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Gender-related differences in the effects of nitric oxide donors on neuroleptic-induced catalepsy in mice

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Abstract

It has been suggested that nigrostriatal dopaminergic transmission is modulated by nitric oxide (NO). Since there is evidence that gonadal hormones can affect extrapyramidal motor behavior in mammals, we investigated the effects of isosorbide dinitrate (ISD), linsidomine (SIN-1) and S-nitroso-N-acetylpenicillamine (SNAP), three pharmacologically different NO donors, on neuroleptic-induced catalepsy in 60- to 80-day-old male and female albino mice. Catalepsy was induced with haloperidol (1 mg/kg, *ip*) and measured at 30-min intervals by means of a bar test. Drugs (or appropriate vehicle) were injected *ip* 30 min before haloperidol, with each animal being used only once. ISD (5, 20 and 50 mg/kg) caused a dose-dependent inhibition of catalepsy in male mice (maximal effect 120 min after haloperidol: 64% inhibition). In the females only at the highest dose of ISD was an attenuation of catalepsy observed, which was mild and short lasting. SIN-1 (10 and 50 mg/kg) did not significantly affect catalepsy in female mice, while a significant attenuation was observed in males at the dose of 50 mg/kg (maximal inhibition: 60%). SNAP (20 mg/kg) significantly attenuated catalepsy in males 120 min after haloperidol (44% inhibition), but had no significant effect on females. These results basically agree with literature data showing that NO facilitates central dopaminergic transmission, although the mechanisms are not fully understood. They also reveal the existence of gender-related differences in this nitrergic modulation in mice, with females being less affected than males.

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Key words
- Neuroleptic-induced catalepsy
- Nitric oxide donors
- Gender differences
- Linsidomine
- SNAP
- Isosorbide dinitrate

Introduction

Antipsychotic drugs like haloperidol and chlorpromazine (the so-called typical neuroleptics) induce abnormal motor behaviors in experimental animals and humans, including catalepsy in rats and mice (1). Neuroleptic-induced catalepsy in rodents is a robust behavioral method for studying nigrostriatal dopaminergic function and its modulation by other transmitter systems (1-4).

Nitric oxide (NO) is recognized as a messenger molecule in the nervous system, where it is synthesized from L-arginine by a nitric oxide synthase (NOS) (5,6). Experimental evidence suggests that central dopaminergic transmission is affected by NO (7,8). This influence is rather complex, since it can in-

volve either dopamine release, uptake or metabolism (9). Some studies suggest that endogenous NO increases dopamine release in the striatum and other areas of the CNS (10-12), while other authors suggest the opposite (13,14). If NO increases the release of dopamine in the striatum, we would expect that drugs that release or are metabolized into NO (i.e., NO donors) are able to attenuate neuroleptic-induced catalepsy, which is mainly caused by blockade of striatal dopaminergic receptors (1). Consistent with this hypothesis, Krzascik and Kostowski (15) showed that molsidomine, a pro-drug of the NO donor linsidomine (SIN-1), attenuated haloperidol-induced catalepsy in male rats, while we have shown that another NO donor, isosorbide dinitrate (ISD), did the same in male mice (16).

There is a large body of evidence showing sexual dimorphisms with regard to some aspects of the extrapyramidal motor system, both in humans and in experimental animals (17,18). In addition, gender-related differences are a common feature of the pharmacological properties of most centrally active drugs (19-21); for instance, drug-induced parkinsonism in humans exhibits a female-to-male predominance ratio of about 2:1 (22). Nevertheless, there is no information concerning gender dimorphism in the effects of nitrergic agents on the phenomenon of neuroleptic-induced catalepsy.

The present study was designed to investigate the effects of three centrally active NO donors, ISD, SIN-1 and S-nitroso-N-acetylpenicillamine (SNAP), on neuroleptic-induced catalepsy in male and female mice. Preliminary accounts of these observations were published in abstract form (23,24).

Material and Methods

Animals

Experiments were performed on 60- to 80-day-old albino mice weighing 26-36 g, obtained from our breeding stock. Animals, separated by sex, were housed two per cage (20 x 18 x 13 cm high) with free access to standard pellet food and filtered water, at 23-26ºC. All observations were made between 9:00 and 17:00 h in a quiet room, with each animal used only once. Female mice were tested without monitoring the estrous cycle, since the major interest of this study was to investigate the possibility of NO donors differentially affecting male and female animals.

Drug administrations

The drugs used were haloperidol (Haldol®, Janssen, São Paulo, SP, Brazil), ISD (Sigma, St. Louis, MO, USA; molecular weight = 236.1), SIN-1 hydrochloride (RBI, Natick, MA, USA; molecular weight = 206.63), and SNAP (Tockris Cookson Inc., Ballwin, MO, USA; molecular weight = 220.25). Haloperidol was diluted with saline (0.9% NaCl). SIN-1 was dissolved by sonication in saline, ISD was dissolved by sonication in a 5% solution (v/v) of ethanol in saline, and SNAP was dissolved in a 20% solution (v/v) of DMSO in saline. The drugs were freshly prepared and solutions were protected from light.

The NO donors were injected *ip* with a 27.5 G needle, in a volume of 6 ml/kg body weight, 30 min before haloperidol. The doses used were ISD: 5, 20 and 50 mg/kg; SIN-1: 10 and 50 mg/kg; SNAP: 20 mg/kg. Whenever possible, the doses were chosen according to those commonly used in the literature. For each experimental group, appropriate vehicle (6 ml/kg, *ip*) was used as control.

Procedure

Catalepsy was induced with haloperidol (1 mg/kg, *ip*) and determined at 30-min intervals by means of a standard bar test (3,16). This dose of haloperidol was chosen to produce a moderate degree of catalepsy so that inhibition or potentiation of catalepsy could

be detected (1,16). The phenomenon was measured as the time the animal maintained an imposed position with both front limbs extended and resting on a 3-cm high wood bar (0.9 cm in diameter). The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. A cut-off time of 720 s was used. The animals were returned to their home cages between determinations.

Statistical analysis

Data are reported as means ± SEM for 10 mice per group. For statistical purposes, the durations of catalepsy were transformed to logarithmic (*ln* x+1) values in order to normalize the data. Significant differences were initially assessed by two-way ANOVA with repeated measures (treatment and time as factors); if significance was detected for treatment x time interactions, one-way ANOVA with repeated measures followed by the two-tailed Dunnett test was performed for each time point to determine differences between control and other experimental groups. The level of significance was set at $P<0.05$.

Results

Effects of isosorbide dinitrate on neuroleptic-induced catalepsy

ISD (5-50 mg/kg) caused a dose-dependent attenuation of neuroleptic-induced catalepsy in male mice (Figure 1A). The maximal effect obtained was 64% inhibition, observed at 120 min after haloperidol. In contrast, for female mice, the doses of 5 and 20 mg/kg of ISD failed to modify the duration of catalepsy. In the females, only at the highest dose (50 mg/kg) did ISD cause an attenuation of catalepsy, which was mild (45% inhibition) and short lasting, since it attained statistical significance only at 90 min after haloperidol (Figure 1B).

Effects of linsidomine on neuroleptic-induced catalepsy

In contrast to male mice, in which the higher dose of SIN-1 significantly inhibited neuroleptic-induced catalepsy by about 60% (Figure 2A), the NO donor did not significantly modify the duration of catalepsy in the females (Figure 2B). In six separate male mice (data not shown), which received 10 mg/kg SIN-1 at 110 min after haloperidol (instead of 30 min before), the NO donor caused a short-lasting but statistically significant attenuation of catalepsy.

Effects of SNAP on neuroleptic-induced catalepsy

Pretreatment of male mice with SNAP (20 mg/kg) caused a short-lasting but statistically significant inhibition (about 44%) of

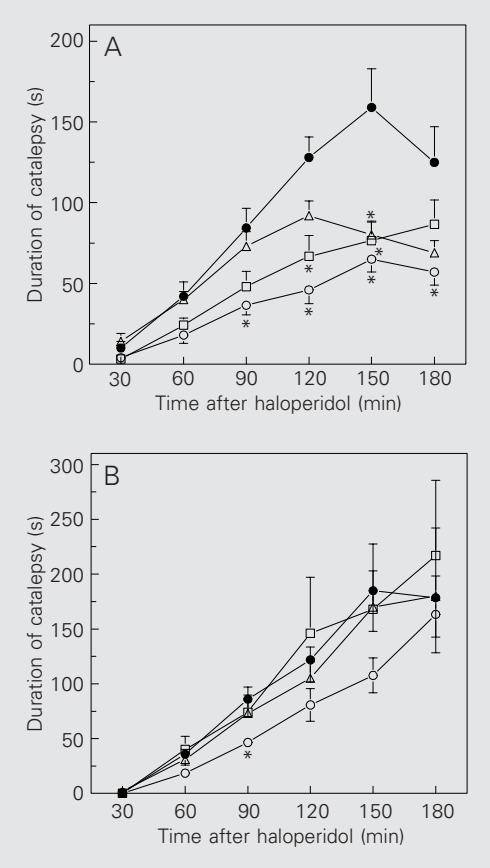

Figure 1. Effects of isosorbide dinitrate (ISD) on neuroleptic-induced catalepsy in male (A) and female (B) mice. ISD, or appropriate vehicle as control, was injected *ip* 30 min before haloperidol (1 mg/kg, *ip*). Data are reported as means ± SEM, N = 10 mice per group. Filled circles, vehicle; triangles, 5 mg/kg ISD; squares, 20 mg/kg ISD; open circles, 50 mg/kg ISD. *P<0.05 compared to vehicle (Dunnett test).

haloperidol-induced catalepsy (Figure 3A), while no significant effect was demonstrable on female animals (Figure 3B).

Discussion

The present data show that drugs able to release NO donors attenuate haloperidol-induced catalepsy in mice. Therefore, this confirms and extends two previous studies showing that such drugs inhibit neuroleptic catalepsy in male rats (15) and mice (16), basically agreeing with the findings that NO facilitates central dopaminergic transmission (10-12). In addition, the present results suggest the existence of gender-related differences in the effects of NO donors (and presumably also of NO) on the nigrostriatal dopaminergic pathway of mice.

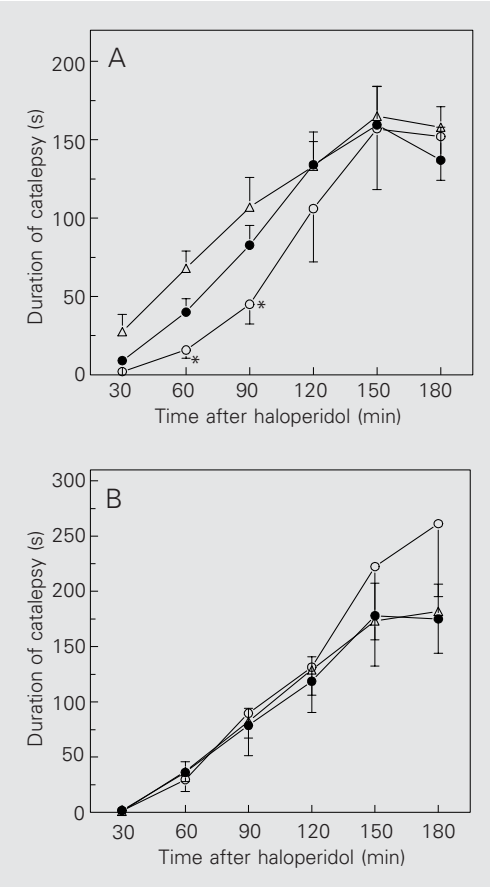

Figure 2. Effects of linsidomine (SIN-1) on neuroleptic-induced catalepsy in male (A) and female (B) mice. SIN-1, or appropriate vehicle as control, was injected *ip* 30 min before haloperidol (1 mg/kg, *ip*). Data are reported as means ± SEM, N = 10 mice per group. Filled circles, vehicle; triangles, 10 mg/kg SIN-1; open circles, 50 mg/kg SIN-1. *P<0.05 compared to vehicle (Dunnett test).

Mechanisms of action and central effects of nitric oxide donors

One possible criticism about our experimental design is that peripheral administration of NO donors does not guarantee the manifestation of a central effect. Therefore, in order to justify our protocol, including the use of three chemically different agents, we would like to briefly discuss some aspects of the pharmacology of the drugs. For instance, organic nitrates (e.g., ISD) and SNAP require *in vivo* transformations to release NO (25). The organic nitrates react with certain endogenous thiols, while SNAP, which is a stable analogue of endogenous S-nitroso compounds, does not need this step to release NO. SIN-1 can release NO directly (no co-factor required) in a complex nonenzymatic way (25). There are also pharmacokinetic differences among NO donors, mainly related to their different lipophilicity (6,25,26). More importantly, in addition to their peripheral vasodilating and antiplatelet effects, there is evidence that most NO-donating compounds can cross the blood-brain barrier after peripheral administration (27-29), a fact that may explain their central effects, including the interference with neuroleptic-induced catalepsy (16). As an example of the complexity of the CNS pharmacology of NO donors, it should be mentioned that peripherally administered sodium nitroprusside, a distinct NO donor, was recently shown to induce catalepsy in male mice, possibly via adenosine release (29).

Sex steroids and the basal ganglia

Our results suggest that, at least in mice, the nigrostriatal pathway of females is less affected by the nitrergic modulation, since a small attenuation of catalepsy was obtained under some circumstances (Figure 1B). Several studies have suggested that gonadal hormones (mainly estrogens) modulate not only dopaminergic but also nitrergic and seroto-

nergic systems (17,18,30,31). Therefore, we may speculate that the reason(s) for the quantitative dimorphism shown here may be related to the action of endogenous estrogens on the extrapyramidal system.

Nitric oxide and dopaminergic transmission

The anatomical association of dopamine neurons and NOS-containing (i.e., nitrergic) interneurons in the rat striatum has been previously described (32). It has been shown that NO released in the striatum is able to control the neuronal activity of midbrain dopamine neurons (12). It seems that, in most cases, NO increases the activity of dopamine neurons (11,12). These latter results, however, were obtained on resting dopamine neurons, whereas after haloperidol administration an excitation of these cells occurs (33). For this reason, the attenuation of catalepsy described here could be due to interference of NO with other neurotransmitter systems and/or with the striatal neurons located post-synaptically with respect to dopaminergic terminals. Concerning the former possibility, it has been shown that NO is able to modulate striatal release of serotonin, glutamate and GABA (9). It has been demonstrated in male mice that NOS inhibitors are able to induce catalepsy and also to potentiate neuroleptic-induced catalepsy (34), an effect that seems to involve serotonin receptors (35). To further emphasize the complexity of this topic, it should be mentioned that, under some circumstances, endogenous NO can decrease the release of dopamine (13,14). Nevertheless, the precise mechanisms involved in the modulation of dopamine release by NO (and NO donors) remain to be determined.

Influence of gender on central nitrergic transmission

There is some evidence that nitrergic activity in some areas of the CNS (e.g., cortex, hippocampus) is higher in male than in female rats (31). However, similar studies in mice, especially involving the striatum, are lacking. It should be mentioned that the literature on this topic is rather confusing; for instance, studies focusing on the neuroendocrine system have shown that the activity of neuronal NOS is increased by estrogen (36) and decreased by androgens (37). In any case, if these influences were present at the level of the nigrostriatal system, they could possibly account for the differential sensitivity of female mice to the 'anticataleptic' effect of NO donors.

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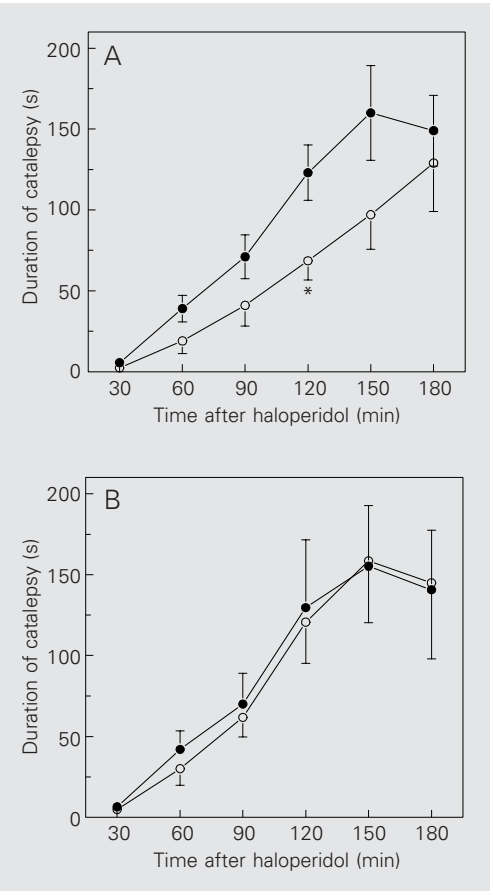

Figure 3. Effects of S-nitroso-N-penicillamine (SNAP) on neuroleptic-induced catalepsy in male (A) and female (B) mice. SNAP, or vehicle as control, was injected *ip* 30 min before haloperidol (1 mg/kg, *ip*). Data are reported as means ± SEM, N = 10 mice per group. Filled circles, vehicle; open circles, 20 mg/kg SNAP. *P<0.05 compared to vehicle (Dunnett test).

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