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Intrastriatal Infusions of Ascorbate Antagonize the Behavioral Response to Amphetamine

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WHITE, L. K., M. MAURER, M. E. KRAFT, C. OH AND G. V. REBEC. *Intrastriatal infusions of ascorbate antagonize the behavioral response to amphetamine.* PHARMACOL BIOCHEM BEHAV **36**(3) 485–489, 1990.—Compared to saline, bilateral infusions of ascorbate (AA) into the neostriatum of freely moving rats attenuated rearing, head bobbing, and sniffing at various times after systemic amphetamine administration. Comparable AA infusions into overlying cerebral cortex failed to alter the amphetamine behavioral response. Intrastriatal AA also enhanced the ability of haloperidol to antagonize amphetamine-induced forepaw shuffling and locomotion. Voltammetric measurements in separate animals revealed a linear increase in neostriatal AA that remained within reasonable physiological limits over the course of the AA infusion. Thus, endogenous AA may modulate behavior via mechanisms intrinsic to the neostriatum.

Amphetamine Ascorbate Haloperidol Intrastriatal infusions

VOLTAMMETRIC techniques, which were developed to measure easily oxidized compounds in the brain (1,12), have revealed an extremely high level of ascorbate (AA)—more than 200 μM—in the extracellular fluid of the neostriatum (14,27). Systemic or intranigral injections of amphetamine approximately double this value (2, 7, 9, 15, 17, 26, 32), and comparable or even greater increases have been observed during spontaneous changes in motor activity (18) and immediately following tail pinch (3). Although the physiological significance of these increases is unknown, they are likely to alter neostriatal function (10,20). In fact, systemic (5) or iontophoretic (6) application of physiological amounts of AA has been shown to accelerate the firing rate of neostriatal neurons, in some cases by as much as 500 percent.

Systemic injections of AA antagonize the behavioral response to amphetamine (28) and also enhance the ability of haloperidol to block this response (22). Comparable results have been obtained with intraventricular AA, ruling out a peripheral site of action (31). Because the neostriatum is known to play a key role in the behavioral response to both of these drugs (21,25), it is conceivable that AA acts in this site to modulate behavior. As an initial test of this hypothesis, we infused AA directly into the neostriatum of rats and monitored the behavioral response to either amphetamine or a combination of amphetamine and a threshold dose of haloperidol. In vivo voltammetry was used in separate animals to monitor the level of extracellular AA in the neostriatum during the AA infusions.

METHOD

Behavior

Behavioral data were obtained from 32 male Sprague-Dawley rats (approximately 400–450 g) housed under standard laboratory conditions. The animals first were anesthetized with chloropent and fitted with a bilateral stainless steel guide cannula through the skull as described elsewhere (31). Following a recovery period of 6–10 days, each animal was placed in a Plexiglas behavioral chamber (32 × 32 × 40 cm), and a 31-gauge stainless steel injector was inserted into each cannula. For intrastriatal infusions (n = 28), the injector was lowered approximately 4.5 mm below dura. In some animals (n = 4), the injector was lowered to a depth of 2.0 mm for cortical infusions. Polyethylene tubing connected the injectors to an infusion pump. Each infusion channel was secured in a rotating holder and attached to a counterbalance arm to allow each animal freedom of movement in all directions.

Following an habituation period of 1–2 hr, each animal received bilateral infusions of either 0.9% saline or 2.0 μg/μl AA sulfate (pH 7.6) at a rate of 0.3 μl/min for 70 min. The AA solution was prepared daily and kept under a steady stream of nitrogen until it was loaded into the infusion apparatus, which further protected AA from oxygen exposure. Ten minutes following the start of the infusion, each animal received a subcutaneous (SC) injection of 1.0 mg/kg d-amphetamine sulfate (free base). Some animals also received (SC) 0.9% saline or 0.025 mg/kg

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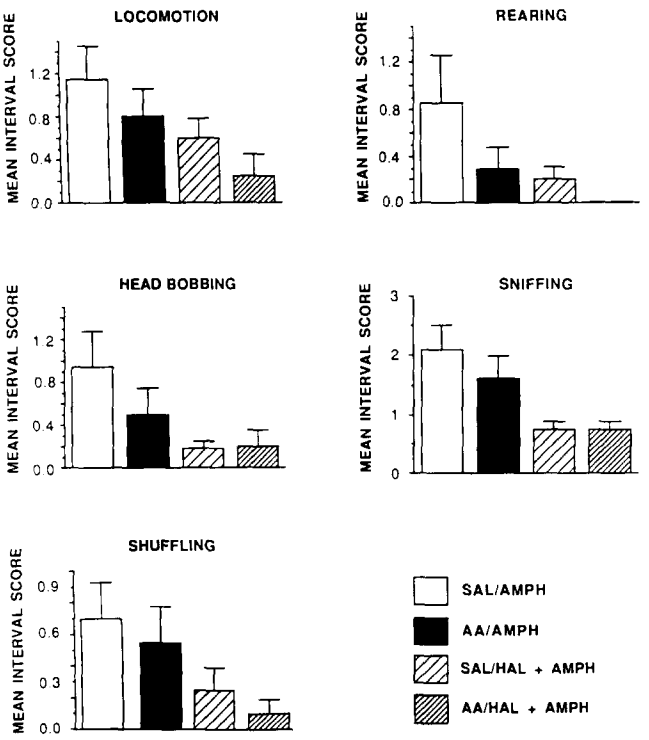

FIG. 1. Mean interval scores averaged across all observation intervals for individual items of behavior produced by 1.0 mg/kg d-amphetamine (AMPH) or 1.0 mg/kg d-AMPH plus 0.025 mg/kg haloperidol in rats that received simultaneous infusions of either physiological saline (SAL) or AA as indicated in the legend. Data are reported for the entire 60-min period following AMPH administration. Each group includes 7 animals. The brackets indicate the standard error of the mean.

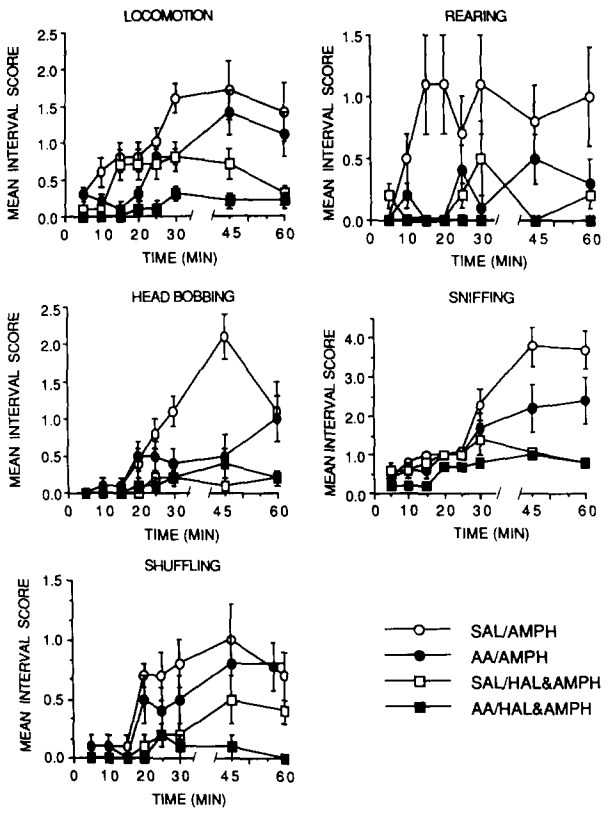

FIG. 2. Time course of individual behavioral items in rats treated with AMPH or an AMPH-HAL combination while receiving infusions of SAL or AA as in Fig. 1. The mean score for each behavior is presented for each observation interval. The brackets indicate the standard error of the mean.

haloperidol at the start of the infusion. Because SC saline pretreatment had no effect on the amphetamine response, these animals were included with the nonpretreated rats.

Independent observers, unaware of the type of infusion, recorded individual items of the amphetamine-induced behavioral response (e.g., locomotion, rearing, sniffing, head bobbing, and forepaw shuffling) every five min for the first 30 min and again at 45 and 60 min. During each observation period, which lasted for one min, behaviors were rated on a four-point intensity scale (0–3) and a two-point duration scale (1 = discontinuous, 2 = continuous). As described elsewhere (20,24), ratings were multiplied to yield a single value for each behavior. This rating procedure is sensitive not only to the multiple behaviors produced by amphetamine but also their antagonism by haloperidol and other neuroleptic drugs (22, 24, 29). Rating procedures that rely on automated measures of behavior or that focus on simple line crossings may miss important drug effects (16,21).

Upon completion of each experiment, animals received a lethal overdose of anesthetic followed by a transcardial perfusion with formosaline. Brains were removed, frozen, sectioned, and stained with cresyl violet for histological analysis. A repeated measures analysis of variance was used to examine the relationship between time after amphetamine and type of infusion. Post hoc analyses were conducted with the Tukey Honestly Significant Difference test.

Voltammetry

Three additional animals were mounted in a stereotaxic frame under urethane anesthesia for in vivo voltammetry according to procedures reported elsewhere (17). An infusion apparatus, connected to a 31-gauge cannula, delivered 2.0 μg/μl AA sulfate to the left neostriatum as described for the behavioral experiments. An electrochemically modified carbon-fiber electrode (approximately 10 μm diameter), which provides an AA wave distinct from other easily oxidized substances in the brain (8,13), was positioned between 0.5–1.0 mm from the cannula tip. The stereotaxic frame served as the auxillary electrode, and a saturated calomel electrode (SCE), attached to the dural surface via a saline bridge, served as the reference.

Staircase potential waveforms, −200 mV to +400 mV vs. SCE, were applied at 2-min intervals during a 10-min baseline period and throughout the course of the infusion. Each scan consisted of 32 steps (25 mV/step) of 66.7 msec duration; the scan rate was 100 mV/sec. A computer (IBM-XT), interfaced with a locally constructed three-electrode potentiostat, generated the waveforms and stored the sampled current. The electrode was tested in a subsequent postcalibration step with a citrate-phosphate buffer that contained 100 μM AA and 20 μM DOPAC, a dopamine metabolite. In vivo concentrations of AA were estimated by normalizing the measured current with respect to postcalibration values. Cannula placements in these animals were verified as previously described (see above).

RESULTS

Histology

Histological analysis revealed that all cannula placements were

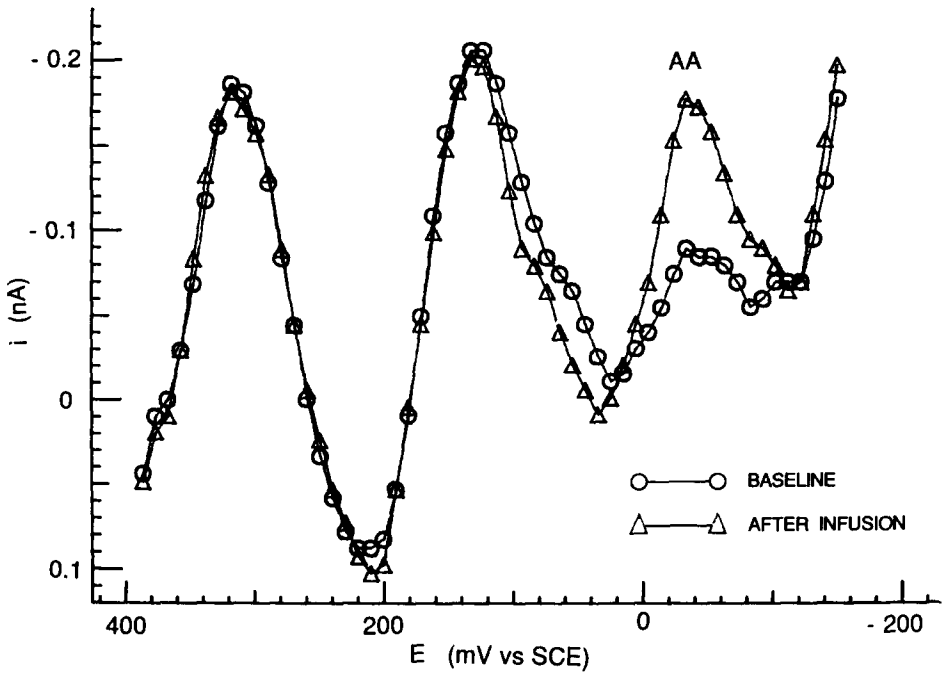

FIG. 3. Voltammetric scans obtained with an electrochemically modified carbon-fiber electrode positioned in the neostriatum between 0.5–1.0 mm from the infusion site. A potential was applied in 10-mV steps from −200 to +400 mV vs. a saturated calomel reference electrode (SCE). The scan rate was set at 100 mV/sec. Note the distinct rise in the AA wave (peak at −20 mV vs. SCE) for the scan obtained during the infusion (in this case at 10 min after infusion onset). AA levels increased linearly throughout the course of the infusion. The catechol wave that occurs at approximately +120 mV vs. SCE represents DOPAC.

located in the anterior neostriatum (2.5–3.0 mm lateral to the midline, 1.0 mm anterior to bregma, and 4.0–5.0 ventral to dura) or in the overlying cerebral cortex (2.0 mm ventral to dura) according to Paxinos and Watson (19). Voltammetric electrodes, lowered to the same neostriatal coordinates, detected a sharp rise in DOPAC, further confirming a neostriatal placement.

Behavior

Figure 1 illustrates the mean interval score for individual behaviors produced by 1.0 mg/kg d-amphetamine or 1.0 mg/kg d-amphetamine plus 0.025 mg/kg haloperidol during intrastriatal infusions of either saline or AA (n = 7 in each group). Note the trend toward a lower behavioral response in the AA-infusion groups compared to the saline controls. This trend occurs with amphetamine alone or with the amphetamine-haloperidol combination. A repeated measures multivariate analysis revealed significant group and time effects ($p<0.01$) and a significant group-by-time interaction ($p<0.02$). Post hoc analysis indicated that compared to saline infusions AA significantly reduced the mean interval score for head bobbing (1.64 ± 0.39 vs. 0.85 ± 0.31, $p<0.05$) and sniffing (3.78 ± 0.57 vs. 2.71 ± 0.52, $p<0.05$) between 30–60 min after amphetamine alone. The mean interval rearing score was reduced significantly during the first 30 min of the amphetamine response in these same animals (0.85 ± 0.34 vs. 0.21 ± 0.25, $p<0.05$). In rats pretreated with amphetamine and haloperidol, post hoc comparisons of saline and AA infusions revealed a significant reduction in the mean interval score for forepaw shuffling (0.42 ± 0.19 vs. 0.14 ± 0.08, $p<0.05$) and locomotion (0.49 ± 0.18 vs. 0.28 ± 0.18, $p<0.05$) between 30–60 min of amphetamine. Other amphetamine-induced behaviors following haloperidol were low in both infusion groups, making it difficult to demonstrate a further effect of AA on these behaviors. The time course of AMPH-induced behavioral changes in each group of animals is shown in Fig. 2.

Animals that received intracortical AA infusions (n = 4) were pretreated with amphetamine alone. Their amphetamine-induced behavioral response was comparable to that of rats receiving intrastriatal saline (see SAL/AMPH group in Fig. 1). None of the behavioral responses to amphetamine differed significantly between these groups at any observation interval.

Voltammetry

Voltammetric recordings (n = 3) indicated that neostriatal AA levels rose linearly during the course of an intrastriatal AA infusion, reaching a final mean maximum value of 837.3 ± 234 μM from a mean preinfusion baseline of 265.7 ± 94 μM. Figure 3 displays representative voltammetric scans from the neostriatum of one animal obtained during the baseline period and at 10 min after the onset of the AA infusion. Note the distinct rise in the AA wave.

DISCUSSION

Our results implicate the neostriatum as an important site of action of AA in modulating the behavioral response to both amphetamine and haloperidol. Intrastriatal infusions of AA reliably decreased amphetamine-induced rearing, sniffing, and head bobbing at various times during the course of the amphetamine

behavioral response. These effects were not mimicked by AA infusions into the overlying cerebral cortex, arguing against a nonspecific action of AA in the forebrain or a direct action on cortical neurons. Although most endogenous AA in the neostriatum arises from corticostriatal terminals (2,9), our results suggest that AA acts in the neostriatum to exert its behavioral effects. That the neostriatum is a likely participant in the amphetamine behavioral response is consistent with evidence that neostriatal neurons are very responsive to amphetamine (1.0 mg/kg) and that these neuronal responses often are linked to locomotion, sniffing, and head bobbing (11,30). Of course, AA also may act in other sites, including the nucleus accumbens, which is known to play a role in amphetamine-induced behaviors (21,25). Interestingly, however, the level of extracellular AA in the nucleus accumbens does not correlate closely with the behavioral changes produced by amphetamine (15).

Our results also implicate the neostriatum in the ability of AA to potentiate the antiamphetamine effects of haloperidol, a dopamine antagonist. AA has been reported to regulate the binding of haloperidol and other dopamine antagonists (4,10), suggesting that, like haloperidol, AA exerts its effects at the level of the dopamine receptor. Other mechanisms, however, cannot be ruled out, including an interaction with neostriatal glutamate (2, 6, 9, 18).

Although our voltammetric measurements were obtained from anesthetized animals, these data serve as useful estimates of extracellular AA during AA infusions in behaving animals. In fact, our reported basal level of AA is comparable to that found in freely moving rats (7,23), suggesting that the basic mechanisms controlling extracellular AA are similar in both cases. Using our voltammetric recordings as a guide, we estimate that extracellular AA levels in the neostriatum remained within reasonable physiological limits during the course of the infusion. The maximum increase of approximately three-times baseline occurred at infusion offset, which coincided with the end of the behavioral observation period. Although undoubtedly higher at the infusion site itself, our observed increases in AA during most of the infusion period approximate the changes produced by amphetamine (2, 7, 26, 32) or that occur during normal circadian rhythms (3,18). Moreover, we observed a significant decline in amphetamine-induced rearing relatively early in the infusion procedure when the level of extracellular AA was well below its final infusion value. This finding suggests that even moderate elevations in neostriatal AA may produce behavioral change. Thus, future experiments may begin to characterize the minimum AA elevation necessary to alter behavior, but it seems evident from our results that the large changes in endogenous AA reported to occur in vivo are capable of exerting dramatic behavioral effects.

It is conceivable that AA infused into the neostriatum eventually reaches the ventricular system where AA could diffuse into cerebrospinal fluid and act at some remote site. Although this possibility cannot be ruled out, it is an unlikely explanation of our results in that AA levels in cerebrospinal fluid are extremely high, almost three times the level in the neostriatum (27). Thus, a large amount of AA would be required to reach the ventricle in order to produce even a relatively small change in the baseline level. Yet our voltammetric measurements indicate that even within the neostriatum AA levels remain below those reported for cerebrospinal fluid for most of the infusion period. Furthermore, all of our behavioral effects were observed during the first hour of the infusion and some were evident within the first 30 min, arguing against a slow diffusion to a distant site. It also is noteworthy that AA did not exert a uniform suppression of all behaviors, ruling out a nonspecific inhibitory effect on neostriatal function. Thus, to the extent that our infusions represent a physiologically relevant fluctuation in extracellular AA, our results suggest an important modulatory role for endogenous neostriatal AA in the amphetamine-induced behavioral response.

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