Amphetamine releases GABA in striatum of the freely moving rat: involvement of calcium and high affinity transporter mechanisms

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Abstract

Using microdialysis the effect was investigated of amphetamine (AMPH) infusions into the striatum on the release of GABA in the freely moving rat. AMPH (5, 10 and 20 μg/μl), infused through a microdialysis probe at the rate of 2.5 μl/min, produced a dose-related increase in extracellular concentrations of GABA. At the highest dose (20 μg/μl), AMPH increased GABA from 0.08 ± 0.01 to 0.67 ± 0.14 μM. Increases in extracellular GABA produced by AMPH were both calcium-dependent and high affinity GABA transporter-mediated. A medium free of calcium reduced the increase of extracellular GABA produced by AMPH by 37%. Nipecotic acid (2, 4 and 8 mM), a specific GABA re-uptake blocker, significantly attenuated increases in extracellular GABA, but not GLU, produced by AMPH (20 μg/μl). This study is the first in vivo evidence showing the release of GABA produced by AMPH through a high affinity transporter mechanism. © 1998 Elsevier Science Ltd. All rights reserved.

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1. Introduction

After the first studies indicating the action of amphetamine (AMPH) on catecholamines (Axelrod et al., 1961), reports have appeared showing the effects of AMPH on multiple neurotransmitter systems. In particular, it has been reported that AMPH increases extracellular concentrations of serotonin (Hernández et al., 1987), diadenosine polyphosphates (Pintor et al., 1995), different peptides (Hurd et al., 1992; Furmidge et al., 1993), ascorbic acid (Pierce et al., 1994), glutamate (GLU) (Mora and Porras, 1993; Del Arco et al., 1994), aspartate (Mora and Porras, 1993), taurine (TAU) (Porras and Mora, 1993; Del Arco et al., 1994) and also GABA (Porras and Mora, 1993). Despite the relatively well known mechanisms by which AMPH releases dopamine (Seiden et al., 1993), very little is known about the mechanisms by which AMPH modifies extracellular concentrations of these other neurotransmitters in the brain.

GABA is a major neurotransmitter in the CNS (Smith and Bolam, 1990). In the striatum, it is one of the most abundant neurotransmitters, located in medium spiny neurons which receive glutamatergic and dopaminergic inputs from cortex and substantia nigra respectively (Smith and Bolam, 1990). Few in vivo or in vitro studies have reported the effects of AMPH in GABAAergic neurotransmission, although it is known that intracerebral or intraperitoneal injections of AMPH produce an increase in extracellular [GABA] in striatum (Besson et al., 1986; Porras and Mora, 1993). An increase in extracellular [GABA] produced by AMPH in striatal slices has also been reported (Bernath and Zigmond, 1989; Lillrank et al., 1991). In contrast, repeated systemic injections of AMPH produced a decrease in extracellular [GABA] in other areas of the brain such as the ventral pallidum or nucleus accumbens (Bourdelais and Kalivas, 1990; Linderfords et al., 1992) but the mechanisms remain uncertain.

In physiological conditions, the release of GABA is assumed to be produced mainly by an exocytotic calcium and TTX-dependent mechanism. In fact, a calcium and TTX-dependent release of GABA after potassium stimulation has been extensively described (Westerink and De Vries, 1989; Bourdelais and Kalivas, 1992; Campbell et al., 1993). However, it has also been reported that GABA can be released in the absence of
external calcium, probably through a high affinity GABA transporter (Bernath and Zigmond, 1990; Campbell et al., 1993; Attwell et al., 1993). In this respect, it has been reported that GLU and dopamine can release GABA by the reversal of the GABA uptake transporter (Bernath and Zigmond, 1989; Belhage et al., 1993; During et al., 1995). The physiological significance of this kind of release remains to be elucidated (Attwell et al., 1993).

This study is designed to investigate the effects of different doses of intracerebral infusion of AMPH on the extracellular concentration of GABA in striatum of the freely moving rat, and the mechanisms through which these effects were produced. Both, the calcium-dependence and the role in this process of the high affinity GABA transporter were studied.

2. Methods

2.1. Animals

Male Wistar rats (2–3 months, 250–350 g weight) were housed in individual wire mesh cages, provided with food and water ad libitum, and maintained in a temperature-controlled room under a light/dark cycle (lights on/off at 8:00 p.m./8:00 a.m.). All in vivo experiments, carried out at the Universidad Complutense of Madrid, were conducted under the dark period of the light/dark cycle, and followed the guidelines of the International Council for Laboratory Animal Science (ICLAS).

2.2. Microdialysis experiments

Bilateral guide-cannulae were stereotaxically implanted in the brain, under Equithesin anaesthesia (2 ml/kg), to accommodate microdialysis probes so that when inserted, the tip of the probes were placed in the striatum: 0.6 mm rostral and 2.5 mm lateral from Bregma; 5 mm ventral from dura mater (König and Klippel, 1967). The guide-cannulae assembly, designed in our laboratory and constructed in the workshop of the Universidad Complutense of Madrid, allowed the fixation of the microdialysis probes during the experiments. Once implanted, guide-cannulae were fixed with two screws and dental cement to the skull.

Three to four days after surgery, the microdialysis probes were inserted and experiments were performed on the freely moving rat. The microdialysis probes were designed and constructed in our own workshop. The probes were of concentric design, with an active dialyzing length of 2 mm. The dialysis membrane (cuprophane) had a molecular-weight cut off of 5000 Da (Hospal). The probes were perfused with artificial CSF (composition in mM: NaCl, 137; CaCl₂, 1.2; KCl, 3; MgSO₄, 1; NaH₂PO₄, 0.5; Na₂HPO₄, 2; glucose, 3; pH = 7.3) at a flow rate of 2.5 μl/min. CaCl₂ was replaced by MgSO₄ (equimolar concentration) for the calcium free experiments. Calcium free CSF was infused an hour before, during, and 50 min after AMPH infusion. The in vitro recovery was determined at room temperature in a solution containing 10 μM of amino acids. The average relative recovery of the probes used in this study was: GABA = 6.9 ± 0.4%; GLU = 8.2 ± 1.4%; GLN = 7.4 ± 0.8%; TAU = 8.6 ± 0.4%; ARG = 7.2 ± 0.7%.

After basal concentrations of amino acids were established, 15-min samples were collected for 240 min and immediately stored at −80°C until analysed. The first four samples were used as control. AMPH (obtained from the National Department of Health) and nipecotic acid (NIP) (Tocris Cookson) were dissolved in CSF before infusion through the microdialysis probe. The in vitro recovery of AMPH (measured with the same chromatographic method described below for amino acids analysis) was calculated at room temperature: 5.5 ± 0.72%. AMPH at doses of 5, 10 and 20 μg/μl was infused for 10 min while NIP (2, 4 and 8 mM) was infused an hour before, during, and 50 min after AMPH infusion, at the rate of 2.5 μl/min. pH was checked. The change in the perfusion medium during experiments was made by a liquid switch (Harvard Apparatus).

At the end of the experiments, the animals were anaesthetized with Equithesin and perfused intracardially with 0.9% saline followed by 10% formalin. The brain was removed and the placement of the microdialysis probe was verified with a cryostat microtome and viewing lens. The microdialysis probes were stored in distilled water between experiments.

2.3. Amino acids analysis

The amino acid content of samples were analysed by reverse-phase HPLC and fluorometric detection according to a method used previously in our laboratory (Segovia et al., 1994; Porras and Mora, 1995). Briefly, precolumn derivatization of 5 μl samples was performed with an o-phthalaldehyde solution. Derivatized samples were injected in a Rheodyne injector running first in a C18 precolumn (Tracer) and then in a C18 column of 5 μm particles and 4 × 150 mm (Tracer). A gradient program of two mobile phases at a flow rate of 1 ml/min was used. Solution A was 95:5 (v/v) mixture of 50 mM sodium acetate buffer (pH = 5.67) and methanol, to which 12.5 ml of isopropyl alcohol per litre was added; solution B was a 70:30 (v/v) methanol/water mixture.

Amino acids were measured by a fluorescence detector (Waters 474). The excitatory filter was set at 340 nm, and the emission filter at 460 nm.
were quantified using the MAXIMA 820 (Waters) software by the internal standard procedure using 0.05 mM homoserine. The detection limit in our 5 μl samples was 0.05 μM.

2.4. Statistical analysis

Data reported were not corrected for in vitro recovery. The effects of NIP were calculated as the difference between the average of samples in which NIP was perfused and the average of four control samples. The effects of AMPH were calculated as the difference between the sample in which AMPH was perfused and the average of four control samples before AMPH infusion. A two factorial test (time-dose; time-treatment) with repeated measures followed by Dunnett’s t-test was performed for multiple comparisons. A linear regression analysis (Pearson’s coefficient) was performed to correlate the extracellular concentration of GABA and doses of AMPH and NIP.

3. Results

3.1. Effects of intracerebral perfusions of amphetamine on extracellular GABA

AMPH (5, 10 and 20 μg/μl) produced a dose-related increase in extracellular concentrations of GABA. At the highest dose (20 μg/μl), AMPH increased extracellular [GABA] from 0.08 ± 0.01 to 0.67 ± 0.14 μM. At the dose of 10 μg/μl, this increase was from 0.09 ± 0.02 to 0.25 ± 0.07 μM. AMPH did not modify extracellular [GABA] at the lowest dose (5 μg/μl) (Fig. 1A). Increases in extracellular concentrations of GABA and different doses of AMPH were statistically correlated (r = 0.64, P < 0.05) (Fig. 1B). The calcium-free medium significantly attenuated (37%, P < 0.01) the increase in extracellular [GABA] produced by AMPH (20 μg/μl) (Fig. 2).

3.2. Effects of intracerebral perfusions of nipecotic acid on extracellular amino acids

NIP (2, 4 and 8 mM) produced an increase in basal extracellular concentrations of GABA. The average increases of basal [GABA] produced by NIP were 0.19 ± 0.04, 0.20 ± 0.05 and 0.61 ± 0.08 μM, respectively (Fig. 3A). Increases in extracellular concentrations of GABA and different doses of NIP were statistically correlated (r = 0.81, P < 0.01) (Fig. 3B).

NIP (8 mM) significantly increased basal levels of TAU but not those of GLU, glutamine (GLN) or arginine (ARG) (Table 1).
Few studies have reported the in vivo or in vitro effects of AMPH on extracellular concentrations of GABA. Besson et al. (1986) showed a release of GABA produced by intracerebral injections of AMPH in the striatum of the rat. Also Bernath and Zigmond (1989), and Lillrank et al. (1991) have shown that AMPH increases [GABA] in striatal slices. More recently, Porras and Mora (1993) reported a release of GABA in striatum produced by intraperitoneal injections of AMPH. In contrast, a decrease of GABA produced by repeated injections of AMPH in nucleus accumbens and ventral pallidum has been reported (Bourdelais and Kalivas, 1990; Linderfords et al., 1992). Presumably the effects of AMPH on the release of GABA is dependent on the route of injection and the neurochemical substrates underlying each structure of the brain. The results reported in this study, in which intracerebral perfusions of AMPH produced a significant release of GABA in vivo, is in line with the results reported by Besson et al. (1986) and Porras and Mora (1993).

As shown in the results section, the release of GABA produced by AMPH was partly dependent on calcium in the perfusion medium. Thus, the increase of GABA produced by AMPH was significantly reduced by about 36% when calcium was replaced in the perfusion medium. A calcium-dependent release of GABA after potassium depolarization has also been described (Westerink and De Vries, 1989; Bourdelais and Kalivas, 1992; Campbell et al., 1993), and this has been shown to come from the vesicular pool of GABA (Belhage et al., 1993; Campbell et al., 1993). It is therefore possible that the calcium-dependent release of GABA produced by AMPH also comes from the vesicular pool of GABA.

Studies using microdialysis have so far not been able to clarify the origin of the extracellular basal concentrations of GABA. In fact, contradictory results have been reported. Thus, the basal extracellular concentrations of GABA may be calcium and TTX-dependent (Campbell et al., 1993; Herrera-Marschitz et al., 1996) or calcium and TTX-independent (Westerink and De Vries, 1989; Morari et al., 1993). Our results, in which no changes in basal concentrations of GABA were found after replacing calcium in the perfusion medium (data not shown), are in agreement with those showing calcium-independent basal levels of GABA. Therefore, the exclusive neuronal contribution to the basal extracellular concentrations of GABA is still controversial (Westerink and De Vries, 1989; Campbell et al., 1993). In fact both neurons and glial cells may contribute to the steady release of GABA as detected by microdialysis.

There are no reports in the literature indicating the possibility that AMPH could act directly on GABA neurons or glia to release GABA. Therefore, the calcium-dependent release of GABA may be mediated by the previous action of AMPH on other neurotransmit-
Table 1

Effects of NIP in extracellular basal concentrations of amino acids in the striatum of the rat

<table>
<thead>
<tr>
<th></th>
<th>GABA</th>
<th>GLU</th>
<th>GLN</th>
<th>TAU</th>
<th>ARG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.20 ± 0.05</td>
<td>1.20 ± 0.22</td>
<td>17.98 ± 2.54</td>
<td>1.50 ± 0.17</td>
<td>1.33 ± 0.15</td>
</tr>
<tr>
<td>NIP 2 mM</td>
<td>0.37 ± 0.06**</td>
<td>1.19 ± 0.27</td>
<td>16.58 ± 2.61</td>
<td>1.56 ± 0.14</td>
<td>1.03 ± 0.13</td>
</tr>
<tr>
<td>NIP 4 mM</td>
<td>0.45 ± 0.09**</td>
<td>1.36 ± 0.23</td>
<td>22.01 ± 1.51</td>
<td>2.32 ± 0.26</td>
<td>1.32 ± 0.26</td>
</tr>
<tr>
<td>NIP 8 mM</td>
<td>0.77 ± 0.10**</td>
<td>1.02 ± 0.15</td>
<td>16.93 ± 2.15</td>
<td>2.88 ± 0.56**</td>
<td>1.18 ± 0.14</td>
</tr>
</tbody>
</table>

** P < 0.01.
Data (mean ± S.E.M.) are presented as absolute values (μM, n = 4).

The calcium-independent release of GABA suggests a release from the cytosolic pool of GABA and a mediation by the reversal of the high affinity GABA transporter (Bernath and Zigmond, 1990; Attwell et al., 1993; Campbell et al., 1993). As shown above, NIP, a specific transportable GABA re-uptake blocker known to be effective in vivo preparations (Lerma et al., 1984; Westerink and De Vries, 1989), significantly attenuated the increase in extracellular GABA, while not modifying the increase in extracellular GLU, produced by AMPH in the striatum. This suggests that AMPH contributes to the release of GABA through a calcium-independent transporter mechanism. Similar results have been reported by Bernath and Zigmund (1989) in striatal slices. It is of interest that increasing doses of NIP produced increasing attenuations of GABA released by AMPH. This is in agreement with the dose-related effect of NIP on GABA release. This fact, together with the lack of effect of NIP on extracellular GLU, gives evidence for the high specificity of NIP acting on the GABA transporter.

The calcium-independent transporter-mediated release of GABA could be produced by GLU previously released by AMPH. In fact, using microdialysis in human hippocampus, During et al. (1995) have shown that GLU induced a calcium-independent transporter-mediated release of GABA probably acting on neuronal and/or glial glutamatergic receptors. Also, Belhage et al. (1993) have shown that GLU acting on glutamatergic receptors can produce a transporter-mediated release of GABA in glutamatergic specific heterotransporters located on GABA neurons (Bonanno and Raiteri, 1994). Nonetheless, the possibility cannot be ruled out that dopamine also produces a transporter-mediated release of GABA (Bernath and Zigmond, 1989).

NIP, which in this study has been shown to increase extracellular GABA, also increases extracellular TAU but not the other amino acids such as GLU, GLN and ARG. The increase in extracellular TAU produced by NIP may be due to the release of GABA. In fact,
Lerma et al. (1985) have reported that intracerebral infusion of GABA increases TAU, probably by a counter-transport process.

In conclusion, this study is the first in vivo evidence showing the action of AMPH on the release of GABA through a high affinity transporter mechanism. The possibility exists that dopamine and GLU, previously released by AMPH, may release GABA in striatum by both calcium-dependent and calcium-independent GABA transporter mechanisms. Further studies will be needed to elucidate whether GLU or dopamine preferentially mediate the release of GABA produced by AMPH through a particular mechanism in the striatum.

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