/cocaine groups yielded the
Effect of 7-NI on the response of animals to acute cocaine administration. Cocaine (15 mg/kg) resulted in a marked increase in ambulatory counts compared with vehicle/saline control group. A two-way ANOVA (drug treatment × time) with time as a repeated measure yielded a significant interaction (P < .0115). Although 15 mg/kg 7-NI (closed dots) partially attenuated cocaine’s effect (P = .0361), a dose of 25 mg/kg (open triangles) completely abolished cocaine-induced locomotion (P = .0085). 7-NI (25 mg/kg; closed triangles) had no significant effect on the locomotor activity of animals.

The effect of cocaine, 7-NI or 7-NI/cocaine on the locomotor activity of animals was next examined after 5 days of drug administration. The comparison between the acute vs. the repeated drug administrations is presented in figure 2. A two-way ANOVA (drug treatment × time) with time as a repeated measure yielded a significant interaction (P = .0004). On day 1, ambulatory counts in the vehicle/cocaine group were significantly higher than the counts registered in the vehicle/saline, 7-NI/saline and 7-NI/cocaine groups (P = .001); the last three groups did not differ significantly from one another. On day 5, ambulatory counts in the vehicle/cocaine group were significantly higher than the counts registered on day 1 for the same group (P < .0001). The vehicle/cocaine-pretreated group was more sensitive to the challenge cocaine injection than the other groups tested (P < .05), which did not differ significantly from one another (fig. 3). These findings suggest that 7-NI blocked the induction of sensitization to the locomotor stimulating effect of cocaine. The results also indicate that the pretreatment with 7-NI alone for 5 days (7-NI/saline group) did not produce a significant change in the locomotor activity of animals in response to a challenge cocaine administration (fig. 3).

To investigate whether 7-NI blocked the development of cross-sensitization to METH, three additional groups of animals pretreated with vehicle/saline, vehicle/cocaine and 7-NI/cocaine were challenged on day 15 to 0.5 mg/kg METH. Results presented in figure 4 show that there was a significant drug-pretreatment effect F [2,27] = 64.98, P < .0001. The vehicle/cocaine-pretreated group was significantly more sensitive to 7-NI/cocaine groups (fig. 3). One-way ANOVA revealed a significant drug-pretreatment effect F[3,36] = 32.36, P < .0001. The vehicle/cocaine-pretreated group was more sensitive to the challenge cocaine injection than the other groups tested (P < .05), which did not differ significantly from one another (fig. 3).

Fig. 1. Effect of 7-NI on the response of animals to acute cocaine administration. Cocaine (15 mg/kg) resulted in a marked increase in ambulatory counts compared with vehicle/saline control group. A two-way ANOVA (drug treatment × time) with time as a repeated measure yielded a significant interaction (P = .0115). Although 15 mg/kg 7-NI (closed dots) partially attenuated cocaine’s effect (P = .0361), a dose of 25 mg/kg (open triangles) completely abolished cocaine-induced locomotion (P = .0085). 7-NI (25 mg/kg; closed triangles) had no significant effect on the locomotor activity of animals.

Fig. 2. Effect of 7-NI (25 mg/kg), cocaine (15 mg/kg) and 7-NI/cocaine administration on mice locomotor activity: comparison between day 1 and day 5. A two-way ANOVA (drug treatment × time) with time as the repeated measure yielded a significant interaction (P = .0004). Bonferroni multiple comparison adjustment was performed to determine specific group differences. On day 1, ambulatory counts in the vehicle/cocaine group were significantly higher than vehicle/saline (*P = .001). On day 5, ambulatory counts in the vehicle/cocaine group were significantly higher than the counts registered on day 1 for the same group (*P = .001).

Fig. 3. Effect of a challenge cocaine injection on day 15. After the 5-day drug treatment period animals remained drug-free for 10 days and on day 15 received a challenge cocaine injection (15 mg/kg). One-way ANOVA yielded a significant drug-pretreatment effect: F[3,36] = 32.36, P < .0001. The vehicle/cocaine pretreated group was more sensitive to the challenge cocaine injection than the other groups tested (P < .05, Neuman-Keuls test) which did not differ significantly from one another.
the METH injection than the two other groups (P < .05), which did not differ significantly from one another. These findings suggest that 7-NI blocked the induction of cross-sensitization to METH.

Effect of 7-NI on the response of animals to acute and repeated METH administration. The administration of 1.0 mg/kg METH to Swiss Webster mice resulted in a marked increase in the locomotor activity of animals, with a peak effect after 30 min and a trough after 60 min. The time course of METH and 7-NI/METH effect on days 1 and 5 is described in figure 5. In subsequent figures where the effect of METH is described, cumulative counts for a 60-min period are reported. Because the dose of 25 mg/kg 7-NI efficiently blocked the induction of sensitization to cocaine, the same dose of 7-NI was used for the experiments with METH. The effect of 7-NI on the acute locomotor effect of METH is described in figures 5 and 6 (left panel). On day 1 (acute effect) the locomotor stimulation produced by vehicle/METH was significantly greater than the one caused by vehicle/saline or 7-NI/METH administration (P = .0001), which suggests that 7-NI inhibited the acute locomotor stimulation caused by METH. Although a trend in increased locomotor activity in the 7-NI/METH group (1,982 ± 321) was observed compared with the vehicle/saline group (788 ± 85), a two-way ANOVA followed by Bonferroni analysis revealed that the difference was not statistically significant. The comparison between animals’ responses on day 1 and day 5 to vehicle/METH and 7-NI/METH is illustrated in figure 6. A two-way ANOVA (drug treatment × time) with time as the repeated measure followed by Bonferroni multiple comparison revealed that on day 5 the vehicle/METH group was significantly more hyperactive than on day 1 (P = .0001). Although on day 5 the 7-NI/METH group was less active than the vehicle/METH group, the comparison between the locomotor activity on days 1 and 5, within the 7-NI/METH group, revealed that on day 5 animals were significantly more active than on day 1 (P = .0001). Thus, unlike the results of the 7-NI/cocaine group (fig. 2), it appears that the 7-NI/METH group became sensitized to METH injection on day 5.

The test for sensitization was performed on day 15, 10 days after the drug treatment was stopped (fig. 7). A challenge
injection of METH (0.5 mg/kg) to vehicle/saline, vehicle/ METH and 7-NI/METH groups revealed a significant drug pretreatment effect $F(2,27) = 56.98$, $P < .0001$. The vehicle/ METH group was significantly more sensitive to the challenge METH injection than both the control group ($P < .05$) and the 7-NI/METH group ($P < .05$). However, the 7-NI/ METH group was significantly more sensitive to METH challenge than the vehicle/saline group ($P < .05$). This finding suggests that 7-NI attenuated, but did not abolish completely, METH-induced sensitization. Results of the cross-sensitization between additional METH-experienced animals and cocaine (fig. 8) confirm this conclusion. A challenge dose of cocaine (15 mg/kg) given to the vehicle/METH group resulted in a sensitized response compared with both the control and 7-NI/METH groups ($P < .05$; fig. 8). However, once again, the 7-NI/METH group was more sensitive to the challenge cocaine than the vehicle/saline control group ($P < .05$; fig. 8).

**Test for conditioned locomotion.** Pairing the injection stimulus with the particular environment of drug administration is known to produce conditioning or context-dependent locomotion. To investigate if the two test sessions (on days 1 and 5) induced conditioned locomotion on day 8, animals were subjected to a saline injection in the test cages. Animals were first allowed a 30-min habituation period in the test cage before a single saline injection was delivered. Locomotor activity was immediately recorded for a period of 30 min. Results presented in figure 9 indicate the following. First, the saline injection resulted in significantly greater locomotor activity in the vehicle/cocaine group than the saline/vehicle-, 7-NI/saline- and 7-NI/cocaine-pretreated groups ($P < .05$ Neuman-Keuls test for the comparison with each of the three groups). Second, no significant differences were observed between the vehicle/saline and 7-NI/cocaine groups. This finding suggests that 7-NI blocked the conditioned locomotion produced by cocaine. Third, ambulatory counts of the vehicle/METH and 7-NI/METH groups were similar (1,749 ± 167 and 1,543 ± 198, respectively) and significantly higher that of the control group (498 ± 60; $P < .05$). This finding suggests that the conditioned locomotion produced by METH was not affected by 7-NI pretreatment.

**Discussion**

The major findings of the present study are: (1) 7-NI completely blocked the induction of behavioral sensitization to cocaine, the cross-sensitization to METH and the conditioned locomotion induced by cocaine. (2) 7-NI partially attenuated the development of behavioral sensitization produced by repeated METH administration, as evident by the responses to challenge injections of METH and cocaine. (3) 7-NI did not affect the conditioned locomotion induced by METH administration. Because 7-NI is considered as a relatively selective inhibitor of the neuronal NOS isoform (Moore et al., 1993), the present study supports the role of brain NOS in the development of behavioral sensitization to psychostimulants.

A few studies have suggested the role of brain NOS in the process of sensitization to the effects of cocaine. Initially, we showed that pretreatment with L-NAME completely blocked the development of increased sensitivity to the convulsive effect of cocaine (cocaine kindling) and the augmentation in lethality rate caused after repeated administration of relatively high doses of cocaine (40 mg/kg) (Itzhak, 1993, 1994). Also, Pudiak and Bozarth (1993) and Kim and Park (1995) reported that L-NAME blocked the development of locomotor sensitization to cocaine. However, two major issues remained to be resolved. First, L-NAME is not a selective inhibitor for the neuronal NOS isoform; inhibition of the endothelial isoform may alter cocaine pharmacokinetiks and hamper the study of the role of brain NOS in the effects of psychostimulants. Second, Pudiak and Bozarth (1993) administered L-NAME and cocaine for a relatively long time, 21 days, and the challenge cocaine injection was given just 72 h after the drug treatments were stopped. The prolonged treatment with L-NAME and the relatively short drug-free period did not assure that on the test day animals were indeed drug-free (e.g., L-NAME-free). Accordingly, L-NAME may have inhibited the expression rather than the induction of sensitization to cocaine. In the present study, not only was the NOS inhibitor administered for a relatively short time (5 days), but most importantly, the challenge injection of cocaine was given 10 days after the drug treatment was stopped. Our...
preliminary studies indicate that at this time point brain
NOS activity was restored to normal levels (Y. Itzhak, unpublished observations). Thus, the current design of drug
administration may rule out potential alteration in cocaine
pharmacokinetics (because of the administration of a nonselective NOS inhibitor), and also assures that on the test day
(day 15) there was no direct effect of the NOS inhibitor on the
behavior of animals. The finding that on day 15 a challenge
cocaine injection given to the 7-NI/cocaine group resulted in
a similar locomotor stimulation as in the vehicle/saline (con-
trol) group (fig. 3) demonstrates that 7-NI blocked the in-
duction of locomotor sensitization to cocaine. By use of a similar
paradigm of drug administration, but a higher dose of co-
caine, we reported that 7-NI also blocked the various stages
in the development of cocaine kindling (Itzhak, 1996). To-
gether, these findings suggest that a common mechanism, in
which NO is involved, may underlie both the induction of
behavioral sensitization to cocaine and cocaine kindling.

The development of cross-sensitization between cocaine
and amphetamines has been well documented (e.g.,
Chaudhry et al., 1988). The finding that 7-NI blocked the
cross-sensitization of cocaine-experienced animals to the
challenge METH injection (fig. 4) supports the involvement
of NO in the psychomotor stimulating effect of METH.

The development of context-dependent sensitization to
psychostimulants has been investigated extensively (Stewart
and Vezina, 1988; Crombag et al., 1996). In the present
study, 7-NI completely blocked the development of cocaine-
induced conditioned locomotion, which suggests a role for NO
in this process. In fact, the major and perhaps the only
difference between the locomotor sensitization generated by
cocaine and METH is the finding that the conditioned loco-
motor induced by METH was resistant to 7-NI administra-
tion. Other than that the effect of 7-NI on cocaine- and
METH-induced locomotor sensitization was quite similar.
First, 7-NI attenuated the acute responses to both cocaine
and METH. Second, 7-NI attenuated the increment in loco-
motor activity produced by repeated cocaine and METH ad-
ministration (day 5). Third, the test for sensitization, on day
15, showed that 7-NI/cocaine and 7-NI/METH groups were
significantly less sensitive to the challenge injection of psy-
chostimulants than animals treated with vehicle/psychostim-
ulant. However, the 7-NI/METH group, but not the 7-NI/
cocaine group, always remained more sensitive to the
psychostimulant challenge than the control animals. This
finding suggests that the co-administration of 7-NI with
METH did not block completely the development of a sensi-
tized response. When the conditioned locomotion induced by
METH was investigated, it appeared that this element of
behavioral sensitization was completely resistant to 7-NI
administration. Thus, it is possible that the incomplete block-
ae of METH-induced locomotor sensitization by 7-NI is
carried by the development of conditioned locomotion that is
not sensitive to 7-NI. The element of behavioral sensitization
carried by METH which was 7-NI-sensitive may perhaps
represent context-independent sensitization.

Several issues still remain unclear, however. For instance,
different mechanisms associated with cocaine- and
METH-induced conditioned locomotion? Or perhaps METH-
induced conditioned locomotion is more resistant to pharma-
cological manipulations than the conditioning caused by co-
caine. Is the magnitude of METH-induced conditioned
locomotion directly proportional to the dosage of METH
used? Would a higher dose of the NOS inhibitor block METH-
induced conditioned locomotion? Despite these questions, our
findings may provide a possible explanation for the apparent
inconsistencies in the literature on the effect of NOS inhibi-
tors against METH (Ohno and Watanabe, 1995; Abe kawa et
al., 1995) or amphetamine-induced sensitization (Stewart et
al., 1994); in these studies conditioned locomotion was not
investigated. Therefore, if the conditioned locomotion pro-
duced by amphetamine was greater than, or masked the
context-independent sensitization, the NOS inhibitors may
be ineffective (e.g., Stewart et al., 1994). Because it has been
reported that under certain circumstances amphetamine may
produce primarily, if not solely, context-dependent loco-
motion (Crombag et al., 1996), it is clear that environmental
factors have a major role in studying the effect of psycho-
stimulants.

Nevertheless, the role of NO in the context-independent
actions of METH is also apparent from our previous studies.
We found that the depletion of striatal dopamine and its
metabolites, as well as the loss in dopamine transporter
binding sites caused by high doses of METH, were blocked by
pretreatment with 7-NI (Itzhak and Ali, 1996). These find-
ings and the present study support the role of NO in both the
development of context-independent behavioral sensitization
to METH and METH-induced neurotoxicity.

The mechanism by which 7-NI attenuates the acute effects
of cocaine and METH and the locomotor sensitization caused
after the repeated administration of these drugs is unclear.
One hypothesis may relate to the interactions between dopa-
mine, glutamate and NO in critical brain regions involved in
the action of psychostimulants. For instance, the interac-
tions between nigrostriatal-dopamine and corticostriatal-gluta-
mate transmission (e.g., Carlsson and Carlsson, 1990) may
provide a basis for the apparent role of the NMDA receptor in
the action of psychostimulants (Karler et al., 1989, 1990,
1994; Itzhak and Stein, 1992; Wolf and Jezierski, 1993; Wolf
et al., 1994; Pulvirenti et al., 1994). Accordingly, activation
of the NMDA receptor after administration of psychostimulants
could lead to the stimulation of brain NOS activity. The
increase in NO levels may further modulate the release of
various neurotransmitters (e.g., dopamine and glutamate)
(Lea et al., 1993; Montague et al., 1994) that contribute to
the sensitization process. However, it is unclear if activation
of the glutamatergic system occurs immediately after the
administration of psychostimulants [e.g., if there is a signif-
icant increase in extracellular glutamate levels after acute
administration of psychostimulants that correlates with psy-
chomotor stimulation (Smith et al., 1995)], or whether the
activation of the NMDA receptors is a slow or delayed process
that parallels the development of sensitization (e.g., Itzhak
and Stein, 1992; Pierce et al., 1996).

Regardless of the “timing” of the glutamatergic input, the
present results indicate that NO is involved in both the
immediate (acute) and long-term effects (sensitization) of the
psychostimulants. In light of the role of dopaminergic neuro-
transmission in both the acute and long-term effects of psy-
chostimulants it is conceivable that direct dopamine/NO in-
teractions may play a role in the acute and sensitized
response to psychostimulants. One possibility, for instance,
is that diminishing brain NO levels may attenuate psycho-
stimulant-induced dopamine release (Bowyer et al., 1995). A
few studies have indicated that NO causes the release of striatal dopamine and that blockade of NOS, in vitro and in vivo, diminished dopamine release (Strasser et al., 1994; Lonart et al., 1993, Zhu and Luo, 1992). However, other studies suggest that NOS inhibitors cause an increase in dopamine release (Silva et al., 1995; Shibata et al., 1996), and our recent studies indicate that 7-NI by itself had no significant effect on the content of striatal dopamine and its metabolites (Itzhak and Ali, 1996). Thus, further studies are required to determine whether, and how, dopamine/NOS interactions or dopamine/glutamate/NOS interactions underlie the mechanism by which NOS inhibitors attenuate the development of locomotor sensitization to psychostimulants.

In summary, the present study supports the role of brain NO in the development of behavioral sensitization to psychostimulants such as cocaine and METH. These findings coupled with our previous studies on cocaine-induced kindling (Itzhak, 1996) and METH-induced neurotoxicity (Itzhak and Ali, 1996) suggest that neuronal selective NOS inhibitors may be useful agents for the treatment of psychostimulant addiction and neurotoxicity.

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References


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