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Ascorbic Acid Reduces Accumulation of [^{3}H]Spiperone in Mouse Striatum *in Vivo* (42576)

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Abstract. [^{3}H]Spiperone was administered (20 μCi/kg, 0.0003 mg/kg, sc) to mice. In agreement with other published reports, 2 hr later the accumulation of tritium was three to four times greater in the corpus striatum than in the cerebellum. Ascorbic acid (100, 1000, 2000 mg/kg, ip, 30 min) reduced the 2-hr accumulation in the corpus striatum 16, 42, and 63%, respectively, with only the highest dose producing any significant (18%) reduction in the cerebellum. The effect was still evident in striatum 18 hr after a single dose of 1000 mg/kg. Striatal minces taken from mice treated 1 or 2 hr earlier with ascorbic acid (2000 mg/kg, ip) showed no reduction in [^{3}H]spiperone binding. However, preincubation of striatal minces for 2 hr with ascorbic acid (10^{-3} *M*) produced an 82% reduction in specific binding while not having any effect on nonspecific binding. While it cannot be certain that the reduction of striatal [^{3}H]spiperone concentrations after ascorbic acid *in vivo* was not a result of some nonspecific alteration in the pharmacokinetics of [^{3}H]spiperone, the *in vitro* observation strongly suggests that it resulted from an alteration of binding characteristics at the receptor level.

It has been common practice to include ascorbic acid as an antioxidant in dopamine receptor binding studies. However, there are reports showing that the agent can interfere with ligand binding (1, 2). This interaction may have more than a technological significance in that the vitamin has now been shown to alter some dopamine-mediated behavioral response *in vivo*. Thus, Rebec *et al.* (3) reported that ascorbic acid potentiated the antiamphetamine and cataleptogenic actions of haloperidol in rats. The catalepsy potentiation was corroborated by the present author, not only in rats but also in squirrel monkeys (4). Tolbert *et al.* (5) also reported antiamphetamine effects of ascorbic acid in rodents and Trulson *et al.* (6) reported an antagonism of what was believed to be a dopaminergically mediated lysergic acid diethylamide-induced response in cats. These various reports strongly suggest that ascorbic acid has an antidopamine action at the receptor level. The ability to garner biochemical evidence for such an action *in vivo* could have significant relevance to the practice of neurology and psychiatry. The present report may be such evidence in that it is consistent with the suggestion that the vitamin is capable of altering ligand binding characteristics of the dopamine receptor under *in vivo* conditions.

Materials and Methods. *Experiments in vivo.* Murrin (7) showed that when [^{3}H]-spiperone was administered to rats ip, its distribution in brain paralleled that of endogenous dopamine. The investigator further showed that the accumulated [^{3}H]spiperone was displaced from striatum, but not from cerebellum, by agonists or antagonists of the dopamine receptor. This technique was modified slightly for the studies reported here. Thus, male mice (25–35 g), Tex:ICR, Timco, Houston, Texas, were given injections (sc) of [^{3}H]spiperone (25.7 Ci/mmole, NEN dissolved in 18% ethanol). The dose was 20 μCi/kg (0.0003 mg/kg) administered in a volume of 100 μl/10 g body wt. After 2 hr the mice were killed, and striata and a piece of cerebellum, equivalent in weight to the striata, were removed and homogenized in 10 ml liquid scintillation cocktail (Beckman Ready-Solv). The fine suspension was transferred to counting vials for scintillation counting. Radioactivity, expressed as dpm/mg tissue, was considered as unchanged [^{3}H]spiperone based on the report of Murrin (7) that 97% of the isotope in striatum could be accounted for as unchanged drug. L-Ascorbic acid (A.C.S., Fisher Scientific Co.), 100–2000 mg/kg in water, was injected ip (100 μl/10 g body wt) 30 min or 18 hr before [^{3}H]spiperone.

Experiments in vitro. Most dopamine receptor binding assays have been carried out using membrane fractions from disrupted cells. The use of such preparations has raised serious questions as to the relevance to the physiological state of tissues (8). For this reason

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TABLE I. EFFECTS OF APOMORPHINE AND HALOPERIDOL ON [^{3}H]SPIPERONE ACCUMULATION IN MOUSE BRAIN

	dpm/mg wet wt		
	Striatum	Cerebellum	Difference
Control	35.08 ± 2.66	10.91 ± 0.66	24.17 ± 2.42
Apomorphine	18.07 ± 1.63[a]	9.46 ± 0.53[b]	8.62 ± 0.97[a]
Haloperidol	12.96 ± 0.55[a]	9.32 ± 0.43[b]	3.64 ± 0.35[a]

Note. Apomorphine (25 mg/kg, sc) or haloperidol (2 mg/kg, ip) was given 20 and 30 min, respectively, before [^{3}H]spiperone (20 μCi/kg, 0.0003 mg/kg, sc). Mice were killed 2 hr after [^{3}H]spiperone. Values are means ± SEM of six animals.

[a] $P < 0.005$ compared to control (two-tailed Student's t test).

[b] Not significantly different from control.

and for simplicity, the following cell preparation was used to determine the effect of ascorbic acid on [^{3}H]spiperone binding *in vitro*.

Corpora striata from individual mice were placed in 3 ml Tris buffer (0.05 *M*, pH 7.7) fortified with salts as follows: NaCl, 120 m*M*; KCl, 5 m*M*; $CaCl_2$, 2 m*M*; and $MgCl_2$, 2 m*M*. Tissues were finely minced with scissors and placed in a Dubnoff metabolic incubator at 37°C and under an O_2/CO_2 (95/5%) atmosphere. After a preincubation period (10 or 120 min) 0.02 μCi (0.96 pmole) [^{3}H]spiperone was added and incubation continued for 120 min (a predetermined time at which binding appeared to approach equilibrium). At the end of the incubation period, tissues were washed with 3 ml cold Tris. After blotting with filter paper, tissues were weighed and homogenized in 1 ml 0.4 *N* perchloric acid. The homogenate was added to 5 ml counting cocktail (Opti-Fluor, Packard) for scintillation counting. Nonspecific binding was estimated by determining the binding that persisted in the presence of *d*-butaclamol (10^{-5} *M*).

Results. *Experiments in vivo.* As further validation for the method employed by Murrin (7), Table I shows that at 2 hr postadministration of [^{3}H]spiperone, the concentration of tritium in the corpus striatum was more than three times that in the sparsely dopaminergically innervated cerebellum. Furthermore, when either apomorphine (25 mg/kg, sc) or haloperidol (2 mg/kg, ip) was given, 20 and 30 min, respectively, before [^{3}H]spiperone, the radioactivity accumulated by the corpus striatum was greatly reduced while that in the cerebellum was essentially unaffected.

Table II shows the effects of ascorbic acid pretreatment, at three different doses, on accumulation of tritium in corpus striatum and cerebellum. There was a dose-dependent reduction in the tritium accumulated by the striatum with only the highest dose producing any significant effect (18%) in the cerebellum.

TABLE II. EFFECT OF 30-min PRETREATMENT WITH ASCORBIC ACID ON [^{3}H]SPIPERONE ACCUMULATION IN MOUSE BRAIN

	dpm/mg wet wt		
	Striatum	Cerebellum	Difference
Control	32.15 ± 1.29	10.19 ± 0.42	21.97 ± 1.08
Ascorbic acid			
100 mg/kg	27.01 ± 1.58[a]	9.43 ± 0.49	17.58 ± 1.29[a]
1000 mg/kg	18.61 ± 1.70[b]	9.96 ± 0.75	8.65 ± 1.37[b]
2000 mg/kg	12.04 ± 0.52[b]	8.34 ± 0.40[a]	3.70 ± 0.40[b]

Note. Ascorbic acid was injected (ip) followed in 30 min by [^{3}H]spiperone (20 μCi/kg, 0.0003 mg/kg, sc). Mice were killed 2 hr after [^{3}H]spiperone. Each value is the mean ± SEM of at least six animals.

[a,b] $P < 0.025$ and 0.001, respectively, when compared to control.

If the amount in the cerebellum represented nonspecific binding and this was subtracted from that in corpus striatum, the percentage reduction in striatal accumulation was as high as 85% with the highest dose of ascorbic acid.

Table III shows a marked reduction in the accumulation of tritium in striatum when [^{3}H]spiperone was administered 18 hr after ascorbic acid (1000 mg/kg).

Experiments in vitro. Pretreatment of mice (2000 mg/kg, ip) for 1 or 2 hr failed to alter [^{3}H]spiperone binding to striatal minces *in vitro.* The inclusion of ascorbic acid (10^{-3} *M*) in the incubation medium during the 10-min preincubation and the 120-min incubation periods failed to alter binding as well (data not shown). However, when tissues were preincubated for 120 min with ascorbic acid (10^{-3} *M*) prior to addition of [^{3}H]spiperone, there was a marked reduction in specific [^{3}H]spiperone binding but no effect on nonspecific binding (Table IV). This observation is in agreement with the report of Chan *et al.* (1) who reported similar results with [^{3}H]-spiperone using a different tissue preparation.

Discussion. As pointed out in the introduction, there are reports showing that ascorbic acid can modify ligand binding characteristics of dopamine receptors *in vitro* (1, 2). These reports, coupled with several reports showing a modification of several dopaminergically mediated behavioral responses by ascorbic acid (3–6), have led to the suggestion that the vitamin might interact, in some way, with dopamine receptors *in vivo.* The present report is evidence in favor of that suggestion. Thus, ascorbic acid pretreatment (30 min or 18 hr) reduced the 2-hr accumulation, *in vivo,* of the dopamine receptor ligand, [^{3}H]spiperone, in the dopaminergic corpus striatum. Binding in the cerebellum was unaffected except at the highest dose and then only marginally.

TABLE III. EFFECT OF 18-hr PRETREATMENT WITH ASCORBIC ACID ON [^{3}H]SPIPERONE ACCUMULATION IN MOUSE BRAIN

	dpm/mg wet wt		
	Striatum	Cerebellum	Difference
Control	34.75 ± 0.50	10.41 ± 0.46	24.35 ± 0.88
Ascorbic acid	24.50 ± 2.64[a]	9.96 ± 0.43	14.54 ± 2.44[a]

Note. Ascorbic acid (1000 mg/kg, ip) was injected. Eighteen hours later [^{3}H]spiperone (20 μCi/kg, 0.0003 mg/kg, sc) was administered and mice were killed after 2 hr. Values are means ± SEM of six animals.

[a] $P < 0.001$ when compared to control.

TABLE IV. EFFECT OF ASCORBIC ACID ON [^{3}H]SPIPERONE BINDING TO STRIATAL MINCES

	dpm/mg wet wt	
	Nonspecific	Specific
Control	277 ± 14	193 ± 17
Ascorbic acid	260 ± 14	36 ± 12[a]

Note. Striatal minces were preincubated for 120 min in the presence of ascorbic acid (10^{-3} *M*) before the addition of [^{3}H]spiperone (0.02 μCi, 20.9 Ci/mmole). The incubation time was also 120 min. Nonspecific binding was defined as that persisting in the presence of *d*-butaclamol (10^{-5} *M*). Specific binding is the difference between total binding and nonspecific binding. Values are means of tissues taken from at least six animals.

[a] $P < 0.0001$ (two-tailed Student's *t* test).

The mechanism of the ascorbic acid-induced reduction in striatal [^{3}H]spiperone accumulation is uncertain. It is consistent with *in vitro* studies (1, 2, and present report) showing reduced binding of dopamine receptor ligands by corpus striatal preparations preincubated with ascorbic acid. However, the possibility must be considered that the vitamin decreased striatal [^{3}H]spiperone accumulation by altering the absorption or distribution of the radioligand. It would seem that, if this had been the case, cerebellar concentrations would also have been reduced. Except for the modicum of effect with the highest dose of ascorbic acid, this was not the case. However, if indeed the effect was on [^{3}H]spiperone distribution, consideration must be given to the possibility that many other drugs might be similarly affected by ascorbic acid. Regardless of the mechanism, it can be said that it involved some semipermanent change in that the effect lasted at least 18 hr.

In conclusion, ascorbic acid decreased accumulation of [^{3}H]spiperone in rat corpus striatum *in vivo,* and in confirmation of other published reports, decreased specific binding of the radioligand to corpus striatal minces *in vitro.* The latter effect required an extended preincubation time. If indeed ascorbic acid interacts with dopamine receptors *in vivo* and

as an antagonist as animal behavior studies suggest (3–6), the reported beneficial effects of vitamin C in schizophrenia (9) are understandable. Furthermore, it would be of interest to know if large doses of ascorbic acid affect the neurology of patients with either tardive dyskinesia or Parkinson's disease. It can be assumed that the condition of the former would be improved while that of the latter would be exacerbated.

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