Pharmacology Biochemistry & Behavior, Vol. 17, pp. 951–954, 1982. Printed in the U.S.A.

Dopaminergic Properties of Nomifensine

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Received 1 April 1982

GIANUTSOS, G., G. MORROW, S. LIGHT AND M. J. SWEENEY. *Dopaminergic properties of nomifensine.* PHARMAC. BIOCHEM. BEHAV. **17**(5) 951–954, 1982.—Nomifensine and a proposed dihydroxy metabolite produced stimulation of motor behavior in mice with nomifensine being more potent. Weak cage-climbing behavior (stereotypy) was also produced. The stimulatory effects were greater in mice in which dopamine receptor sensitivity was increased by long-term haloperidol. Both of the analogs were potent inhibitors of dopamine and norepinephrine uptake in vitro with nomifensine approximately 3 times more potent than the metabolite. In contrast, the two analogs had weak affinity for the post-synaptic dopamine receptor (as measured by displacement of ligand binding in vitro) with dihydroxynomifensine approximately 6 times more potent than nomifensine. These results suggest that the behavioral effects of nomifensine are largely dependent on presynaptic catecholamine mechanisms but that weak direct dopamine agonist properties do exist, particularly in vivo where the drug may be metabolized to a more active form.

Nomifensine Dopamine Uptake Receptors Motor activity

NOMIFENSINE is a clinically active antidepressant [16] whose pharmacological effects appear to differ from classical antidepressants. While the traditional tricyclic antidepressants appear to act by inhibition of the uptake of norepinephrine (NE) and/or serotonin [22], nomifensine demonstrates potent effects on dopamine (DA). Inhibition of DA uptake [11] as well as NE [20] is usually considered to explain the mechanism of action of nomifensine, but a direct stimulation of post-synaptic DA receptors has also been proposed [17].

In this study, we have investigated the dopaminergic properties of nomifensine and a possible active metabolite (3,4-dihydroxy nomifensine), using a variety of in vivo and in vitro means in an effort to analyze the importance of pre- and post-synaptic dopaminergic mechanisms in the action of this drug.

METHOD

Motor Activity

Mice (CD-1 males, Charles River Farms, Wilmington, MA) were used for the behavioral experiments. Activity was measured in a 6 chamber Stoelting Activity Monitor. The mice were allowed a 20 minute period of acclimation in the device and were removed and injected SC with one of the drugs (or saline). The mice were returned to the device and activity was measured for 2 hours. Movements in the chamber which disturbed the radio-frequency field produced by the device were converted to "counts" and accumulated at 30 minute intervals. The cumulated counts were analyzed statistically by analysis of variance followed by Dunnett's test for comparison with control (saline-injected) values ($p<0.05$).

The drug response was also measured in mice maintained on the dopamine antagonist, haloperidol. Haloperidol was administered in the food at a concentration of 0.005% [7] for 8 weeks; drug testing was conducted after the mice were taken off the drug for 3 days. This treatment is believed to induce a supersensitivity of dopamine receptors [7].

Cage Climbing

The intensity of climbing behavior was scored using the five-point rating scale of Baldessarini and coworkers [1]. The mice were allowed a 30 minute period of acclimation to a wire-mesh cage after which time they were removed from the cage, injected and returned to the cage for observation. Ratings were made every 30 minutes for 2 hours. The scores (0–4) for each period were summed (maximum score=16) and analyzed by the non-parametric Mann-Whitney Test ($p<0.05$).

Binding

The activity of drugs on binding to a putative dopamine receptor binding site was measured as described by Costall and coworkers [5] using ^{3}H-N-n-propyl norapomorphine (NPA) as the radiolabelled ligand. A receptor-enriched membrane fraction was prepared from calf-caudate by homogenization in 0.32 M sucrose. After centrifugation and extensive washing and recentrifugation, the resulting pellet was suspended in 50 mM Tris (pH of 7.6) and incubated at 37°C for 20 minutes with NPA (2 nM) and unlabelled drug over a concentration range of 10^{-9} to 10^{-5} M. After incubation, the mixture was rapidly filtered under reduced pressure through a Whatman GF/B filter and washed with 15 ml of ice-cold buffer. The pellet was digested with Protosol and the bound radioactivity was quantified by liquid scintillation. Non-specific binding was defined as label which was not displaced by 10^{-6} M (+) butaclamol. All concentrations were run in triplicate on at least 2 occasions. The displacement of specifically bound NPA was plotted against concentration

and IC_{50} value was defined as the concentration calculated (using linear regression) to displace 50% of the label.

Uptake

Inhibition of synaptosomal uptake of biogenic amines was performed using a modification of the method of Koe [12]. Synaptosomes were prepared from fresh rat brain tissue as described by Gray and Whittaker [8]. Striata (dopamine uptake) or hippocampus + cortex (norepinephrine uptake) were homogenized in 0.32 M sucrose using a teflon/glass homogenizer. The homogenate was centrifuged at 1000 g for 10 minutes and the resulting supernate was centrifuged at 17,000 g for 20 minutes. The resulting P_2 pellet was resuspended in cold, modified Ringers Solution and 100 μL aliquots were added to 1 ml of this buffer and used for uptake studies. The tubes were preincubated for 2 minutes at 37°C and labelled neurotransmitter (^{14}C-dopamine or norepinephrine) was added to yield a final concentration of 0.1 μM. After 8 additional minutes of incubation, the tubes were filtered under reduced pressure and the tissue trapped on the Millipore (0.45 μ) filter was washed with 10 ml cold buffer. The synaptosomes were lysed with Protosol and the radioactivity taken up into the tissue was quantified by liquid scintillation. A blank value, which consisted of label bound under identical conditions but incubated at 0°C, was subtracted from all samples. IC_{50} values were determined after plotting % inhibition of uptake vs log inhibitor concentration.

RESULTS

Behavior

Nomifensine dose-dependently stimulated motor activity in the mice, as previously reported for the rat [4,6]. Doses above 1 mg/kg were active with a peak effect at 8–16 mg/kg (Fig. 1). In addition, nomifensine induced stereotyped cage climbing (Fig. 2), a behavioral effect resulting from stimulation of striatal DA receptors [18], although the magnitude of this effect was substantially less than that produced by other dopaminergic drugs such as apomorphine [18]. Similar effects were produced by dihydroxy nomifensine (Figs. 1 and 2). These behavioral effects were antagonized by prior injection of haloperidol (0.5 mg/kg, data not shown). However, the dihydroxy analog was considerably less potent than nomifensine with 20 mg/kg of dihydroxy nomifensine (0.05 mmoles/kg) producing effects which were roughly equivalent to 8 mg/kg of nomifensine (0.02 mmoles/kg). It is unclear whether this difference is due to an inherent difference in activity between the two compounds or rather to some pharmacokinetic factor(s).

The stimulatory effect of the drugs was enhanced in mice treated chronically with haloperidol (Table 1), a treatment thought to increase DA receptor sensitivity [7]. A normally ineffective dose of nomifensine (1 mg/kg) produced significant stimulation in the chronic-haloperidol group. Dihydroxynomifensine produced even more dramatic changes. A relatively small dose (2 mg/kg), which was less than 1/5 the minimum effective dose in control mice, was active in producing motor stimulation after chronic haloperidol.

Biochemistry

Nomifensine was a potent inhibitor of both DA and NE synaptosomal uptake in vitro being approximately 5–10 times as potent as cocaine (Table 2). Dihydroxynomifensine

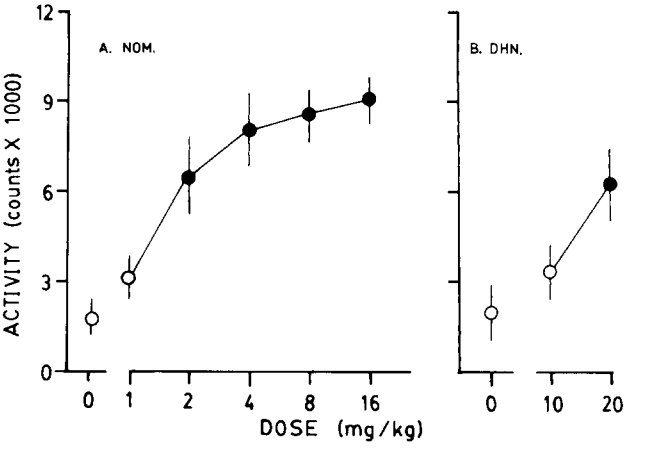

FIG. 1. Effect of nomifensine and dihydroxynomifensine on motor activity. Mice were injected SC with nomifensine (NOM, panel A) or dihydroxy nomifensine (DHN, panel B) and motor activity was monitored for 2 hours. "Counts" represent mean cumulated activity (±1 S.E.) for the period and are the means derived from 10 mice; filled circles represent values which are significantly different ($p<0.05$) from control (0 mg/kg dose). See text for further details.

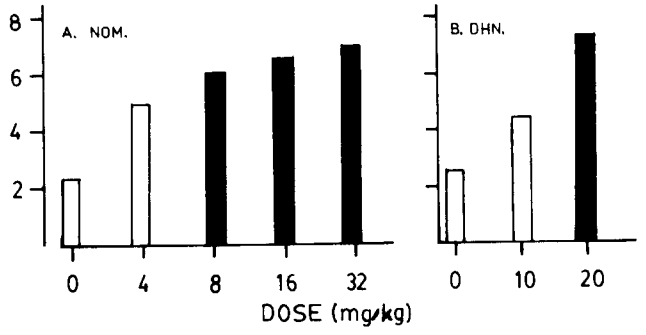

FIG. 2. Effect of nomifensine or dihydroxynomifensine on cage-climbing. Mice were injected SC with nomifensine (NOM, panel A) or dihydroxy nomifensine (DHN, panel B) and climbing was rated at 30 minute intervals for 2 hours (see text). Values represent total cumulated scores for the 4 observation periods (maximum possible score=16) and are the means of results from 6–10 mice; filled columns represent values which are significantly different ($p<0.05$) from control (0 mg/kg dose). See text for further details.

was also active as an uptake inhibitor but was approximately 1/3 as potent as nomifensine. Both analogs were slightly more potent in inhibiting the uptake of NE than DA.

In contrast, the results summarized in Table 2 illustrate that dihydroxynomifensine was approximately 6 times more potent than nomifensine in displacing labelled NPA from binding sites in vitro; this is presumed to reflect a greater affinity of dihydroxy nomifensine for the DA receptor. These results suggest that nomifensine may be metabolized to a compound with a greater ratio of post to presynaptic activity. Both compounds, however, were substantially less potent than apomorphine as DA receptor agonists.

TABLE 1

EFFECT OF DRUGS ON ACTIVITY AFTER CHRONIC HALOPERIDOL

Drug	Dose (mg/kg)	Activity* Control	(Mean counts/hr ±SEM) Chronic Haloperidol‡
Saline	—	2275+386	2833+481
Nomifensine	0.5	2494+633	3901+502
	1.0	3291+401	7377+515†
Dihydroxy nomifensine	2.0	1809+176	5098+535†

*Mean cumulated activity (±SE) for 6–8 mice during the first 2 hr after injection.

†Denotes values which are significantly different from corresponding control (saline).

‡Activity measured 72 hr after withdrawal from long-term drug diet (see text).

TABLE 2

EFFECT OF NOMIFENSINE ON INHIBITION OF CATECHOLAMINE UPTAKE AND RECEPTOR AFFINITY

	A. Uptake Inhibition (IC_{50})* DA	NE	B. Binding Affinity† vs <^{3}H>-NPA (IC_{50})	B/A‡
Nomifensine	0.36	0.11	29.5	81.9
Dihydroxy nomifensine	1.34	0.29	5.0	3.7
Cocaine	3.03	0.60	NM	—
Apomorphine	NM	NM	0.004	—

*Micromolar concentration necessary to inhibit uptake of DA or NE into rat synaptosomes by 50% (see text for details).

†Micromolar concentration necessary to displace specifically bound NPA from caudate membranes by 50% (see text for details).

‡Ratio of IC_{50} on DA uptake to IC_{50} for binding.

NM denotes not measured.

DISCUSSION

Nomifensine produced a powerful behavioral effect in mice in agreement with earlier studies in the rat [3, 4, 6]. These effects, which include stimulation on motor activity and induction of weak cage-climbing behavior, appear to be due to a potent presynaptic action of nomifensine (i.e., DA uptake inhibition) coupled with a weak direct effect on DA receptors. Stimulation of motor behavior in mice has previously been reported for other drugs which block DA uptake [9,19], and this effect is greater than that produced by drugs which act preferentially on the uptake of NE [9].

While an indirect dopaminergic effect (i.e., uptake inhibition) appears to play a major role in the action of nomifensine, direct effects on the DA receptor have been proposed [4,17]. In fact, we found that nomifensine was able to bind to putative DA receptors in vitro, although its affinity for these sites is admittedly far below that for known DA receptor agonists (see [21] for review). However, since nomifensine lacks many of the structural features which are generally considered necessary for DA receptor activity (see [15] for review), such as a catechol function, it is not surprising that its affinity is quite low.

The possibility that nomifensine may be metabolized to an active form must be considered. Heptner and coworkers [10] have identified 7 metabolites (designated M1–M7), in the urine and serum of man and animals. Of these M1 (monohydroxy) and M2 (hydroxy, methoxy) have been reported to be active in vivo with properties similar to nomifensine [4,13]. We investigated the dihydroxy metabolite (M5) which more closely resembles DA. This compound was also active in vivo, but more interesting effects were noted in vitro. Dihydroxynomifensine was approximately 1/3 as potent as nomifensine as an uptake blocker but was approximately 6 times more potent in binding to DA receptors. Poat and coworkers [17] have previously reported that dihydroxy nomifensine was nearly as active as DA in stimulation of adenylate cyclase while nomifensine and its monohydroxy metabolite (M1) were essentially inactive. These results suggest that in vivo nomifensine may be metabolized to a drug which has greater activity as a direct DA receptor agonist and less indirect activity. While this metabolite has been detected in only very small amounts in the urine [10] it is not unlikely that two of the major metabolites (M2 and M3) arise from O-methylation (and possibly deactivation) of the dihydroxy compound.

It must be pointed out that M5 is still several orders of magnitude less potent than apomorphine as a direct agonist, and probably contributes little to the activity of nomifensine in normal subjects. However, in the presence of supersensitive DA receptors, the activity of dihydroxynomifensine in vivo, was markedly enhanced. This may help to explain the small therapeutic effect of nomifensine in Parkinson's Disease [2], particularly since a DA receptor supersensitivity is proposed to develop during the course of the disease [14] along with a loss of presynaptically available DA. In this case, nomifensine and/or its metabolites could produce a much greater effect than might normally be expected, since they may exert an effect directly on these supersensitive receptors.

In summary, nomifensine has potent effects on DA synaptic mechanisms with presynaptic effects playing a major role in its actions but which also includes a postsynaptic effect which should not be ignored, particularly in vivo where metabolism may be a factor.

ACKNOWLEDGEMENTS

This research was supported, in part, by a grant from the United Parkinson Foundation. We are grateful to Hoechst-Roussel Pharmaceuticals (Somerville, NJ) for their generous donation of nomifensine and dihydroxy nomifensine.

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