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BRIEF COMMUNICATION

Interactions of Nicotinamide With Dopamine Receptors In Vivo

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DORRIS, R. L. *Interactions of nicotinamide with dopamine receptors in vivo.* PHARMACOL BIOCHEM BEHAV **33**(4) 915–917, 1989.—[^{3}H]-Spiperone (20 μCi/kg, 0.0003 mg/kg, SC) was administered to mice. Relative decreases in the 2-hr ratio of accumulation of this dopamine receptor radioligand in the dopaminergic corpus striatum ("specific" plus "nonspecific binding") and the nondopaminergic cerebellum ("nonspecific binding" only) were used to evaluate nicotinamide for possible effects on the dopamine receptor. The nicotinamide-treated animals were also observed for signs of catalepsy. Pretreatment for 30 min with IP doses of 200 and 500 mg/kg reduced accumulation in both areas approximately the same as judged from striatum:cerebellum ratios, which did not differ significantly from controls. However, at 1000 mg/kg, although nicotinamide decreased [^{3}H]-spiperone accumulation in both striatum and cerebellum, "specific binding" was affected more than "nonspecific binding," as judged from a statistically significant decrease in the striatum:cerebellum ratio. This dose also produced a cataleptic state. Nicotinamide at high doses might have some antagonistic effect on dopamine receptors in mice as judged from the greater effect on accumulation of [^{3}H]-spiperone in striatum ("specific binding") than in cerebellum ("nonspecific binding") which appeared to correlate somewhat with the production of a cataleptic state.

Nicotinamide Dopamine receptors Catalepsy Mouse

THERE are claims that large doses of vitamins, such as ascorbic acid and nicotinamide, are beneficial in the treatment of schizophrenia (8). The reported antagonism of some dopamine-mediated behavioral effects of ascorbic acid (9–11) are predictive of an antipsychotic potential of this agent, as are reports of antagonism of receptor ligand binding in corpus striatum, both in vitro (2,5) and in vivo (2). The present study was designed to determine if nicotinamide could be shown to interact with the dopamine receptor by determining its effects in vivo on accumulation of the dopamine receptor radioligand, [^{3}H]-spiperone in the mouse dopaminergic corpus striatum relative to that in the nondopaminergic cerebellum, as was done in earlier studies on ascorbic acid (2). Because catalepsy is a behavioral response that has long been associated with dopamine receptor-blocking drugs, nicotinamide-treated animals were also observed for signs of this condition.

METHOD

Biochemical Experiments

As in earlier studies on ascorbic acid (2) the 2-hr accumulation of [^{3}H]-spiperone in mouse corpus striatum ("specific" plus "nonspecific binding") and cerebellum ("nonspecific binding" only) was used to biochemically evaluate nicotinamide for possible effects on the dopamine receptor. Thus, male mice (25–35 g), TEX:ICR, were injected (SC) with [^{3}H]-spiperone, 25.7 Ci/mmole, New England Nuclear, dissolved in 18% ethanol. The dose was 20 μ Ci/kg (0.0003 mg/kg), administered in a volume of 0.1 ml/10 g body wt. Nicotinamide (Sigma Chemical Co.) was dissolved in water and injected IP (0.1 ml/10 g body wt., 30 min before [^{3}H]-spiperone. Mice were killed by exsanguination, under chloroform anesthesia and striata and a piece of cerebellum, equivalent in wt. to the striata, was homogenized in 1.5 ml 0.4 N perchloric acid. The homogenate was transferred to counting vials containing 5 ml liquid scintillation cocktail (Beckman Ready Solv) for scintillation counting. Radioactivity was considered as unchanged [^{3}H]-spiperone based on the reports of Murrin (7) and Laduron *et al.* (6) that more than 90% of the radioactivity in rat brain at 2 hr could be accounted for as unchanged drug. Although this was not validated in mice, the fact that pretreatment with a high dose of haloperidol has been shown to decrease accumulation of administered [^{3}H]-spiperone in mouse striatum to levels near that of cerebellum, without affecting that in cerebellum (2) strongly suggests that under the experimental conditions most of the radioactivity in mouse striatum was either unchanged [^{3}H]-spiperone or most was a metabolite that binds to a site from which it is displaceable by a known dopamine receptor antagonist. The latter possibility seems very unlikely.

Because an equivalent decrease in accumulation in both brain areas studied (which was found to be the case at the lower doses) could mean that nicotinamide had a nonspecific, pharmacokinetic effect, the data were expressed, not only as DPM/mg tissue, but, also, as striatum to cerebellum ratios (4). Thus, a decrease in the

TABLE 1

EFFECT OF NICOTINAMIDE ON [^{3}H]-SPIPERONE ACCUMULATION IN MOUSE CORPUS STRIATUM AND CEREBELLUM

	DPM/mg Wet Wt.		
	Striatum	Cerebellum	Striatum / Cerebellum
Control (11)	31.1 ± 1.1	11.4 ± 0.4	2.75 ± 0.09
Nicotinamide			
200 mg/kg (4)	23.8 ± 0.7*	8.4 ± 0.4*	2.86 ± 0.18^{ns}
500 mg/kg (6)	22.9 ± 1.5*	9.2 ± 0.4*	2.52 ± 0.16^{ns}
1000 mg/kg (7)	14.2 ± 0.6†	7.0 ± 0.3†	2.05 ± 0.12†

Nicotinamide 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. Values are means ± S.E.M. Numbers in parentheses are numbers of animals.

*$p<0.0025$, †$p<0.0005$ when compared to control; ^{ns}not significantly different from control (Student's t-test).

TABLE 2

CATALEPSY AFTER NICOTINAMIDE

	Time After Nicotinamide (min)							
Animal Number	30	60	90	120	30	60	90	120
	500 mg/kg				1000 mg/kg			
1	0	0	0	0.5	6	6	6	6
2	0	0	0	0	1	2	3	3
3	0	0	0	0	2	3	3	2
4	0	0	0	0	0	0.5	3	2
5	6	6	6	6	3	3	6	6
6	0	0	1.5	6	6	6	6	6
Mean ± SEM	1.0 ± 1.0	1.0 ± 1.0	1.3 ± 1.0	2.1 ± 1.2	3.0 ± 1.0	3.4 ± 0.9	4.5 ± 0.7	4.2 ± 0.8

Nicotinamide was injected (IP) and catalepsy determined as described in the Method section. Six sham-injected (water) mice showed no catalepsy at any of the measurement times.

ratio resulting from a greater effect in striatum than cerebellum, would be suggestive of an effect on "specific binding," above that on "nonspecific binding."

Evaluation of Catalepsy

Mice were evaluated for catalepsy by a method similar to that previously used with rats (3). Thus, forepaws were placed, one at a time, on a plastic slide box, 4 cm in height. This was repeated for each hind paw and one-half point was given for each paw that remained on the box for 15 sec. Two boxes were then separated by a distance of approximately 5.5 cm and the mouse's forepaws placed on one box and hind paws on the other. Retention of this position for 15 sec was assigned a value of 2 points. Thus, a

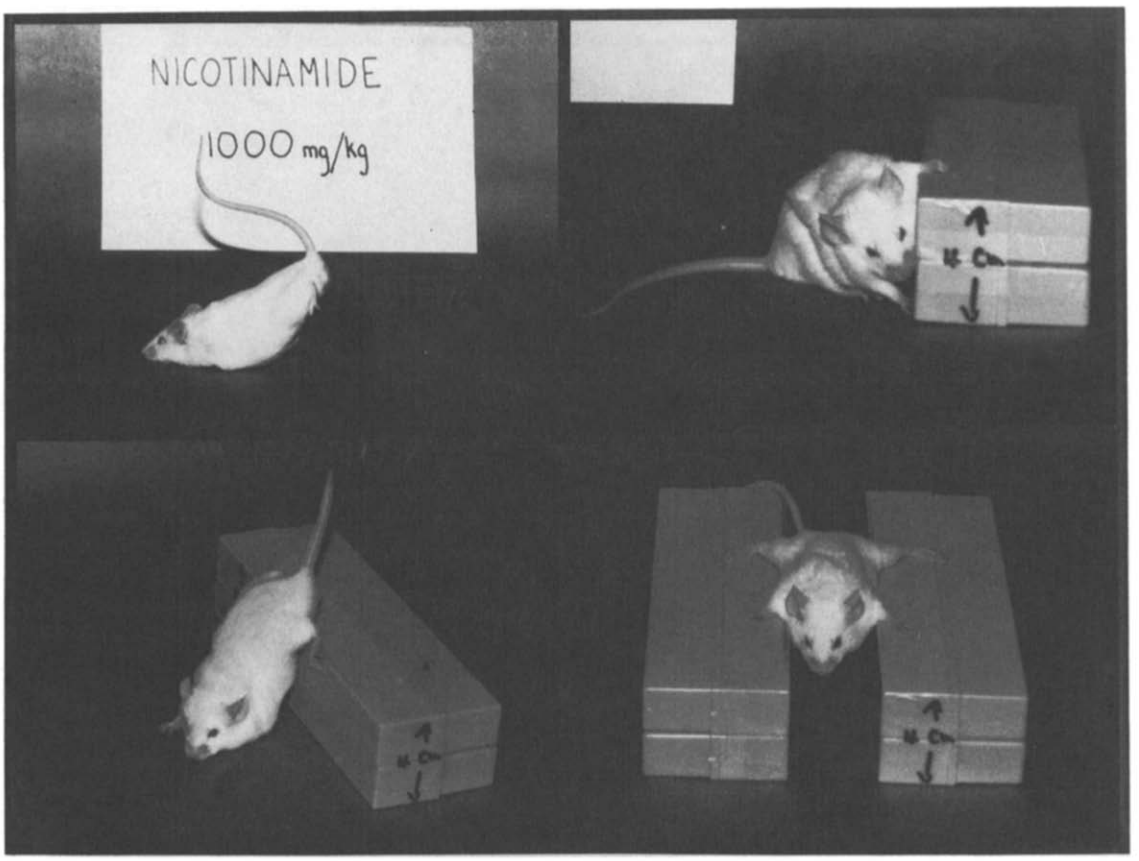

FIG. 1. Nicotinamide-induced catalepsy. The mouse was injected with nicotinamide (1000 mg/kg, IP) and approximately 60–90 min later placed in the positions shown. The positions were maintained for several min.

maximum of 6 points was possible for each mouse at each measurement time. The person administering the test was not apprised of which dose (500 or 1000 mg/kg, IP) of nicotinamide was given. A total of eighteen mice were used in this part of the study, including six water-injected controls.

RESULTS

Accumulation of [^{3}H]-Spiperone

Table 1 shows the effects of 30-min pretreatment with three different doses of nicotinamide. The two lower doses (200 and 500 mg/kg) reduced accumulation in both brain areas and equivalently as indicated from the observation that the striatum to cerebellum ratios did not differ significantly from control. However, at 1000 mg/kg, the dopaminergic striatum was affected more than that of the cerebellum, in that there was a highly significant lowering of the striatum to cerebellum ratio.

Cataleptic Effect

Clearly nicotinamide produced a cataleptic response (Table 2) with 1000 mg/kg being much more effective than the 500 mg/kg dose. All animals in the higher dose group showed at least some level of catalepsy with one-half of them showing maximum responses at 90 min postinjection. At the 500 mg/kg dose, catalepsy was evident in some of the animals, but two-thirds of them did not exhibit any level of catalepsy. For emphasis, photographs were taken (Fig. 1) of a mouse 60–90 min after injection with 1000 mg/kg nicotinamide. These positions, which are not maintained by normal mice, were maintained for several minutes by this animal.

DISCUSSION

There is now much evidence that dopaminergic synapses are involved in the expression of schizophrenic behavior. Virtually all useful antipsychotic drugs are dopamine receptor antagonists and there is a strong correlation between the average clinical doses of these drugs and their ability to inhibit binding of dopamine receptor ligands to membranes taken from a dopaminergic brain area (1). As mentioned in the introduction, there are some who contend that certain vitamins, including ascorbic acid and nicotinamide, are useful in the treatment of schizophrenia (8). Consistent with this contention are reports that ascorbic acid antagonizes several dopamine-mediated behavioral effects (9–11) and that the vitamin decreases dopamine receptor ligand binding in vitro (2,5) as well as in vivo (2).

A previous report (2) showed that ascorbic acid decreased [^{3}H]-spiperone accumulation in vivo in the mouse dopaminergic corpus striatum without altering that in cerebellum. In the present study, nicotinamide decreased accumulation of the ligand in both brain areas. However, at the highest dose (1000 mg/kg) the striatum was affected more than the cerebellum as indicated from the lowering of the striatum to cerebellum ratio with this dose.

It is known that, in animals, catalepsy can result when there is insufficient interaction of dopamine with dopamine receptors in the basal ganglia. Thus, in sufficient dosage, drugs that block dopamine receptors are capable of producing a cataleptic state. It, thus, is of interest that nicotinamide at 1000 mg/kg, a dose that produced a significant lowering of the [^{3}H]-spiperone striatum to cerebellum ratio produced a marked cataleptic state. On the other hand, at 500 mg/kg, the ratio was not significantly changed and cataleptic responses were much less prevalent—being absent in at least one-half of the animals. These observations lend credence to the possibility that, at very high doses, nicotinamide has some dopamine receptor antagonistic properties. Even if this is not the case, the vitamin very clearly altered the distribution of [^{3}H]-spiperone to the brain. This in itself could have clinical significance and consideration should be given to the possibility that many other therapeutic agents might be similarly affected by nicotinamide when administered in the high doses suggested by Pauling (8).

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