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Short communication

Effects of long-term haloperidol treatment on glutamate-evoked ascorbate release in rat striatum

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Abstract

Repeated haloperidol injections increase extracellular striatal ascorbate. Because ascorbate release depends on glutamate uptake, we assessed this mechanism in the haloperidol effect. Linear staircase voltammetry was combined with intrastriatal infusions of L- or D-glutamate or saline in behaving rats after 7 or 21 days of haloperidol (0.5 mg/kg, s.c.). Control animals, receiving either vehicle or no treatment, responded to L-, but not D-glutamate or saline infusion with a 50% increase in ascorbate. In contrast, glutamate-evoked ascorbate release disappeared after 7 but reappeared after 21 days of haloperidol. Thus, increased striatal ascorbate release following chronic haloperidol cannot be explained by an enhanced response to glutamate.

Keywords: Ascorbate; Glutamate; Haloperidol; Striatum; Voltammetry

1. Introduction

Ascorbate, the deprotonated form of vitamin C in the mammalian forebrain (Stamford et al., 1984), is an antioxidant that may function as a neuromodulator (Rebec and Pierce, 1994). Striatal neurons, for example, are highly responsive to fluctuations in extracellular ascorbate (Gardiner et al., 1985), and systemic or intrastriatal ascorbate has been shown to enhance the behavioral effects of haloperidol (Rebec et al., 1985; White et al., 1990), a dopamine receptor antagonist and classical neuroleptic (Kane, 1999). Although this interaction with haloperidol implies a role for dopamine, ascorbate release appears to be linked instead to the uptake of glutamate (O'Neill, 1995), an excitatory amino acid and potential neurotoxin (Götz et al., 1994). Consistent with the stereoselective nature of glutamate transport, application of L-, but not D-glutamate elevates extracellular ascorbate, and this effect is blocked by drugs that prevent glutamate uptake (Grunewald and Fillenz, 1984). These and other lines of evidence (Cammack et al., 1991) suggest that glutamate uptake promotes ascorbate release by heteroexchange.

Multiple injections of haloperidol may enhance this process. Twenty-one days of treatment, for example, elevates extracellular ascorbate in striatum by 200% (Pierce et al., 1994). In fact, such treatment causes a 300% increase in amphetamine-induced striatal ascorbate release, which depends on an intact glutamatergic input from cerebral cortex (Basse-Tomusk and Rebec, 1991). It is conceivable, therefore, that chronic haloperidol facilitates glutamate-evoked ascorbate release. To test this hypothesis, we used linear staircase voltammetry to monitor glutamate-evoked changes in striatal ascorbate in freely behaving rats exposed to varying periods of haloperidol treatment.

2. Materials and methods

Male Sprague–Dawley rats (300–450 g) were anesthetized with either chloropent (0.27 ml/100 g, i.p.) or an anesthetic cocktail (74 mg/kg ketamine hydrochloride, 3.7 mg/kg xylazine hydrochloride, and 0.7 mg/kg acepromazine, i.m.). As previously described (Rebec et al., 1993), a skull hub, designed to mate with a micromanipulator, was cemented in place around a hole overlying the striatum. After a 4-day recovery, rats received single daily injections of 0.5 mg/kg haloperidol or vehicle for either 1 or 3 weeks. A separate group received no injections. Our experimental protocols followed National Institutes of Health

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guidelines (NIH Publication 865-23) and received approval from the Institutional Animal Care and Use Committee.

For voltammetric recording, a 30-μm diameter carbon fiber (ATCO) was inserted into one barrel of a three-barrel glass capillary. Melted bismuth provided electrical contact between the carbon fiber and a stainless steel wire. A 27-gauge needle was inserted into a second barrel to allow drug infusion next to the carbon fiber; the third barrel remained empty. The carbon fiber was electrochemically treated (70 Hz, 3 V triangle wave for 20 s, +1.5 VDC for 10 s, −0.9 VDC for 3 s, +1.5 VDC for 8 s) in citrate–phosphate buffer (180 mM dibasic sodium phosphate, 9 mM citrate, 140 mM sodium chloride; pH 7.4) to separate the oxidation potential of ascorbate from 3,4-dihydroxyphenylacetic acid (DOPAC), a dopamine metabolite and potential interferant (Gonon et al., 1981). Each electrode was tested in vitro with 100 μM ascorbate and 20 μM DOPAC in citrate–phosphate buffer.

A micromanipulator containing the electrochemically modified electrode was placed in the head-mounted hub 1 day after the last haloperidol or control treatment. The micromanipulator and a skull-implanted stainless steel screw were connected via electrically shielded low-noise cable to a mercury swivel. The infusion needle was attached to a pump via flexible polyethylene tubing. A computer-controlled potentiostat of conventional three-electrode design (Ewing et al., 1982) allowed waveform generation and current sampling via the mercury swivel. The carbon fiber served as the working electrode, while the stainless steel screw served as both reference and auxiliary electrode. Voltage was applied to the auxiliary electrode in 6 mV steps from −200 to +600 mV and back at a scan rate of 27 mV/s. One complete scan lasted 1 min.

The animal was placed in an open-field arena (1.3 m^2) inside a sound-attenuating cubicle. After a period of habituation, the recording electrode was lowered into the striatum (~4.5 mm ventral to the brain surface). When the signal stabilized, we established a 10-min baseline followed by infusion of either D- or L-glutamate (4.0 μg/μl) or physiological saline at 5.0 μl/h for 10 min. Pilot studies, adapted from previous glutamate infusions (Pierce and Rebec, 1993), revealed that this glutamate concentration reliably elicited ascorbate release. Post-infusion voltammetric recording continued for up to 20 min. After completion of recording, animals were overdosed with chloropent, and in some cases an electrolytic lesion was made to mark the recording site for subsequent histological verification. After transcardial perfusion with 10% formosaline, brains were removed and fixed for histological analysis. All histologies confirmed an electrode placement in dorsal striatum; placements ranged between 0.8–1.2 mm anterior and 2.3–2.5 mm lateral to bregma (Paxinos and Watson, 1986).

Voltammetric data, based on the amplitude of the ascorbate peak, were expressed as percent change (mean ± S.E.M.) from the baseline period, which was defined as 100%. Data analysis was confined to the post-infusion period because all infusions caused a >50% decline in the ascorbate signal that disappeared immediately after infusion offset. The infusion-induced decline was comparable in all animals, suggesting a dilution of ascorbate at the carbon fiber surface owing to its close proximity to the infusion barrel. Post-infusion scores were calculated for individual animals and mean group values were obtained. These values were compared by a two-way analysis of variance (ANOVA); pairwise differences were determined

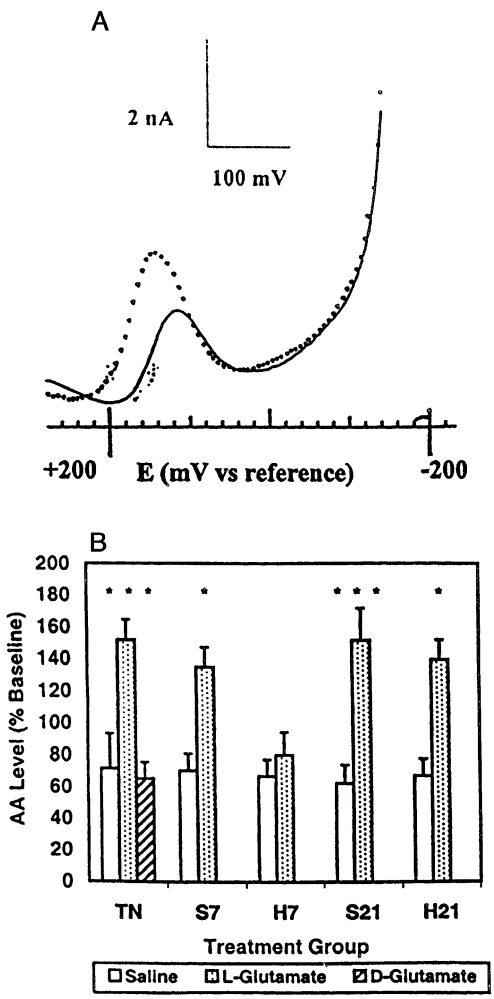

Fig. 1. (A) Representative voltammograms obtained during an experiment. The ascorbate peak occurs between +125 and +150 mV. Solid line = baseline level. Dotted line = oxidative current following 10-min infusion of L-glutamate (4 μg/μl, 5 μl/h). Note the increase in ascorbate following L-glutamate infusion. (B) Effects of chronic drug treatment on glutamate-induced ascorbate release. TN = treatment-naive, Sx = x once daily saline injections (0.9%, s.c.), Hx = x once daily haloperidol injections (0.5 mg/kg, s.c.). Data are expressed as mean (±S.E.M.) percent baseline ascorbate concentration. Note that group H7 showed no ascorbate response to L-glutamate infusion. $^{*}P < 0.05$ versus treatment-naive group receiving saline infusion. $^{***}P < 0.01$.

Table 1
Mean (±S.E.M.) percent change in striatal ascorbate in treatment-naive rats
n = number of rats.

Infused Drug	n	Percent baseline	
Saline	3	71.33	(±30.5)
L-glutamate	4	151.55	(±22.19)*
D-glutamate	4	64.88	(±17.77)

* $P < 0.01$ relative to saline control.

with the Tukey–Kramer post-hoc test. Type of surgical anesthesia had no effect on subsequent ascorbate measurements, and this variable was not considered for further analysis.

3. Results

All animals ($n = 35$) showed rapid habituation to the behavioral chamber (< 30 min). The rats typically rested quietly throughout the experimental session. Casual observation indicated no overt behavioral differences among the three infusion groups in any of the treatment conditions.

During baseline recording, all rats showed an oxidation peak between +125 and +150 mV vs. reference (Fig. 1A), consistent with previous reports of ascorbate oxidation in vivo (White et al., 1990; Gonon et al., 1981; Rebec and Wang, 2001). Both our in vitro tests and direct infusions of ascorbate in untreated pilot animals confirmed this peak as ascorbate.

In treatment-naive rats, the ascorbate signal increased significantly above baseline after infusion of L-glutamate but not after D-glutamate or saline (Table 1), confirming the stereospecifity of ascorbate–glutamate heteroexchange (Grunewald and Fillenz, 1984). The L-glutamate effect was apparent immediately after infusion offset and persisted for up to 20 min. Comparable results were obtained in all treatment controls as well as in rats treated with haloperidol for 21 days. As shown in Fig. 1B, however, this effect disappeared after 7 days of haloperidol.

4. Discussion

Although our results confirm a role for glutamate in striatal ascorbate release, they argue against a role for this mechanism in the enhanced release of ascorbate after chronic haloperidol. Thus, whereas 21 days of haloperidol increased both basal and amphetamine-induced ascorbate release (Pierce et al., 1994), such treatment had no effect on the ascorbate response to glutamate. In fact, we found that 7 days of haloperidol attenuated rather than enhanced glutamate-evoked ascorbate release.

Our results also are interesting in light of observations that chronic exposure to classical neuroleptics enhances glutamate transmission. Repeated haloperidol, for example, increases glutamate release in response to stimulation of glutamatergic corticostriatal neurons (See and Lynch, 1995). One possible substrate for this effect is a decline in glutamate uptake. In fact, long-term haloperidol administration reduces gene expression for the GLT-1 glutamate transporter in striatum (Schneider et al., 1998). Thus, treatment with classical neuroleptics may trigger a decline in glutamate uptake. If such uptake is related to ascorbate release, it is interesting to note that 7 days of haloperidol abolished the ascorbate response to glutamate. That this response reappeared after two more weeks of treatment suggests the induction of additional compensatory mechanisms, including a possible up-regulation of other glutamate transporters. Further evaluation of glutamate-evoked ascorbate release and the relative contribution of individual glutamate transporters to this effect is required. In addition, the recent identification of an ascorbate transporter (Tsukaguchi et al., 1999) suggests that factors other than glutamate uptake may play a role in regulating the level of extracellular striatal ascorbate.

Our observation of a relatively prolonged glutamate-induced increase in ascorbate release (> 10 min) contrasts with the short-lived response (1–3 min) reported previously (Pierce and Rebec, 1993). This discrepancy most likely reflects a methodological difference. Pierce and Rebec (1993) placed an infusion cannula alongside the recording electrode, separating the infusion and recording sites by approximately 500 μm. In the present case, we applied glutamate immediately adjacent to the recording electrode, thereby minimizing variables related to diffusion distance.

Our results show that repeated haloperidol administration does not potentiate the glutamate–ascorbate heteroexchange system. It seems unlikely, therefore, that glutamate plays a direct role in the ability of this neuroleptic to enhance striatal ascorbate release with long-term treatment.

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