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GHB differentially affects morphine actions on motor activity and social behaviours in male mice

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Abstract

There are several reports suggesting that gamma-hydroxybutyric acid (GHB) influences the endogenous opioid system. The present study aimed to investigate the effects of GHB on motor and social activities and to examine its influence on morphine's actions on these behaviours. In a first experiment, several doses of GHB were studied but only the highest (200 and 400 mg/kg) produced a decrease in spontaneous motor activity measured in an actimeter cage. When hyperactivity induced by injecting 50 mg/kg of morphine was evaluated, all the GHB doses efficiently counteracted this morphine action. Using the paradigm of isolation-induced aggression, administration of 200 mg/kg of GHB significantly decreased threat and attack without impairing motor activity and, in addition, increased time spent in social contact. GHB increased morphine's suppression of threat or nonsocial exploratory behaviours. In conclusion, the interaction between GHB and the opioid systems was confirmed, with the drug having an additive effect on morphine-affected social behaviours but counteracting morphine-induced increases in motor activity.

Keywords: GHB; Morphine; Motor activity; Aggression; Social contacts; Mice

1. Introduction

Gamma-hydroxybutyric acid (GHB) naturally occurs in the brain, with GABA being its major precursor (Maitre, 1997; Nicholson and Balster, 2001). A role for GHB as a neuromodulator or neurotransmitter in the mammalian brain has been suggested (Vayer et al., 1987). Although it mainly affects dopaminergic neurons, it also acts on aminoacidergic synapses and the anterior part of the CNS, such as the striatum or the prefrontal cortex (Maitre, 1997). In rats, it has been detected in the frontal cortex, hippocampus, striatum or substantia nigra, although not in concentrations as high as those found in the human brain (Maitre, 1997). GHB presents two classes of binding sites (high and low affinity). When used at low doses, a specific response mediated only by GHB receptors occurs, but at a higher dosage, a $GABA_b$ response is obtained. This GABAergic response could be induced either by GHBergic control of GABA release or probably by the synthesis of GABA from GHB (Maitre, 1997).

Although GHB was originally used in anaesthesia (Laborit et al., 1962) and in the treatment of narcolepsy (Broughton and Mamelak, 1979), more recently, a role for GHB in drug dependence has been hypothesized. At non-hypnotic doses, it may decrease alcohol craving (Gallimberti et al., 1992) or the withdrawal syndrome in both alcohol (Gallimberti et al., 1989) and heroin addicts (Gallimberti et al., 1993, 1994). Furthermore, an increasing number of reports have indicated the growing popularity of GHB as a recreational drug (Stell and Ryan, 1996).

GHB reportedly has a relationship with the endogenous opioid system. Dynorphine or met-enkephalin are increased after GHB administration in structures such as the striatum or frontal cortex (Lason et al., 1983; Gobaille et al., 1994; Schmidt-Mutter et al., 1999). Although it has been suggested that GHB could mediate some of its effects by potentiating some neural opioid mechanisms (Maitre, 1997), no effects on μ-, δ- and κ-opioid receptors have been detected (Feigenbaum and Simantov, 1996). In addition, GHB and morphine induce a number of similar effects, and it has been suggested that most changes induced by GHB can be mimicked by the opiate agonist (Snead and Bearden, 1980, 1982). Conversely, the opiate antagonist naloxone can reverse many of the effects observed after GHB administration

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(Snead and Bearden, 1980; Vayer et al., 1987; Vayer and Maitre, 1989). As GHB does not seem to act directly on the opiate system, it may induce its effects by functionally altering activity of the dopaminergic pathways (Manier et al., 1991; Tang et al., 1983). After an initial attenuation of dopamine levels (Gessa et al., 1966), GHB enhances tyroxine hydroxylase activity and stimulates dopamine release (Morgenroth et al., 1976; Spano et al., 1971).

The present study aimed to determine the effects of GHB on motor and social activities in mice. The influence of this compound on the effects produced by morphine in the previously mentioned behaviours was also studied. The effect of several doses of GHB on spontaneous motor behaviour and on morphine-induced hyperactivity was initially evaluated in male mice. It is generally assumed that the motor stimulant and rewarding effects of drugs of abuse are homologous (Wise and Bozarth, 1987). In concrete, an increase in dopamine release in the nucleus accumbens induced by drug administration causes its motor and rewarding effects (Di Chiara and Imperato, 1988). Subsequently, the effects of a wide range of doses of GHB on social behaviours were assessed. Although GHB has been used to ameliorate the withdrawal symptoms of alcohol or heroin addicts (situations involving an increased irritability or aggressiveness), no specific actions of this drug on aggression have been fully evaluated. Therefore, a known antiaggressive dose of morphine was coadministered with GHB to assess the interaction of these drugs on mouse social behaviour.

2. Materials and methods

2.1. Subjects

Male mice of the OF1 strain (Charles River, Barcelona) were used for the locomotor study (159) and the social interaction test (138). The animals were aged 42 days on arrival at the laboratory and were housed under standard conditions with constant temperature (21 ± 2 °C), a reversed light schedule (white lights on 19:30–07:30 h), and food and water available ad libitum (except during the behavioural test). Animals for locomotor studies and half of those used for the social test (standard opponents) were group housed (four per cage of $28 \times 28 \times 14.5$ cm). Experimental and control animals for social studies were housed in isolation, one per cage (size $23 \times 13.5 \times 13$ cm). Procedures involving mice and their care conformed to national, regional and local laws and regulations, and are in accord with the European Communities Council Directives (86/609/EEC, 24 November 1986).

2.2. Drugs

Gamma-hydroxybutyrate (GHB) (ICN Biomedicals, Aurora, OH), and morphine hydrochloride (Laboratorios Alcaliber, Madrid) were used in these experiments. Both compounds were diluted in physiological saline (0.9% NaCl), which was used as vehicle. A constant volume of drug (10 ml/kg) was injected, the needle being 0.5 mm in diameter and 16 mm in length.

2.3. Procedure and apparatus

After 10 days of adaptation to the laboratory, animals were divided into different groups. In the first experiment to test the effect of GHB on spontaneous motor activity, the animals were divided into five groups ($n = 8$). Four of which received different doses of GHB (25, 100, 200 and 400 mg/kg) and the controls, which received physiological saline only. Other animals were allocated to 10 groups, half receiving 10 and the others 50 mg/kg of morphine. Four subgroups from each of these latter groups also received different doses of GHB (25, 100, 200 and 400 mg/kg). Immediately after drug administration, all animals were placed into the activity cages for 1 h. An actimeter composed of eight cages ($33 \times 15 \times 13$ cm), each with eight infrared lights located in a frame around the cage (distance 2 cm each side), was used to measure spontaneous locomotor activity (CIBERTEC, Spain). Each beam was separated by 2 cm, and was positioned on the horizontal axis, a little higher than the bottom of the cage (body level of mice). The different frames are separated from each other by 4 cm, and since they are opaque, animals cannot see other cospecifics.

In the second experiment, half of the experimental animals were housed individually for 28 days, and the other half (“standard opponents”) was housed in groups of six. Opponents were made temporarily anosmic by intranasal lavage with 4% zinc sulphate solution 1 day before testing (Smoothy et al., 1986). Behaviour was evaluated 20 min after drug administration. Tests consisted of an experimental animal and a standard opponent confronting each other in a neutral cage ($61 \times 30.5 \times 36$ cm) for 10 min, with 1 min of adaptation before the encounter. All tests were carried out under white illumination between the second and fifth hour of the dark phase of the light/dark cycle and were videotaped. The videotapes were analysed using a PC computer and a custom-developed programme (Brain et al., 1989) that facilitated estimation of times allocated to different broad functional categories of behaviour. The study of aggression using laboratory animals has to take into consideration behavioural patterns. The advantage of an ethological approach is that it analyses diverse categories of behaviour, each one consisting of temporally and sequentially organized communicative signals, acts and postures. This kind of analysis also takes into account proximal and triggering antecedents and consequences, each serving different functions (Miczek et al., 2002). In this work, the following behaviours were studied: body care, digging, nonsocial exploration, explore from a distance, social investigation, threat, attack and immobility. A more detailed description of these elements can be found in Rodríguez-Arias et al. (1998).

In addition to these behaviours, latencies and unit of attack and threat (total time of behaviour/number of events) were measured.

2.4. Statistical analyses

Motor activity data were analysed using analysis of variance (ANOVA) with one "within" and two "between" factors. Within factor was the time of recording (hour), with six levels (6 h). Between factors were: factor 1, GHB alone or with morphine (Groups), with three levels (GHB alone; GHB + Morphine 10; and GHB + Morphine 50); factor 2, dose of GHB (Treatment), with five levels (GHB0, GHB25, GHB100, GHB200 and GHB400).

Data of the social encounters were initially analysed using the Kruskal–Wallis test. For the behavioural categories in which this test was significant, differences between groups were examined using the two-tailed Mann–Whitney *U* test. This kind of statistical analysis has been previously used in other studies evaluating aggressive behaviour (Redolat et al., 1991; Rodríguez-Arias et al., 1997, 1999, 2002; Kudryavtseva et al., 1999).

3. Results

3.1. Motor activity

ANOVA reveals significant effect of Group [$F(2,105) = 28.597$; $P < .001$]. Newman–Keuls Post hoc analysis showed that locomotor activity was significantly higher ($P < .01$) in the group that received GHB plus 50 mg/kg of morphine in comparison with the others (see Fig. 1). ANOVA revealed significant effects of Treatment [$F(4,105) = 2.665$; $P < .03$]. Newman–Keuls Post hoc analysis showed that locomotor activity was significantly lower ($P < .05$) in the groups that received the highest GHB dose (400 mg/kg) when compared with controls. ANOVA revealed significant effects of Hour [$F(5,525) = 12.036$; $P < .001$]. Newman–Keuls Post hoc analysis indicated that locomotor activity was significantly lower ($P < .01$) in the first, fifth and sixth hour when compared with the second, third and fourth.

Interactions of Group/Hour [$F(10,525) = 14.755$; $P < .001$], Treatment/Hour [$F(20,525) = 4.155$; $P < .001$] and Group/Treatment/Hour [$F(40,525) = 3.088$; $P < .001$] were significant but not so for Group/Treatment interaction [$F(8, 105) = 1.173$; $P < .3226$].

In the first group (GHB alone), the higher doses (200 and 400 mg/kg) produced a decrease in motor activity during the first and fifth hours ($P < .05$), the dose of 200 mg/kg also inducing an impairment during the third and fourth hour ($P < .05$). In the second group (Mor10 + GHB), no differences were observed at any moment. In the third group (Mor50 + GHB), doses of GHB up to 100 mg/kg were capable of blocking morphine-induced hyperactivity during the first hour ($P < .01$ for Mor50 + GHB400 and Mor50 + GHB200 and $P < .05$ for Mor50 + GHB100), this effect only being observed for Mor50 + GHB400 during the second hour ($P < .05$). In the last hour of recording, this group (Mor50 + GHB400)

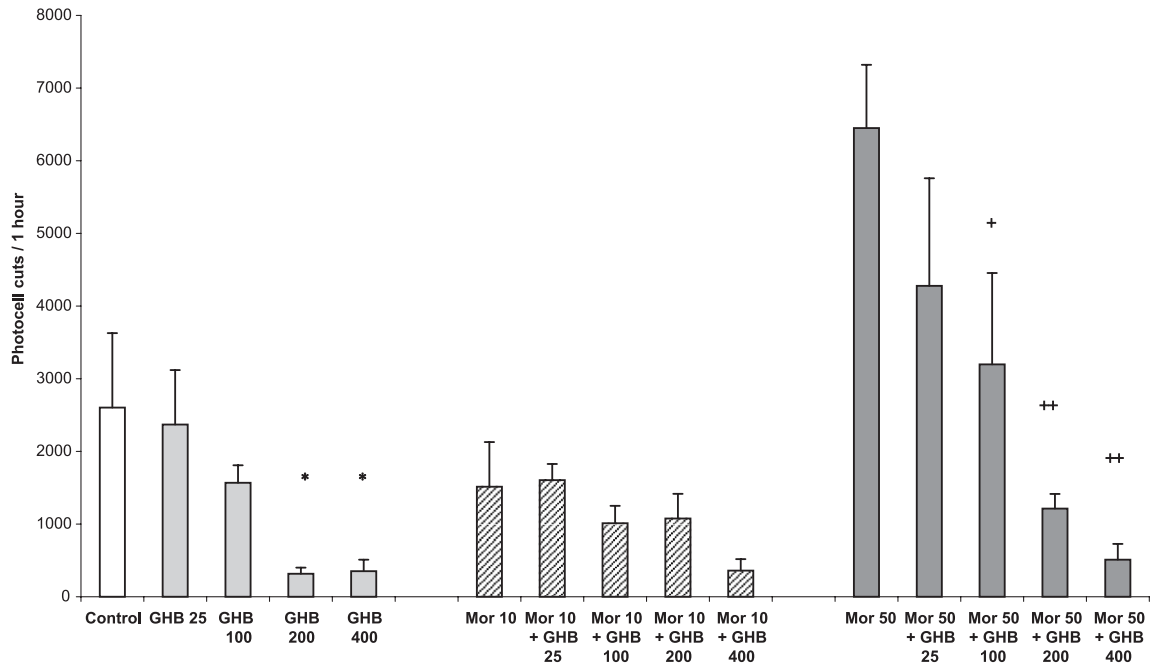

Fig. 1. Means (± S.E.M.) of locomotor activity in activity counts in 1 h shown for 15 groups of mice receiving saline or four different doses of GHB (25, 100, 200 and 400 mg/kg), 10 mg/kg of morphine, or four different doses of GHB (25, 100, 200 and 400 mg/kg) plus the same morphine dose, and 50 mg/kg of morphine, or four different doses of GHB (25, 100, 200 and 400 mg/kg) plus the latter morphine dose. Differences with respect to control group, $^{*}P < .05$; differences with respect to Mor50 group, $^{+}P < .05$; $^{++}P < .02$.

presented a rebound activity in comparison to the rest of the groups ($P<.05$).

When we compare the treatment groups, in those that did not receive any dose of GHB, administration of 50 mg/kg of morphine induced hyperactivity during the first three hours ($P<.01$) in comparison with control or Mor10. During the fourth and fifth hours, Mor10 animals decreased their motor activity in comparison with controls ($P<.05$). Among the animals that received 25 or 100 mg/kg of GHB, hyperactivity was observed during the second hour in those also receiving 50 mg/kg of morphine. The decrease in motor activity shown by animals receiving only 200 mg/kg of GHB was counteracted by morphine administration ($P<.05$) during the first hour. During the two following hours (second and third), hyperactivity was presented in animals which received this GHB dose and 50 mg/kg of morphine. During the third, fourth and fifth hours, administration of 50 mg /kg of morphine plus 400 mg/kg of GHB induced hyperactivity in comparison with GHB administered alone or GHB plus 10 mg/kg of morphine.

3.2. Effects on social activities of GHB alone and in combination with morphine

The highest GHB dose (400 mg/kg) completely abolished behaviour of the animals: they became immobile for most of the time, thus the data are not shown. For the rest of treatment groups, only the most important results (see Table 1) are detailed.

The Kruskal–Wallis test showed significant ($P<.001$) treatment effect on *digging* behaviour. Administration of morphine alone or with any GHB (<400 mg/kg) dose decreased digging in comparison with saline-treated animals ($P<.02$ for Mor10 + GHB 200 and $P<.002$ for the rest).

The Kruskal–Wallis test showed a significant treatment effect ($P<.001$) on *nonsocial exploration*. Animals receiving 25 or 100 mg/kg of GHB in addition to morphine showed a significant increase when compared to controls and groups which received only the corresponding GHB doses ($P<.002$ in all cases).

For *social investigation*, the Kruskal–Wallis test ($P<.005$) showed a significant treatment effect. Animals receiving 200 mg/kg of GHB presented an increase in this behaviour when compared to those treated with saline ($P<.05$).

In any of the behaviours studied related to threat (*threat*, *number of threats* and *threat latency*), differences between groups were observed (Kruskal–Wallis test, $P<.001$ in all cases). On all measures, an additive effect was observed when morphine was coadministered with any GHB dose. Alone, neither was capable of diminishing threat, but the joint administration abolished all threat measures ($P<.02$ for MOR + GHB25 and MOR + GHB100, and $P<.002$ for MOR + GHB 200).

In any of the behaviours studied related to attack (*attack*, *unit of attack* and *attack latency*), differences between groups were observed (Kruskal–Wallis test, $P<.01$ for attack and $P<.001$ for the rest). All groups (except GHB25 and GHB100) showed a significant decrease in attack with respect to controls ($P<.02$ for Mor10 and $P<.002$ for the rest). Unit of attack was only decreased with morphine, alone or with any GHB dose ($P<.002$). Attack latency was longer with GHB 100 mg/kg onwards ($P<.05$ for GHB100, $P<.02$ for GHB200), morphine alone or plus any GHB dose ($P<.02$ for Mor10 and $P<.002$ for the rest).

4. Discussion

In this study, GHB influenced morphine effects in different ways depending on the behaviour studied. Although GHB administered alone reduced spontaneous motor activity only at high doses, it efficiently counteracted morphine-induced hyperactivity. Moreover, GHB reduced aggression and increased social behaviours in male mice

Table 1
Means of accumulated times (in seconds) with SEN allocated to different categories of behaviour

	Control	GHB25	GHB100	GHB200	Mor10	M + GHB25	M + GHB100	M + GHB200
Body care	8.5 ± 2.2	11.2 ± 3.9	8 ± 2	7.6 ± 3.2	15.6 ± 5.1	9.4 ± 2	14.1 ± 2.7	7.5 ± 3.4
Digging (a)	17.5 ± 4.8	6.2 ± 1.4**	9.1 ± 2.8	20.7 ± 10.6	1.8 ± 1.4***	2 ± 1.5***	1 ± 0.5***	9.4 ± 6.2**
Non social exploration (a)	421 ± 14	390 ± 28	457 ± 16	311 ± 61	490 ± 28	525 ± 18***	551 ± 10***	347 ± 78
Explore from a distance	14.5 ± 3	23.4 ± 2.2	12.2 ± 2.2	17.5 ± 4.6	18.5 ± 6.5	15.1 ± 2.5	13.5 ± 4	11.8 ± 3.9
Social investigation (a)	6.9 ± 3.4	16 ± 6.3	9.6 ± 5.6	36.4 ± 11.2*	1.1 ± 0.7	4.3 ± 2.4	0.8 ± 0.8*	15.8 ± 6.2
Threat (a)	30.5 ± 5.9	42 ± 13.2	25.4 ± 3.9	22.3 ± 10.1	26.4 ± 10.8	8.5 ± 5.5**	12 ± 7.2**	3.7 ± 3.2***
Unit of threat (a)	1.3 ± 0.2	1.6 ± 0.3	1.1 ± 0.2	1.1 ± 0.3	0.8 ± 0.3	0.4 ± 0.2**	0.6 ± 0.4**	0.2 ± 0.2***
Threat latency (a)	27 ± 13	83 ± 60	161 ± 68	311 ± 100	371 ± 90	496 ± 68**	479 ± 70**	564 ± 38***
Attack (b)	102 ± 11.9	115 ± 29	76 ± 17	28.8 ± 11**	42 ± 19.5***	14 ± 10***	3 ± 2***	0 ± 0.1***
Unit of attack (a)	3.2 ± 0.4	3.9 ± 0.7	3 ± 0.5	2.6 ± 0.8	2.3 ± 0.6**	0.7 ± 0.5***	0.8 ± 0.5***	01 ± 0.1***
Attack latency (a)	26 ± 13	88 ± 60	174 ± 67*	327 ± 95**	403 ± 91**	506 ± 67***	519 ± 48***	573 ± 28***
Immobility	0 ± 0	0 ± 0	7 ± 6	181 ± 89	5 ± 3	15.2 ± 15.2	7.6 ± 3.5	168 ± 85

Kruskal–Wallis test shows significant variance at (a) $P<.001$, (b) $P<.01$.

* Differs on two-tailed Mann–Whitney U test from control group, $P<.05$.

** Differs on two-tailed Mann–Whitney U test from control group, $P<.02$.

*** Differs on two-tailed Mann–Whitney U test from control group, $P<0.002$.

without affecting motor activity, not suppressing but even potentiating morphine actions on aggression. Thus, present results supported the claimed relationship between the GHBergic and the opiate systems and suggest that this interaction depends on the behaviour evaluated.

Administration of GHB decreased motor activity at doses of 200 mg/kg and upwards, these results supporting others found in mice (Zerbib et al., 1992; Schmidt-Mutter et al., 1998; Cook et al., 2002; Itzhak and Ali, 2002). In addition, hyperactivity was not observed at any time after administration, although it has been reported by other authors (Zerbib et al., 1992; Schmidt-Mutter et al., 1998). The effects of GHB on motor activity can be explained by its actions on the DAergic system (Banvides et al., 1982; Fattore et al., 2000). The GHBergic system seems to participate in the control of the DAergic neurotransmission, mainly by reducing impulse flow in the nigrostriatal and in the meso-corticolimbic pathways (Roth et al., 1980; Nissbrandt et al., 1994). The attenuation of dopamine neurotransmission may underlie the effects of GHB on motor activity (Nicholson and Balster, 2001).

Rodríguez-Arias et al. (2000) reported that 50 mg/kg of morphine induced a clear hyperactivity in mice. It has to be pointed out that, with the exception of the lower dose (25 mg/kg), all the GHB doses used (even those that do not decrease motor activity per se), were capable of counteracting morphine-induced hyperlocomotion, suggesting a specific role of GHB receptors in this action. This blockade was observed mainly during the first hour after drug administration. The mesolimbic DAergic neurons are necessary for the expression of hyperactivity induced by opioids (Iwamoto, 1981). The GHB action on the DAergic system suggests a stronger blockade of this morphine-induced hyperactivity. Additionally, GHB induced the release of endogenous opioids (Lason et al., 1983; Gobaille et al., 1994; Schmidt-Mutter et al., 1999) that could potentiate morphine-induced hyperactivity, but the present results suggest that its impairing actions on the DAergic system are more critical in this behavioural expression.

In social encounters, administration of 200 mg/kg of GHB produced a significant decrease in threat and attack without impairing motor activity. These results are in accord with others (Navarro and Pedraza, 1996) although with a lower range of doses (100 and 120 mg/kg). Even though the same strain of mice was used in both studies, the higher aggressiveness observed in the present animals may explain the necessity of using higher doses to achieve the antiaggressive action. The decrease in aggression observed after GHB administration supports the well-known antiaggressive action of DAergic antagonists in rodents especially in mice (Miñarro et al., 1990; Aguilar et al., 1994; Rodríguez-Arias et al., 1998, 1999). As previously mentioned, GHB can modulate DAergic neurotransmission either acting on the GHB or the GABA receptors. The fact that low doses, with a more specific blockade of GHB receptors, do not affect aggressive or social behaviours of mice suggests that the GHB receptors are not principally responsible for the antiaggressive actions of this compound. At higher doses, $GABA_b$ receptor occupation and activation play a more important role (Nicholson and Balster, 2001). It is well known that activation of the GABA system reduces aggression in animals (Krsiak et al., 1981; Poshivalov, 1981; Puglisi-Allegra et al., 1981) as well in humans (Bjork et al., 2001; Cherek et al., 2002).

GHB at a dose of 200 mg/kg also increases the time spent in social contact (social investigation). An anxiolytic action for GHB has been observed in rats using other paradigms such as the elevated plus-maze (Schmidt-Mutter et al., 1998), being attributed to an action on the $GABA_a$ benzodiazepine receptor complex (Schmidt-Mutter et al., 1998). Although the antiaggressive effects of GHB is similar to the behavioural profile of D_2 dopamine antagonists, this social effect is only observed after the administration of DA D_3 compounds (Rodríguez-Arias et al., 1999).

Although all the nonsedative doses of GHB used affected motor activity measured with the actimeter, no immobility or decrements in nonsocial exploration behaviour (with an essential motor component) were observed during the social encounters. These discrepancies between the results observed with these two different kinds of motor activity measured have been previously found with different DAergic antagonists (Rodríguez-Arias et al., 1998, 1999).

The morphine dose used (10 mg/kg) for the aggression tests clearly decreased attack without affecting other behaviours (except for an abolishment of digging). When administered with GHB, these two behavioural actions were maintained but new effects were observed. Although the time spent by the animals in nonsocial exploration was slightly increased after morphine administration, its coadministration with the two lower GHB doses (25 and 100 mg/kg) produced an additive effect, as a significant increase in this behaviour was observed. Another additive effect was also found in relation to indices of threat: All were decreased (nonsignificantly) by morphine administration alone. Coadministration with any GHB dose (which alone did not affect these behaviours), abolished threat completely. GHB may consequently potentiate the actions of morphine. On the other hand, the increase in social contact observed after administration of 200 mg/kg of GHB was counteracted by morphine.

GHB and opiates have a clear relationship, as many effects of GHB in animals can be mimicked by opioid receptor agonists (Bernasconi et al., 1999) and blocked by opioid receptor antagonists (Snead and Bearden, 1980; Maitre, 1997). In addition, GHB releases endogenous opioids such as proenkephalin (Schmidt-Mutter et al., 1999). Since GHB does not bind to opioid receptors and nor does naloxone bind to GHB receptors (Maitre, 1997; Feigenbaum and Simantov, 1996), these relationships could depend on GHB actions on dopamine or other neurotrans-

mitter systems, such as the GABAergic (Feigenbaum and Howards, 1997). Several studies indicate the existence of an interaction between GABAergic and opiate functions. GABA exert its action on different morphine-induced pharmacological effects, such as endocrine secretions (Maiewski et al., 1985), catalepsy, analgesia (Rattan and Sribanditmongkol, 1994) or anxiolytic behaviour in rats (Sasaki et al., 2002). Particularly interesting is the important role that GABA plays in opiate dependence. GABA administration has been shown to affect the development of tolerance (Ho et al., 1976) and physical dependence on morphine (Zarrindast and Mousa-Ahmadi, 1999; Bexis et al., 2001).

A similar action of GHB and DAergic antagonists has been proposed, specifically with the D_2 antagonists, which do not suppress motor activity (Navarro et al., 1996). The present results confirm that GHB has a similar behavioural profile to raclopride or sulpiride during the social encounters (Aguilar et al., 1994; Redolat et al., 1991). This was not so when the coadministration with morphine was studied. Administered jointly with haloperidol, morphine increases the impairing effect of haloperidol on motor activity but counteracts its antiaggressive actions (Rodríguez-Arias et al., 1997). Thus, although GHB has a similar behavioural profile to DA antagonists, its interaction with the opiate system seems quite different.

In conclusion, the present results confirm and extend the interaction between GHB and opioid systems. A number of papers have pointed out a dissociation between antiaggressive and motor effects of opiates, these behaviours affecting DAergic antagonists in a different way (Winslow and Miczek, 1988; Tidey and Miczek, 1992; Rodríguez-Arias et al., 1997). This dissociation was also observed in the present study when morphine was administered jointly with GHB. This compound had an additive effect in morphine-affected social behaviours but conversely was capable of efficiently counteracting the morphine-induced increase in motor activity. Taking these results and those obtained with DAergic antagonists together, the existence of two different ways controlling social and motor behaviours is suggested.

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