Extracellular Dopamine and Amphetamine After Systemic Amphetamine Administration: Comparison to the Behavioral Response

RONALD KUCZENSKI, WILLIAM P. MELEGA, ARTHUR K. CHO, and DAVID S. SEGAL
Department of Psychiatry, School of Medicine, University of California, San Diego, La Jolla (R.K., D.S.S.) and Department of Medicinal and Molecular Pharmacology, University of California, Los Angeles, Health Sciences Center, Los Angeles, California
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ABSTRACT
To further delineate amphetamine-dopamine pharmacokinetic-pharmacodynamic relationships, we examined extracellular levels of dopamine and amphetamine in caudate-putamen after the s.c. administration of 8 mg/kg amphetamine. In a parallel group of animals, we also assessed caudate-putamen tissue levels of the drug. Extracellular concentrations of the transmitter and the drug exhibited similar temporal profiles, each achieving maximum concentrations within 30 min of drug administration. Tissue levels of amphetamine exhibited a similar, although slightly earlier time to maximum levels. The concentrations of amphetamine and dopamine in the extracellular fluid and amphetamine in tissue rapidly declined with similar rates of elimination. In contrast to the temporal profiles for both dopamine and amphetamine, stereotyped behaviors achieved maximum intensity at about 60 min. In addition, although transmitter and drug declined almost 10-fold from maximum values over the 4-hr interval after amphetamine administration, stereotyped behaviors persisted for at least 3 hr before abating. The results of these studies confirm our previous observation that the temporal profiles for stereotyped behaviors and extracellular dopamine are dissociated, and also extend this dissociation to extracellular amphetamine. In addition, although there was a close correspondence between dopamine and amphetamine within each experimental animal, individual animals exhibited a broad range of maximal dopamine responses, suggesting a differential responsiveness to amphetamine.

The DA transporter plays a prominent role in the dopaminergic effects of stimulants such as COC and AMPH (Kuczenski and Segal, 1994). Thus, the quantitative features of the drug-induced DA response should parallel the synaptic drug concentration at the neuronal membrane. In fact, after COC administration, there is a close temporal correspondence between the extracellular DA response and extracellular COC (Pettit et al., 1990), and, by inference, the synaptic concentrations of these substances. Although comparable direct comparisons between extracellular DA and AMPH kinetics have not been available, our previous pharmacokinetic-pharmacodynamic studies of postmortem tissue levels of AMPH and caudate-putamen extracellular AMPH suggested a similar AMPH concentration-dependent DA efflux process (Melega et al., 1995). Thus, at least after the peak drug response and throughout subsequent elimination times, the temporal profile of the extracellular DA response paralleled that of brain AMPH levels (Melega et al., 1995).

Recently, however, Clausing et al. (1995) characterized extracellular AMPH dynamics in rat caudate-putamen after s.c. administration of a wide range of AMPH doses. Those authors reported a temporal pattern of extracellular AMPH that included peak AMPH concentrations at 60 min after drug administration. In contrast, maximum tissue levels of AMPH and extracellular DA concentrations have generally been reported to occur at substantially earlier times. These data suggest a profound dissociation between extracellular AMPH and DA and would provide evidence that extracellular AMPH may not play a primary role in the extracellular DA response. However, interpretation of their results is difficult because they did not concomitantly evaluate the extracellular DA response profile. It is possible, therefore, that using their experimental procedures, the extracellular DA peak may have occurred at correspondingly later times, correlating with extracellular AMPH. Alternatively the extracellular DA response may be dissociated from extracellular AMPH concentrations, and be more closely linked to intracellular actions of the drug.

To evaluate these possibilities, we concurrently examined extracellular concentrations of AMPH and DA in caudate-

ABBREVIATIONS: AMPH, d-amphetamine; COC, cocaine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; 3MT, 3-Methoxytyramine; serotonin, 5-HT.
putamen, as well as tissue levels of the drug, after the s.c.
administration of AMPH. Our results indicate that extracel-
lar AMPH and DA exhibit highly correlated temporal pat-
terns.

Materials and Methods

Rats obtained from Simonsen Laboratories (Gilroy, CA; 275–300 g) were maintained four per cage on a 14/10 hr light-dark cycle (lights on at 5:00 A.M.) under standard laboratory conditions, with *ad libitum* access to food and water for 2 wk before drug treatment. Animals were then stereotaxically implanted with bilateral guide cannulae using procedures previously described in detail (Kuczenski and Segal, 1989). Guide cannulae extended 2.6 mm below the surface of the skull and were aimed at the caudate-putamen (1.0 mm ante-
rrior to bregma, 2.8 mm lateral and 6.2 mm below dura). After surgery, animals were housed individually and allowed at least 1 wk to recover before receiving any treatment. Amphetamine was dis-
solved in saline and was administered subcutaneously in a volume of 1 ml/kg body weight at about 10:00 A.M. Doses refer to the free base.

Each rat was placed in an experimental chamber and the dialysis probes were inserted on the day before treatment (3:00–4:00 P.M.) to allow for acclimation to the test environment and for adequate equil-
ibrium of the dialysis probes. The behavioral chambers, described in detail elsewhere (Segal and Kuczenski, 1987; Kuczenski and Segal, 1989), were sound-proofed and maintained on a 14/10 hr light-
dark cycle (lights on at 5:00 A.M.), with constant temperature and humidity. Animals had continuous access to food and water. Throughou.

Throughout the behavioral response after drug administration, each dia-
yalyzed animal was videotaped for subsequent quantification of drug-
induced stereotyped behaviors. Concentric microdialysis probes were
constructed of SpectraPor hollow fiber (molecular weight cut-off
6000, o.d. 250 μm) as previously described (Kuczenski and Segal, 1989). The length of the active probe membrane was 3 mm. Probes
were perfused with artificial cerebrospinal fluid (147 mM NaCl, 1.2
mM CaCl2, 0.9 mM MgCl2, 4.0 mM KCl) delivered by a microinfusion
pump (Rheinfelden, Switzerland) via 50 cm of micro-line ethyl vinyl acetate tubing connected to a fluid swivel. Dialysate from one probe for each rat was used for neurotransmitter analyses, and from the opposite probe (counter-balanced) for AMPH analyses. Dialysate was collected through glass capillary tubing into vials for neurotransmitter analyses containing 20 μl of 25% methanol, 0.2 M sodium citrate, pH 3.8. Under these conditions, dialysate DA, 5-HT, and metabolites were stable throughout the collection and analysis interval. Dialysate for AMPH analyses was collected into empty vials and frozen until analysis. Samples were collected outside the experimental chamber to avoid disturbing the animal. Individual probe recoveries were estimated by sampling a standard DA solution *in vitro* and were around 10%. At the end of the experiment, each animal was perfused with formalin for histological verification of probe placements.

Dialysate samples were collected every 10 or 20 min, and were assayed for DA, DOPAC, homovanillic acid, 3MT and 5-hydroxyin-
doleacetic acid by high-pressure liquid chromatography with electro-
chemical detection. In all experiments, solutions of standard re-

where $k_e$ and $k_a$ are rate constants for the appearance of AMPH and A is a constant reflecting dose and an apparent volume.)

Results

Preliminary studies were conducted to compare the caudate putamen dialysate DA responses to AMPH on the left and right sides of the brain. The s.c. administration of 8 mg/kg AMPH resulted in a pattern of changes in extracellular DA and its metabolites, which was typical to this dose range, including a rapid, approximately 30-fold increase in DA, a 20-fold increase in 3MT and a decrease in DOPAC to values near 25% of baseline values. This pattern and the quantitative features of the responses were identical in the right and left caudate-putamen (data not shown). Compari-
sons of the DA responses ($n=8$) on the two sides of the brain revealed highly significant correlations for both the DA peak response ($R^2 = 0.90, P < .001$) and the area under the curve ($0–120$ min) ($R^2 = 0.70, P < .01$).

After administration of 8 mg/kg AMPH, dialysate AMPH and DA concentrations, determined from opposite sides of the brains of the same animals, exhibited parallel temporal profiles (fig. 1). Both achieved maximum concentrations at about 30 min, then gradually declined with half lives of about 45 min (table 1). The mean times to peak dialysate concentra-
tion for DA and for AMPH (31.8 ± 2.8 and 30.5 ± 1.3 min,
respectively) were essentially the same. The temporal profile for AMPH in caudate-putamen tissue is presented in figure 2. The time to peak tissue level (22.4 ± 1.5 min) was significantly earlier than those for both dialysate measures. T max values and rate constants for the appearance and elimination for DA in dialysate, and for AMPH tissue and dialysate levels are summarized in table 1.

The behavioral responses of the dialysis animals consisted of a multiphasic locomotor response that included a prolonged period of intense focused stereotypies, consisting primarily of continuous licking and biting, typical to this dose. The time course of the stereotypy response is presented in figure 2.

The relationship between dialysate AMPH and DA concentrations for individual animals is presented in figure 3. Each of the four animals exhibited a direct relationship between the two substances as a function of time, with correlation R^2 values ranging from 0.72 to 0.92. However, the slopes of the bivariate plots varied by a factor of 4 to 5 (table 2). Thus, although the DA response was proportional to the extracellular AMPH concentration for each animal, the magnitude of the extracellular DA response exhibited wide individual differences.

**Discussion**

Our results indicate that, after the s.c. administration of AMPH, the caudate-putamen extracellular DA response and extracellular AMPH concentrations, as detected by microdialysis, closely parallel each other. Both the transmitter and the drug achieved maximal concentrations at near 30 min after AMPH administration, and both substances exhibited equivalent elimination rates. Those data, together with the highly significant correlations between DA and AMPH con-

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**TABLE 1**

<table>
<thead>
<tr>
<th>Source</th>
<th>k_a (min^-1)</th>
<th>k_app (min^-1)</th>
<th>T_max (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialysate AMPH</td>
<td>0.015 ± 0.002</td>
<td>0.042 ± 0.009</td>
<td>30.5 ± 1.3</td>
</tr>
<tr>
<td>Dialysate DA</td>
<td>0.015 ± 0.003</td>
<td>0.040 ± 0.010</td>
<td>31.8 ± 2.6</td>
</tr>
<tr>
<td>Caudate-Putamen AMPH</td>
<td>0.015 ± 0.001</td>
<td>0.101 ± 0.018</td>
<td>22.4 ± 1.5</td>
</tr>
</tbody>
</table>

*The time-dependent changes in dialysate concentration from each animal was fitted to a two exponential appearance and elimination expression by non-linear regression procedures as described in “Materials and Methods” to estimate rate constant values. The values shown are the means ± S.D. for four animals. The rate constants (± asymptotic S.D.) for changes in brain concentration were estimated from the concentrations of groups of four animals at each time point over the 240-min time interval. The time of peak concentration T_max, is derived from the rate expression, as described previously (Welling, 1986).*
concentrations for individual animals, are indicative of a rapidly equilibrating AMPH concentration-DA response relationship, and suggest an intimate concentration-dependent association between drug levels in the extracellular space and DA response. It is noteworthy that parallel temporal profiles have also been reported for extracellular concentrations of COC and DA after systemic COC administration. Because COC enhances DA transmission strictly through its interaction with the DA transporter at the extracellular surface of the neuronal membrane, a close correspondence between extracellular COC and DA would be expected. A similar association would also be predicted for AMPH, based on an accelerative exchange-diffusion mechanism of action of AMPH at DA terminals (Fischer and Cho, 1979); i.e., the drug-induced exchange of DA should be directly proportional to the extracellular AMPH concentration.

The dynamics of extracellular AMPH and DA demonstrated both similarities to and differences from tissue AMPH. First, all three measures exhibited equivalent rate constants of elimination. These data extend to extracellular AMPH, our previous observation of a parallel decay of AMPH from brain tissue and plasma (Cho et al., 1973; Melega et al., 1995) and lead to the suggestion that, especially at the later time points, all three compartments of AMPH are in rapid equilibrium. Perhaps not surprisingly, extracellular DA exhibited a similar rate constant of elimination, indicating a close association between brain drug levels and the transmitter response. However, peak tissue AMPH was much higher than peak values for extracellular AMPH, and tissue appeared to achieve peak values at times earlier than those for both extracellular AMPH and DA. With respect to the former, because of its high lipophilicity, the free base of AMPH readily penetrates cellular boundaries. In addition, as has been observed for other similar amines (Sawada et al., 1994; Sato and Koshiro, 1995), the cationic species of the drug would be expected to accumulate in glia and neurons by “trapping” in the more acidic environs of the cytoplasmic milieu, as well as by ion pair formation with intracellular anions, e.g., Ericinska et al., 1987; Zaczek et al., 1990). In support of the notion that AMPH accumulates in brain tissue, we have previously shown that in i.v. administration of AMPH leads to a steady-state caudate-putamen tissue/plasma ratio of AMPH concentrations between eight and ten (Melega et al., 1995; Cho et al., 1973). In our study, assuming
rather than with tissue AMPH levels would suggest that the extracellular DA corresponds temporally with extracellular i.v. drug administration (Melega et al., 1995). That peak extracellular AMPH concentration near 2 m is only other recent report of microdialysate AMPH levels (Clausing et al., 1995). However, those authors reported a peak extracellular AMPH concentration near 2 μM (uncorrected for probe recovery) after s.c. administration of 5 mg/kg AMPH, which is in good agreement with the similar values we obtained after 8 mg/kg AMPH. However, they also observed a tissue-to-plasma AMPH ratio near 20:1 at 50 min after drug administration, which is substantially higher than the values we have typically observed (Melega et al., 1995; Cho et al., 1973). The source of this discrepancy is not clear. Additionally, those authors reported peak dialysate AMPH concentrations around 60 min after drug, as opposed to the 30-min time point we obtained. Unfortunately, it is not possible to directly compare the drug-transmitter relationship that we observed in our study to their results. For one, Clausing et al (1995) did not concomitantly evaluate extracellular DA concentrations. In addition, methodological differences between the two studies may also contribute to the apparent discrepancies. For example, those authors began sample dialysate collection 2 hr after probe insertion, whereas our dialysis probes were inserted the previous afternoon to allow for overnight equilibration. Differences in the extent of tissue reequilibration after probe insertion might affect the dynamics of substances in the extracellular space. Additionally, those authors noted that the onset of behavioral activation following AMPH administration was delayed 12 to 15 min. In contrast, using our s.c. drug administration protocol, we typically observe an onset of locomotor activation within 1 to 2 min. Thus, differences in the method of drug administration may have influenced the rates of drug absorption and accumulation into the brain.

Two points regarding our AMPH pharmacokinetics and the drug-induced behavioral response merit some consideration. First, we have previously noted the dissociation between the quantitative features of the behavioral and the caudate-putamen and nucleus accumbens DA responses (for review see Segal and Kuczenski, 1994). This dissociation is most profound in the temporal profiles of the stereotypy and DA responses at higher doses of the drug. Consistent with our past results, this study showed that peak DA concentrations and the onset of oral stereotypies occurred within the first 30 min of the response. However, the magnitude of the stereotypy response was maintained during the subsequent 3 hr, although DA concentrations declined by more than 4-fold to levels that, in response to lower doses of the drug, do not promote oral stereotypies. Our results confirm a dissociation between tissue AMPH levels and the behavior (Kuczenski and Segal, 1994), and extend the dissociation to dialysate AMPH levels as well, i.e., oral stereotypies are maintained at extracellular AMPH levels that are not typically associated with the production of stereotyped behaviors. Thus, although that probe recovery for AMPH was similar to DA (about 10%), the tissue/extracellular AMPH ratio also would be approximately 10. As a consequence, especially at later times, the tissue AMPH pool must represent a drug reservoir and the net movement of drug likely proceeds from tissue to extracellular space to plasma. That tissue AMPH levels remain substantially higher than extracellular and plasma levels does not necessarily conflict with the suggestion that these three pools are in rapid equilibrium. A rapid equilibrium should exist between the intracellular and extracellular space; i.e., the rate constant for the diffusion-dependent movement of AMPH out of the cell would be large compared to the rate constant for overall elimination, and the decline of brain tissue AMPH should parallel the decline from plasma. However, the actual efflux of AMPH from tissue would appear much slower, because this process depends on the concentration gradient of the nonionized species of AMPH between intracellular and extracellular compartments. Thus, despite the rapid equilibrium, intracellular trapping through ionization and ion-pair formation results in and maintains a net accumulation of the drug in tissue.

Regarding the time delay between extracellular AMPH and DA compared to tissue AMPH, a similar delay was also observed between extracellular DA and tissue AMPH after i.v. drug administration (Melega et al., 1995). That peak extracellular DA corresponds temporally with extracellular rather than with tissue AMPH levels would suggest that the DA response is more closely linked to actions of the drug at an extracellular rather than an intracellular site, insofar as tissue measures reflect predominantly intracellular accumulation. However, some evidence suggests that, in the absence of high resolution sampling intervals, the time to peak drug concentration may appear delayed as an artifact of the integrative nature of the microdialysate sampling procedure (Patsalos et al., 1995). Therefore, further studies with greater temporal resolution will be required to address this specific issue.

Our results are not entirely consistent with those of the only other recent report of microdialysate AMPH levels (Clausing et al., 1995). However, those authors reported a peak extracellular AMPH concentration near 2 μM (uncorrected for probe recovery) after s.c. administration of 5 mg/kg AMPH, which is in good agreement with the similar values we obtained after 8 mg/kg AMPH. However, they also observed a tissue-to-plasma AMPH ratio near 20:1 at 50 min after drug administration, which is substantially higher than the values we have typically observed (Melega et al., 1995; Cho et al., 1973). The source of this discrepancy is not clear. Additionally, those authors reported peak dialysate AMPH concentrations around 60 min after drug, as opposed to the 30-min time point we obtained. Unfortunately, it is not possible to directly compare the drug-transmitter relationship that we observed in our study to their results. For one, Clausing et al (1995) did not concomitantly evaluate extracellular DA concentrations. In addition, methodological differences between the two studies may also contribute to the apparent discrepancies. For example, those authors began sample dialysate collection 2 hr after probe insertion, whereas our dialysis probes were inserted the previous afternoon to allow for overnight equilibration. Differences in the extent of tissue reequilibration after probe insertion might affect the dynamics of substances in the extracellular space. Additionally, those authors noted that the onset of behavioral activation following AMPH administration was delayed 12 to 15 min. In contrast, using our s.c. drug administration protocol, we typically observe an onset of locomotor activation within 1 to 2 min. Thus, differences in the method of drug administration may have influenced the rates of drug absorption and accumulation into the brain.

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AMPH levels have a similar time course to DA levels, the behavioral responses do not. That extracellular AMPH and DA levels follow a parallel time course argues that synaptic DA levels follow a similar time course, because the release of DA should be closely dependent on the AMPH concentration. Therefore, these data suggest rapid changes in DA-responsive systems that permit oral stereotypies to continue although synaptic DA levels fall below levels that are required to initiate these behaviors. In this regard, we have recently reported that interruption of a high AMPH dose stereotypy response with a low dose of haloperidol (0.1 mg/kg) rapidly terminates the expression of focused stereotypies and locomotion predominates (Conti et al., 1997), indicating that continued activation of D2 DA receptors is still required for the expression of stereotypies. Furthermore, administration of a low, nonstereotypy producing dose of AMPH (0.5 mg/kg) at the end of a high-dose stereotypy phase reinitiates intense, focused stereotypies, even though DA levels do not rise to values required to initiate stereotypy in a naive animal (R. Kuczenski and D. S. Segal, unpublished data). These observations suggest that DA-responsive postsynaptic mechanisms responsible for the expression of stereotyped behaviors may be sensitized during the acute response.

Second, although a high degree of correlation between extracellular AMPH and DA was obtained within each animal, when individual DA responses were evaluated as a function of dialysate AMPH concentrations (table 2), substantial differences in slopes were observed. These results are suggestive of substantial variability in DA responsivity, and these pharmacodynamic differences may contribute to the broad range of individual differences in behavioral response that we have previously noted (Segal and Kuczenski, 1987). It is unlikely that the individual variability in the DA-AMPH relationship that we observed is an artifact of sampling from opposite sides of the brain, because our previous data (Melega et al., 1995) revealed relatively small regional variations in AMPH levels after systemic drug administration. However, a more accurate approach toward evaluating the relative roles of pharmacokinetic and pharmacodynamic factors in the behavioral response would require the examination of both AMPH and DA from a single dialysis probe, studies that are currently in progress.

In summary, in response to systemic AMPH, extracellular DA concentrations are highly correlated with extracellular drug concentrations. These results are consistent with a primary role for extracellular AMPH acting at the DA transporter in the mechanism of action at the DA terminal, and lend further credence to the view that events in the extracellular space provide an accurate reflection of ongoing psycho-stimulant drug-transmitter dynamics within the synaptic cleft.

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References


Send reprint requests to: Dr. Ronald Kuczenski, Professor of Psychiatry, Psychiatry Department (0603), UCSD School of Medicine, La Jolla, CA 92093,