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Effects of Haloperidol on Amphetamine-Induced Increases in Ascorbic Acid and Uric Acid as Determined by Voltammetry *In Vivo*

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MUELLER, K. AND C. HASKETT. *Effects of haloperidol on amphetamine-induced increases in ascorbic acid and uric acid as determined by voltammetry* in vivo. PHARMACOL BIOCHEM BEHAV 27(2) 231–234, 1987.—Amphetamine produces dramatic changes in extracellular ascorbic acid (AA) and uric acid (UA) in rat caudate; the origin of extracellular AA and UA is being widely investigated. In this study, linear sweep voltammetry with carbon paste electrodes was used to monitor extracellular AA and UA levels in conscious behaving rats. Amphetamine (2 and 4 mg/kg) produced a dose-related increase in UA; the increase in AA was very similar at both doses. Haloperidol (0.2 mg/kg) blocked the amphetamine-induced increase in UA but reduced the AA increase only by about 20%. Thus the amphetamine-induced increase in AA is only partly dependent on dopamine (DA) receptor stimulation whereas the amphetamine-induced increase in UA is completely dependent upon DA receptor stimulation.

Ascorbic acid Uric acid Haloperidol Caudate

DURING the past few years, voltammetric recording *in vivo* has revealed dramatic changes in extracellular ascorbic acid (AA) and uric acid (UA) in the brain after administration of psychoactive drugs such as amphetamine. The origins and functions (if any) of extracellular AA and UA are receiving increasing attention. We now report that, in anterior caudate, UA levels more closely mirror functional changes in dopamine (DA) systems than do AA levels. Our data also suggest that the amphetamine-induced increase in AA is only partly dependent upon DA receptor stimulation.

Amphetamine produces a dramatic increase in extracellular AA in caudate [6, 20, 23]. A reasonable first hypothesis seemed to be that the amphetamine-induced increase in AA is either directly or indirectly related to DA activity. Whether the amphetamine-induced increase in AA is dependent upon the integrity of DA neurons is controversial [6,7]. High doses of haloperidol, which would be expected to enhance DA release, have been reported to either decrease [4] or have no effect on AA levels in caudate [2]. Thus the relationship, if any, between DA systems and extracellular AA is not particularly clear.

Extracellular UA has been much less extensively studied. Preliminary data have suggested that extracellular UA levels may reflect activity in purinergic neuromodulatory systems [17]. On the other hand, extracellular UA could simply be a nonspecific metabolic index. UA is the end product of purine metabolism in most mammals. Thus in circumstances of increased energy use (e.g., increased firing, increased neurotransmitter release, increased neurotransmitter reuptake, etc.) one night well expect to see increased UA as energy-related purines are metabolized.

If the amphetamine-induced increase in both extracellular AA and UA levels is due to enhanced binding of DA to its receptor (and/or to activation of neurons "downstream" from the DA receptor) as was earlier hypothesized, one would expect haloperidol to block the amphetamine-induced increase in both AA and UA. We found instead that haloperidol is far more effective in blocking the amphetamine-induced increase in UA than it is in blocking the amphetamine-induced increase in AA.

METHOD

In each of the following experiments male Wistar rats were housed singly with free access to food and water. A 12 hour light-dark cycle was maintained; testing always occurred two hours after lights-on.

Carbon paste working electrodes were constructed as described previously [14]. Two working electrodes were implanted in both the right and left anterior caudate (2.8 mm anterior to bregma, 2.8 mm lateral, 5.2 mm beneath the cortex) [19]. Electrode positions were verified post-mortem by examination of frozen sections and data obtained from animals with misplaced electrodes were discarded.

Extensive testing indicates that these electrodes monitor AA and UA [14,15]. The putative UA signal is dramatically reduced by allopurinol (a UA synthesis inhibitor) and is eliminated by microinfusions of uricase. The putative UA signal

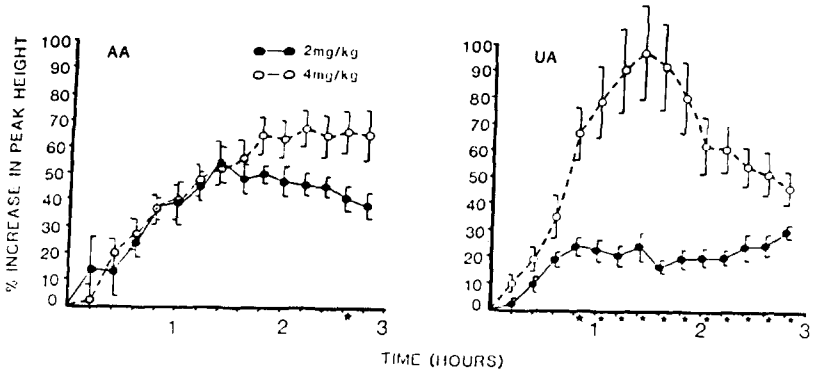

FIG. 1. The effects of d-amphetamine on AA and UA in caudate. Percent increase from baseline (recording prior to administration of drug) is shown. ★ Indicates statistically significant differences between groups (ANOVA).

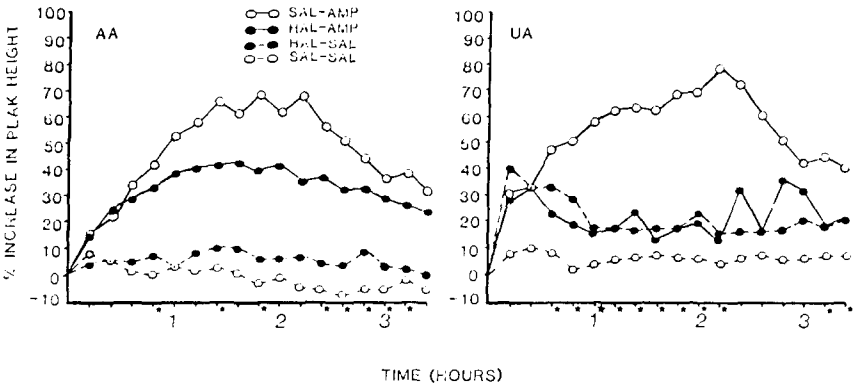

FIG. 2. The effect of haloperidol (0.2 mg/kg) on the amphetamine-induced (3 mg/kg) increase in AA and UA. Percent increase from baseline (recording prior to administration of drug) is shown. ★ Indicates statistically significant differences between groups (ANOVA).

is unaffected by the serotonin synthesis inhibitor p-chlorophenylalanine (PCPA) and PCPA has no effect on the amphetamine-induced increase in the putative UA signal (data submitted for publication). However, the amphetamine-induced increase in the putative UA signal is abolished by microinfusion of uricase.

A DCV5 voltammetry controller (BAS) remotely controlled by an Apple II+ microcomputer was used to conduct semidifferential linear sweep voltammetry. The applied potential increased from −100 mV to 500 mV at 10 mV per second. Electrodes were scanned every 12 minutes. To ensure a stable baseline, recording was conducted for two hours before drug administration. Results are presented as percent deviation from the pre-injection baseline (baseline is defined as the mean peak height of the three scans prior to the first injection).

In the first experiment seven animals were injected with d-amphetamine, 2 mg/kg SC (mean body weight=334 g), and nine animals were injected with d-amphetamine, 4 mg/kg SC (mean body weight=398 g). All animals were tested between the third and the fifth days after surgery.

In the second experiment eight animals were pretreated with saline or haloperidol (0.2 mg/kg IP) and thirty minutes later were injected with amphetamine (3 mg/kg) or saline. Each animal was injected with all four combinations of drugs but the order of drug conditions was varied. Drug treatments were separated by a period of 48 hours.

RESULTS

Figure 1 shows the effects of d-amphetamine on extracellular AA and UA. In general, both doses of amphetamine (2 mg/kg and 4 mg/kg) produced an increase in extracellular AA. However there were virtually no differences between doses until 2 hours after amphetamine. In contrast, amphetamine produced an obvious dose-related increase in extracellular UA.

Figure 2 shows the effects of haloperidol (0.2 mg/kg) on the amphetamine-induced increase in AA and UA. Haloperidol inhibited the amphetamine-induced increase in AA by about 20%. Haloperidol alone had little effect on AA levels as compared with saline controls. Interestingly, haloperidol completely inhibited the amphetamine-induced increase in UA.

DISCUSSION

If release of extracellular AA were dependent upon DA

receptor stimulation as was once suggested, amphetamine should produce a dose-related increase in extracellular AA levels and haloperidol should block this increase. Instead we found only small dose-related changes in extracellular AA and only a small effect of haloperidol on these changes. On the other hand, UA levels seemed to be highly dependent upon these indirect manipulations of DA release and DA receptor activation.

AA is found in high concentrations in mammalian brain [13]. Because AA has been reported to modulate binding of a variety of neurotransmitters [5, 9, 11, 21] and because AA is released from synaptosomes [8] and modulates the release of other neurotransmitters from synaptosomes [12], the hypothesis that AA may function as a neuromodulator requires careful attention. Because of the effect of amphetamine on DA neurons, an association between DA and AA seemed likely.

Although the higher dose of amphetamine produced a longer duration of increased AA, the dose-related effect was much smaller than we had anticipated. One might wonder whether 2 and 4 mg/kg are sufficiently different to produce dramatic dose-related effects on extracellular AA. However, these two doses of amphetamine produce dramatically different behavioral effects. The lower dose enhances locomotions; the higher dose reduces locomotions and produces intense stereotypy with licking and biting of the cage.

Another explanation for the small dose-related effect of amphetamine on AA might be that near maximal amounts of AA are released by the lower dose of amphetamine. Since AA is not synthesized in brain tissue AA may be fairly easily depleted. However, pilocarpine rapidly produces an 80% increase in extracellular AA in caudate [16]; therefore, some stimuli are capable of increasing extracellular AA to a greater degree than 4 mg/kg amphetamine.

Since behavior is so clearly dose-related and since UA levels are so clearly dose-related in this range of doses, the small dose-related effect of amphetamine on extracellular AA is quite striking. Although a previous study has found dose-related effects of amphetamine on AA levels, the identity of the electrochemical species contributing to the voltammetric signal in that study is unclear [10].

In addition, haloperidol produced only a modest reduction of the amphetamine-induced increase in AA. Thus a postsynaptic origin of the majority of the amphetamine-induced increases in AA seems unlikely, as does an origin from other neurons "downstream" from the DA receptor.

Co-release of DA and AA would be consistent with the results of the current study, however, other data suggest that co-release of AA and DA is unlikely [22]. Another alternative is that the amphetamine-induced release of AA is associated with a pathway other than the nigrostriatal pathway. For example, others have suggested that extracellular AA in caudate may be associated with a cortico-striatal pathway that is non-dopaminergic [18].

On the other hand, the amphetamine-induced increase in extracellular UA was both dose-related and blocked by haloperidol. Thus in this study, UA levels seem to be associated with DA receptor stimulation in anterior caudate. Since UA is ubiquitous in the body, associating UA exclusively with DA is premature. For example, O'Neill has suggested that changes in UA might be related to variations in adenosine [17].

But these data are also consistent with the hypothesis that UA may be a nonspecific metabolic index. For example, amphetamine indirectly results in DA receptor stimulation in caudate which in turn produces a great deal of metabolic activity in postsynaptic neurons. Likewise adenosine may increase metabolic activity in postsynaptic neurons by interacting with its receptor. (Such metabolic activity need not occur only in the immediate postsynaptic neuron but would also occur in other neurons further "downstream" from the receptor in the area of the brain in question.) In the presence of the appropriate receptor blocker this metabolic activity cannot occur. However at present the data are insufficient to select among these hypotheses.

There has been some controversy regarding the ability of voltammetry to distinguish between UA and 5-hydroxyindoleacetic acid (5HIAA). Carbon fiber electrodes do seem to produce a compound signal consisting of UA+5HIAA [1,3]. However, the data previously discussed strongly suggest that carbon paste electrodes monitor UA with little or no contribution from 5HIAA [14,15]. Since intracerebral dialysis confirms that the striatal extracellular fluid contains over 10 times more UA than 5HIAA [23] we have interpreted the data presented here in terms of UA rather than 5HIAA. However, there may have been some slight contribution of 5HIAA.

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