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Modulatory Effects of Ascorbate, Alone or With Haloperidol, on a Lever-Release Conditioned Avoidance Response Task

JOSHUA M. GULLEY AND GEORGE V. REBEC

Program in Neural Science, Department of Psychology, Indiana University, Bloomington, IN 47405

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GULLY, J. M. AND G. V. REBEC. *Modulatory effects of ascorbate, alone or with haloperidol, on a lever-release conditioned avoidance response task.* PHARMACOL BIOCHEM BEHAV **63**(1) 125–129, 1999.—Pretreatment with ascorbate, a modulator of dopamine transmission in the striatum, enhances the ability of haloperidol, a dopamine antagonist, to induce catalepsy and block the motor-activating effects of amphetamine. The present study extended this line of work to a lever-release version of the conditioned avoidance response (CAR) task, which is highly sensitive to changes in striatal dopamine. Adult male rats were trained to avoid footshock by releasing a lever within 500 ms of tone onset. Ascorbate (100 and 1000 mg/kg, IP) or vehicle was tested either alone or in conjunction with haloperidol (0.01 and 0.05 mg/kg, SC). Compared to vehicle pretreatment, 1000 mg/kg ascorbate alone or in combination with haloperidol impaired CAR performance by increasing avoidance latency. Latency to escape footshock was not impaired, ruling out a generalized motor deficit. In contrast, 100 mg/kg ascorbate alone or in combination with haloperidol had no consistent efects on CAR performance, even at a haloperidol dose (0.005 mg/kg, SC) known to potentiate dopamine transmission by preferentially blocking autoreceptors. Collectively, these results support an antidopaminergic action of ascorbate on striatal function, but suggest that this effect requires relatively high systemic doses. © 1999 Elsevier Science Inc.

Ascorbate Conditioned avoidance response Dopamine Glutamate Haloperidol Striatum

ASCORBATE, the endogenous form of vitamin C, is found in high concentration in the basal ganglia, especially in the striatum, where it appears to function as an extracellular neuromodulator [for review, see (24)]. Pretreatment with 1000 mg/kg ascorbate, for example, elevates striatal ascorbate in conjunction with an increase in striatal neuronal activity (5). Neuronal excitations also have been reported in response to ascorbate iontophoresis, confirming a direct action of ascorbate in the striatum (7,11,19). Behaviorally, 1000 mg/kg ascorbate has been reported to potentiate both the cataleptic and antiamphetamine effects of haloperidol (23), a dopamine antagonist, and similar results have been obtained with direct intrastriatal applications of ascorbate (34). It appears, therefore, that an elevation in the level of extracellular ascorbate opposes dopamine function in the striatum.

This conclusion, however, contrasts with evidence that administration of relatively low systemic doses of ascorbate (50–200 mg/kg) enhances the motor-activating effects of amphetamine (28). In addition, pretreatment with 100, but not 500, mg/kg ascorbate has been shown to potentiate both amphetamine-induced conditioned place preference and amphetamine-induced striatal dopamine release (20). Although a likely explanation for these behavioral findings is that low and high doses of ascorbate exert opposing effects on striatal dopamine transmission, it would be useful to test this hypothesis on a task that does not require amphetamine administration to assess dopaminergic mechanisms. In fact, because of its ability to alter both ascorbate and dopamine release in the striatum [for review, see (12)], amphetamine is likely to complicate any interpretation of how fluctuations in striatal ascorbate influence dopamine-mediated behavior.

To address this issue, we trained rats on a lever-release version of the conditioned avoidance response (CAR) task. Even relatively modest alterations in striatal dopamine transmission, such as a 15% dopamine depletion, can impair lever-release CAR performance (26). Furthermore, whereas task performance is impaired by dopamine antagonists (30,32), drugs that potentiate dopamine transmission have the oppo-

Requests for reprints should be addressed to George V. Rebec, Program in Neural Science, Department of Psychology, Indiana University, Bloomington, IN 47405.

site effect (29). Thus, the lever-release CAR task is well suited to assess the potential of high- and low-dose ascorbate pretreatment to attenuate or facilitate, respectively, dopamine-dependent motor functions. Rats were tested with either ascorbate alone or in conjunction with threshold doses of haloperidol.

METHOD

Subjects

A total of 19 male, Sprague–Dawley rats, weighing between 300–430 g at the start of training, served as subjects. They were bred in our animal colony from source rats supplied by Harlan Industries (Indianapolis, IN). All animals were housed individually under standard laboratory conditions, including a 12-h light/dark cycle, with lights on at 0700 h. Animal care and experimentation followed National Institutes of Health guidelines, and all protocols were approved by the local Institutional Animal Care and Use Committee.

Apparatus and Training Procedure

The apparatus and training procedure for the lever-release CAR task has been described in detail elsewhere (33). Briefly, the CAR apparatus is housed inside a sound-attenuating cubicle and consists of a modified operant chamber having a rectangular, 18-bar grid floor connected to a neon grid scrambler and shock generator. This arrangement allows for manual or computer-controlled (IBM-XT compatible) delivery of footshock (0.6-mA, 60-Hz square wave; maximum 1-s duration). The grid floor is surrounded by Plexiglas and stainless steel walls with a wall-mounted lever located 10 cm above the floor. The computer also provides information on the status of the lever (idle or depressed) and quantifies the presence or absence of a lever-release response (percent) and its latency (ms).

Animals were trained by successive approximation to approach, depress, and hold the lever down with their forepaw. After reaching this level of performance, animals received an auditory signal (85-dB buzz) followed by footshock. To avoid the shock, animals were required to release the lever within 500 ms of signal onset (avoidance response). Failure to do so results in a footshock that continued until the lever was released (escape response) or until 500 ms had elapsed. The intertrial interval (ITI) varied randomly between 9–12 s. If animals did not return to hold the lever down with 3–5 s of the end of the ITI, which occurred during the early days of training, a manually delivered footshock induced the animal to return to the lever. Only one training session was administered per day, with the number of trials/session increasing gradually up to as many as 120. A criterion of 80% avoidance responses for five consecutive sessions was required before animals were advanced to drug testing.

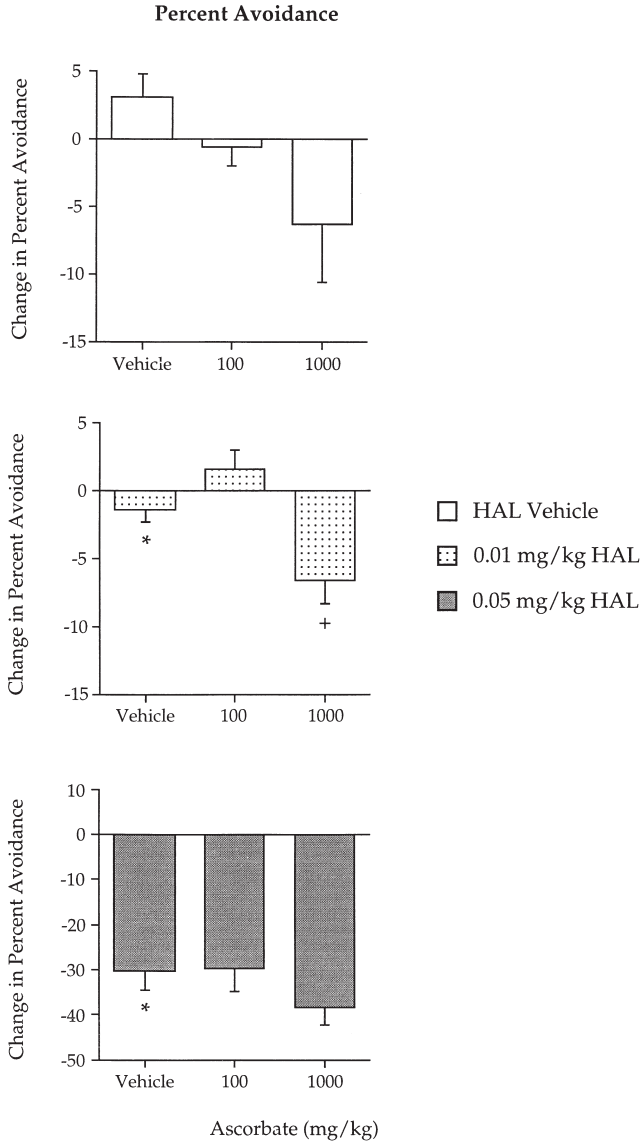

FIG. 1. Change in percent avoidance responses in rats treated with 0 (vehicle), 100, or 1000 mg/kg ascorbate (AA). In Figs. 1 and 2, AA was administered in conjunction with haloperidol (HAL) or vehicle, as indicated in the legend. Data are expressed as mean difference between post- and predrug performance. Brackets represent the standard error of the mean. $^{*}p < 0.05$ compared to AA and HAL vehicles; $^{+}p < 0.05$ compared to AA vehicle and 0.01 mg/kg HAL.

Test Sessions

Each test session consisted of 70 predrug trials, the first 10 of which served as practice trials and were not included for analysis, and 60 postdrug trials. After predrug testing, 13 rats received sodium L-ascorbate (0, 100, or 1000 mg/kg, IP), followed 30 min later by haloperidol (0, 0.01, or 0.05 mg/kg, SC); the 0 dose indicates the appropriate vehicle. The nine different drug combinations were administered in successive test sessions in pseudorandom order such that each combination appeared first in the series at least once. In another test of the potential for low-dose ascorbate to facilitate task performance, a separate group of six animals was given sodium L-ascorbate (0 or 100 mg/kg, IP) followed 30 min later by 0.005 mg/kg haloperidol (SC) or its saline vehicle in a counterbalanced order. Approximately 30 min after the last drug injection, animals were returned to the CAR apparatus for postdrug testing. At least 3 days elapsed between test sessions to minimize cumulative drug effects.

Data Analysis

Drug effects were assessed by comparing pre- and postdrug performance as measured by percent avoidance responses and by the latency (ms) to avoid or escape footshock. An overall, one-way, repeated-measures analysis of variance

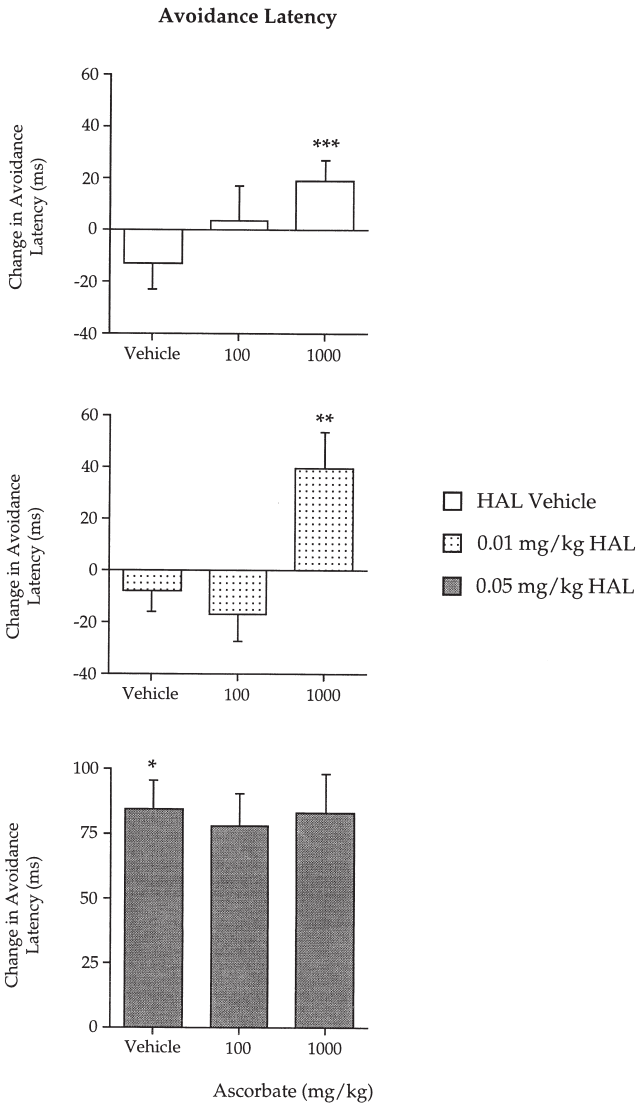

FIG. 2. Change in avoidance latency in rats treated with AA and HAL. Data are presented as in Fig. 1. *** $p < 0.01$ compared to AA and HAL vechiles; ** $p < 0.02$ compared to AA vehicle and 0.01 mg/kg HAL; * $p < 0.05$ compared to AA and HAL vehicles.

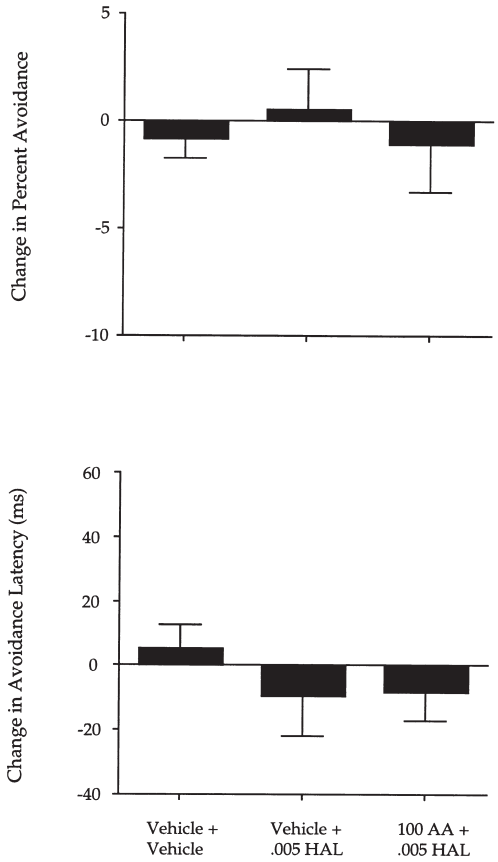

FIG. 3. Change in percent avoidance responses (top) and avoidance latency (bottom) in rats treated with the combination of HAL and AA vehicles, vehicle and 0.005 mg/kg HAL, or 100 mg/kg AA and 0.005 mg/kg HAL. Data are expressed as mean difference between post- and predrug performance, with brackets representing the standard error of the mean.

(ANOVA) was performed on the mean difference scores (post- minus predrug) followed by post hoc comparisons based on repeated-measures *t*-tests. Startle responses (i.e., avoidances <100 ms) were not included for analysis.

RESULTS

The mean percent avoidance for all animals in all predrug test trials was 92.6 ± 0.42%, while the mean avoidance and escape latencies were 254 ± 6.23 and 53 ± 4.15 ms, respectively. For the 13 rats tested with high and low doses of ascorbate and haloperidol, statistical analysis indicated a significant overall effect of drug treatment on both percent avoidance, $F(8, 96) = 27.10$, $p < 0.001$, and avoidance latency, $F(8, 96) = 15.68$, $p < 0.001$. Such treatment failed to alter the escape response, $F(8, 96) = 1.94$, $p > 0.05$. Note in this regard that the mean escape latency for the high dose of ascorbate plus haloperidol vehicle (25 ± 16.0 ms) is comparable to, if not lower than, the value for treatment with 0.05 mg/kg haloperidol and ascorbate vehicle (26 ± 9.92 ms), arguing against even a trend toward a generalized behavioral debilitation with ascorbate. Post hoc analyses of individual treatments are described in the following sections and summarized in Figs. 1 and 2.

Effects of Haloperidol Alone

Consistent with previous results (30), haloperidol, in combination with ascorbate vehicle, impaired CAR performance. This was especially evident at 0.05 mg/kg, in which both percent avoidance and avoidance latency were dramatically altered ($p < 0.001$ in each case). At 0.01 mg/kg, haloperidol caused a relatively modest decline in percent avoidance ($p < 0.05$) without altering avoidance latency ($p > 0.05$).

Effects of Ascorbate Alone

Unlike 100 mg/kg ascorbate, which had no consistent effect on either percent avoidance or avoidance latency when given with haloperidol vehicle, 1000 mg/kg ascorbate clearly impaired CAR performance. This dose significantly increased avoidance latency ($p < 0.01$) and also caused a decline, albeit not statistically significant ($p < 0.09$), in percent avoidance.

Ascorbate–Haloperidol Combinations

Whereas 100 mg/kg ascorbate failed to alter the response to either dose of haloperidol, the high dose of ascorbate enhanced the effect of 0.01 mg/kg haloperidol. Compared to this dose of haloperidol alone, this ascorbate–haloperidol combination significantly decreased percent avoidance ($p < 0.02$) and avoidance latency ($p < 0.02$). Pretreatment with 1000 mg/kg ascorbate, however, failed to potentiate the effects of 0.05 mg/kg haloperidol.

Figure 3 presents data from six rats tested to evaluate the hypothesis that an autoreceptor-selective dose of haloperidol, which enhances dopamine transmission, interacts with low-dose ascorbate to enhance CAR performance. In contrast to this hypothesis, treatment with 100 mg/kg ascorbate and 0.005 mg/kg haloperidol or either drug's saline vehicle did not significantly alter either percent avoidance, $F(2, 10) = 0.34$, $p > 0.05$, avoidance latency, $F(2, 10) = 0.69$, $p > 0.05$, or escape latency, $F(2, 10) = 0.53$, $p > 0.05$.

DISCUSSION

A dose of ascorbate previously shown to potentiate the action of haloperidol in blocking the behavioral response to amphetamine also attenuated performance on the lever-release CAR task. This effect was apparent either with 1000 mg/kg ascorbate administered alone or in combination with 0.01 mg/kg haloperidol. By itself, ascorbate increased CAR latency without altering the latency to escape shock, arguing against a generalized motor impairment (see also below). When combined with a threshold dose of haloperidol, ascorbate significantly altered both avoidance latency and percent avoidance compared to either substance alone. At 0.05 mg/kg haloperidol, CAR performance was sufficiently impaired such that ascorbate supplementation had no further effect. Because the lever-release CAR task is exquisitely sensitive to manipulations of striatal dopamine transmission (26), as our haloperidol data confirm (30,31), our results lend further support to the view that ascorbate antagonizes dopamine function. This conclusion also is consistent with evidence of an ascorbate-induced impairment on a shuttle-box version of the CAR paradigm (3).

Our results, however, do not support an opposing action of ascorbate at low doses. Pretreatment with 100 mg/kg ascorbate, for example, failed to enhance lever-release CAR performance. Although a relatively high predrug rate of avoidance in our subject sample (~93%) may have obscured any further improvement on this measure, avoidance latency, which can decline by as much as 50 ms in response to dopamine agonists (29), was unaffected by 100 mg/kg ascorbate. This dose also failed to alter the effect of either 0.01 or 0.05 mg/kg haloperidol. In fact, even when dopamine transmission was enhanced by 0.005 mg/kg haloperidol, which selectively blocks inhibitory autoreceptors (14,17,21), low-dose ascorbate failed to enhance CAR performance. Although this dose of haloperidol by itself did not significantly alter either percent avoidance or escape latency, both these variables moved in the direction of enhanced CAR performance, which is characteristic of dopamine agonists (29). Adding low-dose ascorbate failed to have a further effect. Taken together, these findings indicate that if low-dose ascorbate has a direct potentiating action on dopamine transmission, it was not evident at the dose used in our CAR task.

The mechanism by which high-dose ascorbate pretreatment antagonizes dopamine-mediated behavioral responses remains unclear, though several possibilities exist. Although we cannot rule out ascorbate-induced behavioral toxicity, this possibility seems unlikely in view of evidence that 1000 mg/kg ascorbate alone fails to block either spontaneous or amphetamine-induced behavioral activity (23). Moreover, the ability of ascorbate to potentiate the motor-suppressing effects of haloperidol occur with intrastriatal administration of ascorbate, ruling out complications associated with nonselective peripheral effects (34). Perhaps the most direct explanation for our results with high-dose ascorbate is a blockade of dopamine receptors. Certainly, this interpretation is consistent with our haloperidol data, and indeed, most in vitro evidence on this topic suggests that ascorbate inhibits dopamine binding in striatal tissue [(9,10)], but see also (1)]. The inhibition, however, is complex, and appears to involve an allosteric modulation of the receptor site (27). It also is possible that ascorbate may influence dopamine transmission indirectly by acting on other neurochemical systems that, in turn, modulate the dopamine system. Although some evidence indicates that ascorbate may interact with opioid peptides (4) and perhaps sigma receptors (18), the most likely interaction appears to involve glutamate-containing neurons. High doses of ascorbate, for example, have been proposed to block *N*-methyl-D-aspartate (NMDA) receptors in vitro (13), although it is unclear if a single systemic ascorbate injection can elevate striatal ascorbate to the millimolar level required for NMDA antagonism. In fact, ample evidence suggests that glutamate and ascorbate act cooperatively to enhance glutamate transmission. We have found that under behaviorally relevant conditions coiontophoresis of ascorbate potentiates the activation of striatal neurons induced by glutamate alone (11,19). Consistent with this result, increases in extracellular ascorbate have been proposed to modulate glutamate uptake (2,8,15). According to this model, glutamate uptake depends on the outward movement of ascorbate via a heteroexchange process at the glutamate transporter. Thus, an increase in extracellular ascorbate may potentiate the synaptic action of glutamate by interfering with its normal uptake (6,22,24). To the extent that glutamate opposes dopaminergic function in the striatum, as some behavioral evidence suggests (16,25), it is possible that an ascorbate-induced increase in striatal glutamate transmission underlies, at least in part, the ability of ascorbate to impair lever-release CAR performance.

In summary, our results support an antagonistic role for high-dose ascorbate in a test of dopamine-mediated behavior that does not require amphetamine administration for its assessment. Follow-up work should focus on a possible interaction of ascorbate with dopamine receptors and the striatal glutamate system.

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