TIAPRIDE-INDUCED CATALEPSY IS POTENTIATED BY GAMMA-HYDOXYBUTYRIC ACID ADMINISTRATION

JOSE FRANCISCO NAVARRO, CARMEN PEDRAZA, MERCEDES MARTIN, JUAN M. MANZANEQUE, GUADALUPE DAVILA and ENRIQUE MALDONADO

Area de Psicobiología, Facultad de Psicología, Universidad de Málaga, Málaga, Spain

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Abstract


1. The effect of administration of gammahydroxybutyrate (GHB) and tiapride, either alone or in combination, on catalepsy behavior was examined in male mice.
2. Catalepsy was measured by bar and grid tests. Two successive evaluations were carried out 30 and 60 min after injections.
3. Tiapride (175 and 200 mg/kg) and gammahydroxybutyrate (200 mg/kg) provoked an increase of catalepsy scores, exhibiting different time courses. GHB produced a marked but short lasting catalepsy with a peak of action at 30 min, while tiapride produced a catalepsy state with a peak of action at 60 min.
4. Tiapride-induced catalepsy was potentiated by gammahydroxybutyrate administration at 30 min (bar test) and 60 min (bar and grid tests).
5. These results underline the view that GHB interacts with central dopamine D2 transmission.

Keywords: catalepsy, dopamine gammahydroxybutyric acid, tiapride

Abbreviations: gamma-aminobutyric acid (GABA), Gammahydroxybutyric acid (GHB)
Introduction

Gammahydroxybutyric acid (GHB) is a metabolite of gamma-aminobutyric acid (GABA) which is able to traverse the blood-brain barrier after peripheral administration (Tunnicliff, 1992; Cash, 1994). This substance is present in micromolar quantities in all brain regions investigated as well as in several peripheral organs (Maître, 1997). In rodents, GHB administration produces a wide range of pharmacological effects, including sedation (Laborit, 1964), hypothermia (Kaufman et al, 1990), a decrease of aggression (Navarro and Pedraza, 1996) and bilaterally synchronous spike wave discharges associated with behavioral changes resembling those of petit mal or generalized absence seizures (Banerjee et al, 1993). The existence of specific brain receptor as well as brain mechanisms for synthesis, release and uptake provide a marked support to the hypothesis that GHB may act as a neuromodulator on specific neuronal populations (Vayer et al, 1987; Hechler et al, 1992; Cash, 1994). In fact, it has been proposed that GHB might exert a regulatory influence on different neurotransmitter systems, especially on dopaminergic neurons (Diana et al, 1991; Hechler et al, 1993).

There is experimental evidence suggesting the existence of a central interaction between GHB and dopamine receptor. Thus, the GHB system appears to exert a neuromodulatory action on the dopaminergic nigrostriatal and mesolimbic systems via specific GHB receptors. In this sense, several studies have focused on the role of GHB as a regulator of dopaminergic activity in the nigrostriatal pathway. For instance, Hechler et al. (1992) have documented the presence of GHB high-affinity binding sites in substantia nigra. This result could support the GHB action of dopaminergic activity. These authors have also suggested that GHB participates in the regulation of global dopaminergic activity in the brain. Likewise, Hechler et al. (1993) have demonstrated that GHB ligands exhibit an antidopaminergic activity using neuropharmacological tests which can usually predict an anti-D₂ profile, such as apomorphine-induced stereotypes and hypothermia. GHB and analogs of GHB also induce catalepsy in rodents which appears to be dose-dependent. This state of immobility has been clearly described in rats (Snead and Bearden, 1980; Hechler et al, 1993) and mice (Navarro et al, 1996) using different types of behavioral tests. Moreover, GHB-induced catalepsy is reduced in a dose-dependent manner by administration of NCS-382, a GHB receptor antagonist (Schmidt et al, 1991). The regulating properties of the endogenous GHB system on the dopaminergic pathways are a cause for the recent interest in synthetic ligands acting specifically
Catalepsy induced by tiapride and GHB coadministration

at GHB receptors and devoid of any role as metabolic precursor of GABA in brain (Maitre, 1997).

Tiapride is a benzamide with a specific antagonism of D\textsubscript{2} receptors and without affinity for other receptors (Peters and Faulds, 1994). This neuroleptic drug possesses antiaggressive (Navarro and Manzaneque, 1997) and anxiolytic properties (Costall et al, 1987). Furthermore, tiapride is also able to induce catalepsy in mice (Navarro et al, 1997), especially at high doses. From a clinical point of view, it is considered as an alternative treatment to benzodiazepines or chlormethiazole in patients at risk of severe alcohol withdrawal, being recommended as an anxiolytic drug in elderly patients (Steele et al, 1993).

From these experimental data, it can be predicted that administration of GHB will perhaps potentiate the neuroleptic-induced catalepsy. For this purpose the authors compared the effects of GHB (200 mg/kg) and tiapride (175 and 200 mg/kg), either alone or in combination, on catalepsy evaluated by the bar and grid tests in male mice.

**Methods**

**Animals**

300 OF.1 strain albino male mice weighing 25-30 g were obtained from "Servicio de Animales de Laboratorio", Granada, Spain. Animals arrived in the laboratory at 42 days of age and were housed in transparent plastic cages (24 x 13.5 x 13 cm) in groups of five under standardized lighting conditions (light: 20:00-8:00), a constant temperature and laboratory chow and tap water available *ad libitum*. All animals underwent a seven-day adaptation period to the laboratory before experimental treatments began.

**Drug Administration**

GHB and tiapride (Sigma Laboratories, Madrid, Spain) were diluted in saline to provide appropriate doses for injection (i.p). Animals were assigned to six different experimental groups (24-26 mice per group) receiving:

1. GHB (200 mg/kg) + saline
2. Tiapride (175 mg/kg) + saline
3. Tiapride (200 mg/kg) + saline
4. GHB (200 mg/kg) + tiapride (175 mg/kg)
5. GHB (200 mg/kg) + tiapride (200 mg/kg)
6. Saline group. Mice were treated with two injections of 0.9 % NaCl.

Experimental Procedure
Catalepsy was measured by means of the bar and grid tests. In the bar test, an aluminium bar of 5 mm in diameter was placed 4 cm above the floor. Animals’ forepaws were gently put on the bar and the time it took the animal to place at least one paw on the floor was measured. If 1 min elapsed without movement, the test was interrupted.

In the grid test, mice were placed head down, approximately midway on a wood-framed (40 cm x 30 cm) wire grid at an angle of 45 degrees with the table surface. Under these conditions, normal undrugged mice quickly scurried down usually within 1-2 sec. Testing was terminated when any limb moved or when 1 min had passed.

Successive behavioral evaluations of catalepsy were carried out 30 and 60 minutes after administration of drugs. Between determinations, the mice were kept in their home cages. Individual animals were tested in a random order.

Data Analysis
Nonparametric Kruskal-Wallis were used to assess the variance of catalepsy over different treatment groups. Subsequently, appropriate paired comparisons were carried out using Mann-Whitney U-tests to contrast the behavior in the different treatment groups. A value of p<0.05 was considered to be statistically significant.

Results
Table 1 illustrates medians (with ranges) of catalepsy scores shown by animals after administration of GHB, tiapride or GHB + tiapride in both catalepsy tests.
Kruskal-Wallis analysis showed that there was significant variance in catalepsy scores over different treatment groups (p<0.001).

When tested at 30 min, paired comparisons revealed that catalepsy scores increased significantly following acute injection of GHB (p<0.01) in both bar and grid tests, as compared with the control group. Tiapride (200 mg/kg) increased significantly catalepsy scores only in the bar test (p<0.05). Both groups of animals treated with GHB+tiapride also showed a significant increase in catalepsy scores, in comparison with saline group (p<0.001), as well as in comparison with tiapride and GHB groups (p<0.001).

When tested at 60 min, paired comparisons indicated that catalepsy scores (in bar and grid tests) increased significantly after injection of both doses of tiapride, as compared with saline group (p<0.01). Likewise, both groups of mice treated with GHB+tiapride showed a significant increase in catalepsy scores, in comparison with saline group (p<0.01), as well as in comparison with animals receiving only GHB (p<0.01). Furthermore, when tested in grid test mice treated with GHB+tiapride exhibited significantly more catalepsy than mice treated with tiapride+saline and GHB+saline (p<0.01).

No significant differences were found between mice treated with 175 and 200 mg/kg of tiapride in both behavioural tests.

Discussion

Tiapride (175 and 200 mg/kg) increased catalepsy of mice, especially at test carried out 60 min after injection. Therefore, in agreement with previous studies, it has been demonstrated that D2 receptors are clearly involved in the mediation of catalepsy (Navarro et al, 1997). On the other hand, GHB (200 mg/kg) also provoked an increase of catalepsy scores at 30 min after injection. This result is also in concordance with recent studies in which a dose-dependent effect on catalepsy was described after GHB administration (Navarro et al, 1996). GHB and tiapride were found to display a different time course in the induction of catalepsy. GHB produced a marked but short lasting catalepsy with a peak of action within 30 min, while tiapride produced a catalepsy state with a peak of action at 60 min. Muscular tone did not seem to be different in all groups examined.
Table 1

Median Scores of Catalepsy (with ranges) after Treatment with GHB (200 mg/kg), Tiapride (T1: 175 mg/kg; T2: 200 mg/kg), GHB+T1 and GHB+T2. Tests at 30 and 60 min after Injection.

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Differs from saline group on Mann-Whiney U-tests, *p<0.001; **p<0.01; ***p<0.05
This cataleptogenic effect of GHB can indicate an antidopaminergic and neuroleptic-like activity of this substance and underlies the potential antipsychotic activity of GHB agonists, opening up the possibility that GHB agonists (or analogs) could be used as antipsychotics. Nevertheless, GHB has already been tested without success on schizophrenic patients (Levy et al., 1982; Schuls et al., 1981).

GHB and opiates induce a number of similar effects, including catalepsy. Therefore, the GHB-induced cataleptic effect could be produced by increased release of opioid substances, since it has been reported that naloxone and naltrexone abolish the cataleptic effect of GHB, suggesting that GHB might produce some of its central actions by acting as an opiate agonist (Schmidt et al., 1991). However, Feigenbaum and Simantov (1996) have recently examined the effects of GHB on mu, delta and kappa-opioid receptor binding and their findings indicated that GHB was clearly inactive at every dose examined and, consequently, it was not a direct opiate receptor agonist.

Thirty min after injection, coadministration of GHB + tiapride produced a clear potentiation of their actions on catalepsy in bar and grid tests. As Table 1 shows, catalepsy scores were markedly increased in animals treated with both drugs. In fact, the induction of catalepsy following coadministration of GHB + tiapride was significantly higher than the sum of catalepsy scores observed after administration of GHB and tiapride separately. This potentiation of tiapride-induced catalepsy was also evident 60 min after injection when mice were tested in the grid test.

Catalepsy is considered as an appropriate animal model for extrapyramidal side-effects in humans receiving antipsychotics (Sanberg et al., 1988). Therefore, from a clinical point of view, our data suggest that in patients treated simultaneously with neuroleptic drugs and GHB a careful caution should be required because of a possible increase in the frequency of extrapyramidal effects. Although the therapeutic utility of GHB is still limited, it has been successfully used in the treatment of withdrawal syndrome produced by alcohol and opiates and to alleviate symptoms associated to narcolepsy (Cash, 1994; Maitre, 1997). In any case, the number of patients simultaneously treated with both drugs will be presumably reduced.
Conclusion

Tiapride-induced catalepsy was clearly potentiated by GHB administration (200 mg/kg). These results are in agreement with the view that GHR receptors interact with central dopamine D2 transmission. Further studies are needed to clarify the interaction mechanisms between GHBergic and dopaminergic systems.

References


Inquiries and reprint requests should be addressed to:

Dr. José Francisco Navarro
Area de Psicobiología, Facultad de Psicología, Universidad de Málaga
Campus de Teatinos, 29071 Málaga, Spain. E-mail: navahuma@uma.es