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BRIEF COMMUNICATION

Potentiation of Haloperidol-Induced Catalepsy by Ascorbic Acid in Rats and Nonhuman Primates

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DORRIS, R L AND R E DILL *Potentiation of haloperidol-induced catalepsy by ascorbic acid in rats and nonhuman primates* PHARMACOL BIOCHEM BEHAV **24**(3) 781–783, 1986 —Ascorbic acid was examined for potentiative effects on the catalepsy induced by haloperidol in rats and squirrel monkeys In both animal species pretreatment with ascorbic acid (1000 mg/kg) markedly potentiated catalepsy induced by haloperidol It is suggested the vitamin binds to, and inactivates, some brain dopamine receptors and in so doing potentiates an otherwise minimally cataleptogenic dose of haloperidol

Catalepsy Potentiation Ascorbic acid Monkey Rats

IT has been common practice to use ascorbic acid as an antioxidant in incubation media in dopamine receptor ligand binding studies There are now reports showing that the use of an antioxidant is not always necessary and that the inclusion of ascorbic acid in binding assays can result in lowered ligand binding [2, 3, 5] The studies by Tolbert *et al* [11], showing that the vitamin inhibits amphetamine induced stereotypy, as well as apomorphine induced hypothermia, suggest that the influence of the vitamin on the dopamine receptor may be more than of methodological significance We now report additional evidence that ascorbic acid can influence a functional dopaminergic activity

METHOD

The rats (female, Sprague Dawley) used in this study weighed between 150–200 g Catalepsy in these animals was evaluated by a method similar to that utilized by Morpurgo [6] Thus, forepaws were placed, one at a time, on a wooden block 7 5 cm in height This was repeated for each hind paw and one-half point was given for each paw that remained on the block for 10 sec Homolateral fore- and hind paws were then placed simultaneously on the block and a point was given for each of the two sides that remained on the block for 10 sec Two wooden blocks were then separated by a distance of approximately 10 cm and the rats forepaws placed on one block and hind paws placed on the other Retention of this position for 10 sec was assigned a value of 2 points Thus, a maximum of 6 points was possible for each rat at each measurement time Ascorbic acid was dissolved in water and given IP (1000 mg/kg) followed in 15 min by haloperidol (0 2 mg/kg, SC) Control rats were injected with equivalent volumes of acidified saline (pH 2 3) in lieu of ascorbic acid Each rat was used twice, separated by at least 6 days and were counterbalanced as to treatment with ascorbic acid and acidified saline Catalepsy determinations were done at 30 min intervals for up to 120 min after haloperidol administration

The three adult male squirrel monkeys (*Saimiri sciureus*) used in the study weighed between 1 0 and 1 1 kg Each monkey was alternately given either ascorbic acid (1000 mg/kg, IP) or an equal volume of solvent and placed in a clear plastic observation cage The cage measured 30×28×60 cm (width, depth, height) with a perch made from 9 mm dia rod placed horizontally 20 cm above the floor and 14 cm from the front of the cage One hour after administration of either ascorbic acid or the solvent, animals were given 0 1 mg/kg haloperidol SC and returned to the observation cage Every fifteen minutes for the first hour, every 30 min the second hour and every hour thereafter the animals were tested for signs of catalepsy This was achieved by placing the animal on the perch and observing the manner of

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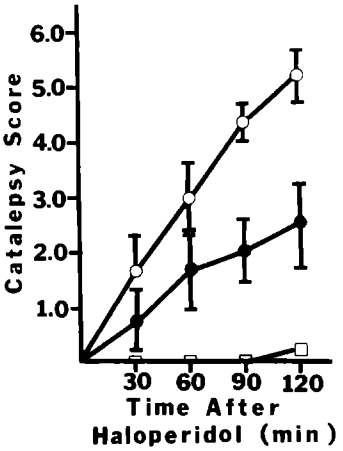

FIG 1 Potentiation of cataleptogenic effects of haloperidol by ascorbic acid Rats were injected with ascorbic acid (1000 mg/kg, IP, open circles) or an equivalent volume of acidified saline (pH 2 3, HCl, closed circles) Fifteen min later haloperidol (0 2 mg/kg, SC) was administered Some rats were treated with ascorbic acid alone (open squares) Each point is the mean±S E M of 8–12 animals Values at 90 and 120 min for ascorbic acid vs acid saline pretreatment are significantly different from each other ($p<0$ 0025, Student's t-test)

dismounting the perch Catalepsy was determined as being present if the animal slowly sagged to a position such that the head and trunk were below the level of the perch and remained in that position with at least two limbs on the bar for at least one minute Such posture is not seen in the normal monkey The onset and duration of catalepsy were recorded as measures of the intensity of catalepsy

Ascorbic acid was dissolved in sterile demineralized water at a concentration of 250–300 mg/ml Control monkeys were injected with sterile 0 9% saline adjusted to a pH of 3 0 Commercial haloperidol (Haldol® injectable, McNeil Labs) was diluted with sterile physiologic saline to a concentration of 0 5 mg/ml for use in monkeys For injection in rats, powdered haloperidol (McNeil Labs) was dissolved in 0 01 N HCl (2 mg/ml) and then diluted with water to 0 1 mg/ml The dose of haloperidol selected was one that produced minimal catalepsy In rats, exact doses were determined experimentally but were based on the report of Shore and Dorris [10] Previous studies with squirrel monkeys [4] indicated that 0 1 mg/kg haloperidol produced only marginal catalepsy

RESULTS

Haloperidol alone produced very little catalepsy in rats and ascorbic acid produced essentially no catalepsy (Fig 1) However, when the two drugs were combined the animals became progressively more cataleptic throughout the time period examined

As in rats, ascorbic acid alone produced no detectable catalepsy in monkeys but markedly increased the catalepsy induced by haloperidol (Table 1) Animal No 83-1 became "plastic" after the combined ascorbic acid-haloperidol treatment and it was possible to place this monkey in bizarre positions outside the cage (Fig 2) Such positions were maintained for two to five minutes and were not possible in animals which received only haloperidol Clearly ascorbic acid potentiated the catalepsy-inducing properties of haloperidol in these three primates

TABLE 1

POTENTIATION OF CATALEPTOGENIC EFFECTS OF HALOPERIDOL BY ASCORBIC ACID IN SQUIRREL MONKEYS

Animal No	Saline + Haloperidol		Ascorbic acid + Haloperidol	
	onset	duration	onset	duration
83-1	15 min	30 min	15 min	6 hr
83-2	no catalepsy		1 hr	2 hr
83-3	no catalepsy		15 min	>6 hr

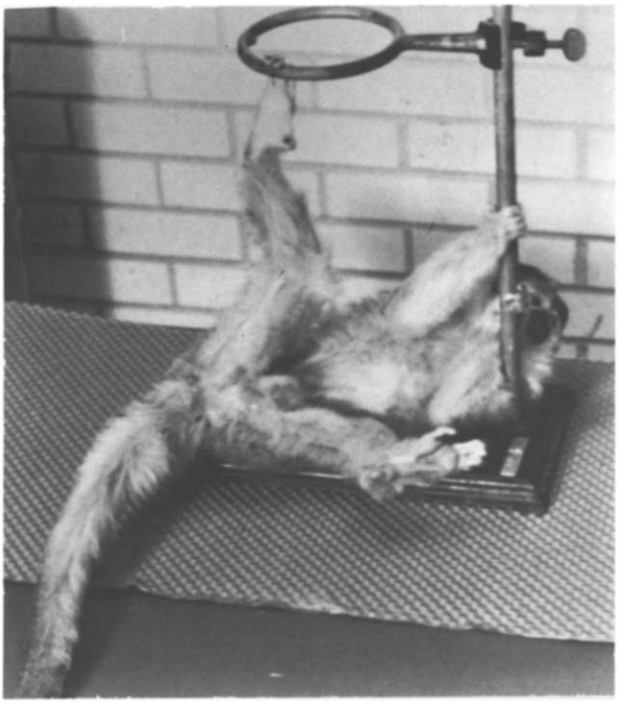

FIG 2 Monkey treated with ascorbic acid-haloperidol combination The monkey was injected with ascorbic acid (1000 mg/kg, IP) followed in 1 hr by haloperidol (0 1 mg/kg, SC) Subsequently the animal was removed from the observation cage and placed in this position The abnormal position shown here was maintained for several min

DISCUSSION

It is known that in animals catalepsy can result when there is insufficient interaction of dopamine with dopamine receptors in the basal ganglia This is probably the counterpart of rigidity in human parkinsonism Thus, in sufficient dosage, drugs that block the interaction of the neurotransmitter with its receptor are capable of producing a cataleptic state In this present study haloperidol was used in a dose that had a minimal effect on striatal dopaminergic transmission and thus produced only marginal catalepsy The thought was that if ascorbic acid also produced some marginal effect

on transmission, this dopamine receptor blocking drug should exhibit potentiated effects when administered along with the vitamin This was shown to be the case in both the rodent and primate models

It is realized that the dose of ascorbic acid used in this study was very large However, it has been reported [1] that the rise in extracellular brain ascorbic following systemic injection of this dose of ascorbic acid is approximately the same as that released endogenously by the dopamine agonist, amphetamine Furthermore, some authorities have recommended taking gram quantities of the vitamin [8] which could conceivably result in cumulative tissue concentrations comparable to those attained with these single large doses For that matter some people have taken daily doses (100 g) equivalent to these doses for several days [7]

The question could be raised as to whether ascorbic acid potentiated haloperidol by modifying pharmacokinetics This seems unlikely in that ascorbic acid was given IP, whereas, haloperidol was given SC Furthermore, control rats were given equivalent volumes of saline adjusted to a pH approximating that of the ascorbic acid solution

The results of this present report support the contention by others [11] that ascorbic acid may be capable of a pharmacological/toxicological action on dopamine systems and suggest a mechanism for the purported beneficial effects of large doses of ascorbic acid in schizophrenia [8], a condition known to improve by dopamine-dopamine receptor intervention In light of these findings, however, it is also suggested that parkinsonian patients, who have compromised striatal dopaminergic function, might be worsened by large doses of vitamin C, a possibility that would seem to warrant investigation

It should be added that as this manuscript was nearing completion a report by Rebec *et al* [9] was published on findings similar to those reported here, but in a rat model only

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