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Effects of a Cold Environment or Age on Methamphetamine-Induced Dopamine Release in the Caudate Putamen of Female Rats

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BOWYER, J. F., B. GOUGH, W. SLIKKER, JR., G. W. LIPE, G. D. NEWPORT AND R. R. HOLSON. *Effects of a cold environment or age on methamphetamine-induced dopamine release in the caudate putamen of female rats.* PHARMACOL BIOCHEM BEHAV 44(1) 87–98, 1993.—Extracellular levels of dopamine (DA) and metabolites as well as serotonin [5-hydroxytryptamine (5-HT)] and 5-hydroxyindoleacetic acid (5-HIAA) were determined in the caudate putamen (CPU) of either 6- or 12-month-old female rats using microdialysis and high-performance liquid chromatography with electrochemical detection (HPLC-ED) before, during, and after four consecutive injections (given at 2-h intervals) of methamphetamine (METH). In 6-month-old rats administered 4 × 5 mg/kg METH at an environmental temperature (ET) of 23°C, peak extracellular DA levels (between 50 and 150 pg/10 μl) were attained 30–45 min after each dose of METH while dihydroxyphenylacetic acid (DOPAC) decreased steadily after the first doses of METH until it reached a plateau at 50% of control (550–700 pg/10 μl) levels. Increases in 5-HT levels during METH administrations paralleled DA increases while 5-HIAA decreases paralleled DOPAC decreases. The total CPU DA and 5-HT content of these rats was about 65% of control at 3 days post-METH. Reducing the ET to 4°C during dosing decreased the peak and average DA levels attained during the 4 × 5 mg/kg METH administration to about 50% of that observed at a 23°C ET. Increasing the dose to 4 × 10 mg/kg METH (4°C ET) increased peak and average CPU DA levels to 200% that observed during 4 × 5 mg/kg METH at a 23°C ET. However, no significant decreases in total CPU DA content of any rats dosed with METH at a 4°C ET were observed 3 days post-METH. In 12-month-old rats dosed with 4 × 5 mg/kg METH (23°C ET), the peak and average extracellular DA levels were only 30–60% that of 6-month-old rats. However, the CPU DA content of older rats was significantly decreased both 3 (30% control) and 14 (60% control) days post-METH. In summary, METH toxicity may not be predicted solely by the extracellular levels of DA attained during METH administration; age and ET also greatly influence METH neurotoxicity.

Methamphetamine Microdialysis Caudate putamen Dopamine Amino acids Neurotoxicity Aging

AMPHETAMINE- and methamphetamine (METH)-induced neurotoxicity were initially reported by Seiden et al. (26), Ellison et al. (10), Fuller and Hemrick-Luecke (12), and Hotchkiss and Gibb (14) after high to moderate doses of amphetamine or METH. This neurotoxicity took the form of long-term reductions in serotonin [5-hydroxytryptamine (5-HT)] and dopamine (DA) content of the caudate putamen (CPU). The mechanism(s) of this amphetamine neurotoxicity are not clearly understood. Seiden and colleagues suggested that an amphetamine-induced flood of DA release overwhelms the normal catecholamine metabolic machinery in the brain, leading to production of toxic by-products of DA, especially 6-hydroxydopamine (6-OHDA) (27). A second hypothetical mechanism relates amphetamine toxicity to stimulation of the NMDA receptors (30,31).

In support of a dopaminergic mechanism, Seiden detected 6-OHDA in the CPU of METH-exposed rats [albeit in small amounts and only after high doses of METH (26)]. Conversely, Sonsalla's group has been able to block METH neurotoxicity by blocking the NMDA receptor with MK 801, suggesting that METH is working through a now-classic excitotoxic mechanism. Recently, a third group used cerebral mi-

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crodialysis to show that there is a late surge of DA release in the CPU of young male rats given four consecutive doses of METH, that the magnitude of this surge is correlated with the degree of subsequent neurotoxicity, and that both this surge and the neurotoxicity are blocked by MK 801 (33). In further support of a glutaminergic/excitotoxic mechanism, this laboratory recently reported a downregulation of glutamate-evoked DA release 24 h post-METH, suggesting that METH may have triggered an increase in the release of glutamate at the time of exposure or shortly thereafter (6). Because glutamate can presynaptically evoke the release of DA through stimulation of NMDA receptors (17) and DA release mediated by METH and glutamate are additive (5), increased glutamate release from cortical striatal neurons during or shortly after METH administration could increase DA release. Therefore, NMDA antagonists could block glutamate-evoked DA release and reduce the formation of 6-hydroxy-DA; in such a case, the proposed mechanisms of neurotoxicity of Sonsalla et al. (31) and Seiden and Vosmer (27) would be interdependent.

If METH toxicity is related to dopamine release, or if a late DA surge is a biomarker for this toxicity, then one would predict that the magnitude of toxicity would be correlated with the magnitude of METH-induced DA release. Similarly, if METH-induced stimulation of the NMDA receptor (presumably through enhanced release of glutamate) is the toxic mechanism, then there should be a correlation between glutamate release and neurotoxicity. We report here two experiments conducted to test the above predictions. Both used microdialysis to measure METH-induced CPU DA and glutamate release under conditions thought to alter METH toxicity.

The first such condition was METH exposure in the cold. Temperature has been implicated as a factor in the neurotoxicity produced by MDMA (25) and has long been known to be a factor in the lethality of amphetamines (1,8,9,11,13,15,36). We recently reported that repeated administration of moderate doses of METH to adult, male rats can produce decreases in CPU dopamine and serotonin content and these decreases can be blocked by exposure at environmental temperatures (ETs) of 4°C (6). If METH toxicity is related to either DA or glutamate release, then one or both should be reduced when METH exposure occurs in the cold. We test this hypothesis by comparing the effects of 4 × 5 mg/kg METH doses at 2-h intervals on caudate microdialysate at room temperature to the effects of equal or higher METH doses at 4°C.

The second condition thought to alter METH toxicity was age at exposure. Older rats are typically more susceptible to neurotoxins, and this enhanced susceptibility could also be linked to alterations in METH-induced release of DA or glutamate. This possibility was assessed by microdialysis in the CPU of older (12 month) and younger (6 month) female rats given 5 mg/kg METH four times at 2-h intervals. In an attempt to replicate reports of a late DA surge following the fourth 5-mg/kg dose (20,33), we also tracked monoamine levels in CPU dialysate of older rats for 6 h after the last of four METH doses.

METHOD

Animals

All animals used in these experiments were female Sprague-Dawley rats from the NCTR colony and were approximately of either 6 or 12 months of age. They were housed in clear 45 × 22 × 20-cm acrylic cages with wood chips for bedding. Food and water were available ad lib. Rats were caged in a vivarium on a 12 L:12 D cycle with lights on 6:00 a.m. Temperature (23 ± 1°C) and humidity (53 ± 15%) were closely controlled.

Experimental Design

Two experiments were conducted. Both involved collecting microdialysate from probes implanted in the head of the CPU, and in both cases METH was administered IP four times (×) at 2-h intervals. The first experiment utilized female rats greater than 5 but less than 7 months of age and was a 2 × 2 factorial design, crossing meth exposure with ambient temperature. Rats were either controls or exposed to four doses of 5 mg/kg METH, and were dosed at either room temperature (23°C) or in a walk-in cold room (4°C). A fifth group was dosed with 4 × 10 mg/kg METH at 4°C but were not dosed with 4 × 10 mg/kg METH at a 23°C ET because of the lethality produced at this ET. In this first experiment microdialysate samples were collected at 15-min intervals for 8 h and analyzed for monoamine content.

The second experiment utilized both "younger" (6 months) and "older" (11–13 months) female rats. Again, this experiment was a 2 × 2 factorial design, crossing METH exposure with age, that is, each age group was divided into controls rats or those that received 4 × 5 mg/kg METH. In this second experiment, microdialysate samples were collected at 20-min intervals and analyzed for monoamine and amino acid levels. Collection continued for 6 h after the last injection. Both designs are shown in Table 1, with sample sizes.

Brain Dialysis and METH Dosing

To implant the microdialysis guide cannulae, animals were anesthetized with sodium pentobarbital (50 mg/kg, IP) and placed into a stereotaxic frame (Köpf, Topanga, CA). The level dorsal skull surface was exposed and a small hole was drilled to allow implantation of the intracerebral guide cannula (Carnegie Medicine, Stockholm, Sweden) in the caudate. Coordinates were AP 0.2 mm, Lat 3 mm, DV 5.0 mm relative to bregma (21). The guide cannula was fixed to the skull of the rat with dental acrylic and two anchor screws. The cannula was closed with a tight-fitting obturator between implantation and installation of the microdialysis probe. Body temperature during surgery and recovery from anesthesia was maintained at 37°C with a small heating pad (Deltaphase Isothermal Pad, Braintree Scientific, Braintree, MA) under the animal. To avoid effects of anesthesia and allow recovery from surgical trauma, the dialysis experiments were started no sooner than 3 days after surgery.

On the morning of the test, the animal was hand held and the dialysis probe was inserted through the guide cannula and into the underlying caudate. Microdialysis probes used in this

TABLE 1
EXPERIMENTAL DESIGN

	Experiment 1		Experiment 2	
	No. Tested at 23°C	No. Tested at 4°C	No. of Young Rats	No. of Old Rats
Control	5	6	8	3
5 mg/kg METH	5	6	9	8
10 mg/kg METH	–	6	–	–

study were CMA/12 [Bioanalytical Systems (BAS), West Lafayette, IN] and measured 14 mm in length and 0.65 mm in diameter. The membrane tip was 2.0 × 0.5 mm. The probes had an in vitro recovery efficiency of between 12 and 14% for either 5-HT and DA or amino acids at a flow rate of 1.0 μl/min. The two types of Ringers solutions used for these experiments, either a glucose-free pH 6.2 Ringers solution of 147 mM Na^+, 2.3 mM Ca^+, 4 mM K^+, 155.6 mM Cl^- (12), pH 6.2, in experiment 1 or a glucose-containing pH 7.4 Krebs-Ringer solution of 140 mM Na^+, 1.25 mM Ca^{2+}, 1.5 mM Mg^{2+}, 4.5 mM K^+, 121 mM Cl^-, 25 mM CO_3^-, 10 mM *D*-glucose, 100 μM ascorbate, pH 7.4, in experiment 2, were perfused at a flow rate of 1.0 μl/min using a CMA/100 microinjection pump (Carnegie Medicine). The pH 7.4 Krebs-Ringer buffer solution containing glucose was used only on many animals in experiment 2 to determine if a more physiologically buffered solution decreased basal amino acid release or altered monoamine release (18). Animals undergoing testing were housed in an awake animal system (20 in diameter, 12 in. deep glass bowl with balancing arm) with wire tether and single-channel swivel. Dialysates were collected at either 15-min intervals (if just monoamine levels were to be determined) or 20-min intervals (if both monoamines and amino acids levels were determined) into 250-μl conical tubes. In animals in which the pH 7.4 Krebs-Ringer solution was used, samples were injected immediately after collection to avoid degradation of monoamines and metabolites. After relatively stable baseline values for catecholamines were established (less than 15 pg/10 μl microdialysate, which occurred 1–2 h after initiation of microdialysis insertion), animals were injected with METH. All rats dosed with METH received four doses of either 5 or 10 mg/kg METH (IP) spaced 2 h apart. Animals dosed at room temperature received only 4 × 5 mg/kg METH. Those dosed in the cold received either 4 × 5 or 4 × 10 mg/kg METH. Some control rats were injected with saline (150 mM NaCl) according to the same dosage regimen. However, not all control rats received saline injections because pilot studies could detect no effect of saline injection on dialysate levels of monoamines or their metabolites. Rats receiving injections at 4°C were first placed in a walk-in cold room 30 min prior to administration of saline or METH and remained in the cold until 1 h after the last injection.

Most rats used for microdialysis were sacrificed 1, 3, or 14 days after either METH or saline administration. Animals were sacrificed by decapitation and brains were removed and immediately immersed in 4°C normal saline. The right and left cerebral hemispheres were then separated, and the right cerebral hemisphere was fixed in formalin (10%)/normal saline solution for later histological verification of cannulae and probe location. The CPU and hippocampus of the left hemisphere were immediately dissected and stored at −70°C until total monoamine content could be determined.

Determination of Microdialysate Monoamine and Metabolite Levels

To determine the levels of monoamines in the microdialysate, 10 μl (either one half of the 20-μl microdialysate collected for each time interval when both monoamines and amino acid levels were determined or 67% of the microdialysate collected when only monoamines were determined) of the collected microdialysate was injected onto the high-performance liquid chromatography (HPLC) column. Analysis of the DA, dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA), 5-HT and homovanillic acid (HVA) by HPLC with electrochemical detection (HPLC-ED) (BAS 460 systems, BAS) in the microdialysate was determined using a Phase-II cartridge column (BAS, 100 × 3.2 mm, 3 μm). The mobil phase consisted of 0.15 M monochloroacetic acid, 0.86 mM sodium octyl sulfate, 0.67 mM EDTA, and 2.5% acetonitrile, pH 3.0. Flow on the column was 1.0 ml/min at 40°C with an electrode operating at an applied voltage of 0.8 V. Retention time for elution of all compounds under investigation was less than 10 min; each sample was assayed immediately after collection to prevent decomposition. Pilot studies in the rat and monkey showed that in our system 3-methoxytryptamine (3-MT) formed a separate peak and did not coelute with serotonin.

Determination of Monoamine and Metabolite Content in the CPU and Hippocampus

Tissues were weighed and diluted with a measured volume (1:10, w/v) of 0.1 N perchloric acid. The cells were then disrupted by ultrasonication for 15 s in a conical tube that was immersed in a small ice bath. Cell debris was removed by centrifugation of the sonicate at 10,000 × *g* for 10 min at 4°C. The supernatant was then filtered in the cold (4°C) through a 0.45-μm microfilter (MF-1 microcentrifuge filter, BAS). Aliquots of the filtrate were injected directly onto an HPLC-ED system for separation of monoamines and their metabolites.

DA, 5-HT, and their metabolites were measured using HPLC with a reverse-phase Supelcosil^R LC-18 column and electrochemical detection (17). A mobile phase (pH 3.0) consisting of 70 mM KH_2PO_4, 1 mM sodium 1-heptanesulfonate, 0.1 mM EDTA, and 8% methanol was used to separate DA, DOPAC, HVA, 5-HT, and 5-HIAA.

Determination of the Amino Acid Levels in the Microdialysates

The 10-μl aliquots used for analysis of the amino acid levels in the microdialysates were derivitized with *o*-phthaldialdehyde and separation on HPLC-ED using methods similar to Zielke (39) as modified from Lindroth and Mopper (16). The amino acid levels are reported as their concentration (μM) in the microdialysate.

Statistical Analysis

Both experiments used factorial designs with repeated measures. Accordingly, both were analyzed with analysis of variance (ANOVA) using a 2 × 2 factorial design that tested for main effects of dose, room temperature, and their interaction (Experiment 1) or for main effects of age, dose, and their interaction (Experiment 2). In both cases, the repeated-measures (dialysate samples over time) nature of the design also provided a main effect of time and two-way interaction between time and both main effects. Because there were typically more measurements over time than there were subjects, a multivariate analysis of the repeated measures was not possible. Hence, all tests of time effects, and all interaction terms that included the repeated-measures factor, were conducted using the Huynh–Feldt adjustment for autocorrelation. Alternatively, single treatment conditions (5 mg/kg METH at 23°C, e.g.) were sometimes analyzed over time by using within-subject *t*-tests to compare pre-METH baseline values with same-subject values at specific times during METH exposure.

In addition, a fifth condition (10 mg/kg METH at a 4°C ET) was used in the first experiment. This unbalanced condi-

tion was not included in the factorial design. Rather, mean dialysate levels averaged over the entire time course were analyzed separately in a one-way ANOVA together with the four other dose conditions (control or 5 mg/kg METH at room temperature or 4°C). If this one-factor, five-level ANOVA was significant, Duncan's test was used for posthoc comparisons among the five dose conditions.

RESULTS

Experiment 1

At 23°C, each of the four injections of 5 mg/kg METH produced a peak in the CPU microdialysate levels of DA and 5-HT (see Fig. 1 for DA and Fig. 2 for 5-HT), this peak occurring from 30–45 min postinjection. The four consecutive monoamine peaks produced by METH showed a dampened oscillation, with each peak tending to be lower than the last. Consequently, for DA only the first two peaks were significantly above baseline (within-subjects *t*-test), while for 5-HT, which did not show as pronounced a dampening over doses, only the fourth and last peak did not significantly exceed the same-subject baseline. Between peaks, the microdialysate levels of both DA and 5-HT decreased slowly but never to levels seen in same-subject baseline or in nontreated controls. DOPAC and 5-HIAA microdialysate levels showed a different response pattern (see Fig. 3 for DOPAC; 5-HIAA results not shown). Both metabolites were depressed by METH but this depression did not show the pronounced peaks and valleys obtained for DA and 5-HT. Because this depression reached almost 50% of control after the first dose, and declined slowly thereafter, each of the four doses produced a significant depression of DOPAC and 5-HIAA as compared to the same-subject baseline.

When ambient temperature and METH effects were compared in a dose (control or 5 mg/kg METH) × temperature (23 or 4°C) factorial design with time as a repeated measure (30 consecutive 15-min microdialysate samples), room temperature had a pronounced effect on DA levels in the microdialysate. Beginning with microdialysate DA levels (Fig. 1—the cold-room control values were not different from the 23°C controls and so were not shown to reduce visual clutter), there were main effects of METH [METH increased DA release in a dampened pulsatile pattern over doses, at both temperatures, $F(1, 19) = 45.5$, $p < 0.0001$], of ambient temperature [DA release was reduced in the cold, $F(1, 19) = 4.9$, $p < 0.039$], and a significant dose × temperature interaction [METH-induced DA release was lower at 4 than at 23°C, while control values were not altered by ambient temperature,

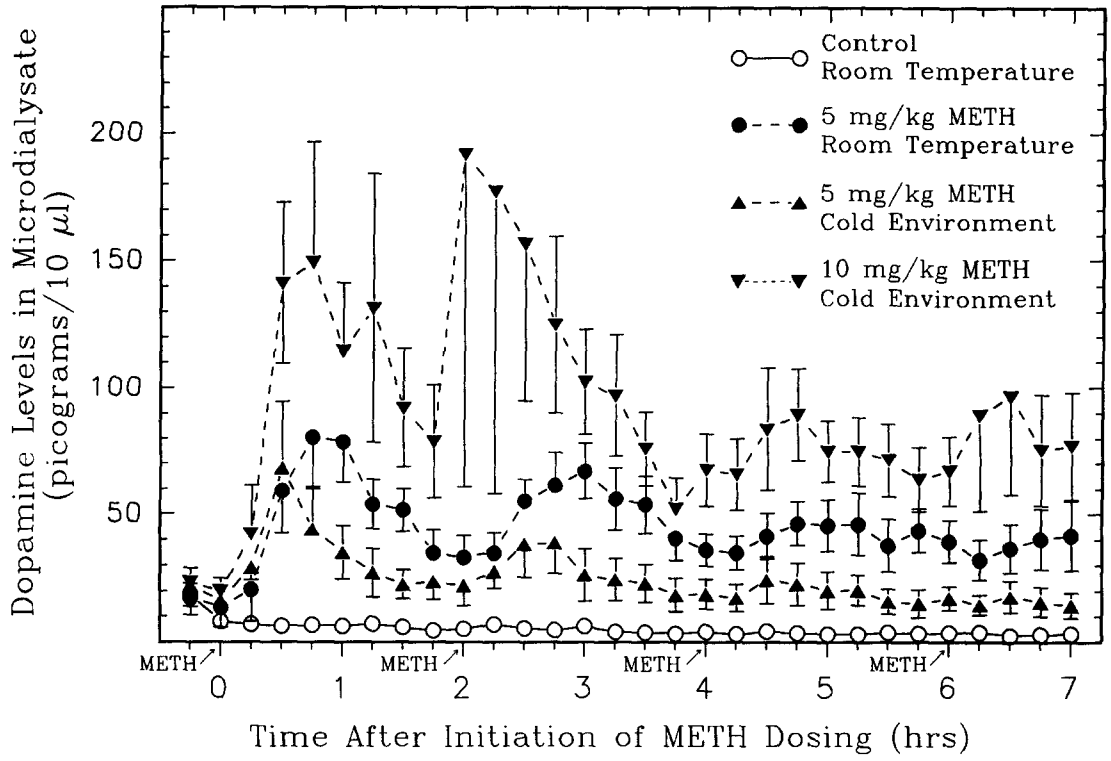

FIG. 1. Interactions of a cold environmental temperature with methamphetamine (METH)-induced increases in extracellular dopamine (DA) levels in the caudate putamen (CPU). Changes in the extracellular levels of DA during METH administration in 6-month-old rats are shown as fluctuations of DA levels in microdialysate collected at 15-min intervals. The administration of METH occurred just prior to collection of the microdialyste and is indicated by the arrows on the *x*-axis. Only the room temperature control (O) and not the cold room controls, is shown for clarity of presentation of METH effects. The release of DA differed significantly over the 7-h time. For levels averaged over all 7 h, the 10-mg/kg METH groups' levels at 4°C environmental temperature (ET) were significantly greater than all other groups, and the 5-mg/kg METH levels were significantly higher at 23 compared to 4°C ET (see the text). The number of rats per groups was five for control, five for 4 × 5 mg/kg METH at 23°C ET, six for 4 × 5 mg/kg METH at 4°C, and six for 4 × 10 mg/kg METH at 4°C ET.

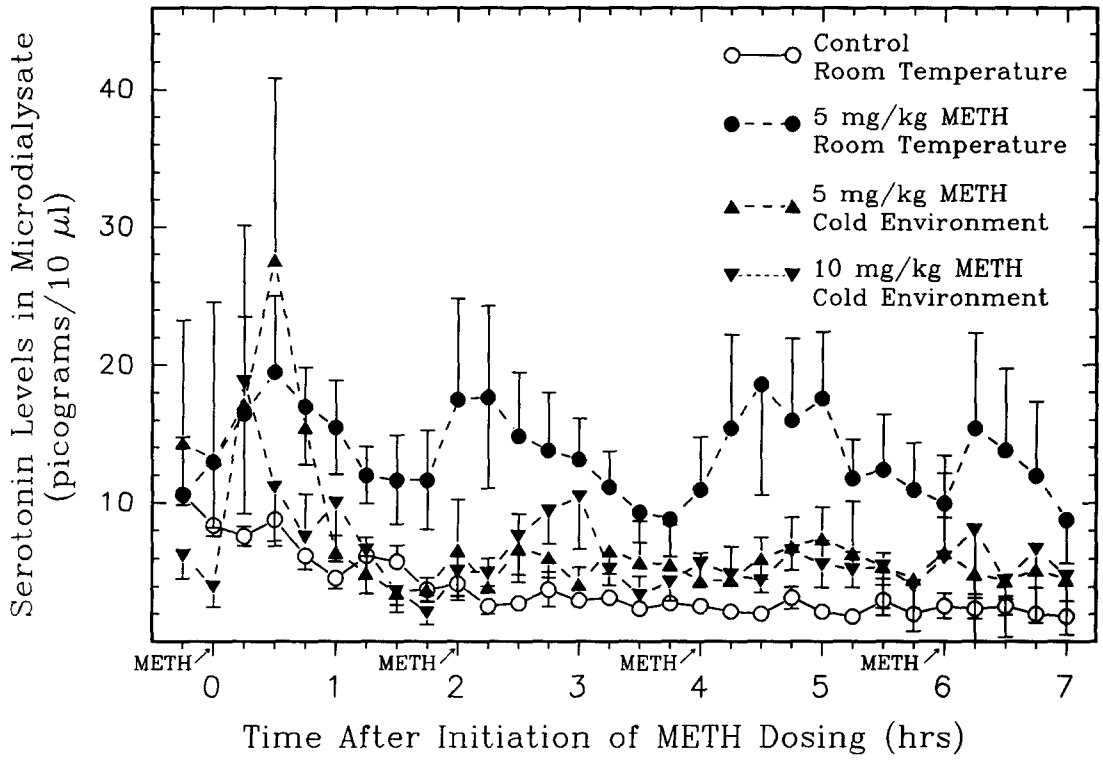

FIG. 2. Interactions of a cold environmental temperature with methamphetamine (METH)-induced increases in extracellular serotonin 5-hydroxytryptamine (5-HT) levels in the caudate putamen (CPU). Changes in the extracellular levels of 5-HT during METH administration to 6-month-old rats are shown as fluctuations in the 5-HT of microdialysate collected at 15-min intervals. The mean release of 5-HT in the 5-mg/kg METH group at room temperature was significantly higher than for either control ($p < 0.025$); the 5-HT release after either 5 or 10 mg/kg METH at 4°C did not differ from that caused by 5 mg/kg at room temperature.

$F(1, 19) = 5.5, p < 0.035$]. The main effects of time and the time × dose interaction were also significant [$F(29, 551) = 5.0, p < 0.001$, for time; $F(29, 551) = 5.5, p < 0.0005$, for the time × dose interaction, this latter effect indicating that the METH-induced and control DA values responded differently over time]. Similar dose effects were obtained for 5-HT [Fig. 2—METH increased 5-HT levels, $F(1, 18) = 11.2, p < 0.004$], but temperature effects were not significant and none of the interaction terms reached significance.

The primary metabolites of DA and 5-HT also showed substantial effects of ambient temperature. CPU microdiaylsate levels of DOPAC (Fig. 3) and 5-HIAA (data not shown) revealed a main effect of room temperature [for DOPAC, $F(1, 18) = 7.6, p < 0.015$; for 5-HIAA, $F(1, 15) = 6.7, p < 0.025$]. In both cases, levels of these two metabolites were reduced by exposure to cold in the METH and control groups equally given that there was not a significant dose × temperature interaction for either metabolite. DOPAC but not 5-HIAA also showed a main effect of dose, $F(1, 18) = 11.4, p < 0.005$. The main effects of sampling over time were significant for both compounds [DOPAC, $F(29, 551) + 5.0, p < 0.001$; 5-HIAA, $F(29, 522) = 31.9, p < 0.0001$] as was the time × dose interaction [DOPAC, $F(29, 522) = 20.1, p < 0.0001$; 5-HIAA, $F(29, 435) = 4.2, p < 0.005$]. This finding simply means that the metabolites in the METH condition declined steadily over time, while controls did not change much in the 7.5 h of recording. Finally, 5-HIAA but not DOPAC also showed a significant time × ambient temperature effect, $F(29, 435) = 3.8, p < 0.01$, with microdialysate levels dropping more rapidly at the 4°C ET.

METH exposure at 4°C had a substantial protective effect on drug-induced mortality, so much so that at this temperature rats tolerated a 4 × 10 mg/kg METH regimen that was lethal at 23°C. When this condition was added to the four conditions analyzed above (5 mg/kg METH or control at 4°C or 23°C), the higher dose substantially increased DA release (Fig. 1) to levels (averaged over all 30 samples) that were twice as high as those seen under any other conditions, including 5 mg/kg METH at 23°C [10 mg/kg METH at 4°C = 93.2; 5 mg/kg METH at 23°C = 44.4; 5 mg/kg METH at 4°C = 24.7; 23°C control = 5.2; 4°C control = 3.8 (all in units of pg/10 μl microdialysate), $F(4, 23) = 8.3, p < 0.0003$]. This substantial increase in DA was not mirrored in 5-HT, DOPAC, or 5-HIAA. For none of these latter compounds were overall levels elicited by the 10-mg/kg METH condition at 4°C higher than those seen with 5 mg/kg METH at room temperature (Fig. 3 for DOPAC, Fig. 2 for 5-HT, data not shown for 5-HIAA).

In rats dosed with 4 × 5 mg/kg METH at 23°C, a significant decrease in the total CPU DA content occurred at sacrifice 3 days after METH, while the metabolites of DA and both 5-HT and 5-HIAA were not significantly reduced (Table 2). No significant decrease in total CPU DA content was observed 3 days post-4 × 10 mg/kg METH at 4°C ET. The total CPU DA content was not determined in 6-month-old rats at 14 days post-METH because we have previously been

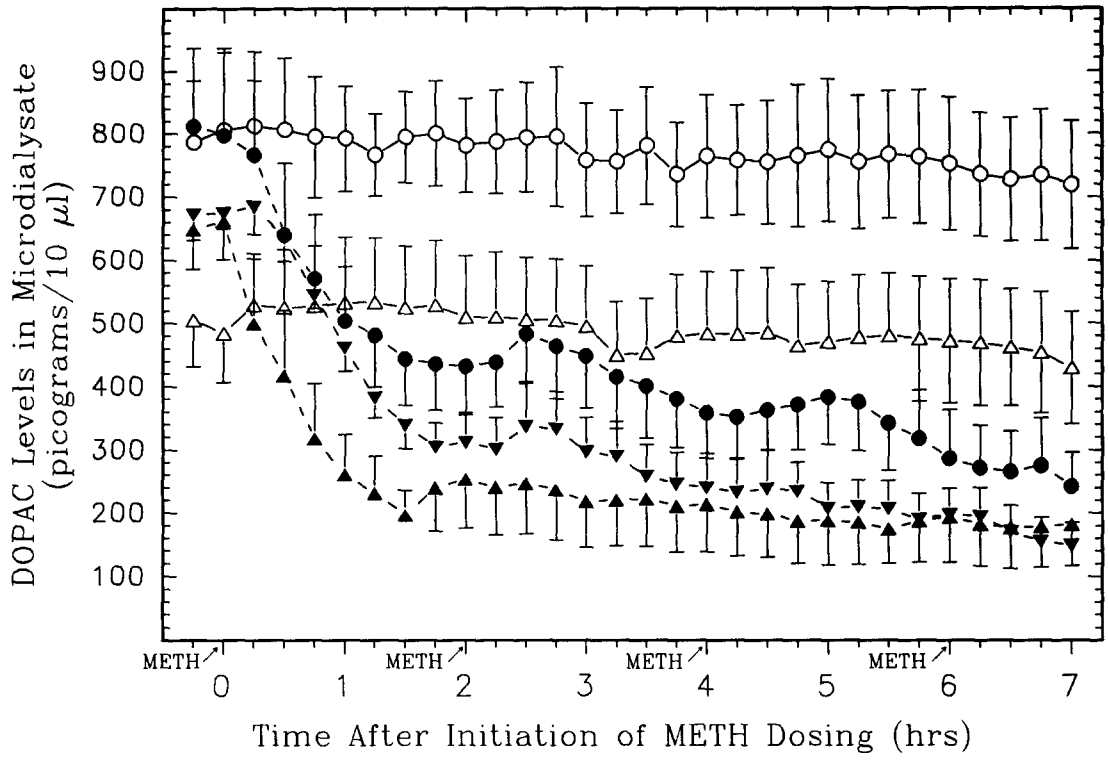

FIG. 3. Interactions of a cold environmental temperature with methamphetamine (METH)-induced decreases in extracellular dihydroxyphenylacetic acid (DOPAC) levels in the caudate putamen (CPU). Changes in the extracellular levels of DOPAC during METH administration in 6-month-old rats are shown as fluctuations of DOPAC in microdialysate collected at 15-min intervals. The symbols represent the same groups as in Fig. 1 (control, ○); 5 mg/kg METH at 23°C ET, ●; 5 and 10 mg/kg METH at a 4°C ET, ▲, ▼, except the control at a 4°C ET are also represented (△). For the two controls and two 5-mg/kg METH groups, the release of DOPAC was significantly ($p < 0.005$) reduced by METH within 1 h of the initiation of dosing at either a 23 or 4°C environmental temperature (ET). The release of DOPAC was also significantly, ($p < 0.02$) reduced by cold, and there was no dose × temperature interaction, showing that METH release was reduced equally in dosed and control animals at 4°C ET. The mean release of DOPAC in 4°C rats given 10 mg/kg METH did not differ from either 5 mg/kg METH group.

unable to show significant decreases at this post METH time point in female rats (unpublished data).

Experiment 2

In this second experiment, the effect of METH (4 × 5 mg/kg) on DA levels in the microdialysate from the CPU of 6-month-old rats was similar to that seen in the first experiment. Again, each injection triggered a DA peak around 40 min postinjection, with peak response declining monotonically across the four doses and trough levels remaining substantially above baseline (Fig. 4A). The extracellular CPU DA levels were not as substantially elevated by 4 × 5 mg/kg METH at 23°C ET in 12-month old rats as compared to younger animals (Fig. 4A). DA levels averaged over all 23 of the 20-min samples in the 12-month-old METH-treated rats were only 19.4 ± 4.1 ρg/10 μl compared to 54.8 ± 8.3 ρg/10 μl for younger rats. Further, average DA levels in older controls (1.8 ± 0.3 ρg/10 μl) were considerably lower than at 6 months (13.5 ± 6.1 ρg/10 μl). A two-factor age × dose analysis with time (23 consecutive 20-min samples) as a repeated measure verified that age and dose had significant and independent effects on DA levels in CPU microdialysate. As the above figures indicate, age decreased overall DA levels, $F(1, 19) = 9.1, p < 0.01$, while METH increased DA levels, $F(1, 19) = 11.3, p < 0.003$. There was no significant age × dose interaction, demonstrating that DA was decreased proportionally in both older controls and older METH rats. Finally, there were also significant effects of time, $F(22, 418) = 3.3, p < 0.007$, and a significant time × dose interaction for CPU DA levels, $F(22, 418) = 3.1, p < 0.02$.

The reduction of extracellular levels of CPU DA in 12- compared to 6-month old rats was accompanied by a similar reduction in the DOPAC levels of older rats (Fig. 4B). Again, a factorial analysis revealed a significant main effect of age, $F(1, 18) = 8.4, p < 0.02$, without an age × dose interaction.

In older rats, the levels of 5-HT were not quantifiable either before or after METH. This suggests that the decline in extracellular DA was accompanied by a decline in 5-HT, although this issue cannot be resolved by the present study.

Extracellular DA and DOPAC levels were monitored for 6 h after the last dose of METH in older rats. In seven of eight of the 12-month-old rats, there was no late surge in DA release. Instead, DA levels tended slowly and smoothly downward over that period (Fig. 4A). In only one of the older METH-treated rats were increases in DA levels observed 5 h after the last dose of METH. This rat had already developed respiratory difficulties and had to be euthanized 16 h after the

TABLE 2

CPU CONTENT OF DOPAMINE, SEROTONIN, AND METABOLITES AFTER METH ADMINISTRATION

Dose (mg/kg)	Age (months)	Days After Treatment to Sacrifice	Dopamine (ng/100 mg)	DOPAC (ng/100 mg)	HVA (ng/100 mg)	5-HT (ng/100 mg)	5-HIAA (ng/100 mg)	n
Control	6	3	1,025 ± 57	71 ± 5	41 ± 4	45 ± 3	18 ± 2	5
5*	6	3	683 ± 101†	58 ± 6	42 ± 9	26 ± 7	17 ± 6	7
10‡	6	3	1,032 ± 72	78 ± 17	50 ± 18	60 ± 17	26 ± 4	4
Control	12	3 & 14	1,092 ± 29	72 ± 3	38 ± 6	49 ± 9	20 ± 3	4
5*	12	3	327 ± 87†	33 ± 5†	15 ± 3†	16 ± 2†	11 ± 2†	5
5*	12	14	626 ± 251†	47 ± 13†	28 ± 8	26 ± 7†	10 ± 3†	4

All values are nanograms per 100 mg tissue wet weight, ±SEM.
*Dosed at a 23°C ET.
†Content significantly less than control ($p < 0.05$).
‡Dosed at a 4°C ET.

last dose of METH. Although only three 6-month-old rats were monitored out to 6 h after the last dose of METH, there were also no late increases in DA levels in these rats (data not shown).

Despite the lower METH-induced release of DA in the CPU of older rats, and in the absence of a late DA release (surge), METH toxicity was enhanced. Thus, at sacrifice 3 days post-METH CPU DA content was only 30% of same-age controls in 12-month-old rats, while these levels were 67% of controls in younger rats (Table 2). Further, at 2 weeks post-METH CPU tissue levels were still depressed to 57% of control in the older rats. Similar effects were seen for total CPU 5-HT content (Table 2). Note that no such long-term effect of METH on either DA or 5-HT CPU content was seen in younger rats. It is also important to note that despite lower levels of DA release in the CPU of 12-month-old rats the CPU DA and 5-HT content were almost the same in the two ages. This suggests that the lowering of the extracellular CPU DA release seen in 12-month-old rat is a regulatory phenomenon, not an effect of DA cell death or terminal degeneration.

Extracellular CPU glutamate and taurine levels (determined in the same microdialysate samples that produced the monoamine data in Fig. 4) are shown in Fig. 5. These figures have been simplified by the exclusion of 12-month-controls, which did not differ from younger controls. It is clear that taurine levels were relatively stable over the entire time course, that these levels showed no apparent response to METH administration, and that there were no substantive age effects on extracellular CPU taurine levels (Fig. 5B). In contrast, average glutamate levels were initially high and then dropped precipitously over the next 7 h in all groups. Hence, for glutamate too there were no overall effects of dose or age (Fig. 5A).

The apparent steady decline in glutamate levels is, however, somewhat misleading. The group means conceal the fact that there were two distinct glutamate patterns. In one set of rats (about 50% of all animals, irrespective of dose or age), initial glutamate levels were high. No rat in this group had an initial glutamate level of less than 10 μM at the 0.0 time point ($n = 13$ rats). In this group, the initial high levels were followed, after varying time intervals, by a rapid drop of all amino acids, except taurine, to levels less than 0.25 μM. In a second group, initial glutamate levels were low, averaging only 4.5 μM at time point 0.0 ($n = 15$ rats). In this subset, levels were more stable and did not rapidly drop to levels below detection during the entire time course (see control group, Fig. 6A). However, there were no clear differences in the taurine levels in control rats that initially had low glutamate levels and all rats tested (compare Figs. 5B and 6B).

The reason for this dichotomy in glutamate levels is unclear. There was no correlation with age (5 of the 11 older rats were in the high glutamate group while 7 of the 17 younger rats had high initial glutamate levels). There was also no correlation between initial DA and glutamate levels ($r = 0.02$). Further, the use of a more physiological dialysis buffer (a bicarbonate-buffered KRH containing physiological concentrations of mono- and divalent cations and glucose) did not alter monoamine or amino acid levels in the microdialysate at any time.

If only those rats with initially low levels of glutamate are considered (Fig. 6), there was a tendency for extracellular CPU glutamate levels to increase over the time course of METH dosing, in particular in older rats (Fig. 6A). However, these effects were not statistically significant in part due to the small sample size resulting from division of our total sample into high-glutamate and low-glutamate subgroups.

DISCUSSION

There are numerous studies of the effects of a single administration of amphetamine and METH on extracellular increases in CPU DA, 5-HT, and their metabolites (23,28,29, 33,35,37,38). However, only recently have the effects of multiple doses of METH on DA and 5-HT extracellular levels using the techniques of microdialysis been implemented as a means to further understand how multiple administration of METH may produce neurotoxicity (20).

Using the techniques of microdialysis, O'Dell et al. (20) reported that a surge in the extracellular CPU DA levels occurs 1–2 h after the last dose of METH (using the same dosing paradigm we employed and 4 mg/kg METH/dose) in 2- to 3-month old male rats and that this surge can be attenuated with the noncompetitive glutamate/NMDA antagonist MK 801 (32). This DA surge might be the result of a surge in the extracellular levels of glutamate that could cause further increases in DA release over the DA release evoked by the direct presynaptic actions of METH. These results appear to further elucidate how MK 801 and competitive NMDA antagonists protect against METH toxicity (30,31). MK 801 does not appear to directly block the METH-induced DA release

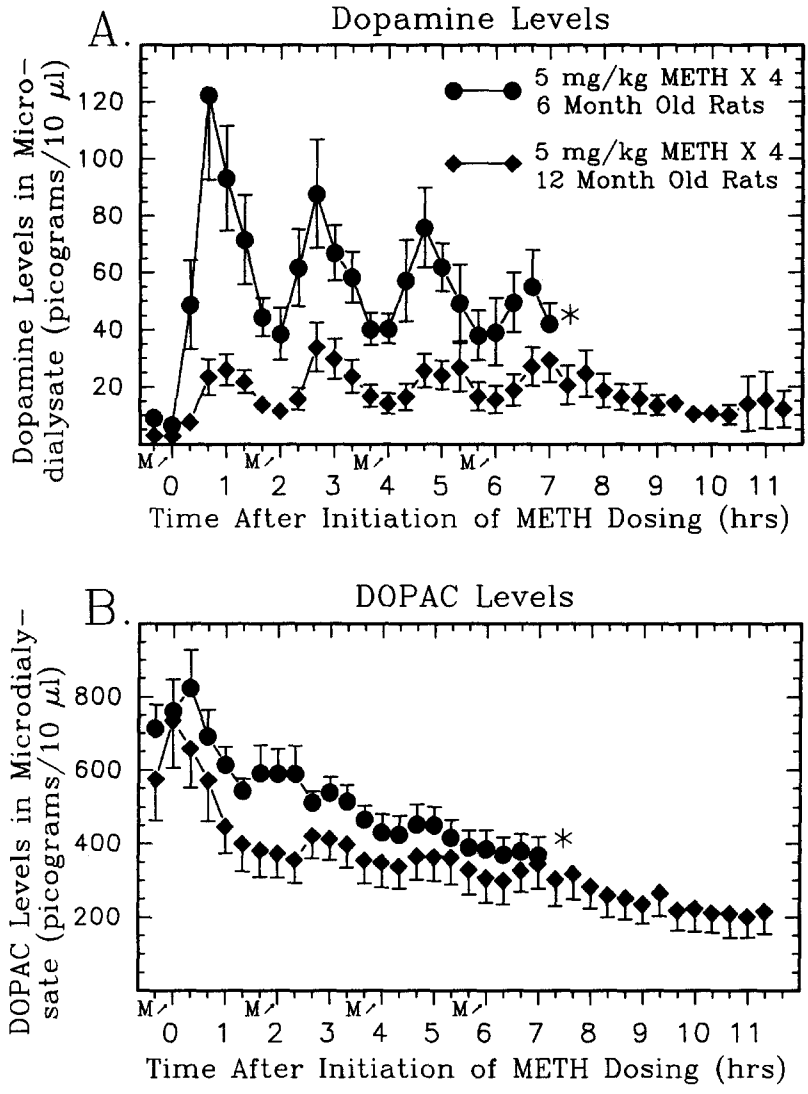

FIG. 4. Differences between 12- and 6-month-old rats in the extracellular caudate putamen (CPU) levels of dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) during methamphetamine (METH) administration. Changes in the extracellular levels of DA (A) and DOPAC (B) during METH administration [23°C environmental temperature (ET)] are shown as fluctuations of either DA or DOPAC in microdialysate collected at 20-min intervals. The M and the arrows on the *x*-axis indicate the time of METH administration. The levels of DA for 12-month-old rats were significantly ($p < 0.0075$) less over the time course of METH administration. Mean DA levels for controls over 7 h were 1.8 pg/10 μl from older rats and 16.5 pg/10 μl for younger animals. Although METH decreased DOPAC levels to the same extent in both age groups, the initial DOPAC levels were lower in 12-month-old rats prior to METH ($p < 0.006$) The number of METH-exposed rats was nine for 6-month-old rats and eight for 12-month-old rats. *Levels for 6-month-old rats not determined past this time point.

that results from the reversal of the DA uptake transporter (5), one of the primary means by which amphetamines evoke DA release and block DA reuptake (22,24). However, because (presynaptically) glutamate- and METH-evoked DA release are primarily additive (5) the increased release of glutamate during or shortly after in vivo METH could increase DA release and possibly the formation of neurotoxins such as 6-hydroxy-DA (27). Further, it has been observed that there is a decrease (downregulation?) in glutamate-evoked DA release 1 day post-METH, suggesting that excessive glutamate/NMDA stimulation of presynaptic DA terminals may occur during or shortly after METH (6).

We did not observe a surge in DA at any time during or up to 6 h after the last dose of METH in seven of the eight 12-month old female rats (Fig. 4A). It might be expected that 6-month-old female rats would not show a post-METH DA

or glutamate surge because they only had moderate decreases in CPU DA content at 3 days, and no decreases at 14 days, post-METH. However, in 12-month-old rats significant short- and long-term changes (neurotoxicity?) in the CPU DA content occurred (Table 2) despite the lack of an extracellular DA surge (Fig. 4A). Therefore, although the late surge of DA observed in male rats may either explain why male rats are more susceptible to METH neurotoxicity or predict the occurrence of neurotoxicity this surge may not be necessary to produce or precede neurotoxicity. Because our rats were also older than those tested by O'Dell et al. (20), an increase in age may also explain the absence of a post-METH DA surge.

Although a surge in extracellular DA was not observed during METH dosing, it was found that glutamate levels tended to rise to higher levels in rats (particularly the 12-month-olds) 5 h after initiation of dosing in the subsets of rats that had lower initial glutamate levels (Fig. 6A). However, when those rats with high initial glutamate levels (between 10

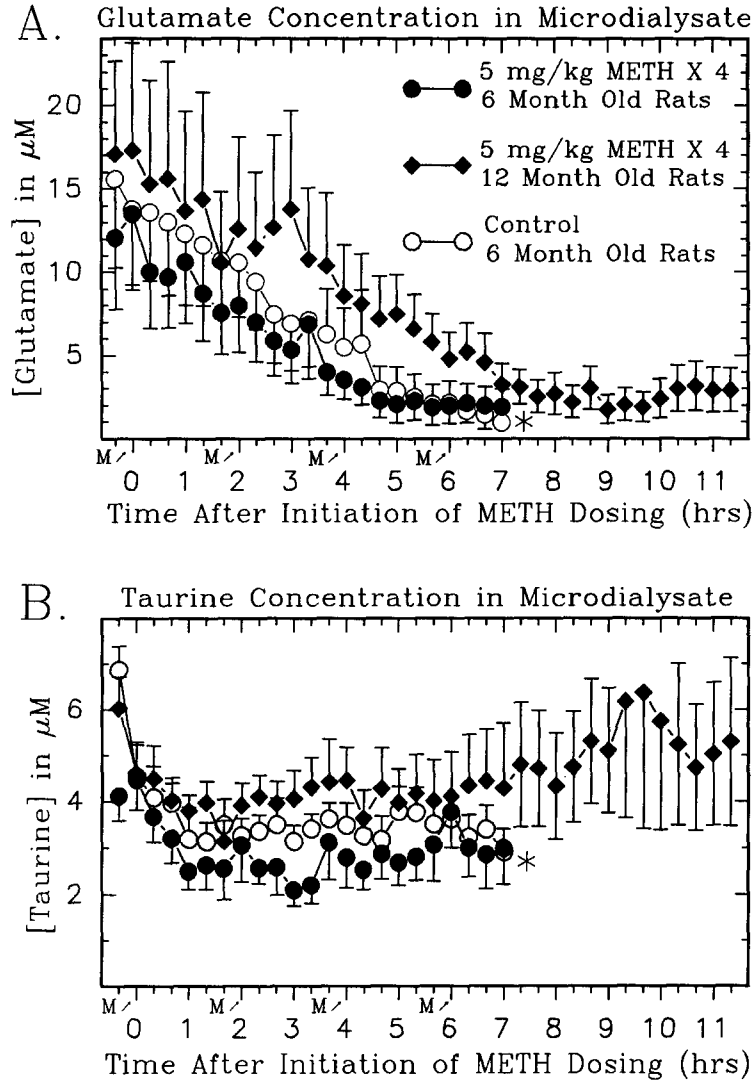

FIG. 5. Extracellular CPU levels of glutamate and taurine in 6- and 12-month-old rats administered methamphetamine (METH). Changes in the extracellular levels of glutamate (A) and taurine (B) during METH administration at a 23°C environmental temperature (ET) are shown as fluctuations in the concentrations of glutamate and taurine in the microdialysate determined at 20-min intervals [only 10 of the 20 μl microdialysate collected were used to determine the glutamate or taurine with the remaining 10 μl being used to generate the dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) levels for Fig. 4]. There were no significant differences between METH and control group (glutamate or taurine) at any time point. The number of rats per groups was eight for 6-month-old controls and nine for 6-month-old 4 × 5 mg/kg METH groups while eight rats were in the 12-month-old 4 × 5 mg/kg METH group. *Levels for 6-month-old rats not determined past this time point.

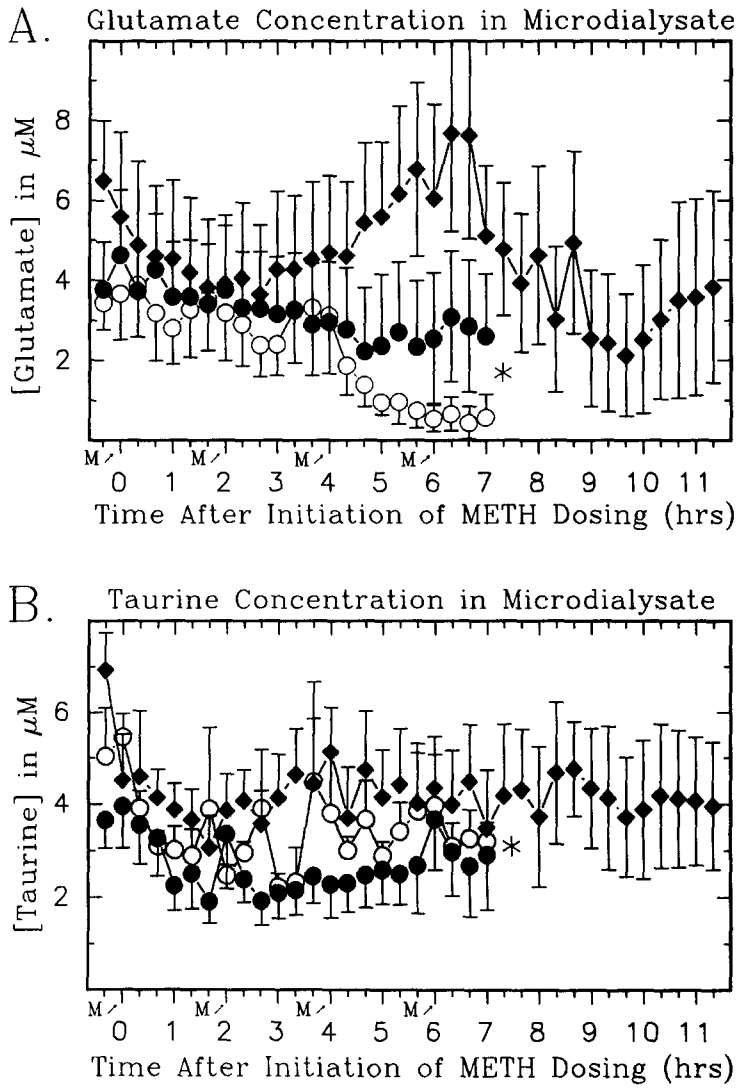

FIG. 6. Extracellular caudate putamen (CPU) levels of glutamate and taurine in 6- and 12-month-old rats, with low glutamate 2 h after initiation of microdialysis, administered methamphetamine (METH). A subgroup of rats used to generate Fig. 5 that had low levels of glutamate prior to METH administration were used to generate Fig. 6. There were no significant increases in the extracellular levels of either glutamate or taurine during the course of METH administration, although glutamate did tend to increase over the course of METH exposure in younger rats. The number of rats per groups was four for 6-month-old controls and 5 for 6-month-old 4 × 5 mg/kg METH groups while four rats were in the 12-month-old 4 × 5 mg/kg METH group. *Levels for 6-month-old rats not determined past this time point.

and 22 μM) are combined with the groups in Fig. 6A (Fig. 5A) the effect was not notable. This could either be due to the increased background of glutamate levels obscuring the ability to detect glutamate increases or it may be that prolonged high extracellular CPU levels of glutamate prevent the subsequent rise. Because glutamate has been implicated in the NMDA-mediated presynaptic release of DA (17), a concomitant increase in extracellular DA levels might be expected 5–7 h after initiation of METH dosing due to possible stimulation of NMDA receptors. Again, this was not observed (Fig. 4A). However, there was also no correlation between DA and glutamate levels at time point 0.0 in individual rats. It is possible that the elevation of extracellular glutamate in the CPU for several hours could affect any NMDA-mediated DA release that would normally occur or that other damage incurred during probe implant has blocked the normal regulation of DA release by glutamate. An alternative explanation for the lack of correlation between DA and glutamate levels in the micro-

dialysate is that at the probe tip DA levels are a reflection of the average DA levels within several millimeters of the probe while the glutamate levels may only reflect the glutamate levels in the close vicinity of the probe tip and not overall CPU levels.

The DA release (extracellular CPU DA levels detected by microdialysis) during the entire time course of METH administration does not necessarily predict changes in DA terminals of the CPU because much less DA is released by METH (4 × 5 mg/kg at a 23°C ET) in older rats yet this group showed the most change in CPU DA content. In addition, within the 6-month-old age group DA levels attained during METH administration do not necessarily predict short-term decreases in CPU DA content. We observed significantly greater extracellular CPU DA levels in rats dosed with 4 × 10 mg/kg METH at a 4°C ET than those dosed with 4 × 5 mg/kg METH at a 23°C ET. Nonetheless, significant changes in total CPU DA content 3 days post-METH were only seen in rats dosed at room temperature. It may be argued that, although the cold environment enables rats to survive higher doses of METH that produce significantly greater DA release, the effects of this cold ET were only shown in the present experiments to affect short- and not long-term neurotoxicity. However, 6-month-old male rats receiving 4 × 5 mg/kg METH at 23°C ET showed significant decreases while the same age receiving 4 × 10 mg/kg METH at 4°C ET were not affected with respect to total CPU DA content 14 days post-METH (6). Further, the results presented herein and in Bowyer et al. (6) indicate that, although a 4°C ET may inhibit both basal and METH-evoked DA release in the CPU, the cold environmental temperature is protecting against METH neurotoxicity by mechanisms other than just inhibition of DA release. Although our results are compatible with the proposal that 6-hydroxy-DA is involved in METH toxicity, they indicate that the magnitude of the increases in extracellular DA levels during METH dosing may not be as important as other factors in the formation of 6-hydroxy-DA.

Evaluating the extracellular CPU levels of DA, 5-HT, and amino acids using the techniques of microdialysis is one of the most advanced techniques yet available to monitor in vivo neurotransmitter release. However, because the probe tips displace millions of neurons and glia and some capillaries a significant amount of damage to neurons, glia, and the blood–brain barrier probably occurs during the probe implant. Although a review by Benveniste (3) cites results indicating that the blood–brain barrier was intact within 30 min after probe implant, it is difficult to envision all the capillary damage resolving within that time frame. Using a more "physiological" dialysis buffer (a bicarbonate-buffered KR containing physiological concentrations of mono- and divalent cations and glucose) apparently does not reduce the possible damage as indicated by lack of any reduction in amino acid levels over the entire time course of microdialysis (see the Results section). We do not believe that the lower Ca^{2+} level in this buffer affected DA release, based upon direct comparisons between the two buffers, as well as the report by Westerink et al. (34) that METH-induced DA release is not affected by Ca^{2+} concentration in the microdialysis buffer. Although it might be argued that one should wait up to 8 h after probe implant before initiation of the experiment, this may only allow more time for the damage induced by probe to exacerbate because of the glutamate stimulation of both NMDA and non-NMDA receptors that occurs over the first 2 h. Further, if microglial infiltration was significant within 18 h or less a window of opportunity for microdialysis exists before prominent gliosis may occur [although there is some evidence that this effect is not as prominent or immediate with microdialysis probes compared to push–pull cannulae (3)]. Therefore, experiments evaluating the extracellular levels of monoamines and amino acids over a 12-h time period were initiated 2–3 h after probe insertion.

Despite any damage produced by probe implantation a slight surge in extracellular glutamate after METH administration was observed in extracellular CPU glutamate around the time of the final dose of METH in rats with initially low glutamate levels. Large increases in the extracellular levels of glutamate, like that observed in cerebral ischemia (4), might not be expected with METH neurotoxicity because only nerve terminal damage and not soma cytotoxicity has been reported (26). In addition, during METH administration the scavenging of released glutamate by glial cells (2,32) could reduce the ability to detect either physiological or toxicological increases in glutamate release. Therefore, the perceived magnitude of the extracellular glutamate increases may be less than those that actually occur at the synapse.

In summary, the short- and long-term effects of METH on DA terminals in the CPU may not always be predicted solely by either a) the extracellular levels of DA attained during METH administration or b) the magnitude of a 2- to 5-h post-METH administration surge of DA. Further, both age and ET also greatly influence METH-induced changes in the DA terminals of the CPU. Although an elevation of extracellular CPU glutamate may occur during METH dosing, it is not completely clear that it is sufficient to either produce or be involved in METH toxicity.

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