Effects of amphetamine, methylphenidate, and apomorphine on regional brain serotonin and 5-hydroxyindole acetic acid

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Abstract. Electrophysiological and cytofluorometric data suggest that doses of amphetamine which enhance locomotor activity and promote focused stereotypies produce pronounced effects on serotonin pathways in the CNS. However, the biochemical evidence regarding changes in serotonergic function produced by moderate doses of this drug is inconsistent. Therefore, the present study was designed to further examine the effects of amphetamine (1–5 mg/kg) on regional brain serotonin and its metabolite and to compare these effects to behaviorally comparable doses of methylphenidate and apomorphine. At doses which produce a multiphasic behavioral response pattern, including a stereotypy phase consisting primarily of repetitive head movements and occasional oral stereotypies, amphetamine (3 mg/kg) and methylphenidate (30 mg/kg) increased levels of 5HIAA in striatum and frontal cortex, two brain regions which receive serotonergic projections from the dorsal raphe nucleus. In contrast, these drugs decreased or had no effect on 5HIAA levels in hippocampus, a brain region which receives its serotonergic innervation from the median raphe nucleus. A moderate dose of apomorphine (0.5 mg/kg) produced a comparable pattern of neurochemical effects. These data are consistent with electrophysiological and cytofluorometric data suggesting enhanced dorsal raphe serotonergic function following amphetamine-like stimulants. Pretreatment of animals with z-methyltyrosine at a dose sufficient to prevent the locomotor stimulation and stereotypy promoted by amphetamine, or by haloperidol, failed to prevent the amphetamine-induced increase in 5HIAA, indicating that these serotonergic effects are not secondary to the amphetamine facilitation of dopaminergic transmission. The results of this study suggest that serotonin may play a modulatory role in the behavioral effects of amphetamine-like stimulants which is dependent for its expression on an intact dopamine system.

Key words: Amphetamine – Stereotypy – Serotonin – 5HIAA

Most biochemical evidence indicates that amphetamine (AMPH) is considerably less potent in its effects on serotonin (5HT) systems than on dopamine (DA) systems (Ross et al. 1977; Raiteri et al. 1977). For example, the early in vivo studies by Fuxe and colleagues revealed effects on DA accumulation and release at doses of AMPH as low as 0.5–1.0 mg/kg, but no effect at 10 mg/kg on 5HT accumulation (Fuxe et al. 1967; Fuxe and Ungerstedt 1968). Consequently, more recent biochemical studies have focused on high doses of AMPH, and have attempted to relate changes in 5HT metabolism to abnormal behavioral syndromes (Sloviter et al. 1978a; Lees et al. 1979; Fernando et al. 1980).

In contrast to these high dose effects of AMPH, electrophysiological (Foote et al. 1969; Gallagher and Aghajanian 1976; Baraban et al. 1978; Rebec and Curtis 1983) and cytofluorometric (Geyer et al. 1975) data indicate profound effects on apparent 5HT function at moderate doses of AMPH which enhance locomotor activity or promote focused stereotypies. Further, some evidence suggests a role for 5HT in modulating both AMPH-induced locomotor activity (Neill et al. 1972; Mabry and Campbell 1973; Breese et al. 1974; Costall and Naylor 1974) and stereotypies (Segal 1976; Sloviter et al. 1978b; Lucki and Harvey 1979). However, the available biochemical data is inconsistent with regard to changes in 5HT function at lower doses of AMPH, in part because many of the earlier studies examined whole brain 5HT metabolism using techniques with limited sensitivity.

To further assess the possible role of the various 5HT systems in the AMPH response, in the present study we determined the effects of low to moderate (1.0–5.0 mg/kg) doses of AMPH on hippocampus, striatum and frontal cortex 5HT and its metabolite. The hippocampus and striatum were chosen because most evidence suggests they receive their respective 5HT projections exclusively from median raphe (Jacobs et al. 1974; Lorens and Guldberg 1974; Bobillier et al. 1975; Geyer et al. 1976) or dorsal raphe (Jacobs et al. 1974; Miller et al. 1975; Bobillier et al. 1976; Bunney and Aghajanian 1976; Geyer et al. 1976; Fibiger and Miller 1977; Pasquier et al. 1977; Azmitia 1978). The frontal cortex receives 5HT projections from both of these 5HT nuclei. We also examined the effects of methylphenidate (MP) and...

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Abbreviations: DA, dopamine; AMPH, S(+)amphetamine; 5HT, serotonin; MP, methylphenidate; APO, apomorphine; 5HIAA, 5-hydroxyindoleacetic acid; zMT, z-methyltyrosine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanilllic acid; HAL, haloperidol
Table 1. Effects of AMPH on regional levels of 5HT and 5HIAA. Groups of rats (n = 6) were administered saline or AMPH, SC, and were sacrificed at the indicated times. Values are the means ±SEM in µg/g tissue.

<table>
<thead>
<tr>
<th></th>
<th>5HT</th>
<th>AMPH</th>
<th>5HIAA</th>
<th>AMPH</th>
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<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>1.0 mg/kg AMPH, 30 min</td>
<td>Saline</td>
<td>5.0 mg/kg AMPH, 90 min</td>
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<tr>
<td>Striatum</td>
<td>0.45 ± 0.02</td>
<td>0.49 ± 0.02*</td>
<td>0.51 ± 0.02</td>
<td>0.51 ± 0.02</td>
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<tr>
<td>Frontal cortex</td>
<td>0.57 ± 0.02</td>
<td>0.62 ± 0.02*</td>
<td>0.23 ± 0.01</td>
<td>0.22 ± 0.01</td>
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<tr>
<td>Hippocampus</td>
<td>0.40 ± 0.01</td>
<td>0.41 ± 0.01</td>
<td>0.36 ± 0.02</td>
<td>0.32 ± 0.01*</td>
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Significantly different from saline, * P < 0.05, ** P < 0.001

Methods

Male Sprague-Dawley rats, weighing 200–250 g, were obtained from Harlan Industries, Cumberland, Indiana, and were housed five per cage under standard laboratory conditions for at least 1 week before being subjected to experimental manipulation. S(+)-Amphetamine sulfate, haloperidol, and apomorphine HCl were administered SC, and D-methyltyrosine methyl ester (Sigma Chemical Co.) and methylphenidate HCl (a gift from CIBA Pharmaceutical Co.) were administered IP in saline, or, in the case of APO, in 0.1% ascorbic acid. All doses are expressed as the free base.

Animals were sacrificed by decapitation, the brain removed, placed on a cold plate, and sliced according to Segal and Kuczenski (1974) with modification to obtain specific brain regions. These were immediately frozen on dry ice and stored at -70 °C prior to biochemical analyses. Samples were analyzed by high pressure liquid chromatography with electrochemical detection for monoamines and metabolites by modification of the method of Magnusson et al. (1980). Briefly, tissue was homogenized in 0.1 N perchloric acid containing N-methyl dopamine as an internal standard, centrifuged at 8000 g for 3 min, filtered through a 0.2 g filter and injected into a Bio-Analytical Systems LC304 system equipped with an ODS-C18 guard column, a Biophase ODS C18 5 µ analytical column (250 x 4.6 mm) maintained at 35 °C and a glassy carbon electrode maintained at +0.8 V. The mobile phase consisted of 0.08 M citrate buffer, pH 5.2, 5% methanol, and 0.9 mM hexane sulfonate, pumped at a flow rate of 1.5 ml/min. Peak areas were quantitated with a Hewlett-Packard 3390A integrator.

Behavior was monitored with the use of residential activity chambers (RAC) as previously described (Segal et al. 1980). Response profiles included indices of general activity (locomotion, rearing, intercompartment entries), exploration (frequency and duration of contact with environmental stimuli), and ingestion (duration of eating and drinking). In addition animals were videotaped and their patterns of stereotypy were scored by trained raters who were unaware of the specific treatment conditions (Segal et al. 1980).

Data were analyzed by analysis of variance and subsequent Neumann-Keuls test.

Results

Response of regional 5HT metabolism to AMPH

The administration of AMPH resulted in complex, region-specific changes in 5HT and 5HIAA levels in the brain. In striatum all doses tested (1.0–5.0 mg/kg) significantly increased 5HT levels, with the duration and magnitude of the increase proportional to the dose of the drug. AMPH at 3 mg/kg or greater also significantly increased 5HIAA levels in this brain region. In contrast, in hippocampus, AMPH had no effect on 5HT levels, whereas all doses tested decreased 5HIAA levels, with the decrease proportional to the dose of the drug. Levels of 5HT increased in frontal cortex, whereas 5HIAA levels exhibited a biphasic response, decreasing at early time points and increasing at later time points (Fig. 1, Table 1).

In parallel behavioral studies, following 1 mg/kg AMPH, animals exhibited enhanced locomotor activity in the absence of focussed stereotypies (data not shown). In contrast, following 3 mg/kg AMPH (Fig. 3) or 5 mg/kg AMPH (data not shown), animals exhibited a typical multiphasic behavioral response, including a period of reduced locomotion, during which the animals were engaged, depending on the dose, in focussed sniffing, repetitive head movements, and oral stereotypies.

Effects of methylphenidate on regional 5HT metabolism

To examine the generality of these effects, groups of animals were administered 30 mg/kg MP, a dose which promoted a multiphasic response profile, similar to the profile observed following 3 or 5 mg/kg AMPH, and including a period of reduced locomotion accompanied by the appearance of focussed stereotypies (data not shown). Animals were sacrificed at various times afterwards for determination of 5HT and 5HIAA levels. The data are presented in Fig. 2. In contrast to AMPH, MP significantly decreased 5HT lev-