An Evaluation of l-Ephedrine Neurotoxicity with Respect to Hyperthermia and Caudate/Putamen Microdialysate Levels of Ephedrine, Dopamine, Serotonin, and Glutamate

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l-Ephedrine is an active ingredient in several herbal formulations with a mechanism of action similar to amphetamine and methamphetamine. However, its potential to damage dopaminergic terminals in the caudate/putamen (CPUs) has yet to be fully evaluated. The studies here used in vivo brain microdialysis experiments to determine the systemic doses and extracellular brain levels of l-ephedrine necessary to produce similar increases in CPU extracellular dopamine and marked hyperthermia that were previously shown necessary for amphetamine-induced neurotoxicity in male Sprague-Dawley rats. At an environmental temperature of 23°C, a single 40 mg/kg intraperitoneal (ip) dose of l-ephedrine produced marked hyperthermia (≥40°C), peak microdialysate ephedrine levels of 7.3 ± 1.2 μM, and a 20-fold increase in microdialysate dopamine levels. Twenty-five mg/kg produced a lesser degree of hyperthermia, peak microdialysate ephedrine levels of 2.6 ± 0.4 μM, and a 10-fold increase in dopamine levels. Three doses of 40 mg/kg given at 3-h intervals or 4 doses of 25 mg/kg l-ephedrine given at 2-h intervals were compared with 4 doses of 5 mg/kg d-amphetamine given at 2-h intervals. Multiple doses of either ephedrine or amphetamine caused severe hyperthermia (≥41.3°C) but striatal tissue levels of dopamine 7 days after dosing were reduced only 25% or less by ephedrine compared to the 75% reductions produced by amphetamine. The increases in CPU microdialysate levels of serotonin produced by either 4 × 25 mg/kg l-ephedrine or 4 × 5 mg/kg d-amphetamine did not significantly differ, but elevation of dopamine levels by d-amphetamine were over 2-fold times the level caused by l-ephedrine. Microdialysate glutamate levels were elevated to the same extent by either 25 mg/kg l-ephedrine or 4 × 5 mg/kg d-amphetamine. l-Ephedrine may not be as neurotoxic to dopaminergic terminals as d-amphetamine, because non-lethal doses of l-ephedrine do not sufficiently increase the CPU dopamine levels within nerve terminals or the extracellular space to those necessary for a more pronounced long-term dopamine depletion.

Key Words: neurotoxicity; microdialysis; ephedrine; striatum; dopamine; serotonin.

The medicinal use of ephedrine obtained from plant extracts dates back thousands of years (Chen and Schmidt, 1930).

1 To whom correspondence should be addressed at NCTR, HFT-132, Jefferson, AR 72079-9502. Fax: (870) 543-7745. E-mail: jbowyer@nctr.fda.gov.
mia during drug exposure (Bowyer et al., 1992, 1993, 1994, 1998; Bowyer and Holson, 1995; Eisch and Marshall, 1998; Miller and O’Callaghan, 1994; Schmued and Bowyer, 1997). However, little is known about whether ephedrine produces neurotoxicity similar to that produced by amphetamine and methamphetamine.

The current study was designed to determine the systemic doses of l-ephedrine necessary to produce hyperthermia in the rat, and the extracellular concentrations of ephedrine in the CPu after systemic doses of l-ephedrine which produce hyperthermia. In addition, increases in CPu extracellular levels of dopamine, 5-HT, and metabolites, as well as glutamate, after either l-ephedrine or d-amphetamine doses that cause hyperthermia, were determined. Finally, the potential for neurotoxicity to dopaminergic terminals was determined by measuring striatal tissue dopamine content 7 days after multiple doses of either l-ephedrine or d-amphetamine were administered. In vivo brain microdialysis was used to monitor changes in CPu extracellular levels of ephedrine and norephedrine, as well as changes in dopamine, serotonin, and glutamate levels. The effects of multiple doses of l-ephedrine that produced acute hyperthermia, and increases in CPu extracellular dopamine levels on striatal tissue dopamine levels 7 days after dosing were compared to the effects of neurotoxic doses of 4 × 5 mg/kg d-amphetamine. In addition, the effects of doses 4 × 25 mg/kg of l-ephedrine on CPu microdialysate levels of dopamine, serotonin (5-HT), and glutamate were compared to 4 × 5 mg/kg d-amphetamine. Comparing the effects of ephedrine with amphetamine should aid in evaluating the neurotoxic potential of ephedrine in humans. The more active (l) isomer of ephedrine was selected, rather than the (d) isomer (Chen and Schmidt, 1930), and was compared with the more active (d) isomer of amphetamine.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Crl:COBS CD [SD] BR), 4–6-months-old, were obtained from the breeding colony of the National Center for Toxicological Research (NCTR). In experiments not involving microdialysis, rats were pair-housed in acrylic cages (45 × 22 × 20 cm) on wood shavings bedding until the day before treatment, at which time each was individually housed until sacrifice 7 days later. Rats used for microdialysis were individually housed starting on the day of guide-cannula implantation and until sacrifice at 7 days post l-ephedrine treatment. To collect brain microdialysates, each rat was transferred into a microdialysis bowl 3 h before either l-ephedrine or d-amphetamine administration. Experiments were carried out at room temperatures of 22–23°C. Rectal temperatures (core body temperatures) were recorded hourly in animals not undergoing microdialysis, as previously described by Bowyer et al. (1994). In rats undergoing microdialysis, to avoid damaging the microdialysis equipment and implanted probe, rectal temperatures were only taken when rats became sluggish or collapsed from hyperthermia. To prevent the lethal effects of severe hyperthermia, rats in which temperatures exceeded 41.3°C were placed unrestrained on crushed ice for 15 to 25 min either in the microdialysis bowl (for subjects in the microdialysis experiments) or in the home cage. The Institutional Animal Care and Use Committee of the NCTR approved all the procedures involving animals. Studies were carried out in accordance with the declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Drugs. For these experiments, l-ephedrine HCl, l-norephedrine HCl, and d-amphetamine sulfate were purchased from Sigma Chemical Co. (St. Louis, MO). Amphetamines were dissolved in normal saline and injected ip. Concentrations of dosing solutions of l-ephedrine were verified using HPLC techniques described later.

Single doses of l-ephedrine. The dose of l-ephedrine necessary to produce hyperthermia (core body temperatures of greater than 39.5°C) and pronounced hyperactivity in a 22–23°C environment was determined. Rats were treated with 0, 20, or 40 mg/kg ip l-ephedrine, and rectal temperatures were recorded hourly. Activity levels were monitored after 20 and 40 mg/kg doses using 6 drug-naıve animals at each dose. The apparatus used to determine activity was a Plexiglas cube (46.5 × 46.5 × 46.5 cm) bisected with photo beams and interfaced with a computer (Ferguson et al., 1996). Each rat was weighed and placed into the cube for 60 min to determine the pre-drug (baseline activity), then injected with either 20 or 40 mg/kg l-ephedrine and observed for 2 h to determine post-drug activity. The activity was recorded as the number of beam breaks per h. Subsequently, a 40 mg/kg single dose of l-ephedrine was selected to be administered to rats implanted for the first microdialysis experiment (see below for details of microdialysis procedures), and levels of ephedrine and its metabolite norephedrine (phenylpropanolamine) were measured, as well as of dopamine, 5-HT, and their metabolites.

Multiple doses of either l-ephedrine or d-amphetamine. As determined above, in the first set of neurotoxicity experiments a dose of 40 mg/kg l-ephedrine was chosen for repeated administration with dosing intervals of 3 h, because it produced a hyperthermia above 39.5°C or greater in all the animals tested. The controls were given 3 doses of 1 ml/kg saline. Although dosing intervals of 2 h were used for previous methamphetamine and amphetamine studies, an interval of 3 h was selected, since the half-life of ephedrine in the microdialysate appeared to be about 50% longer than that previously observed with amphetamine (Clausing et al., 1995). Initially, 3 instead of 4 doses of 40 mg/kg (3 × 40) l-ephedrine were given to rats not implanted for microdialysis, so that the total time of ephedrine exposure would be as long as with amphetamine. Body temperature was measured hourly for 9 h from the start of the l-ephedrine dosing. Rats were monitored for behavioral activity every 30 min, and for signs of behaviors commonly elicited by 5-HT receptor stimulation (e.g., head weaving, forepaw treading, retrograde propulsion; Jacobs et al., 1976) as well as other stereotypic behavior. Seven days later, rats were sacrificed for measurement of striatal aromatic monoamine levels (described below). Later, 3 groups of non-implanted animals were administered 4 doses, each dose given 2 h apart, of either 1 ml/kg saline, 25 mg/kg (4 × 25) l-ephedrine, or 5 mg/kg (4 × 5) d-amphetamine. These animals were also sacrificed 7 days post-dosing for striatal aromatic monoamine levels.

Early extreme hyperthermia and extensive cooling intervention was necessary with the 3 × 40 mg/kg l-ephedrine dosing paradigm. Because the cooling procedures used to prevent lethal hyperthermia can drastically interfere with in vivo microdialysis, a second dosing regimen of 4 × 25 mg/kg l-ephedrine was selected for the second microdialysis experiment to determine ephedrine, norephedrine and dopamine, 5-HT, and metabolite levels in CPu microdialysate. Also, this dosing paradigm produced temperature changes more commonly seen with 4 × 5 mg/kg d-amphetamine in a 22–23°C environment. The third microdialysis experiment directly compared the effects of either 4 × 25 mg/kg l-ephedrine or 4 × 5 mg/kg d-amphetamine on CPu microdialysate levels of dopamine, 5-HT, and glutamate. Seven pairs of rats were run, with one of the pair receiving 4 × 25 mg/kg l-ephedrine, while the other was given 4 × 5 mg/kg d-amphetamine.

Brain microdialysis. CPu microdialysis was carried out in the manner as previously described (Clausing et al., 1995). CMA microdialysis equipment (Carnegie Medicine, Stockholm, Sweden) was used and CMA/12 guide cannulae were implanted into the CPu using the coordinates AP 0.2 mm, LAT 3.0 mm, DV 5.5 mm relative to bregma (Paxinos and Watson, 1995). The artificial cerebrospinal fluid (ACSF) was composed of: 145 mM NaCl, 1.5 mM KCl, 1.5 mM MgCl₂, 6H₂O, 1.25 mM CaCl₂, 2H₂O, 1 mM glucose, 1.5 mM K₂HPO₄,
adjusted to pH 7.0 with HCl. After surgery, each rat was allowed a recovery period of 7 days.

On the morning of the experiment, each rat was hand-held as the CMA/12 dialysis probe (2-mm probe tip) was carefully inserted through the guide cannula into the right CPu. Microdialysate flow rate was held at 1.0 µl/min throughout, and fractions were collected every 20 min. In order that the aromatic monoamine levels in the microdialysate reached a relatively stable baseline, dosing did not begin until 2 h or more after probe insertion. Tubes that collected the fractions each contained 2 µl of 0.25 M phosphoric acid to acidify and stabilize the aromatic monoamines in the microdialysate as the fraction was collected. Each 20-min aliquot was immediately halved into 11-µl aliquots and frozen on dry ice. The frozen aliquots were then transferred to a −150°C freezer until analysis.

After microdialysis, each rat was sacrificed and the brain removed and fixed in 4% formalin for later verification of microdialysis probe location. In vitro probe recovery was performed to assess the functionality of the individual probes and to exclude non-functional probes from the experiment after each probe, was used in vivo. Percent in vitro probe recovery for ephedrine and norephedrine was determined as [concentration in collected sample × 100/ concentration in standard solution] at 23°C and 1 ml/min flow rate. The estimated average in vitro probe recoveries for ephedrine and norephedrine for the probes used in these experiments ranged from 15 to 22%.

**HPLC-quantitation of ephedrine and norephedrine.** Analyses of ephedrine and norephedrine were performed using modified high performance liquid chromatography (HPLC) methods previously developed to detect fenfluramine and norfenfluramine levels in plasma, brain, and microdialysate. Details of this HPLC method have been described (Clausing et al., 1997). The only alteration necessary was a change in the HPLC elution gradient, which is shown in Table 1.

Ephedrine and norephedrine levels were determined by fluorescent detection after derivatization with dansyl chloride; excess dansyl chloride was removed with a strong anion-exchange resin. A Supelcosil LC 18-mm column, running a step gradient with 50% KH₂PO₄ (0.05 M, pH 5.5) + 50% acetonitrile (mobile phase A) versus 25% KH₂PO₄ (0.05 M, pH 5.5) + 75% acetonitrile (mobile phase B) was used for HPLC isolation. Eleven µl of the microdialysate plus phosphoric acid was derivatized directly, and the quantitation limits of this method were 1 pmol in microdialysate. Because this method does not distinguish between d- and l-enantiomers, ephedrine and norephedrine levels are described without designating the enantiomers as (l).

**TABLE 1**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Gradient slope</th>
<th>Buffer A (%)</th>
<th>Buffer B (%)</th>
</tr>
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<tbody>
<tr>
<td>0–5</td>
<td>Isocratic</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>5–7</td>
<td>Step</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>7–12</td>
<td>Linear</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>12–14.5</td>
<td>Linear</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>15</td>
<td>Step</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>22</td>
<td>Step</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>End run</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

In vitro –150°C freezer until analysis.

**HPLC-quantitation of glutamate and amphetamine levels in CPu microdialysate.** In the final microdialysate experiment, glutamate and amphetamine levels were determined along with dopamine, 5-HT, and metabolites in 11-µl aliquots of the CPu microdialysate. Methods adapted by Bowyer et al. (1995b), which were originally developed as methods for detecting amino acids (Godel et al., 1984), were used for detecting both glutamate and amphetamine. Unfortunately, this method cannot be used to detect ephedrine. The only changes necessary to detect glutamate, as well as amphetamine, were an alteration of the elution gradient and a shift to a more basic pH. In brief, AMPH levels were determined by fluorescent detection after o-phthalaldehyde/3-mercaptopropionic acid derivatization and separation on a Supelcosil LC 18 column (4.6 mm × 15 cm), running a gradient with 95% KH₂PO₄ (0.05 M, pH 7.4) + 5% methanol (mobile phase A) versus 35% KH₂PO₄ (0.05 M, pH 7.4) + 65% methanol (mobile phase B). Flow rate was 1.5 ml/min, and the fluorescent detector was set at λₑx = 340 nm and λₑm = 440 nm. Microdialysis samples were derivatized and injected directly using a CMA/200 autosampler.

**FIG. 1.** HPLC separation and fluorescent detection of ephedrine and norephedrine in CPu microdialysate. (A) A chromatogram of a dansyl chloride derivative of 11 µl of microdialysate (plus phosphoric acid) collected just prior to 40 mg/kg l-ephedrine administration. The derivatized microdialysate was resin cleaned before separation on HPLC. The chromatogram shows the changes in the fluorescence of the HPLC eluate (see Materials and Methods for further details). (B) A chromatogram of a dansyl chloride derivative of 11 µl of microdialysate containing (spiked with) l-ephedrine and l-norephedrine (10 µM was the final concentration for both). The derivatized microdialysate was resin-cleaned before separation on HPLC. (C) A chromatogram of derivatized microdialysate collected 1.67 hrs after a single dose of 40 mg/kg i.p. l-ephedrine. The labeled peaks indicate the 7.9 µM ephedrine and 0.2 µM norephedrine calculated to be present in microdialysate. (D) A chromatogram of derivatized microdialysate collected 2 h after the fourth dose of 4 x 25 mg/kg ip l-ephedrine. The labeled peaks indicate the 8.4 µM ephedrine and 0.9 µM norephedrine calculated to be present in microdialysate.
similar to Stephans
5-HT, and 5-HIAA were determined using reverse-phase HPLC by methods
nanoamperes while a 0.5- or 1-nanoampere range was used for microdialysate.
detection. For tissue samples, the detector was set at a sensitivity range of 5
amperometric detector with a BAS-LC-17 oxidative flow cell was used for
mm, Supelco, Bellefonte, PA) was used for separation, and a BAS-LC4B
HPLC/EC system.

Centrifugation, the supernatant was removed and injected directly onto the
immediately injected onto the HPLC for analysis.

Methylamines (aspartate through alanine) as well as glutamate and amphetamine, but
quantitated at 0.5 pg/10

The HPLC gradient consisted of the multiple timed segments, as shown in Table 2.
This gradient was capable of separating the first 10 most hydrophilic amino
carbons (aspartate through alanine) as well as glutamate and amphetamine, but
only the glutamate and amphetamine levels are reported in this paper.

**HPLC-quantitation of aromatic monoamine levels in microdialysate and striatum.** Striatal tissue levels of dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), and 5-hydroxy-indole acetic acid (5-HIAA) were analyzed by electrochemical detection. For tissue striatal levels, each striatum was weighed and diluted with a measured volume (20% w/v) of 0.2-M perchloric acid containing 100 ng/ml 3,4-dihydroxybenzylamine (Sigma) as internal standard. After sonication and centrifugation, the supernatant was removed and injected directly onto the HPLC/EC system.

Samples were analyzed under the conditions previously described (Bowyer et al., 1995a) using reverse-phase HPLC. The isocratic mobile phase consisted of 92% KH₂PO₄-buffer (0.07 M, pH 3.0) and 8% methanol, containing 1 mM Na₁-heptanesulfonic acid and 0.2 mM Na₂-EDTA per liter, and its flow rate was 1.0 ml/min. A Supelcosil LC18 3 μm analytical column (7.5 cm × 4.6 mm, Supelco, Bellefonte, PA) was used for separation, and a BAS-LC4B amperometric detector with a BAS-LC-17 oxidative flow cell was used for detection. For tissue samples, the detector was set at a sensitivity range of 5 nanomperes while a 0.5- or 1-nanopere range was used for microdialysate.

Microdialysate levels of dopamine, DOPAC, HVA, 3-methoxytyramine, 5-HT, and 5-HIAA were determined using reverse-phase HPLC by methods similar to Stephans et al. (1998). Dopamine, DOPAC, and 5-HIAA could be quantitated at 0.5 pg/10 μl microdialysate while 5-HT and HVA were measurable at the 1 pg/10 μl levels and 3-methoxytyramine at the 2 pg/10 μl level. For the 10-μl microdialysate plus 1 μl of 0.25 M phosphoric acid aliquots, 15 μl of HPLC mobile phase was added to adjust the pH, and the sample immediately injected onto the HPLC for analysis.

**Statistics.** Data are presented as arithmetic mean ± standard error of the mean (SEM) unless otherwise indicated. Multiple groups were analyzed by either a one- or two-way analysis of variance (ANOVA) or a repeated measures two-way ANOVA. A post hoc Tukey’s least-significant-difference test was applied if significant main effects were observed.

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**RESULTS**

**Single Doses of l-Ephedrine**

A single dose of 20 mg/kg l-ephedrine produced hyperactivity relative to control (Table 3) along with some stereotypic behavior and a maximal elevation in body temperature from control levels of 37.8 ± 0.4 to 39.4 ± 0.4°C (n = 6). However, the 40 mg/kg l-ephedrine produced a more pronounced and prolonged hyperactivity (Table 3) and stereotypy along a maximal elevation in body temperature from control levels of 38.0 ± 0.5 to 40.3 ± 0.6°C (n = 6).

Subsequently, rats in the first microdialysis experiment were administered a single dose of 40 mg/kg l-ephedrine. Peak microdialysate ephedrine concentrations (7.3 ± 1.2 μM, n = 8) were reached at 1 to 1.33 h after administration (Fig. 2, top). The half-life of ephedrine in the microdialysate ranged from 0.55 to 1.59 h with a mean half life of 1.1 ± 0.1 h (n = 8). Mean area under the curve was 15.3 ± 3.2 μM*h. Norephedrine levels were more than an order of magnitude less (0.3 ± 0.1 μM) than ephedrine levels, and peak concentrations were not reached until 2 to 3 h after dosing (Fig. 2, top). Peak dopamine levels (151 ± 52 nM) were reached 1 h after dosing, a time that coincided with peak ephedrine levels (Fig. 2). DOPAC levels rapidly declined from pre-dosing levels (1338 ± 202 nM) but did not plateau until over 2 h after dosing (405 ± 79 nM; Fig. 2, bottom). 5-HT levels increased only slightly to peak levels of 164 ± 50% of control approximately 1 h after dosing, while 5-HIAA levels were not significantly changed (data not shown).

**Multiple Doses of l-Ephedrine**

After each of the 3 doses of 40 mg/kg, extensive hyperactivity and stereotypic behavior such as grooming, licking, chewing, head nodding, and vertical exploration were observed. Serotonergic-type stereotypy (head-weaving) was seen only in 1 of 8 rats. Five of the 8 rats dosed became severely hyperthermic (above 41.5°C) and required cooling on crushed ice by the second dose of l-ephedrine. The cooling procedure, coupled with an extra hour (3 rather than 2 h) between l-ephedrine injections relative to previous experiments resulted in wide individual swings in body temperature after the second and third doses. Nonetheless, the extent of the hyperthermia produced by the 3 × 40 mg/kg l-ephedrine was substantial (Fig. 3, top).

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**TABLE 2**

<table>
<thead>
<tr>
<th>Chromatographic Gradient Conditions for HPLC Determination of Glutamate and Amphetamine OPA/3-Mercaptopropionic Derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time (min)</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>0–3</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>9.1</td>
</tr>
<tr>
<td>22</td>
</tr>
<tr>
<td>22.1</td>
</tr>
<tr>
<td>30</td>
</tr>
</tbody>
</table>

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**TABLE 3**

<table>
<thead>
<tr>
<th>Dose of l-ephedrine</th>
<th>Total beam breaks 1 h prior to l-ephedrine</th>
<th>Total beam breaks in the first h after l-ephedrine</th>
<th>Total beam breaks in the second h after l-ephedrine</th>
<th>Number tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mg/kg</td>
<td>364 ± 88</td>
<td>874 ± 225</td>
<td>1139 ± 261</td>
<td>12</td>
</tr>
<tr>
<td>40 mg/kg</td>
<td>152 ± 30</td>
<td>1237 ± 184</td>
<td>779 ± 172</td>
<td>8</td>
</tr>
</tbody>
</table>
The hyperthermia produced by the 4 × 25 mg/kg \( l \)-ephedrine dosing regimen in rats not implanted for microdialysis was almost identical to that produced by 4 × 5 mg/kg \( d \)-amphetamine in non-implanted rats (Fig. 3, bottom). Behavioral changes resulting from 4 × 25 mg/kg \( l \)-ephedrine were very similar to those exhibited after 3 × 40 mg/kg \( l \)-ephedrine with only 2/8 rats showing the 5-HT behavior of forepaw treading. Despite the extensive increase in CPu dopamine levels and severe hyperthermia after multiple doses of \( l \)-ephedrine (3 × 40 mg/kg and 4 × 25 mg/kg), total striatal dopamine content decreased only 19 and 26% 7 days post-dosing (Table 4). This was significantly less than the greater than 72% decrease seen after 4 doses of 5 mg/kg \( d \)-amphetamine (Table 4). A 2-way ANOVA, with experiment and drug as factors, showed no significant differences between experiments. However, there was a significant drug effect, indicating that the 4 × 25 mg/kg and 3 × 40 mg/kg \( l \)-ephedrine treatments significantly reduced dopamine levels \((p = 0.005, \text{Tukey’s test})\). The depletions produced by 4 × 5 mg/kg \( d \)-amphetamine were much more pronounced and were significantly greater \((p = 0.001, \text{Tukey’s test})\) than the depletions produced by either \( l \)-ephedrine dosing.
the first dose to 12.1

metabolites as well as glutamate during exposure to either 4

increased to almost 200% of baseline (data not shown). The

in the group) that showed serotonergic behavior, 5-HT levels

time point were not different from initial levels. In the 2 rats (8

l

25 mg/kg

-ephedrine dose with an apparent plateau reached after the

third dose (Fig. 5). DOPAC levels in the microdialysate con-

levels in the microdialysate increased after the first and second

l

-ephedrine while 5-HIAA levels at this

value of F[19, 209] = 10.5). The post-hoc tests showed there

DOPAC levels of dopamine in the 4
depth of ephedrine and norephedrine as well as dopamine, 5-HT,

l

3

40 mg/kg

sate levels of 5-HT and glutamate were not significantly less in

the 4 × 25 mg/kg l-ephedrine group compared to the 4 × 5

mg/kg d-amphetamine group (Fig. 6, bottom; Fig. 7, top). However, there were indications that, regardless of treatment,

the animals with the greatest responses to hyperthermia and
dopamine increases showed the greatest increases in 5-HT and

5-HIAA did not differ between the 2 groups (data not shown).

A more rapid rate of rise and decline of these neurotrans-
mitters is seen in the CPu microdialysate of the 4 × 5 mg/kg
d-amphetamine rats than in the 4 × 25 mg/kg l-ephedrine

groups (p < 0.05).

significantly less than control (p < 0.05).

paradigm. Also, all 8 rats in the d-amphetamine group dis-

played forepaw treading and head weaving, and 6 of the 8

exhibited retrograde propulsion.

In the second microdialysis experiment, 4 × 25 mg/kg

l-ephedrine was administered, and the CPu microdialysate

levels of ephedrine and norephedrine as well as dopamine, 5-HT,

and metabolites were determined over the 4 doses (Figs. 4 and

5). Ephedrine levels ranged from 2.6 ± 0.4 μM (n = 8) after

the first dose to 12.1 ± 2.0 μM after the fourth dose. Dopamine

levels in the microdialysate increased after the first and second

l-ephedrine dose with an apparent plateau reached after the

third dose (Fig. 5). DOPAC levels in the microdialysate con-

continued to drop steadily throughout the time course until 2 h

after the last dose of l-ephedrine while 5-HIAA levels at this
time point were not different from initial levels. In the 2 rats (8

in the group) that showed serotonergic behavior, 5-HT levels

increased to almost 200% of baseline (data not shown). The

remainder of the rats showed less than 50% increase in 5-HT.

A third (final) microdialysis experiment compared the

changes in CPu microdialysate levels of dopamine, 5-HT, and

metabolites as well as glutamate during exposure to either 4 ×

25 mg/kg l-ephedrine or 4 × 5 mg/kg d-amphetamine. All of

the 7 rats in the d-amphetamine group displayed prominent

serotonergic behavior, and all but 1 rat showed signs of hy-

perthermia. Four of these rats required cooling to prevent lethal

hyperthermia. Only 2 of the 7 rats in the l-ephedrine group

displayed some serotonergic behavior but all showed signs of

hyperthermia. Six of these rats required cooling to prevent

lthal hyperthermia.

The results of this experiment showed that the increases in

CPu microdialysate levels of dopamine, 5-HT, and

metabolites is seen in the CPu microdialysate of the 4

3

25 mg/kg

The Long-term Effects of Multiple Doses of l-Ephedrine or

d-Amphetamine on Striatal Tissue Dopamine Content

<table>
<thead>
<tr>
<th>Dosing paradigm and compound</th>
<th>Striatal dopamine levels (ng/mg tissue)</th>
<th>Number tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 × 1 ml/kg saline</td>
<td>1119 ± 72</td>
<td>5</td>
</tr>
<tr>
<td>3 × 40 mg/kg l-ephedrine</td>
<td>907 ± 81</td>
<td>6</td>
</tr>
<tr>
<td>4 × 1 ml/kg saline</td>
<td>1260 ± 50</td>
<td>6</td>
</tr>
<tr>
<td>4 × 25 mg/kg l-ephedrine</td>
<td>926 ± 161*</td>
<td>6</td>
</tr>
<tr>
<td>4 × 5 mg/kg d-amphetamine</td>
<td>324 ± 110*</td>
<td>7</td>
</tr>
</tbody>
</table>

*Significantly less than control (p < 0.05).

**TABLE 4**

FIG. 4. Time course of the microdialysate levels of ephedrine and norephedrine after multiple doses of 25 mg/kg l-ephedrine. Levels of ephedrine and norephedrine in microdialysate are plotted on a logarithmic scale versus time of microdialysate fraction collection on a linear scale during the admin-
istration of 4 consecutive doses, given at 2-h intervals, of 25 mg/kg ip of

l-ephedrine. Peak norephedrine levels are less than 1/10th of the ephedrine

levels. Microdialysate levels of ephedrine and norephedrine levels did not peak

until after the third and fourth doses.

FIG. 5. Striatal dopamine levels (ng/mg tissue) 20, 40, and 60 min after both the first and second doses, as well as at 40 min after the third dose. The increases in CPu microdialys-
ephedrine or $4 \times 5$ mg/kg $d$-amphetamine animals during the cooling used to reduce the hyperthermia (Fig. 6). In the rat with the greatest increases in microdialysate dopamine and 5-HT levels and hyperthermia during $4 \times 5$ mg/kg $d$-amphetamine exposure, the cooling procedure suppressed dopamine and 5-HT levels over 1 h. A similar suppression of microdialysate levels of dopamine and 5-HT after cooling can also be seen in the rat with the greatest responses to $4 \times 25$ mg/kg $l$-ephedrine. Cooling may have also had an inhibitory effect on the gluta-

Following these increases, extensive increases in CPu extracellular dopamine, supported by continued synthesis of dopamine within nerve terminals during methamphetamine exposure, have been implicated as necessary for long-term dopamine depletions (Gibb and Kogan, 1979; O’Dell et al., 1991; Schmidt et al., 1985; Weihmuller et al., 1992). Subsequent, (within 24 h) to this tremendous increase in intracellular and extracellular dopamine, there is evidence of decreased striatal tyrosine hydroxylase activity and immunoreactivity, dopamine depletion, and reactive gliosis (Bowyer and Holson, 1995; Hotchkiss and Gibb, 1980; Seiden and Sabol, 1995). Prominent increases in CPu extracellular glutamate levels have also been suggested to play a critical role in the long-term dopamine depletions produced by amphetamine and methamphetamine (Nash and Yamamoto, 1992; Sonsalla et al., 1989; Stephans and Yamamoto, 1994; Weihmuller et al., 1992). However, body temperature during exposure to amphetamine or meth-

![FIG. 5. Time course of the microdialysate levels of dopamine, DOPAC, and 5-HIAA after multiple doses of 25 mg/kg $l$-ephedrine. Levels of dopamine, DOPAC and 5-HIAA in microdialysate are plotted on a logarithmic scale versus time of microdialysate fraction collection on a linear scale during administration of 4 consecutive doses, given at 2 h intervals, of 25 mg/kg ip of $l$-ephedrine. Peak dopamine levels are less than 1/30th the initial DOPAC levels. Microdialysate levels of dopamine peaked after the second dose while DOPAC levels continued to decline until the fourth dose. 5-HIAA levels remained relatively constant.](image1)

ephemer or $4 \times 5$ mg/kg $d$-amphetamine animals during the cooling used to reduce the hyperthermia (Fig. 6). In the rat with the greatest increases in microdialysate dopamine and 5-HT levels and hyperthermia during $4 \times 5$ mg/kg $d$-amphetamine exposure, the cooling procedure suppressed dopamine and 5-HT levels over 1 h. A similar suppression of microdialysate levels of dopamine and 5-HT after cooling can also be seen in the rat with the greatest responses to $4 \times 25$ mg/kg $l$-ephedrine. Cooling may have also had an inhibitory effect on the glutamate levels (Fig. 7, top). However, cooling did not appear to suppress increases in microdialysate levels of either amphetamine (Fig. 7, bottom) or 5-HIAA levels (data not shown).

**DISCUSSION**

The factors that are important in the generation of the long-term depletion of striatal dopamine and other neurotoxicities that occur after exposure to either amphetamine or methamphetamine should also be important with respect to $l$-ephedrine neurotoxicity. Extensive increases in CPu extracel-

![FIG. 6. Comparison of the increases in CPu microdialysate levels of dopamine and 5-HT produced by either $4 \times 25$ mg/kg $l$-ephedrine or $4 \times 5$ mg/kg $d$-amphetamine. The number of rats ($n = 7$) was the same for both groups (large open symbols), and the means $\pm$ SEM for the groups are shown. Individual data shown with solid symbols and no SEM, and with the time points where the individual rats were cooled on crushed ice indicated by arrows (see Materials and Methods for details). (Top) Group and individual CPu microdialysate dopamine levels are shown over the course of 4 doses of either 25 mg/kg $l$-ephedrine or $4 \times 5$ mg/kg $d$-amphetamine. (Bottom) Group and individual CPu microdialysate 5-HT levels are shown over the course of 4 doses of either 25 mg/kg $l$-ephedrine or $4 \times 5$ mg/kg $d$-amphetamine.](image2)
long-term dopamine depletion differences seen with \( l \)-ephedrine and to \( d \)-amphetamine.

From the initial phases of the studies, the minimal serotonergic behavior (such as head weaving, forepaw treading, and retrograde propulsion; Jacobs, 1976) produced by \( l \)-ephedrine indicated that less 5-HT release might occur during \( l \)-ephedrine, compared to \( d \)-amphetamine, treatment. Although dopamine release has been implicated in the long-term depletions in 5-HT produced by methylenedioxyamphetamine (Schmidt et al., 1992), the role of 5-HT release or receptor stimulation on long-term dopamine depletions produced by methamphetamine and amphetamine is unclear. It has been postulated that fluoxetine enhances methamphetamine neurotoxicity by elevating brain levels of methamphetamine (Ricaurte et al., 1983) but the effects of fluoxetine on extracellular 5-HT levels also might be a factor.

However, the increases in 5-HT levels seen in CPu microdialysate after 4×25 mg/kg \( l \)-ephedrine treatment were not significantly less than those produced by 4×5 mg/kg \( d \)-amphetamine despite the fact that serotonergic behaviors were rarely observed in the ephedrine group. Thus, we could find no direct evidence that a decrease in the extracellular CPu 5-HT levels during \( l \)-ephedrine exposure was a factor in the reduced long-term dopamine depletions produced by \( l \)-ephedrine compared to \( d \)-amphetamine. Nonetheless, increases in extracellular 5-HT levels may play a role in the long-term dopamine depletions, since they were significantly greater in animals that had the greatest increases in microdialysate dopamine and body temperature during exposure to either amphetamine or ephedrine. In contrast, 4×5 mg/kg \( d \)-amphetamine produced more than 2-fold greater increases in dopamine levels in the CPu microdialysate than 4×25 mg/kg \( l \)-ephedrine in the side-by-side comparison. Also, the rate of increase in CPu microdialysate dopamine was faster after amphetamine than after ephedrine, due to the more rapid rate of rise of amphetamine compared to ephedrine in the brain.

Although the 3×40 mg/kg dose of \( l \)-ephedrine should produce CPu extracellular dopamine levels more comparable to the 4×5 mg/kg \( d \)-amphetamine dose, the excessive cooling necessary to prevent lethality would obviate dopamine and 5-HT levels. The reduction in dopamine and 5-HT levels is not the only mechanism by which cooling would reduce long-term dopamine depletions. We have previously observed that animals dosed with 4×10 mg/kg \( d \)-amphetamine in a cold environment have higher CPu microdialysate dopamine levels than animals dosed with 4×5 mg/kg \( d \)-amphetamine at 23°C temperature but no significant long-term dopamine depletions (Bowyer et al., 1993). The cooling should also reduce oxidative stress, since indices of oxidative stress produced by quinones of dopamine are decreased when hyperthermia does not occur during methamphetamine exposure (LaVoie and Hastings, 1999). Increased oxidative stress and reactive oxidative species of dopamine, such as 6-hydroxy-dopamine and quinones of dopamine, have been postulated to be mediators of

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**FIG. 7.** Comparison of the increases in CPu microdialysate glutamate levels produced by 4×25 mg/kg \( l \)-ephedrine and 4×5 mg/kg \( d \)-amphetamine. The same rats (\( n = 7 \)) used to generate the data in Figure 6 were used for both the group and individual data in Figure 7. The means ± SEM for the groups (large open symbols) are shown, while individual data is shown with solid symbols and no SEM. The time points where the individual rats were cooled on crushed iced are indicated by arrows (see Materials and Methods for details). (Top) Group and individual CPu microdialysate glutamate levels are shown over the course of 4 doses of either 25 mg/kg \( l \)-ephedrine or 4×5 mg/kg \( d \)-amphetamine. (Bottom) Group and individual CPu microdialysate amphetamine levels are shown. The levels of ephedrine are not shown, since the derivatization process used to detect amino acids will only work with primary amines like amphetamine.
Ephedrine and CPU dopamine

dopamine neurotoxicity in the CPu (Graham, 1978; O’Dell et al., 1991; Seiden and Sabol, 1995; Stokes et al., 1999; Yamamoto and Zhu, 1998). Thus, from these previous studies, it seems likely that the greater the dopamine release and hyperthermia the greater the generation of reactive dopamine-like species. However, it is possible that the increase of dopamine within the dopaminergic terminals of the CPu is primarily mediating long-term neurotoxicity (LaVoie and Hastings, 1999).

CPu microdialysate levels of glutamate in the 4 × 5 mg/kg d-amphetamine group compared to the 4 × 25 mg/kg l-ephedrine group were not statistically different. Thus, differences in glutamate release and extracellular levels may not explain why ephedrine produces less dopamine depletion than amphetamine. Nonetheless, the data indicates that increased extracellular glutamate levels could play an important role in the long-term dopamine depletions since, like 5-HT levels, they were significantly greater in animals with the greatest increases in microdialysate dopamine and body temperature during exposure to either amphetamine or ephedrine.

The more than 4-fold increase in peak ephedrine levels in the microdialysate that occurred between the first and fourth doses of 25 mg/kg l-ephedrine cannot be explained by the t_{1/2} of 1.1 h obtained from a single dose of 40 mg/kg l-ephedrine. A 4-fold increase would be expected if the t_{1/2} was more than 3 h. It is possible that the prolonged hyperthermia produced by multiple doses has more of an effect on the pharmacokinetics than the shorter hyperthermia produced by a single dose. Hyperthermia has been shown to increase bioavailability and plasma levels of ephedrine in humans (Vanakoski et al., 1993). Also, amphetamine treatment increases lactic acid levels in the brain (Nahorski, 1980; Zalis et al., 1967) and microdialysate (Stephans et al., 1998). It is possible that lactic acid accumulation in brain during amphetamine exposure leads to an increased t_{1/2} of amphetamine in microdialysate, particularly after the third and fourth doses (Fig. 7, bottom; Clauising and Bowyer, unpublished data). A similar phenomenon may produce the elevation of l-ephedrine after multiple doses. Further studies examining the time course of plasma and brain tissue, as well as microdialysate levels of ephedrine and norephedrine, will be necessary to determine the potential alteration in ephedrine t_{1/2} that occurs during multiple dosing.

In summary, the doses of l-ephedrine tested produced parent-compound levels in the CPu microdialysate of 2 to 10 μM with the metabolite levels of norephedrine being less than 10% ephedrine. The peak levels of CPu microdialysate ephedrine were reached 40 to 80 min after dosing, which was 20 to 40 min after peak levels of amphetamine levels were attained; however, the pharmacokinetic mechanisms behind this difference were not determined. The increases in CPu microdialysate 5-HT levels as well as the hyperthermia induced after multiple doses of l-ephedrine did not significantly differ from that seen during amphetamine exposure. Also, microdialysate glutamate levels were not statistically different. However, the increase in microdialysate dopamine levels was significantly less in the 4 × 25 mg/kg l-ephedrine-treated rats compared to the 4 × 5 mg/kg d-amphetamine-treated rats. From the research of other investigators, it is possible that a reduced increase of dopamine within nerve terminals, which would also result in reduced extracellular levels, could be the prime factor in the lesser long-term striatal dopamine depletions produced by l-ephedrine. The enhancement of CPu dopamine levels produced by higher doses of l-ephedrine (3 × 40 mg/kg) may not increase dopamine depletions because of the early and extensive cooling necessary to prevent lethal hyperthermia.

REFERENCES


