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EFFECTS OF $GABA_A$/ω/Cl- AGONISTS ON BODY WEIGHT IN ISOLATED MALE MICE

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Numerous studies have demonstrated that $GABA_A$/ω/Cl- receptor agonists may increase body weight in grouped rodents. However, there is no evidence in relation to the possible actions of these drugs under isolation conditions. In this study, we examine the acute and subchronic effects of several doses of benzodiazepines (diazepam, clobazam, bentazepam and midazolam), cyclopyrrolones (zopiclone) and imidazopyridines (zolpidem) on body weight in isolated male mice. All animals were housed individually in transparent plastic cages during 30 days (non-drug period) and weighted weekly. After isolation period, drugs were administered acutely or daily for 10 consecutive days and body weights measured. Results showed that benzodiazepines, cyclopyrrolones and imidazopyridines did not vary significantly body weight, as compared with their saline groups. It is concluded that housing conditions can play a critical role in the modulation of $GABA_A$/ω/Cl- receptor agonists actions on body weight in mice.

Numerosos estudios han demostrado que los agonistas del receptor $GABA_A$/ω/Cl- pueden incrementar el peso corporal en ratones agrupados. Sin embargo, no existe evidencia respecto al efecto de dichos fármacos bajo condiciones de aislamiento. En el presente trabajo, examinamos el efecto de la administración aguda y subcrónica de varias dosis de benzodiazepinas (diacepam, clobazam, bentacepam y midazolam), ciclopirrolonas (zopiclona) e imidazopiridinas (zolpidem) sobre el peso corporal en ratones machos aislados. Todos los animales fueron aislados en jaulas transparentes durante 30 días y pesados semanalmente. Tras el período de aislamiento, todos los fármacos fueron administrados aguda o subcrónicamente (10 días), registrándose los pesos de los animales. Los resultados mostraron que ninguno de los fármacos modificó significativamente el peso corporal, en comparación con sus respectivos grupos controles. Se concluye que las condiciones de aislamiento pueden desempeñar un papel clave en la modulación de los efectos de los agonistas del receptor $GABA_A$/ω/Cl- sobre el peso corporal en ratones.

It is a well-documented fact that benzodiazepines cause increased food consumption in many mammalian species (Cooper, 1991; Nasure, 1994; Berridge and Peciña, 1995). Presumably, the benzodiazepine-induced hyperphagia is mediated by actions at central benzodiazepine (omega) recognition sites, since it is reversed by the receptor antagonist flumazenil (Cooper et al, 1985). Moreover, it has been suggested that omega receptors located in the parabrachial nucleus might be a site of action for the effects of benzodiazepines on ingestive behaviour

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(Higgs and Cooper, 1996). It has been proposed that the benzodiazepine-induced increase in food intake is due to an increase in the palatability or hedonic value of food. Likewise, partial agonists at omega receptors (such as bretazenil) can also enhance the ingestion of food (Clifton and Cooper, 1996). On the other hand, zopiclone (10 mg/kg) does not seem to alter body weight in rats, although this action has been only observed in females.

In contrast, zolpidem (that binds selectively to ω1 receptor) does not affect food consumption. Therefore, there is not unreasonable to suggest that the hyperphagia effect might be mediated by the type 2 receptor (ω2) (Sanger and Zivkovic, 1988; Cooper, 1991). Barbiturates, which share many of the pharmacological properties of benzodiazepines, produce a strong stimulation of food intake, indicating that drug action at an alternative site in the $GABA_A$/ω/Cl- receptor complex can also lead to hyperphagia (Cooper and Moores, 1985).

Although a considerable number of studies have demonstrated that $GABA_A$/ω/Cl- receptor agonists may increase body weight, all these investigations have been carried out using grouped animals, and there is no evidence in relation to the possible actions of these drugs under isolation conditions.

The aim of this study was to examine the acute and subchronic effects of different $GABA_A$/ω/Cl- receptor agonists (benzodiazepines, cyclopyrrolones and imidazopyridines) on body weight in isolated male mice.

Method

Animals

276 OF.1 strain albino male mice aged approximately 42 days (Servicio de Animales de Laboratorio, Granada, Spain) in arriving to the laboratory were used. Mice were housed under standard laboratory conditions: constant temperature (21 Cº), a reversed light schedule (lights on:20:00-08:00 hrs), normal lab chow (Panlab, Barcelona, Spain) and tap water available "ad libitum". Mice were housed individually in transparent plastic cages (24 x 13.5 x 13 cm) during 30 days (isolation period), being employed as experimental or control animals.

Procedure

Non drug phase

Animals, divided into four groups (except with diazepam) in each experiment (12 per group), were isolated for 30 days and changes in body weight were weekly measured (at the same hour of the day and once a week) using a digital balance.

Drug treatment phase

After 30 days without pharmacological treatment, mice were given intraperitoneally acute or daily injections (during 10 days) of diazepam (0.5 and 2 mg/kg), clobazam (0.75, 1.5 and 3 mg/kg), bentazepam (1, 3 and 5 mg/kg), midazolam (0.5, 1 and 1.5 mg/kg), zopiclone (1.5, 3 and 6 mg/kg) and zolpidem (0.75, 1.5 and 3 mg/kg), or physiological saline (0.9% NaCl) and were monitored daily for body weight. All drugs were administered in a volumen of 10 ml/kg.

As statistical analysis, Mann-Whitney *U*-tests were used.

Results and Discussion

Body weight did not vary significantly between experimental and control groups during non-drug phase.

Figures 1 to 6, and table 1, illustrate the evolution of mean body weight in all groups during the drug treatment. As can be observed, no significant differences between experimental and control groups were found for the six drugs used.

Benzodiazepines (diazepam, clobazam, bentazepam, midazolam), cyclopyrrolones (zopiclone) and imidazopyridines (zolpidem) did not modify body weight of isolated male mice after acute or subchronic treatment, as compared with their control groups. These results indicate that stress produced by isolation appears to affect feeding responses and, consequently, to body weight of animals. Therefore, housing conditions (isolation or grouping) may influence differentially body weight of mice (Martín-López and Navarro, 1996). This action has been previously described with other substances, such as morphine, which decreased body weight in isolated mice but did not affect to grouped mice (Espert et al, 1996).

Although an appropriate interpretation of isolation effects on body weight is unclear, it can be suggested that social isolation could lead pronounced changes in $GABA_A/\omega/Cl$- receptor functions in the brain. In fact, Early and Leonard (1977) have found lower levels of GABA in striatum, hyppocampus and amigdala of isolated albino mice in comparison with grouped mice. Nevertheless, others authors have found that social isolation does not alter brain regional benzodiazepine binding site numbers and affinity in rats (Morinan et al, 1992).

Prolonged isolation can produce marked changes in brain neurotransmitter systems. Thus, it has been reported that isolation provokes an increase of brain dopamine turnover, a slight decrease of brain noradrenaline turnover and a large decrease of brain serotonin turnover (Valzelli, 1978). Moreover, a decrease of glutamic acid decarboxylase activity in the brain of isolated mice has also been reported (Blindermann et al, 1979), as compared with grouped mice. More recently, it has been demonstrated that social isolation also can modify metabolic activity of the hippocampus, a limbic region in which GABAergic neurons are clearly present (Cimadevilla et al, 1997).

In conclusion, housing conditions can play an important role in the modulation of $GABA_A/\omega/Cl$- receptor agonists actions on body weight. Likewise, further studies are necessary in order to attempt to clarify the interactions between GABA and other brain neurotransmitters in the control of the effects of social isolation on body weight.

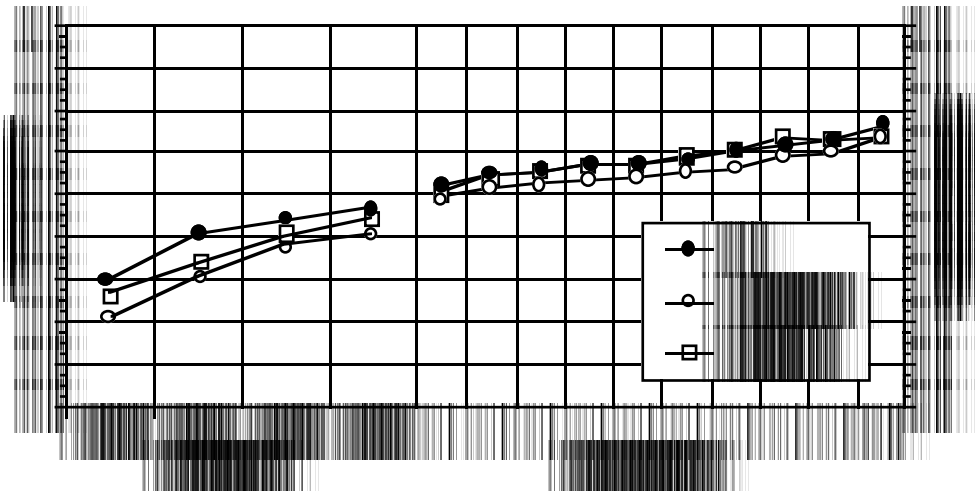

Figure 1. Effects of diazepam on body weight in male mice.

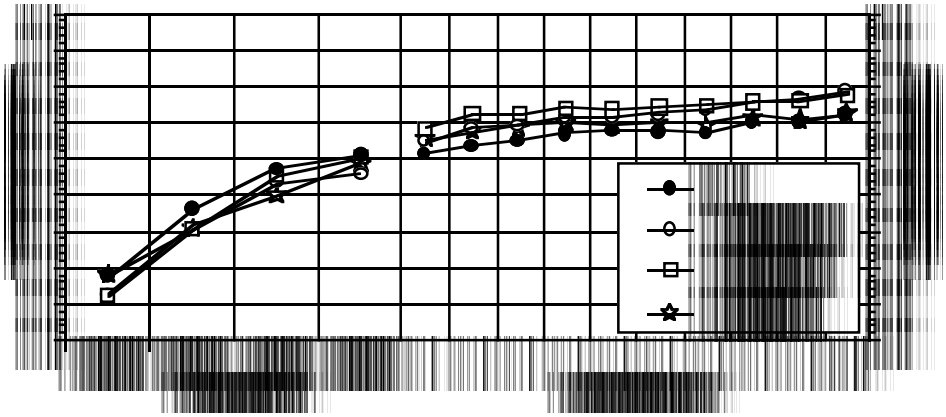

Figure 2. Effects of clobazam on body weight in male mice.

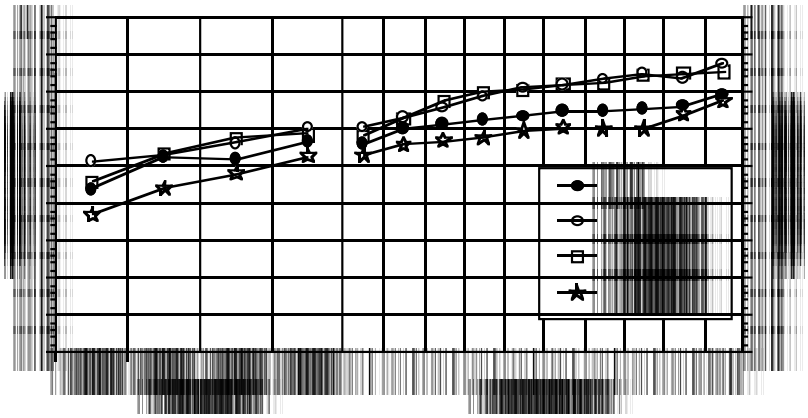

Figure 3. Effects of bentazepam on body weight in male mice.

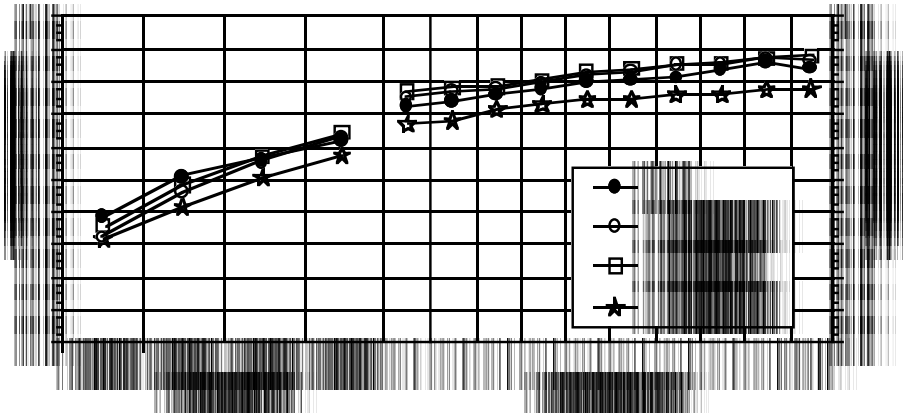

Figure 4. Effects of midazolam on body weight in male mice.

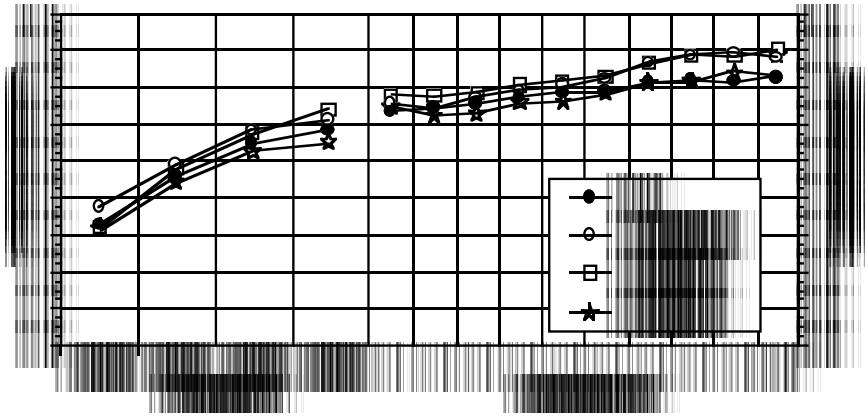

Figure 5. Effects of zopiclone on body weight in male mice.

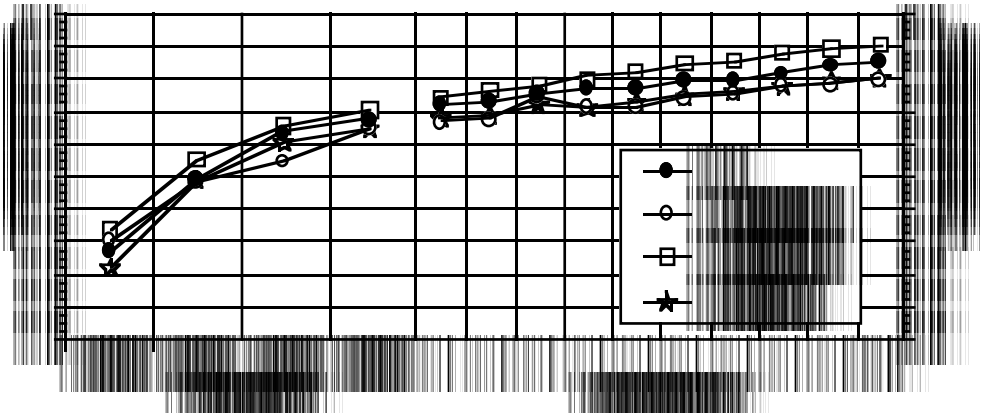

Figure 6. Effects of zolpidem on body weight in male mice.

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TABLE 1

	NONDRUG PHASE				DRUG PHASE (Days)									
Diazepam	Week 1	Week 2	Week 3	Week 4	1	2	3	4	5	6	7	8	9	10
Veh	28,2±2,2	30,2±2,3	30,9±2,1	31,4±2,1	32,4±2,6	32,9±2,5	33,3±2,5	33,3±2,5	33,3±2,5	33,6±2,3	34±2,7	34,2±2,6	34,4±2,8	35±3,1
0,5 mg/kg	26,4±1,69	28,2±2,4	29,7±2,9	30,2±2,8	31,8±2,8	32,3±2,4	32,4±2,7	32,6±2,6	32,8±2,7	33±2,9	33,2±2,7	33,7±3	33,8±2,8	34,7±3,5
2 mg/kg	27,5±2,7	28,9±3,7	30,1±3,6	31±3,4	32,3±3,2	32,8±3,3	33,1±3,4	33,3±3,4	33,4±3,4	33,8±3,4	33,9±3,4	34,5±3,5	34,3±3,1	34,6±3
Bentazepam	Week 1	Week 2	Week 3	Week 4	1	2	3	4	5	6	7	8	9	10
Veh	30,5±2,9	32,3±2,6	32,2±2,5	33,1±2,5	33,1±2,4	33,8±2,6	34±2,7	34,3±2,6	34,5±2,5	34,8±2,8	34,8±2,7	34,9±2,7	35±2,9	35,6±2,8
1 mg/kg	32,1±2,8	32,5±2,7	33,1±2,7	33,9±2,7	33,9±2,6	34,5±2,5	35±2,4	35,7±2,4	36±2,3	36,3±2,6	36,5±2,6	36,9±2,4	36,7±2,4	37,4±2,7
3 mg/kg	31±3	32,6±2,4	33,4±2,4	33,7±2,2	33,5±1,5	34,4±1,9	35,4±1,6	35,9±1,9	36±2	36±2,2	36,3±1,8	36,8±1,8	36,9±1,9	37±2,6
5 mg/kg	29,4±2,6	30,8±2,1	31,5±2,1	32,4±2,1	32,4±1,3	33,1±1,6	33,2±1,7	33,4±1,4	33,8±1,7	34±1,8	34±1,5	34±1,4	34,7±1,9	35,4±2
Clobazam	Week 1	Week 2	Week 3	Week 4	1	2	3	4	5	6	7	8	9	10
Veh	25,4±4	29,3±2,4	31,4±2	32,2±1,9	32,3±2,7	32,8±2,7	33,1±2,6	33,5±3	33,7±2,5	33,6±2,8	33,5±2,9	34,2±3,3	34,1±3,2	34,5±3,1
0,75 mg/kg	24,4±2,4	28,2±3,7	30,7±1,9	31,3±2,4	33±2,3	33,7±2,5	33,8±2,4	34,2±2,3	34,5±2,3	34,7±2,5	34,8±2,6	35,2±2,9	35,4±2,8	35,7±2,5
1,5 mg/kg	24,6±3	28,3±2,6	31,3±2,2	32,3±2	33,8±2,2	34,7±2,5	34,7±2,2	34,9±2,1	34,9±2	35±2,1	35,1±2,1	35,3±1,9	35,4±2,1	35,5±2,2
3 mg	25,7±2,5	28,2±3	30,1±2,3	32±2	33,2±2,2	33,5±2,1	33,8±2,2	33,9±2	33,9±2,1	34,1±1,9	34±1,9	34,3±2	34,3±2,3	34,4±2
Midazolam	Week 1	Week 2	Week 3	Week 4	1	2	3	4	5	6	7	8	9	10
Veh	29,5±3,4	32±3	33±2,7	34,2±2,9	36,3±3,6	36,7±3,6	37±3,3	37,3±3,3	37,8±2,9	38±3	38,2±3	38,6±3,2	38,9±3	38,6±3
0,5 mg/kg	28,4±2,6	31,2±2,2	33,1±2,8	34,5±3,8	36,7±4,5	37,4±4,8	37,4±4,7	37,7±4,5	38±4,8	38,4±5	38,8±5	38,9±4,8	39,2±4,9	39,2±5
1 mg/kg	29,3±3,1	31,6±2,3	33,3±2,3	34,8±1,9	37,2±2,3	37,5±2,5	37,6±2,6	38±2,6	38,6±2,6	38,7±2,5	38,5±2,5	38,9±2,5	39,2±2,7	39,6±2
1,5 mg/kg	28,4±2,4	30,3±1,7	32,2±1,6	33,5±1,5	35,4±1,2	35,6±1,1	36,2±1,1	36,5±1,3	36,8±1,5	36,8±1,5	37,1±1,3	37,1±1,3	37,4±1,3	37,6±1,5
Zoplclone	Week 1	Week 2	Week 3	Week 4	1	2	3	4	5	6	7	8	9	10
Veh	28,6±2,1	31,1±1,8	33±2	33,7±2,2	34,7±2,4	34,9±2,2	35,2±2,4	35,4±2,6	35,7±2,5	35,8±2,6	36,2±2,7	36,3±2,7	36,2±2,5	36,5±2,5
1,5 mg/kg	29,5±2,4	31,8±1,6	33,6±1,8	34±2,7	35,1±2,5	34,9±2,4	35,6±2,9	35,9±2,7	36,1±2,8	36,5±2,9	37,4±2,9	37,7±3,1	37,8±2,8	37,6±2,9
3 mg/kg	28,5±3	31,5±2,5	33,4±3	34,7±3,5	35,6±3,3	35,6±3,3	35,8±3,1	36,3±3,2	36,4±3,2	36,7±3,5	37,4±3,7	37,7±3,7	37,7±3,5	38,1±3,2
6 mg/kg	28,3±2,4	30,8±2,4	32,5±2,8	33±3,1	35±3,8	34,4±3,4	34,5±3,2	35,1±3	35,2±3,3	35,6±3,5	36,1±3,5	36,3±3,5	36,8±3,9	36,6±3,9
Zolpidem	Week 1	Week 2	Week 3	Week 4	1	2	3	4	5	6	7	8	9	10
Veh	27,4±2,8	31,8±1,8	34,4±1,8	35,4±1,9	36,2±2,2	36,5±2,1	37±2,2	37,3±2,2	37,4±2,2	37,8±2,4	37,8±2,3	38,3±2,4	38,6±2,2	38,8±2,5
0,75 mg/kg	28,2±2,5	31,7±1,3	33±1,4	35±1,6	35,3±1,6	35,5±1,3	36,9±1,5	36,2±1,5	36,3±1,6	36,8±1,5	37,1±1,6	37,5±1,7	37,6±1,6	37,9±1,4
1,5 mg/kg	28,7±3,5	33,3±2,7	35,1±1,7	36±1,8	36,8±2	37,3±1,8	37,6±1,7	38±1,9	38,4±2,1	38,9±2,1	39,2±1,8	39,5±2,2	39,7±2,3	40±2,3
3 mg/kg	26,5±2,5	32±2,7	34±2,2	35±2,2	35,6±2,3	35,8±2,1	36,3±2,2	36,4±2	36,7±2	37±2,1	37,3±2	37,7±2	37,6±2	38,2±2