

Modulation of the Neurotoxic Effects of Methamphetamine by the Selective κ -Opioid Receptor Agonist U69593

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Abstract: κ -Opioid receptor agonists prevent alterations in dopamine neurotransmission that occur in response to repeated cocaine administration. The present microdialysis study examined whether administration of the selective κ -opioid receptor agonist U69593 with methamphetamine prevents alterations in dopamine levels produced by neurotoxic doses of methamphetamine. Swiss Webster mice were injected intraperitoneally with methamphetamine (10.0 mg/kg) or saline, four times in 1 day, at 2-h intervals. Prior to the first and third injection, they received U69593 (0.32 mg/kg s.c.) or vehicle. Microdialysis was conducted 3, 7, or 21 days later. Basal and K^+ -evoked (60 and 100 mM) dopamine overflow were reduced 3 days after methamphetamine administration. These effects were long-lasting in that they were still apparent 7 and 21 days after methamphetamine treatment. Intrastratial (5.0 and 50 μM) or systemic (1.0–10.0 mg/kg) administration of methamphetamine increased dopamine concentrations in control animals. In mice preexposed to methamphetamine, methamphetamine-evoked dopamine overflow was reduced. In animals that had received methamphetamine with U69593, basal dopamine levels did not differ from those of vehicle-treated controls. U69593 treatment attenuated the decrease in K^+ -evoked dopamine produced by prior methamphetamine exposure. The reduction in methamphetamine-evoked dopamine levels was also attenuated. The administration of U69593 alone did not modify basal or stimulus-evoked dopamine levels. These data demonstrate that repeated methamphetamine administration reduces presynaptic dopamine neuronal function in mouse striatum and that co-administration of a selective κ -opioid receptor agonist with methamphetamine attenuates these effects. U69593 treatment did not modify the hyperthermic effects of methamphetamine, indicating that this κ -opioid receptor agonist selectively attenuates methamphetamine-induced alterations in dopamine neurotransmission. **Key Words:** Methamphetamine—Dopamine—Microdialysis— κ -Opioid receptors—U69593. *J. Neurochem.* **74**, 1553–1562 (2000).

Methamphetamine is a psychomotor stimulant with high liability for abuse. Increasing evidence suggests that this psychomotor stimulant is also neurotoxic to dopaminergic neurons. The repeated administration of meth-

amphetamine to rodents or nonhuman primates decreases striatal concentrations of dopamine (DA) and its metabolites in several brain regions (Wagner et al., 1980; Seiden and Ricaurte, 1987). Degeneration of DA nerve terminals and reductions in DA uptake and the activity of tyrosine hydroxylase have also been reported (Hotchkiss and Gibb, 1980; Schmidt and Gibb, 1985; Bowyer et al., 1994). These alterations occur within days following the cessation of drug treatment and persist for months or years thereafter (Ricaurte et al., 1980; Wagner et al., 1980).

The acute administration of methamphetamine increases extracellular DA levels via the transporter-dependent reverse transport of DA and the displacement of DA from vesicular stores (Liang and Rutledge, 1982; Sulzer et al., 1993). Several studies suggest that alterations in DA levels underlie the neurotoxic effects of methamphetamine. The magnitude of methamphetamine-induced DA overflow is correlated with subsequent decreases in DA neurotransmission (O'Dell et al., 1993). Furthermore, DA receptor antagonists or synthesis inhibitors attenuate methamphetamine-induced neurotoxicity (Axt et al., 1990; O'Dell et al., 1993). DA uptake inhibitors also attenuate methamphetamine-induced deficits in DA neuronal activity, suggesting a critical role of the DA transporter in the mediation of neurotoxicity (Schmidt and Gibb, 1985; Marek et al., 1990). Consistent with this hypothesis, methamphetamine-induced neurotoxicity is not observed in mice lacking the DA transporter (Fumagalli et al., 1998).

The acute administration of methamphetamine is also associated with a marked and prolonged increase in glutamate release (Nash and Yamamoto, 1992; Abekawa et al., 1994). An involvement of excitatory amino acids

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Abbreviations used: aCSF, artificial cerebrospinal fluid; AUC, area under the curve; DA, dopamine; U69593, (5 α ,7 α ,8 β)-(+)N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide.

in the modulation of DA neuronal activity (Roberts and Anderson, 1979; Clow and Jhamandas, 1989) and methamphetamine-induced neurotoxicity has been suggested. NMDA receptor antagonists decrease methamphetamine-induced DA overflow and prevent decreases in tyrosine hydroxylase activity and DA levels produced by repeated methamphetamine administration (Sonsalla et al., 1989, 1991; Weihmuller et al., 1992). Taken together, these findings suggest that increases in DA and excitatory amino acid neurotransmission may underlie the neurotoxic effects of methamphetamine.

κ -Opioid receptor agonists decrease dialysate levels of DA and glutamate in several brain regions (Spanagel et al., 1990; Hill and Brotchie, 1995). Evidence that κ -opioid receptor agonists modulate DA D2 receptors has also been obtained (Izenwasser et al., 1997). Interactions of κ -opioid receptor agonists with psychostimulants have previously been documented. Selective κ -opioid receptor agonists prevent alterations in DA neurotransmission that occur following the repeated administration of cocaine (Heidbreder and Shippenberg, 1994). κ -Opioid receptor agonist treatment also attenuates the development of cocaine-induced behavioral sensitization (Heidbreder et al., 1995; Shippenberg et al., 1996). Administration of dynorphin 1–13, the postulated endogenous ligand for the κ -opioid receptor (Chavkin et al., 1982), attenuates the development of D -amphetamine-induced behavioral sensitization (Toyoshi et al., 1996). The ability of these agents to attenuate alterations in DA dynamics that occur following the administration of neurotoxic doses of methamphetamine, however, is unknown.

The present study used *in vivo* microdialysis in the mouse to address this issue. Animals received repeated injections of methamphetamine either alone or in combination with the κ -opioid receptor agonist ($5\alpha,7\alpha,8\beta$)-(+)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide (U69593). This methamphetamine treatment regimen was that previously shown to be neurotoxic to DA neurons (O'Callaghan and Miller, 1994; Hirata et al., 1998; Tsao et al., 1998). Basal and stimulus-evoked DA levels were quantified in the dorsal striatum 3 days following the cessation of drug treatments. The duration of the methamphetamine-induced alterations in basal and stimulus (K^+)-evoked DA levels was also examined. As pharmacological treatments that decrease the hyperthermic effects of methamphetamine attenuate the neurotoxic effects of this psychostimulant (Bowyer et al., 1994), the influence of U69593 treatment on methamphetamine-induced hyperthermia was also assessed.

MATERIALS AND METHODS

Animals

Male Swiss Webster mice (Taconic Forms, Germantown, NY, U.S.A.; 30–40 g) were housed five per cage in a colony room. They were maintained on a 12-h light/dark cycle with food and water available *ad libitum*. Animals were acclimatized

to the colony for at least 1 week prior to commencement of experiments. All animal care and experimental procedures were conducted according to the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the local Animal Care and Use Committee of the National Institute on Drug Abuse (NIDA).

Implantation of microdialysis guide cannula

Mice were anesthetized with a combination of xylazine (8.0 mg/kg *i.p.*) and ketamine (80 mg/kg *i.p.*) and positioned in a stereotaxic frame modified for the mouse (David Kopf, Topanga, CA, U.S.A.). The skull was exposed and a 1.0-mm-diameter hole drilled for placement of a microdialysis guide cannula (CMA 11; CMA, Acton, MA, U.S.A.) into the dorsal striatum (anteroposterior: +0.4 mm; lateral: (\pm 2.1 mm; dorsoventral: 2.1 mm) according to the atlas of Slotnick and Leonard (1975). The guide cannula was fixed to the skull with cranio-plastic cement (Geristore, Santa Maria, CA, U.S.A.). Surgery was conducted 5 days prior to the commencement of drug treatment.

Drug treatment

Mice received four intraperitoneal injections of methamphetamine (10 mg/kg/injection) or saline at 2-h intervals commencing at 9:00 a.m. The methamphetamine treatment regimen employed was that previously shown to be neurotoxic to striatal DA neurons (O'Callaghan and Miller, 1994; Hirata et al., 1998; Tsao et al., 1998). Vehicle or U69593 (0.32 mg/kg *s.c.*) was administered 15 min before the first and third injection of saline or methamphetamine. As previous studies (Heidbreder and Shippenberg, 1994) have shown that as few as two injections of U69593 are effective in preventing alterations in behavior (e.g., sensitization) that occur in response to repeated psychostimulant administration, only two injections of U69593 were used in the present studies.

Microdialysis procedure

Dialysis experiments were conducted 3, 7, or 21 days following the cessation of the various drug treatments. Twelve hours before the commencement of experiments, a microdialysis probe (CMA 11; 2.0-mm membrane length) perfused with artificial cerebrospinal fluid (aCSF; 145 mM NaCl, 2.8 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.25 mM ascorbic acid, and 5.4 mM *D*-glucose, pH 7.4) at a flow rate of 0.6 μ l/min was inserted into the guide cannula. The probe was connected to a microinfusion pump (Instech, Plymouth Meeting, PA, U.S.A.) via a single-channel quartz-lined swivel (Instech). On the morning of experiments, the aCSF was replaced with fresh aCSF and the probes were allowed to equilibrate for 90 min. Three consecutive dialysate samples were then collected at 30-min intervals for determination of basal DA levels.

K^+ -evoked DA levels

Basal dialysate samples were obtained in animals ($n = 4$ /treatment condition) as described above. The aCSF was then replaced with that containing 60 mM KCl (87.8 mM NaCl, 60 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.25 mM ascorbic acid, and 5.4 mM *D*-glucose), and one 30-min dialysate sample was collected. The aCSF was then replaced with physiological aCSF, and two additional samples were obtained. This sequence was repeated with aCSF containing 100 mM K^+ .

Methamphetamine-induced DA levels

The response to intrastriatal infusion of methamphetamine (5.0 and 50 μ M) was assessed in parallel groups of animals ($n = 4$ /group). Basal DA levels were determined as above. The

dialysis probe was then perfused with aCSF containing 5.0 μ M methamphetamine for 30 min, and one dialysate sample was obtained. Normal aCSF was then perfused through the probe, and two consecutive dialysis samples were collected. This sequence was then repeated with aCSF containing 50 μ M methamphetamine.

Additional groups of mice ($n = 4$ –8/treatment condition) received an intraperitoneal injection of saline (1.0 ml/100 g) followed 90 min later by an intraperitoneal injection of 1.0 mg/kg methamphetamine. They then received an intraperitoneal injection of 3.0 mg/kg followed 90 min later by an intraperitoneal injection of 10.0 mg/kg. Three consecutive dialysate samples were collected after each injection. Control animals received three injections of saline.

Duration of methamphetamine-induced alterations in DA levels

In view of the marked reductions in basal and stimulus-evoked DA overflow observed 3 days after methamphetamine administration, additional studies were conducted to determine the duration of this effect. Animals ($n = 4$ /group/abstinence time point) received four injections of methamphetamine or saline as described above. Basal DA levels and K^+ (60 mM)-evoked DA overflow were assessed 7 and 21 days after the cessation of these treatments. It was hypothesized that if the changes in basal and stimulus-evoked DA levels observed 3 days after methamphetamine exposure reflected neurotoxic effects, then similar changes should also be evident weeks after drug exposure.

Histology

Following completion of experiments, animals were deeply anesthetized with sodium pentobarbital and killed by decapitation. The brains were quickly removed from the cranium and immediately frozen. Probe placement was verified histologically via coronal sections (20 μ m) using a cryostat (Hacker Instrument, Fairfield, NJ, U.S.A.). Probe placements were localized using the atlas of Slotnick and Leonard (1975).

Quantification of dialysate DA levels

A BAS (Bioanalytical Systems, West Lafayette, IN, U.S.A.) PM-80 solvent delivery pump and an LC-4C amperometric detector with a working electrode set at +750 mV vs. Ag/AgCl were used in combination with a six-port rotary injection valve (model 7125; Rheodyne, Berkeley, CA, U.S.A.). Chromatographic separations were performed with a BAS-C18 column (100 \times 2.0 mm; 3 μ m). The mobile phase consisted of 0.15 M sodium phosphate, 1.6 mM sodium octanesulfate, 1.0 mM EDTA, and 12% methanol (vol/vol), adjusted to pH 5.0. The mobile phase was filtered through a 0.22- μ m filter (Millipore Corp., Bedford, MA, U.S.A.), degassed under vacuum, and delivered at a flow rate of 0.5 ml/min. The retention times of standards were used to identify peaks, and peak heights were used to calculate recovery of internal standard and amounts of DA.

Measurement of core body temperature

Animals were housed in their home cages (3/cage) throughout the experiment. Mice received four intraperitoneal injections of methamphetamine (10 mg/kg/injection) or saline at 2-h intervals commencing at 9:00 a.m. Vehicle or U69593 (0.32 mg/kg s.c.) was administered 15 min before the first and third injection. Body temperature was determined at 15-min intervals for 1 h prior to the commencement of injections and at 15-min intervals thereafter. Core body temperature was assessed using a BAT-12 thermometer and thermocoupled microprobe (Physiotem Instruments, Clifton, NJ, U.S.A.) coated with mineral oil. Mice were removed

from the cage and gently restrained by the scruff of the neck. The microprobe was inserted 2.5 cm into the rectum for 1–2 min, and body temperature was recorded. After removal of the probe, the animal was returned to its home cage.

Drugs and reagents

Methamphetamine hydrochloride (NIDA Drug Supply, Rockville, MD, U.S.A.) was prepared in saline and administered in a 1.0-ml/100 g volume. U-69593 (Lahti et al., 1985) was obtained from NIDA Drug Supply and prepared in saline containing 20% propylene glycol. Chemicals used in the preparation of aCSF, HPLC standards, and mobile phase were analytical grade and obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) or Fluka (Ronkonkoma, NY, U.S.A.).

Statistical analysis

Only data from animals with accurate probe placements in the dorsal striatum were used for subsequent statistical analysis. DA levels (nM) in basal dialysate samples were assessed using ANOVA followed by the Scheffé test. Time course data are expressed as the mean \pm SEM DA concentration per 30-min interval. The effects of methamphetamine and K^+ were analyzed by calculating cumulative area under the curve (AUC) values for each animal and stimulus condition relative to the last basal value preceding drug administration (Zar, 1974). The resultant data are expressed as DA concentration (nM) per time interval (Gray et al., 1999). This method permits analysis of overall changes in DA levels rather than changes at individual time points and is not confounded by group differences in basal levels. AUC data were analyzed using a univariate multifactorial ANOVA with repeated measures over drug challenge. Following confirmation of significant main effects or interactions by the overall ANOVAs, simple effects probes were used to compare the means of selected levels of a factor or combination of factors. The Greenhouse–Geisser correction for repeated measures was used (Zar, 1974). Body temperature data were analyzed using a three-factor (U69593 \times methamphetamine \times time) ANOVA with repeated measures over time. The accepted level of significance for all tests was $p \leq 0.05$.

RESULTS

Basal DA levels

Figure 1 shows basal dialysate levels of DA in the four treatment groups 3 days following injections of methamphetamine or saline. DA levels in the three consecutive samples collected did not differ within groups and were thus pooled for between-group comparisons. DA levels in methamphetamine-treated animals were significantly reduced relative to control animals ($p \leq 0.0001$). As shown in Table 1, the reduction in basal DA levels was long-lasting. A significant decrease in DA levels was still apparent 7 and 21 days after the injections of methamphetamine.

In animals that had received methamphetamine in combination with U69593, no reduction of basal DA levels was seen relative to animals that had received injections of vehicle and saline 3 days after injections ($p \geq 0.1$). However, DA levels in animals that had received U69593 in combination with methamphetamine were significantly lower than in U69593/saline-treated animals at this same abstinence time point ($p \leq 0.03$). No difference in basal DA levels was observed between the

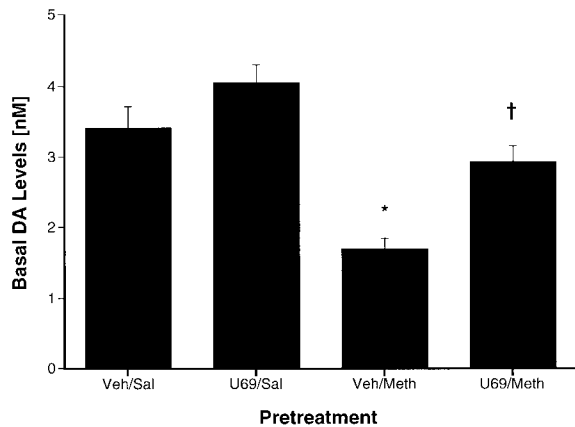


FIG. 1. Influence of prior U69593 (U69) and methamphetamine (Meth) treatment on basal dialysate DA levels in striatum. Animals received four injections of saline (Sal) or methamphetamine. Fifteen minutes prior to the first and third injection, they received U69593 or its vehicle (Veh). Basal DA levels were quantified 3 days later. Treatment condition is shown on the abscissa, and DA concentration (nM), uncorrected for in vitro recovery, is shown on the ordinate. The data were obtained from 12–16 mice/group. ANOVA revealed a significant difference between treatment groups [$F(3,61) = 17.4, p \leq 0.001$]. * $p \leq 0.0001$, significant difference between the vehicle/saline and vehicle/methamphetamine group and the U69593/saline and vehicle/methamphetamine groups (Scheffé test). † $p \leq 0.03$, significant difference between U69593/saline and U69593/methamphetamine groups (Scheffé test).

vehicle/saline and U69593/saline treatment groups ($p \geq 0.3$).

K⁺ perfusion

Figure 2 shows the effects of K⁺ perfusion (60 and 100 mM) on dialysate DA levels. Perfusion of K⁺ significantly increased DA levels from preinfusion values in

TABLE 1. Time course of methamphetamine-induced changes in DA levels

Treatment	Day after treatment	Basal DA (nM)	60 mM K ⁺ AUC (DA × 90 min)
Vehicle/saline	7	3.0 ± 0.1	896 ± 30 ^c
	21	3.2 ± 0.1	
Vehicle/methamphetamine	7	2.1 ± 0.1 ^a	442 ± 21 ^b
	21	2.3 ± 0.1 ^a	613 ± 6 ^b

Mice received four injections of methamphetamine (10.0 mg/kg) or saline as described in Materials and Methods. Dialysis studies assessing basal and K⁺-evoked DA levels were conducted 7 and 21 days later in separate groups of animals ($n = 4$ /group).

^aBasal DA overflow was reduced 7 or 21 days after methamphetamine treatment [day 7: $F(1,6) = 39, p \leq 0.001$; day 21: $F(1,6) = 75.0, p \leq 0.001$].

^bK⁺-evoked DA levels were also reduced 7 or 21 days after the methamphetamine treatment regimen [day 7: $F(1,6) = 321, p \leq 0.0001$; day 21: $F(1,6) = 305, p \leq 0.001$].

^cPooled value.

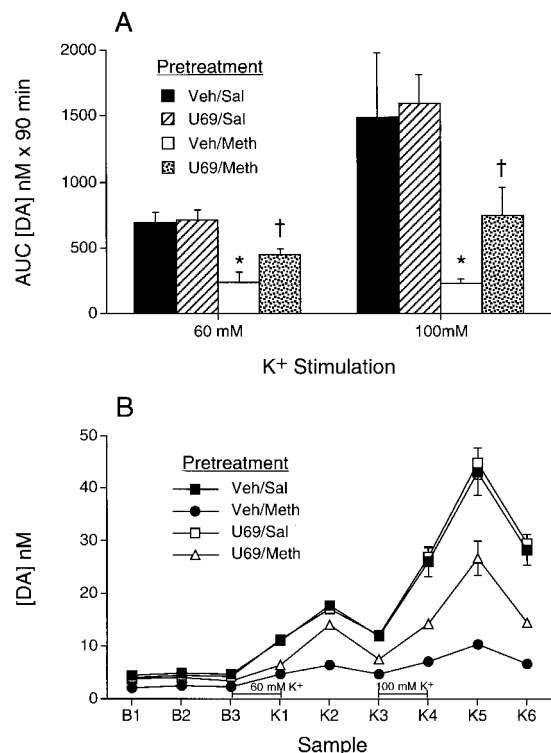


FIG. 2. Influence of prior U69593 (U69) and methamphetamine (Meth) treatment on K⁺-evoked DA levels. ACSF containing 60 or 100 mM K⁺ was perfused through the probe for 30 min. Samples were collected during K⁺ perfusion and for 60 min thereafter. **A:** Histograms of AUC values are expressed as means ± SEM. ANOVA revealed significant main effects of methamphetamine [$F(1,12) = 78, p \leq 0.001$], U69593 [$F(1,12) = 9.2, p \leq 0.01$], and K⁺ [$F(1,12) = 75.5, p \leq 0.001$] and significant K⁺ × methamphetamine [$F(1,12) = 28.9, p \leq 0.001$] and U69593 × methamphetamine [$F(1,12) = 5.4, p \leq 0.04$] interaction effects. The effects of K⁺ were concentration dependent in control [vehicle (Veh)/saline (Sal) group: $F(1,3) = 14.2, p \leq 0.03$; U69593/saline group: $F(1,3) = 135, p \leq 0.001$] but not methamphetamine-treated rats [vehicle/methamphetamine group: $F(1,3) = 0.03, p \geq 0.9$; U69593/methamphetamine group: $F(1,3) = 8.8, p = 0.06$]. *Significant effect of methamphetamine treatment relative to vehicle/saline and U69593/saline groups. †Significant difference between vehicle/methamphetamine- and U69593/methamphetamine-treated mice. **B:** DA concentration (nM) as a function of time after K⁺ stimulation. Each point represents the mean ± SEM of $n = 4$ animals and is uncorrected for in vitro probe recovery.

control (vehicle/saline and U69593/saline) mice, and the magnitude of this effect was related to the perfusate concentration (vehicle/saline: $p \leq 0.03$; U69593/saline: $p \leq 0.001$). A peak increase in DA levels was observed 30 min following the cessation of K⁺ perfusion. This time lag has been observed in other studies and is most likely attributable to the length and dead volume of the dialysis tubing and the resulting delay between introduction of the perfusate and subsequent probe perfusion (Shippenberg and Thompson, 1997).

Prior methamphetamine exposure resulted in a blunted response to K⁺ stimulation that was evident 3 as well as 7 and 21 days after exposure (Fig. 2; Table 1). The increase

in DA levels observed in methamphetamine-preexposed animals was significantly less than that of controls. In addition, raising the K^+ concentration from 60 to 100 mM did not result in a further increase of DA levels in methamphetamine-pretreated animals ($p \geq 0.9$). Table 1 shows that the blunted response to K^+ perfusion was long-lasting. It was still apparent 21 days after methamphetamine exposure. In animals, however, that had received U69593 with methamphetamine 3 days previously (Fig. 2), the increase in DA levels evoked by K^+ did not differ from that of controls. The administration of U69593 to saline-treated animals did not modify K^+ -evoked DA levels.

Methamphetamine perfusion

Figure 3 shows the influence of intrastriatal perfusion of methamphetamine (5.0 and 50 μM) on dialysate DA levels in the four treatment groups. Methamphetamine significantly increased DA levels in control animals, and the magnitude of this effect was related to the concentration of methamphetamine added to the perfusate (vehicle/saline: $p \leq 0.004$; U69593/saline: $p \leq 0.005$). Methamphetamine perfusion also resulted in a concentration-dependent increase in DA levels in methamphetamine-exposed animals ($p \leq 0.001$). This increase, however, was significantly less than that observed in control (vehicle/saline) animals or those that had received injections of U69593 in combination with saline (5.0 μM : $p \leq 0.001$; 50 μM : $p \leq 0.001$). U69593 pretreatment failed to modify the attenuated response to 5.0 μM methamphetamine. Thus, the increase in DA in these animals was less than that of control animals and did not differ significantly ($p \geq 0.2$) from that of animals that had previously received methamphetamine alone. The blunted response observed in response to the higher concentration of methamphetamine was, however, attenuated in animals that had previously received U69593 in combination with methamphetamine. Thus, DA levels evoked by 50 μM methamphetamine were significantly greater in U69593/methamphetamine as compared with vehicle/methamphetamine-treated mice ($p \leq 0.001$). The effects of 50 μM methamphetamine did not differ in vehicle/saline- and U69593/saline-treated animals ($p \geq 1.0$).

Systemic methamphetamine challenge

DA levels were stable throughout the 180-min experimental session (data not shown). No difference between groups in saline-evoked DA levels was seen. The systemic administration of methamphetamine significantly increased DA levels (Fig. 4). The administration of methamphetamine to control or U69593/saline-treated mice resulted in a significant increase in DA overflow, and the magnitude of this effect was linearly related to dose (vehicle/saline: $p \leq 0.002$; U69593/saline: $p \leq 0.03$). Although methamphetamine increased DA levels in methamphetamine-treated animals, a blunted response was seen. Thus, DA overflow evoked by doses of 3.0 and 10.0 mg/kg methamphetamine was significantly less than that in

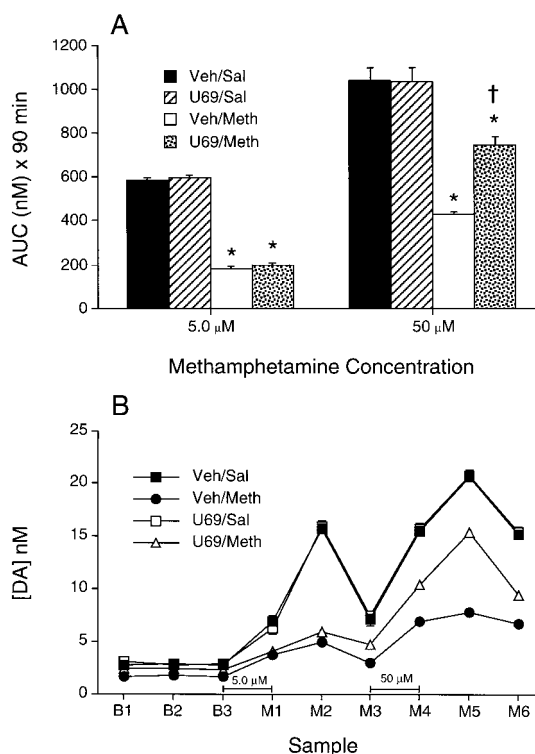


FIG. 3. Influence of prior U69593 (U69) and methamphetamine (Meth) treatment upon DA levels evoked by intrastriatal perfusion of methamphetamine. ACSF containing 50 and 100 μM methamphetamine was perfused through the dialysis probe for 30 min. Samples were collected during methamphetamine perfusion and for 60 min thereafter. **A:** Histograms of AUC values are means \pm SEM. ANOVA revealed significant main effects of methamphetamine [$F(1,12) = 290$, $p \leq 0.001$] and U69593 pretreatment [$F(1,12) = 11.6$, $p \leq 0.005$] as well as methamphetamine perfusion [$F(1,12) = 359$, $p \leq 0.001$]. Methamphetamine increased DA levels in all treatment groups, and this effect was concentration dependent [vehicle (Veh)/saline (Sal) group: $F(1,3) = 66.6$, $p \leq 0.004$; vehicle/methamphetamine group: $F(1,3) = 223.1$, $p \leq 0.001$; U69593/saline group: $F(1,3) = 56.2$, $p \leq 0.005$; U69593/methamphetamine group: $F(1,3) = 267.1$, $p \leq 0.001$]. Prior methamphetamine exposure resulted in a blunted response to methamphetamine perfusion [5.0 μM : $F(1,6) = 742$, $p \leq 0.001$; 50 μM : $F(1,6) = 111$, $p \leq 0.001$]. U69593 treatment failed to modify the response to 5.0 μM methamphetamine [$F(1,12) = 1.7$, $p \geq 0.2$] when administered in combination with either saline or methamphetamine. The effect of 50 μM methamphetamine did not differ in vehicle/saline- and U69593/saline-treated animals [$F(1,6) = 0.003$, $p \geq 0.96$]. The increase in DA evoked by 50 μM methamphetamine was greater in U69593/methamphetamine-exposed animals relative to vehicle/methamphetamine animals [$F(1,6) = 77.6$, $p \leq 0.001$]. *Significant difference from control animals. †Significant difference between vehicle/methamphetamine and U69593/methamphetamine groups. **B:** DA concentration (nM) as a function of time after methamphetamine perfusion. Each point represents the mean \pm SE of four animals and is uncorrected for in vitro probe recovery.

control or U69593-treated mice. The prior administration of U69593 alone did not modify the effects of a subsequent methamphetamine (1.0–10.0 mg/kg) challenge. Methamphetamine (3.0 mg/kg)-evoked DA overflow did not differ in methamphetamine- and

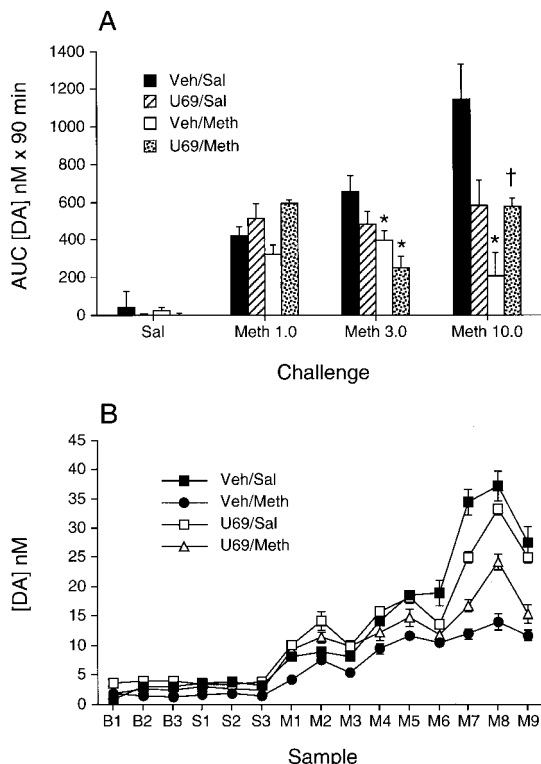


FIG. 4. Influence of prior U69593 (U69) and methamphetamine (Meth) exposure on methamphetamine (i.p.)-evoked DA concentrations. **A:** Histograms of AUC values are means \pm SEM. ANOVA revealed significant main effects of methamphetamine treatment [$F(1,26) = 14, p \leq 0.001$] and challenge [$F(3,78) = 59.2, p \leq 0.001$] and significant U69593 \times challenge [$F(3,78) = 3.56, p \leq 0.05$] and methamphetamine \times challenge [$F(3,78) = 18.8, p \leq 0.001$] interactions. Methamphetamine increased DA overflow in all groups. The magnitude of this effect was linearly related to dose in control [$F(1,7) = 21.7, p \leq 0.002$] and U69593/saline (Sal)-treated animals [$F(1,3) = 15.1, p \leq 0.03$]. Methamphetamine-treated animals exhibited a blunted response to challenge doses of 3.0 and 10.0 mg/kg methamphetamine. U69593 treatment did not alter the blunted response to the 3.0-mg/kg dose of methamphetamine [$F(1,16) = 1.1, p \geq 0.3$]. The blunted response to 10.0 mg/kg methamphetamine was attenuated [$F(1,16) = 14.1, p \leq 0.002$]. *Significant difference from control [vehicle (Veh)/saline and U69593/saline] values. †Significant difference between vehicle/methamphetamine and U69593/methamphetamine groups. **B:** DA concentration as a function of time after intraperitoneal injection of saline or 1.0, 3.0, or 10.0 mg/kg methamphetamine. Each point represents the mean \pm SEM of four animals and is uncorrected for in vitro recovery.

U69593/methamphetamine-treated mice ($p \geq 0.3$). However, the blunted response to the 10.0-mg/kg dose of methamphetamine was significantly attenuated ($p \leq 0.002$).

Core body temperature

Figure 5 shows that the administration of methamphetamine to control animals increased body temperature relative to that produced by the injection of saline. A significant increase in body temperature was also observed in animals that received U69593 prior to the first

and third injection of methamphetamine, and there was no difference between groups in the magnitude of this effect. No alteration in body temperature was observed in animals that received U69593 injections in combination with saline. ANOVA revealed a significant effect of methamphetamine treatment ($p \leq 0.001$) but no effect of U69593 treatment ($p \geq 0.8$).

DISCUSSION

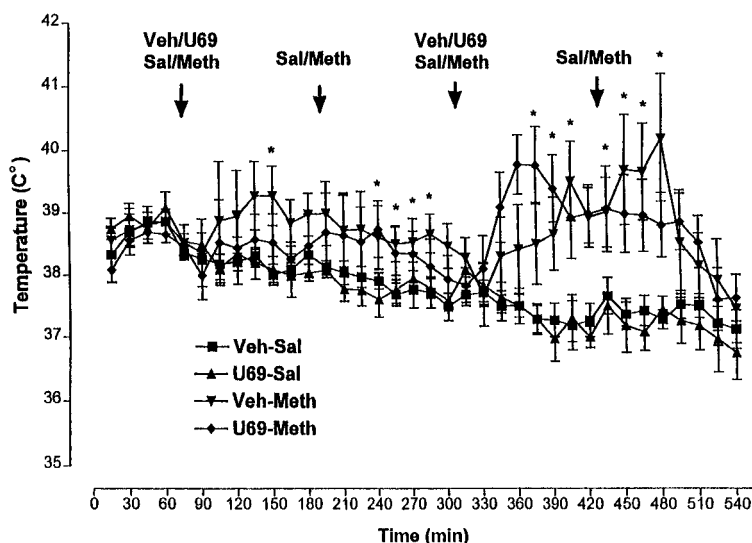
This study demonstrates that a methamphetamine treatment regimen, previously shown to be neurotoxic to DA neurons (O'Callaghan and Miller, 1994; Hirata et al., 1998; Tsao et al., 1998), decreases basal, K^+ -, and methamphetamine-evoked DA levels in mouse striatum. In animals, however, that received the same methamphetamine treatment regimen in combination with a selective κ -opioid receptor agonist, these alterations in presynaptic DA neuronal activity were attenuated.

Dialysate DA levels in the striatum were decreased relative to control values 3 days following the cessation of repeated methamphetamine exposure. This finding is in accord with that of a previous study (Cass, 1997) in rats and indicates that the basal activity of mesostriatal DA neurons is reduced following repeated methamphetamine administration. A reduction in basal DA levels was still apparent 7 and 21 days after the cessation of methamphetamine treatment, suggesting that the methamphetamine treatment regimen employed produces long-lasting decrements in presynaptic DA function. Consistent with this hypothesis, a significant decrease in DA transporter number is also observed 21 days following the same methamphetamine treatment regimen (T. S. Shippenberg et al., manuscript in preparation).

Previous studies have shown that the repeated administration of methamphetamine to rodents and nonhuman primates decreases DA uptake and the activity of tyrosine hydroxylase. DA terminal degeneration and reductions in the density of dopamine and vesicular monoamine transporters have also been reported, suggesting a neurotoxic effect of methamphetamine on DA neurons (Wagner et al., 1980; Brunswick et al., 1992; Frey et al., 1997). In this regard, it is important to note that although dialysate DA levels provide an estimate of extracellular levels, dialysate levels are affected by pharmacological treatments that alter DA clearance or release (Parsons and Justice, 1994; Thompson and Shippenberg, 1997). Therefore, the precise mechanism(s) underlying the reduction in dialysate DA levels observed in this and a previous study (Cass, 1997) is unclear. As, however, dialysate DA levels as well as DA uptake and release are reduced following repeated methamphetamine administration, it is likely that extracellular DA levels are also decreased.

K^+ -evoked DA overflow was decreased in methamphetamine-treated mice. This finding is consistent with previous in vivo voltammetry and microdialysis data obtained in the rat (Cass, 1997) and suggests that depolarization-induced DA release is reduced. Basal dialysate

FIG. 5. Influence of U69593 (U69) and methamphetamine (Meth) exposure upon body temperature. Body temperature was measured at 15-min intervals prior to and after injections of methamphetamine or saline (Sal). U69593 or vehicle (Veh) was administered prior to the first and third injections of methamphetamine or saline. Injections are indicated by the arrows. ANOVA revealed a significant effect of methamphetamine injection [$F(1,10) = 27.4, p \leq 0.001$] and time [$F(4,40) = 3.2, p \leq 0.02$] as well as a significant methamphetamine \times time interaction [$F(4,40) = 10.4, p \leq 0.001$] but no significant effect of U69593 treatment [$F(1,10) = 0.14, p \geq 0.7$]. Ordinate: Body temperature ($^{\circ}\text{C}$). Abscissa: Time after injection (min). Each point represents the mean \pm SEM of three or four mice per group. *Significant difference of vehicle/methamphetamine group from vehicle/saline or U69593/saline groups.



levels of DA as well as tissue DA content (Wagner et al., 1980) are also reduced in methamphetamine-treated animals, suggesting that the calcium-dependent releasable pool of DA is decreased following the repeated administration of methamphetamine. Previous studies (Marek et al., 1990; O'Callaghan and Miller, 1994) have shown that the methamphetamine treatment regimen used produces axonal degeneration and a loss of DA terminals. Therefore, the decrease in basal and K^+ -evoked DA levels most likely reflects these neurotoxic effects of methamphetamine.

Prior exposure to methamphetamine also decreased methamphetamine-evoked DA overflow. Thus, the increase in DA levels following the local application of methamphetamine was significantly less in methamphetamine- as compared with saline-treated controls. Methamphetamine exposure also resulted in a blunted response to systemically administered methamphetamine (3.0 and 10.0 mg/kg). Interestingly, however, the increase in DA overflow evoked by a lower dose of methamphetamine (1.0 mg/kg) did not differ from controls.

The amphetamines increase DA levels via a calcium-independent, carrier-mediated exchange diffusion mechanism that requires the presence of the DA transporter (Jones et al., 1998). Evidence that the amphetamines promote the redistribution of DA from synaptic vesicles to the cytosol, thereby promoting reverse transport of DA, has been obtained (Sulzer et al., 1993, 1995). DA neuronal degeneration and DA transporter loss in the striatum have been observed in studies employing a methamphetamine treatment regimen similar to that used in the present study (Wagner et al., 1980; O'Callaghan and Miller, 1994). Therefore, the decreased response to methamphetamine may reflect a reduction of DA transporters in this brain region. The decreased response to higher doses of methamphetamine, together with the decrease in basal DA levels, may also indicate a smaller pool of DA available for displacement by a subsequent

methamphetamine challenge. Finally, calcium-dependent effects of moderate to high doses of amphetamine on dialysate DA levels have been observed in several studies (Benwell et al., 1993; Gray et al., 1999). Evidence that DA efflux is greater in cells transfected with DA and vesicular monoamine transporters than with the DA transporter alone has also been presented (Piffl et al., 1995). Therefore, the present results may indicate an effect of methamphetamine treatment on vesicular (action potential-dependent) release. Furthermore, the differential response to local versus systemic methamphetamine challenge may suggest that a calcium-dependent, transsynaptic activation of DA neurons mediates this effect.

In mice that had received methamphetamine in combination with the selective κ -opioid receptor agonist U69593 (Lahti et al., 1985), dialysate DA levels did not differ from those of mice that had received injections of vehicle and saline. However, basal DA levels in U69593/methamphetamine-treated animals were still 25% less than those of animals that had received U69593 in combination with saline, suggesting that U69593 may attenuate, rather than prevent, the decrease in basal DA levels produced by methamphetamine. The attenuated response to methamphetamine cannot be attributed to a U69593-induced increase in DA because no alteration in DA levels occurred in mice that had received two injections of U69593 alone. Furthermore, the acute administration of κ -opioid receptor agonists decreases dialysate DA levels (Spanagel et al., 1990).

U69593 treatment also attenuated the effects of repeated methamphetamine administration upon K^+ -evoked DA overflow. Thus, in animals that had received U69593 with methamphetamine, the magnitude of the K^+ -evoked increase in DA overflow did not differ from that in control animals. Furthermore, the concentration dependency of K^+ -evoked DA overflow was restored. These effects cannot be attributed to a κ -agonist-induced

compensatory increase in DA release as the response to K^+ was unaltered in animals that had previously received U69593 alone.

U69593 failed to modify the decreased responsiveness of DA neurons to local perfusion of 5.0 μM methamphetamine. However, the decreased response of DA neurons to a higher concentration of methamphetamine (50 μM) was prevented. Similarly, U69593 treatment failed to modify the blunted response produced by the systemic administration of a low dose of methamphetamine. Again, however, the increase in DA levels produced by a high dose of methamphetamine was significantly greater in animals that had previously received U69593 with methamphetamine than in those that had received the methamphetamine treatment regimen alone. These findings are consistent with recent studies showing that κ -opioid receptor agonist administration attenuates alterations in basal and cocaine-evoked DA overflow that occur during abstinence from cocaine (Heidbreder et al., 1994; Chefer et al., 1999). They further indicate that a selective κ -opioid receptor agonist is also effective in attenuating decreases in basal and stimulus-evoked DA levels that occur in response to neurotoxic doses of methamphetamine. Evidence that synthetic κ -opioid receptor agonists or dynorphin 1–13, the postulated endogenous κ -opioid receptor ligand (Chavkin et al., 1982), prevent alterations in behavior (e.g., sensitization) that occur following the repeated administration of these psychostimulants has also been presented (Heidbreder et al., 1995; Shippenberg et al., 1996; Toyoshi et al., 1996). Taken together, these findings suggest a more global role of κ -opioid receptor systems in modulating adaptations in behavior and DA neurochemistry that occur as a consequence of repeated psychostimulant use.

The mechanism(s) mediating the interaction of U69593 and methamphetamine is unclear. Methamphetamine elevates extracellular concentrations of DA and also results in a prolonged increase of extracellular glutamate concentrations in striatum (Stephans and Yamamoto, 1994). Several lines of evidence suggest that these actions contribute to the neurotoxic effects of methamphetamine (Axt et al., 1990; Marek et al., 1990; Sonsalla et al., 1991; Weihmuller et al., 1992). Autoradiographic and in situ hybridization studies have revealed dense κ -opioid receptor expression and κ -opioid receptor mRNA in the dorsal as well as medial and ventral portions of the striatum (Nock et al., 1988; Unterwald et al., 1991; Mansour et al., 1996). Evidence that κ -opioid receptors in these regions are located presynaptically on or in close proximity to DA and glutamatergic neurons has also been presented (McGinty et al., 1995; Steiner and Gerfen, 1998). κ -Opioid receptor agonists decrease DA release (Spanagel et al., 1990). These agents also decrease basal and stimulus-evoked glutamate overflow in striatum (Hill and Brotchie, 1995; Rawls and McGinty, 1998). Therefore, the opposing effect of U69593, as compared with methamphetamine, on DA and glutamatergic release may underlie the protective effects of U69593 observed in the present study. Furthermore, the findings that U69593 was efficacious in attenuating the blunted response

to high, but not low, doses of methamphetamine may indicate a selective effect of U69593 treatment on those effects of methamphetamine that are calcium dependent. Although speculative, indirect evidence in support of this hypothesis has recently been obtained. κ -Opioid receptor activation decreases calcium influx by preventing the opening of presynaptic P- or N-type calcium channels (Tallent et al., 1994; Kanemasa et al., 1995) or via activation of presynaptic K^+ channels (Grudt and Williams, 1993). In addition, recent microdialysis studies (Rawls and McGinty, 1998; Gray et al., 1999) have shown that the acute administration of U69593 attenuates the calcium-dependent increase in glutamate overflow evoked by the transport inhibitor *L-trans*-pyrrolidine dicarboxylate. It also inhibits the calcium-dependent component of amphetamine evoked DA and glutamate overflow in rat striatum.

U69593 treatment attenuated decreases in basal and stimulus-evoked DA levels in the striatum but did not prevent them. Several explanations may account for the limited efficacy of κ -opioid receptor agonist treatment. First, U69593 was administered only prior to the first and third injection of methamphetamine. Although this treatment regimen was selected on the basis of previous studies (Heidbreder et al., 1993), showing that two injections of a κ -opioid agonist can prevent the behavioral effects of repeated cocaine administration, the duration of action of these psychostimulants differs (Abekawa et al., 1994; Heidbreder et al., 1998). Therefore, in contrast to cocaine, the administration of U69593 prior to each injection of methamphetamine may have been required for prevention, rather than attenuation, of methamphetamine's effects. In addition, previous studies examining the interaction of κ -opioid receptor agonists with psychostimulants (Heidbreder et al., 1993, 1995) have been conducted in the rat. Therefore, species differences may also underlie the limited effectiveness of U69593 observed in this as compared with previous studies. Finally, increases in glutamate as well as DA neurotransmission have been implicated in mediating the neurotoxic effects of methamphetamine. Evidence that the amphetamines may, depending on the dose administered, increase DA levels via a stimulation of DA release or an inhibition of DA uptake has also been presented (Benwell et al., 1993; Pifl et al., 1995; Gray et al., 1999). If U69593 prevents some, but not all, of these actions of methamphetamine, then attenuation, rather than abolition, of the neurotoxic effects of methamphetamine would be seen.

In conclusion, this study demonstrates that the systemic administration of a selective κ -opioid receptor agonist attenuates alterations in striatal DA dynamics that occur in response to a neurotoxic methamphetamine treatment regimen. Previous studies have shown that the repeated administration of methamphetamine is associated with a marked increase of prodynorphin gene expression in the striatum (Hanson et al., 1988; Smith and McGinty, 1994). In view of the protective effects of U69593 observed in the present study, we hypothesize that this increase in gene expression may be compensatory and opposes the development of methamphetamine-induced neurotoxicity. Studies examining the influence of dynorphin and other prodynorphin-

derived peptides on the neurotoxic effects of methamphetamine are currently in progress.

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