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Anxiogenic-like effects of gamma-hydroxybutyric acid (GHB) in mice tested in the light-dark box

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Gamma-hydroxybutyric acid (GHB) is a drug with abuse potential, popularly known as «liquid ecstasy». It is an endogenous compound of the mammalian brain which satisfies many of the criteria for consideration as a neurotransmitter or neuromodulator. In this study, the effects of acute administration of GHB (40, 80 and 120 mg/kg, ip) on anxiety, tested in the light/dark box, were examined in male mice of the OF.1 strain. Likewise, we compared the behavioural profile of GHB with that induced by mCPP (1 mg/kg, ip), a compound with known anxiogenic actions. GHB-treated mice spent notably less time in the lit area (40 and 80 mg/kg) and more time in the dark area (all doses), whereas the total number of 'rearings', transitions and latency were significantly reduced. A very similar behavioural profile was observed in mCPP-treated animals. Overall, these findings indicate that GHB exhibits anxiogenic-like properties in male mice. It is suggested that the anxiogenic effects of GHB could be related to its ability to modulate GABA and/or dopaminergic receptors.

Efectos ansiogénicos del ácido gamma-hidroxibutírico (GHB) en ratones evaluados en el test del «light-dark». El ácido gamma-hidroxibutírico es una droga con potencial de abuso popularmente conocida como «éxtasis líquido». Es un compuesto endógeno presente en el cerebro de mamíferos que cumple muchos de los criterios para ser considerado como neurotransmisor o neuromodulador. En este estudio examinamos el efecto de la administración aguda de GHB (40, 80 y 120 mg/kg, ip) sobre la ansiedad evaluada en el test de «light-dark» en ratones machos de la cepa OF.1. Asimismo, comparamos el perfil conductual del GHB con el inducido por mCPP (1 mg/kg, ip), un compuesto con conocidos efectos ansiogénicos. Los animales tratados con GHB pasaron significativamente menos tiempo en el compartimento iluminado (40 y 80 mg/kg) y más tiempo en el compartimento oscuro (con todas las dosis), mostrando además una reducción significativa del número total de «rearings», transiciones y latencia. En los animales que recibieron mCPP se observó un perfil conductual muy similar. En conjunto, estos resultados indican que el GHB exhibe propiedades ansiogénicas en ratones machos. Se sugiere que los efectos ansiogénicos del GHB podrían estar relacionados con su capacidad para modular los receptores GABAérgicos y/o dopaminérgicos.

Gamma-hydroxybutyric acid (GHB) is an endogenous constituent of the mammalian brain, with GABA being its major precursor, which readily and rapidly crosses the blood brain barrier. Nevertheless, GHB is not merely a by-product of GABA metabolism. It satisfies many of the criteria for consideration as a neurotransmitter at the central nervous system. In fact, GHB has its own high and low affinity binding sites in different species, also modulating dopaminergic and GABAergic activity (Maitre, 1997; Crunelli, Emri, & Leresche, 2006; García, Pedraza, & Navarro, 2006).

GHB has gained notoriety as a drug of abuse (popularly known as «liquid ecstasy»). Typically, it is ingested with the intention of inducing euphoria and relaxation during music festivals or «rave parties». However, numerous cases of GHB intoxication have been documented, and symptoms of tolerance, dependence, and withdrawal have been reported (Drasbek, Christensen, & Jensen, 2006). Furthermore, GHB has earned a reputation as a «date rape» drug, since its color- and odorless appearance goes unnoticed in the drinks of the victims, thereby leading to sedation by GHB as a prelude to the sexual assault (Varela, Nogue, Oros, & Miro, 2004; Crunelli et al., 2006).

Exogenously administered GHB induces a wide range of pharmacological effects in rodents, including sedation, catalepsy (Navarro et al., 1996; 1998), a decrease of aggression (Navarro & Pedraza, 1996; Pedraza, Dávila, Martín-López, & Navarro, 2007), memory impairment (Sircar & Basak, 2004; García, Pedraza, Arias, & Navarro, 2006), seizures or an increase in sleep stages 3 and 4 (Crunelli et al., 2006). Schmitt-Mutter et al. (1998) reported an anxiolityc-like effect of GHB in male rats tested in the «elevated plus maze». However, preliminary results in mice using the same procedure suggested an anxiogenic-like action of GHB (Navarro et al., 2000). In this study, an attempt was made to clarify the anxiolytic/anxiogenic profile of GHB (40, 80 and 120 mg/kg,

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ip) by examining its effects of behaviour of male mice in the light-dark box, a paradigm routinely used to study anxiety-related behaviour in mouse (Hascoët, Bourin, & Dhnonnchadha, 2001). Likewise, we also analyzed the effects of mCPP (1 mg/kg) on anxiety, a compound with an anxiogenic action demonstrated in rodents (Griebel, Misslin, Pawlowski, & Vogel, 1991).

Materials and methods

Animals

55 albino male mice of the OF.1 strain weighing 25-30 g were used. Animals were housed in groups of five in plastic cages (24×13.5×13 cm) under standardized lighting conditions (white lights on 20:00-8:00), a constant temperature (20 ºC) and food and tap water available ad libitum, except during behavioural tests. Cage maintenance was undertaken twice weekly, but never on the day of testing. Mice were housed 7 days before the experiment.

This experiment was carried out in accordance with the guiding principles for care and use of Laboratory Animals approved by the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Drug administration

Five groups of mice were used. Animals were randomly allocated to one control group (n= 11) receiving physiological saline and three experimental groups (n= 10-12) receiving GHB injections. An additional control group of animals treated with mCPP (1 mg/kg; n= 11) was used. GHB (Sigma Laboratories) was diluted in physiological saline to provide appropriate doses for injections and administered in three doses: 40, 80 and 120 mg/kg. These doses were selected on the basis of previous experiments carried out in our laboratory with mice. Drugs or saline were injected intraperitoneally in a volume of 10 ml/kg. Tests were performed 30 min after injections.

Apparatus

The light-dark aversion test was used according to Belzung et al. (1987) procedure. The apparatus consisted of two glass boxes (27×21×24 cm) with an interconnecting grey plastic tunnel (7x10 cm). One of the boxes was painted in black, being weakly lit by a red 25-W bulb (0 lux). The other box was lit by a 60-W desk lamp (400 lux) placed 30 cm above the box, which provided the only laboratory illumination. The floor was lined into 9 cm squares. The apparatus was positioned on a bench 70 cm above the floor.

Experimental procedure

The test was performed in a quiet, darkened room. Tests were carried out between 09:00 and 15:00 h. After injection (saline or treatment) mice were placed in their home cage. At the beginning of the test, naïve mice were placed individually in the middle of the light area facing away from the opening, and were videotaped during 5 min using a Sony V8 camera. The following parameters were recorded (Maldonado and Navarro, 2000): (a) number of exploratory rearings in the light and dark sections; (b) number of transitions between the lit and dark areas; (c) time spent in the light and dark areas; (d) latency of the initial movement from the light to the dark area, and (e) motor activity in both areas. A mouse was considered to have entered the new area when all four legs were in this area. None of these animals were used on more than one occasion. The group order was counterbalanced according to a Latin square design. At the conclusion of the test period, mice were returned to their cages, and another animal was placed into the box. The floor of each box was cleaned between sessions. Behavioural analysis was performed by a trained experimenter who was unaware of treatment of the groups.

Data analysis

Nonparametric Kruskal-Wallis tests were used to assess the variance of the behavioural measures over different treatment groups. Subsequently, appropriate paired comparisons were performed using Mann-Whitney U-tests to contrast the parameters in the different treatment groups. The analysis was performed using nonparametric statistics since the criteria for parametric statistics were not met by the data.

Results

Table 1 illustrates medians (with ranges) of the parameters used. Kruskall-Wallis analysis showed that there was significant variance in the parameters of latency, number of transitions, rearings, time in lit and dark areas, and motor activity ($p<0.001$-0.05).

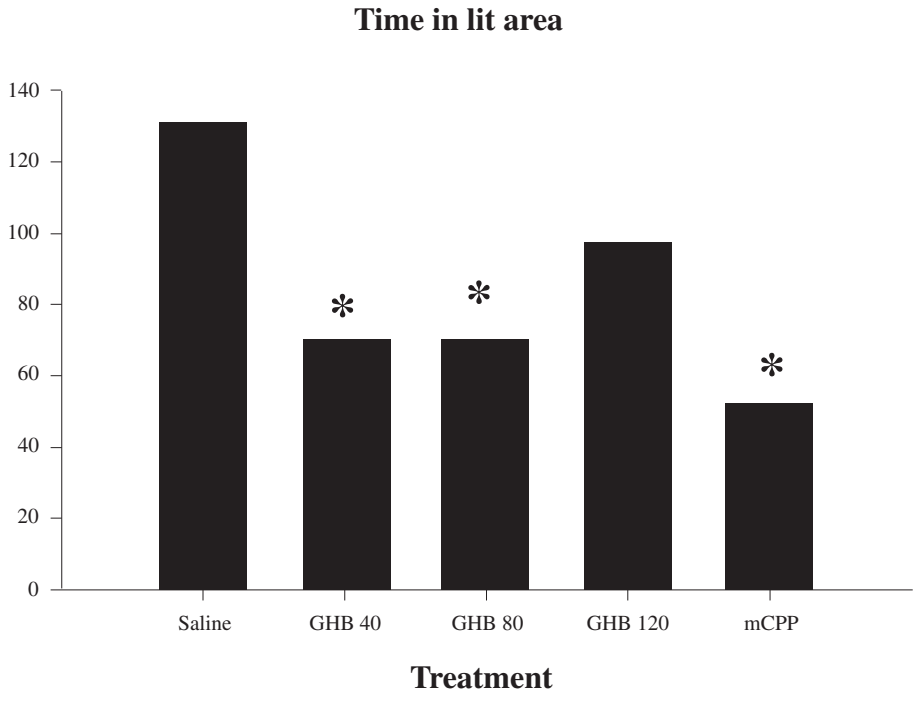
Paired comparisons revealed that GHB (80 and 120 mg/kg) significantly reduced the number of transitions between the lit and dark areas, as compared with the control group ($p<0.01$-0.001). Latency (40 and 80 mg/kg), time spent in lit area (40 and 80 mg/kg), motor activity (120 mg/kg), as well as total number of rearing (80 and 120 mg/kg), rearings in dark area (120 mg/kg) and

Table 1
Effects of GHB on behavioural parameters in the light/dark test in mice (median values with ranges)

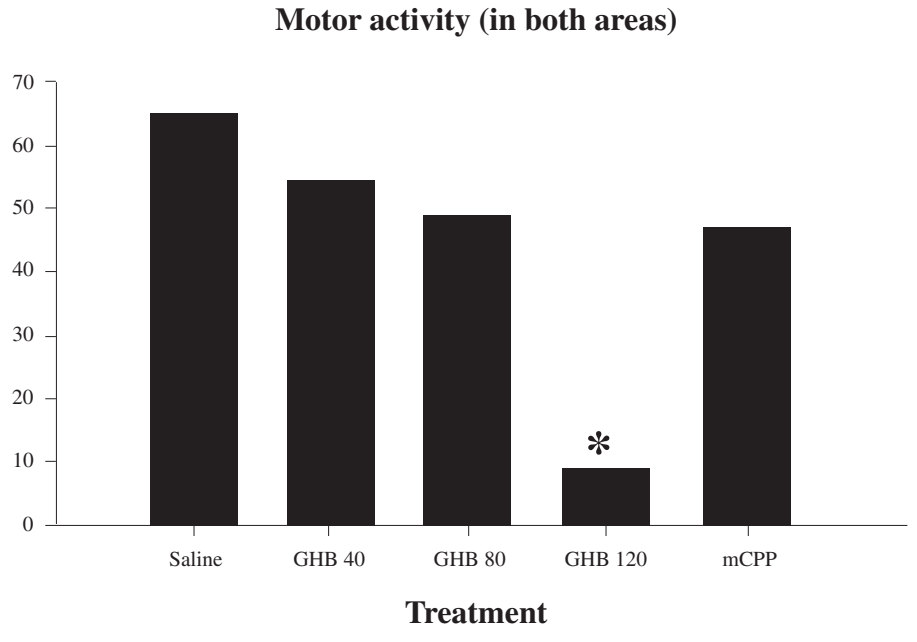
		GHB (mg/kg)			mCPP (mg/kg)
Parameters	**Saline**	**40**	**80**	**120**	**1**
Latency (in sec) [a]	20 (2-123)	5.5* (2-39)	7* (1-87)	25.5 (1-300)	9* (2-30)
Number of transitions [a]	17 (2-31)	11 (1-25)	3* (1-29)	1* (0-25)	7* (1-20)
Rearings (lit area) [a]	10 (0-31)	5.5* (0-23)	0* (0-26)	0* (0-15)	5* (0-22)
Rearings (dark area)[a]	16 (0-20)	15.5 (0-23)	8 (0-25)	3* (0-25)	11 (0-20)
Total number of rearings [a]	26 (0-49)	22 (0-46)	16* (0-28)	4.5* (0-40)	16.5* (0-42)
Time in lit area (in sec) [a]	131 (28-232)	70.5* (2-110)	69* (1-140)	97 (1-300)	52.5* (3-119)
Time in dark area (in sec) [a]	68 (29-174)	132.5* (73-282)	118* (56-293)	109.5* (0-298)	133* (67-279)
Motor activity (sec) (in both areas) [a]	65 (5-85)	54.5 (4-89)	49 (3-105)	9* (0-77)	46.5 (0-63)

Kruskal-Wallis test showed significant variance, [a] $p<0.001$-0.05
Differs from control on Mann-Whitney U-tests, * $p<0.001$-0.05

rearings in lit area (all doses) were also significantly reduced after treatment with GHB, in comparison with the control group ($p<0.05$-0.001). Finally, GHB increased time spent in dark area (all doses) ($p<0.05$-0.001). On the other hand, mCPP (1 mg/kg) significantly reduced latency, number of transitions, rearings and lit area and total number of rearings as well as time in lit area ($p<0.05$-0.001), whereas increased time spent in dark area ($p<0.01$), as compared with the control group.



*** p<0.05-0.001 in comparison with control group**



*** p<0.05-0.001 in comparison with control group**

Figure 1. *Medians (time in sec) in lit area and motor activity (in both areas) in mice treated with saline, GHB (40, 80, 120 mg/kg) or mCPP (1 mg/kg)*

Discussion

The light/dark paradigm is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of the animals, applying mild stressors, i.e. novel environment and light. Thus, in a two compartment box, rodents will prefer the dark area. Drugs with anxiogenic properties should increase the time spent in the dark compartment, also reducing the time spent in the lit compartment. Consequently, transitions between the compartments and time to explore each of them are interpreted as indicators of anxiety and have shown to be sensitive to anxiety-affecting drugs. The Swiss strain of mice has been found a suitable strain to be used in this test (Hascoët et al., 2001; Hascoët & Bourin, 1988; Ohl, 2003).

As Table 1 shows, GHB produced a significant decrease in exploratory activity, in concordance with an anxiogenic-like profile of the drug. In this sense, GHB-treated mice spent significantly less time in the lit area (40 and 80 mg/kg) (see figure 1) as well as more time in the dark area (all doses). Likewise, the total number of 'rearings' and transitions was reduced in mice treated with GHB, as compared with the control group. These effects were selective for 40 and 80 mg/kg of the drug, whereas animals receiving 120 mg/kg showed a marked reduction of motor activity. Therefore, motility was significantly decreased in mice treated with the highest dose of GHB (120 mg/kg) (see figure 1), suggesting that, at this dose, the effects of GHB on anxiety are not specific. This reduction of motor activity has been also described in other studies with male mice (Navarro et al., 1996; 1998). As a positive reference, mCPP (1 mg/kg) was tested in the same experiment. The behavioural profile of GHB (40 and 80 mg/kg) was very similar to that observed after administration of mCPP, a compound with an anxiogenic action repeatedly demonstrated in several animal models of anxiety, including the light-dark test (Griebel et al., 1991).

These results are in concordance with a preliminary study in which GHB also produced anxiogenic-like effects in male mice tested in the elevated plus maze (Navarro et al., 2000), but they differ from Schmidt-Mutter et al. (1998), who found an anxiolytic action of GHB (150 and 250 mg/kg, i.p) in the elevated plus maze in rats, suggesting an interaction of GHB with the GABA-A receptor complex since this action was blocked by the benzodiazepine receptor antagonist, flumazenil. This discrepancy might represent a species difference. In fact, administration of drugs such as MDMA induces usually anxiogenic-like effects in mice (Maldonado & Navarro, 2000; 2001; Navarro & Maldonado, 2002; Navarro et al., 2004), whereas in rats produces anxiolytic or anxiogenic-like effects, dependent on the basal anxiety level or the test situation employed (Green & McGregor, 2002). Likewise, the anxiolytic action of GHB described by Schmidt-Mutter et al. (1998) was evident with higher doses (150 and 250 mg/kg) than those used in our experiment (40 and 80 mg/kg). Another possible explanation for the discrepancy between both studies may be related with the biphasic effects of GHB on GABA synapses (Maitre, 1997; Drasbek et al., 2006): at low doses (40 and 80 mg/kg), GHB exerts a feedback inhibition on GABA synapses via GHB receptors and reduces GABA release (anxiogenic effects), whereas at higher doses (150-250 mg/kg) GHB receptors are down-regulated and high doses of GHB interact with GABA-B receptors, provoking a potentiation of the general GABA tone in brain (anxiolytic effects).

On the other hand, GHB interacts with central D2 receptors (Pedraza et al., 2007). The anxiogenic-like activity found in our study might be mediated by the dose-related biphasic effect on dopamine neurotransmission. Low doses of GHB (40 and 80 mg/kg) would exert an excitatory effect on dopamine release, whereas relatively high doses (150-250 mg/kg) inhibit release (Howard & Feigembaum, 1997; Pedraza et al., 2007). In fact, administration of D2 receptor agonists (such as quinpirole) has been associated with an anxiogenic-like profile in mice (Navarro & Maldonado, 1999) and D2 receptor antagonists (such as tiapride or sulpiride) with an anxiolytic-like action (Barry, Costall, Kelly, & Naylor, 1987).

In conclusion, GHB (40 and 80 mg/kg) produced a selective reduction in exploratory activity of mice (rearings and transitions), also decreasing the time spent in lit area and increasing the time spent in dark area. This behavioural profile found in the light/dark test is indicative of an anxiogenic-like action of GHB at these doses. It is suggested that the anxiogenic effects of GHB could be related to its ability for modulating GABA and/or dopaminergic receptors. Although is difficult to extrapolate to humans the results obtained in mice, our findings suggest that the consumption of GHB at low doses could be associated to an increase of the anxiety. Further studies are needed to clarify our understanding of GHB actions on anxiety.

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