Psicothema
2000. Vol. 12, nº 1, pp. 113-115

ISSN 0214 - 9915 CODEN PSOTEG

Effect of gammahydroxybutyric acid administration on catalepsy behaviour in female mice

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This study was designed to examine the effect of acute administration of gammahydroxybutyric acid (GHB; 150-250 mg/kg, i.p), a central neurotransmissor or neuromodulator, on catalepsy behaviour in female mice. Catalepsy was measured by means of the bar test. An aluminium bar of 5 mm in diameter was placed 4 cm above the floor. Animal`s 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 interrumped. Catalepsy was evaluated 30 and 60 min after administration of GHB or saline (control group). The results indicated that GHB (225 and 250 mg/kg) significantly increased catalepsy of female mice, an action probably mediated by the interaction between GHBergic and dopaminergic neurons in striatum.

Efectos de la administración de ácido gammahidroxibutírico sobre la conducta de catalepsia en ratones hembras. El objetivo de este trabajo fue examinar el efecto de la administración aguda de ácido gammahidroxibutírico (GHB; 150-200 mg/kg, i.p), un neurotransmisor o neuromodulador central, sobre la conducta de catalepsia en ratones hembras. Para la evaluación de la catalepsia se utilizó el «test de la barra» (de 5 mm de diámetro y colocada a 4 cm sobre el suelo). Las patas de los animales fueron suavemente colocadas sobre dicha barra, contabilizándose el tiempo que el animal tardaba en situar al menos una pata sobre el suelo. Si transcurría 1 minuto sin movimiento, la prueba era interrumpida. La catalepsia fue evaluada 30 y 60 minutos tras la administración de GHB o suero salino (grupo control). Los resultados indicaron que el GHB (225 y 250 mg/kg) incrementó significativamente la catalepsia de los ratones hembras, una acción probablemente mediada a través de las interacciones entre neuronas GHBérgicas y dopaminérgicas en el estriado.

Gammahydroxybutyric acid (GHB) is a GABA metabolite which can traverse the blood-brain barrier after peripheral injection. It satisfies many of the criteria for consideration as a neurotransmitter, being present in micromolar quantities in numerous brain regions as well as in several peripheral organs. GHB high affinity receptors are located only in neurons, specially in hippocampus, cortex, striatum, olfactory bulbs and tubercles, substantia nigra and area tegmental ventral (Cash, 1994; Maitre, 1997; Tucnnicliff, 1997).

From a clinical point of view, GHB has been used as anesthetic drug, for treatment of narcolepsy (Lammers et al., 1993) and to alleviate the alcohol/opiate dependence (Gallimbeti et al., 1989). Likewise, this compound is a reliable stimulant of slow-wave sleep in normal subjects, simultaneously enhancing sleep related growth hormone secretion (van Cauter et al., 1997). Recently, it has been characterized as an emerging drug of abuse that cause physical dependence (Galloway, 1997). In this sense, its prolonged use at high doses may lead to a withdrawal syndrome, which resolves without sequelae.

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In animals, it has been demonstrated that GHB posessess rewarding properties (evaluated by a conditioned place preference paradigm) (Martellotta, Fattore, Cossu and Fratta, 1997). Moreover, GHB exhibits a wide range of pharmacological effects, including hypothermia (Kaufmann, Porrino and Nelson, 1990), epilepsy seizures (Banerjee, Hirsch and Snead, 1993), a decrease of aggression (Navarro and Pedraza, 1996), anxiolytic properties (Schmidt et al., 1998) and catalepsy (Navarro et al., 1996; 1998). This cataleptic behaviour observed in rodents after GHB administration is reverted by NCS-382, a GHB receptor antagonist (Schmidt et al., 1991).

Although catalepsy has been observed in rats (Snead and Bearden, 1980; Hechler et al., 1993) and mice (Navarro et al., 1996; 1998) using different types of behavioural tests, all studies have been carried out in male rodents. Therefore, this experiment was designed to examine the effect of acute administration of GHB (150-250 mg/kg) on catalepsy behaviour in female mice.

Materials and methods

Animals

60 CD strain albino female mice weighing 25-35 g were obtained from CRIFFA (Barcelona, Spain). Animals were housed in transparent plastic cages (24 x 13.5 x 13 cm) in groups of five un-

der standardised 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 (Sigma Laboratories, Madrid, Spain) was diluted in saline and administered acutely in five doses (150, 175, 200, 225 and 250 mg/kg, i.p) to female mice. Control animals received 0.9% sodium chloride (vehicle).

Behavioural test

Catalepsy was measured by means of 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. Successive behavioural evaluations of catalepsy were carried out 30 and 60 minutes after the administration of GHB or saline. Between determinations, the mice were kept in their home cages. Individual animals were tested in a random order.

Estrous cycle

A determination of the phase of the estrous cycle was immediately made for each animal after the catalepsy test . This determination was carried out according with the procedure described by Waynforth and Flecknell (1992).

Statistical analysis

Nonparametric Kruskal-Wallis tests were used to assess the variance of the catalepsy scores 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. Wilcoxon tests were also employed to contrast the catalepsy scores of mice at different behavioural evaluations used. A value of $p<0.05$ was considered to be statistically significant.

Results and Discussion

Table 1 illustrates the mean scores of catalepsy shown by female mice after single administration of GHB. Kruskal-Wallis analysis showed that there was significant variance in catalepsy scores over different treatment groups ($p<0.05$). Paired comparisons using Mann-Whitney *U*-tests revealed that bar catalepsy scores (225-250 mg/kg) increased significantly following GHB administration, in comparison with mice treated with saline, at test carried out 30 min after injection ($p<0.02$). Catalepsy was never observed in saline-treated mice.

Furthermore, Wilcoxon tests showed that catalepsy scores (225 and 250 mg/ kg) for GHB-treated female mice were always clearly higher when tested at 30 min, as compared with mice tested 60 min after administration of the drug ($p<0.05$).

As Table 1 shows, GHB (225 and 250 mg/kg) provoked a marked but short lasting catalepsy with a peak of action within 30 min. However, 60 min after GHB administration this cataleptic effect had disappeared. These results are in concordance with a recent study using male mice in which a dose-dependent effect on catalepsy was described (Navarro et al., 1996). Moreover, like in our study, a clear catalepsy was also observed in male mice from 225 mg/kg of the drug.

Catalepsy is considered as an appropriate rodent model for detecting the extrapyramidal side effects of antipsychotic/neuroleptic drugs (Hoffman and Donovan, 1995; Navarro, Manzaneque, Martín and Vera, 1997), an action mainly mediated by dopamine neurons located in striatum (Sanberg, 1980). The cataleptogenic effect of GHB could be related to its action on striatal dopaminergic neurons. In fact, GHB is considered to be an inhibitor of striatal dopamine release in awake animals (Howard and Feigenbaum, 1997) and it has also been shown to have antidopaminergic activity (Navarro and Pedraza, 1996) as well as effects similar to those of typical antipsychotics (Beardsley, 1996). In sum, our findings might be in aggreement with the view that GHB receptors interact with central dopamine D2 transmission.

The phase of estrous cycle (proestrus, estrus, diestrus or metaestrus) was specifically identified in vaginal smears taken immediately after the experiment in each mouse. During catalepsy test, most of female mice were either in diestrus or in metaestrus (progestational stages). Although it cannot be definitely excluded that the cataleptic effect of GHB in females may be partially modulated by sexual hormone changes that occur during the estrous cycle, catalepsy behaviour does not appear to vary significantly during phases of the cycle in rodents (Kazandjian et al., 1988).

Table 1
Catalepsy scores (in sec) after acute treatment with GHB (150-250 mg/Kg) in female mice (medians with ranges). Tests at 30 and 60 min after injection

	Doses of GHB (mg/kg)					
Test	Saline	150	175	200	225	250
30 m	0 (0-0)	0 (0-4)	0 (0-15)	0 (0-7)	5.5 (0-60)*	23 (0-60)*
60 m	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-39)	0 (0-3)

As compared with saline, *$p<0.02$ (Mann-Whitney U-tests)

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Aceptado el 20 de abril de 1999