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Evidence of interaction between fluoxetine and isosorbide dinitrate on neuroleptic-induced catalepsy in mice

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Abstract

Drugs which influence 5-HTergic mechanisms can modify neuroleptic-induced catalepsy (NC) in rodents, a phenomenon produced by striatal dopamine (DA) receptor blockade. Previous research also suggests a role for endogenous nitric oxide (NO) in the modulation of striatal DAergic neurotransmission; in addition, NO seems to play a role in the 5-HT reuptake mechanism. It is known that clomipramine potentiates NC in mice, but the reported effects of selective 5-HT reuptake inhibitors (SSRIs) in this model are rather contradictory. We then decided to re-address this issue, investigating the effect of fluoxetine (FX), an SSRI, on NC. In view of the ubiquitous role of NO as a central neuromodulator, we also studied the effect of isosorbide dinitrate (ID), a centrally active NO donor, and how both drugs interact to affect the phenomenon of NC. Catalepsy was induced in male albino mice with haloperidol (H; 1 mg/kg, *ip*) and measured at 30-min interval by means of a bar test. Drugs (FX, ID and FX + ID) or saline (controls) were injected *ip* 30 min before H, with each animal used only once. FX (5 mg/kg) significantly reduced NC, with maximal attenuation (about 74%) occurring at 150 min after H. ID (5 mg/kg) also inhibited NC (150 min: 62% attenuation). The combined drugs (FX + ID group), however, caused a great potentiation of NC (4.7-fold at its maximum, at 90 min). The effect observed with ID is compatible with the hypothesis that NO increases DA release in the striatum. The attenuation of NC observed with FX may be due to a preferential net effect on the raphe somatodendritic synapse, where inhibitory 5-HT_{1A} autoreceptors are operative. The enhancement of NC caused by combined administration of FX and ID suggests the presence of a pharmacodynamic interaction, whose mechanism, still unclear, may be related to a decrease in striatal DA release.

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- Serotonin reuptake inhibitors
- Dopaminergic transmission

Administration of typical neuroleptics (e.g., haloperidol, fluphenazine) to rats and mice can induce catalepsy, a state of abnormal postural immobility which is mainly produced by blockade of postsynaptic striatal dopamine D_1 and D_2 receptors (1). This experimental phenomenon is a useful method to evaluate the propensity of antipsychotic agents to exert extrapyramidal side effects, since the motor components of the 'neuroleptic syndrome' in patients (induced by therapeutic doses of neuroleptic drugs) are

believed to be its clinical correlate. In addition, neuroleptic-induced catalepsy (NC) also continues to be a robust behavioral method for studying central dopaminergic function and its modulation by cholinergic, serotonergic and other neurotransmitter systems (2-7). For instance, we previously reported that clomipramine, a non-selective serotonin (5-HT) reuptake inhibitor, potentiates NC in mice (4). Since there is a tonic 5-HTergic inhibition of dopamine (DA) release (5,6), clomipramine could be acting by increasing this inhibition, thus causing a reduction in DA release, which increases catalepsy. However, the effects of selective 5-HT reuptake inhibitors (SSRIs) on the levels of DA in the striatum are rather contradictory (8). We then decided to re-address this issue, investigating the effect of fluoxetine (FX), an SSRI, on neuroleptic catalepsy. It is also known that endogenous nitric oxide (NO) can act as a neuromodulator on several neurotransmitter systems (see 9), including the 5-HT transport mechanism (10). For this reason, we also studied the effect of isosorbide dinitrate, a therapeutically used NO donor (11), and how both drugs interact to affect the phenomenon of NC.

Experiments were performed on male adult albino mice weighing 26-36 g. Animals were housed individually in Perspex cages (20 x 18 x 13 cm) 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. Catalepsy was induced with haloperidol (H; 1 mg/kg, *ip*) and determined at 30-min intervals by means of a standard bar test (7). This dose of H was chosen to produce a moderate degree of catalepsy so that inhibition or potentiation of catalepsy could be detected (5,7). 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 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. The animals were returned to their home cages between determinations. Drugs used were haloperidol (Haldol®, Janssen, São Paulo, Brazil), fluoxetine (a donation from Eli Lilly, São Paulo, Brazil) and isosorbide dinitrate (Sigma Chemical Co., St. Louis, MO). Haloperidol was diluted from ampoules with saline. Fluoxetine (FX; 5 mg/kg) and isosorbide dinitrate (ID; 5 mg/kg), dissolved by sonication in saline, were injected intraperitoneally with a 27.5-G needle, in a volume of 3.2-3.5 ml/kg body weight, 30 min before H. The doses were chosen according to those commonly used in the literature. Saline (0.9%, w/v, 0.13 ml/animal, *ip*) was used as control (CL). Thus, experimental groups were FX, ID, FX + ID and CL. Data are reported as means ± SEM for 9 mice per group and were analyzed by two-way ANOVA (groups x time) with repeated measures, followed by one-way ANOVA for each time. Dunnett's multiple comparison test was then used to determine statistical differences between CL and other experimental groups, for each time. Differences between means were considered significant when $P<0.05$.

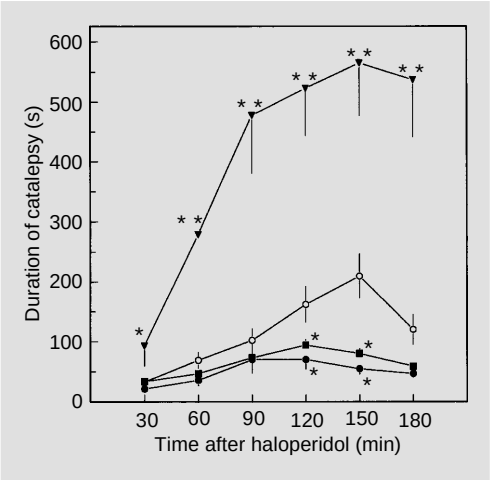

Figure 1 - Effects of fluoxetine (FX), isosorbide dinitrate (ID) and combined FX + ID on neuroleptic-induced catalepsy in mice. Catalepsy was induced by haloperidol (H; 1 mg/kg, *ip*). The drugs tested, or saline for the control group, were injected *ip* 30 min before H. Groups: control (open circles), 5 mg/kg FX (filled circles), 5 mg/kg ID (squares) and 5 mg/kg FX + 5 mg/kg ID (inverted triangles). Data are reported as means ± SEM for 9 mice per group. *P<0.05 and **P<0.01 compared to control group (Dunnett's *t*-test after ANOVA).

The results (Figure 1) showed that FX (5 mg/kg) significantly reduced NC, with maximal attenuation (about 74%) occurring at

150 min after H. The NO donor ID (5 mg/kg) also inhibited NC (150 min: 62% attenuation). In both cases the attenuation was short-lasting, since there was no statistically significant difference from the control at 180 min. Surprisingly, combined drugs (FX + ID group) caused an impressive potentiation of catalepsy (4.68-fold at its maximum, at 90 min after H). In six separate experiments, FX or ID alone (up to 15 mg/kg *ip*) did not induce a cataleptic state in the mice (data not shown).

It is now accepted that organic nitrates such as glyceryl trinitrate and ID, which act through NO release (11), can easily cross the blood-brain barrier after peripheral administration (12,13). It was also reported that locally produced NO, acting via cGMP, increases 5-HT transport into presynaptic terminals in the brain (10). Taking into account those observations and the existence of a tonic 5-HTergic inhibition of DA release (5,6), we can hypothesize that systemic administration of ID releases NO in the striatum (area involved in the extrapyramidal motor behavior) and that NO, through the increment in 5-HT reuptake, reduces the synaptic concentration of 5-HT, with a subsequent increase of DA release, which obviously reduces NC. Phenomenologically speaking, if an increase in central production of NO reduces NC, then inhibition of its formation should cause the opposite effect. In fact, it has been recently shown that L-NOARG, an inhibitor of NO synthase, induced catalepsy in mice, which was reversed by L-arginine (14).

According to the above model, we should expect that FX, an SSRI drug, should reduce DA release and then potentiate NC. However, we found that FX (5 mg/kg) attenuated this behavior. Since potentiation of NC with clomipramine in mice was found at a dose of 5 mg/kg (4), it seems that the 'failure' of FX to increase NC is not due to an insufficient dose. A possible explanation for this apparent contradiction is that, under the present conditions, FX increased the extracellular levels of 5-HT in the raphe nuclei, which activated somatodendritic 5-HT_{1A} autoreceptors, causing a reduction in the tonic 5-HTergic inhibition of DA release (15). However, since serum levels of haloperidol were not measured before and after the drug treatments, the possibility of a pharmakokinetic interaction among the drugs cannot be discarded.

The potentiation of NC observed when FX was combined with ID is quite intriguing. To better understand this issue, a crucial question is: how do NO and FX interfere with DA release in the striatum? The answer can be rather complex: it seems that, depending on the experimental conditions, FX (8) and NO (16) can either increase or reduce the levels of DA in the striatum. In the present conditions, we may speculate that, in some way, the release of NO in the striatum (caused by ID) enhanced the inhibitory effect of FX on DA release, surpassing its initial effect on the somatodendritic 5-HT_{1A} autoreceptors. However, the precise mechanism responsible for such interaction remains to be determined. Since both drugs are extensively used in clinical practice, and considering that there is already evidence of parkinsonism and other extrapyramidal side effects following FX in patients (17), the possibility of a clinically relevant interaction between these drugs cannot be discarded and deserves further investigation.

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