INTRODUCTION

The γ-aminobutyric acid (GABA)-ergic system seems to play an important role in the aetiology of alcoholism, although certain mechanisms of interaction between ethanol and especially GABA receptor ligands are not completely understood (Burt and Kamatchi, 1991; Samson and Harris, 1992; Boyle et al., 1993; Grant, 1994; Korpi, 1994; De Witte, 1996; Hoffman and Tabakoff, 1996). It is well known that some GABA receptor agonists may be used in treatments aimed at relieving the symptoms of chronic alcohol intoxication, and benzodiazepines (BZ) are a group of such drugs whose effectiveness in that respect seems to be quite well established (Tabakoff, 1995; Lewis, 1996; Mayo-Smith, 1997). Yet they do cause some side-effects (amnesia, tolerance, dependence) (Lister, 1975; Curran, 1986; Izquierdo and Medina, 1991, 1995; Lader, 1994; Plaznik, 1995; Kalueff and Nutt, 1996–1997) and therefore the search for new drugs is justified. Recent publications suggest that a new group of GABA\textsubscript{A} agonists from the imidazopyridine group could be used for that purpose, mainly due to their lack of side-effects characteristic of BZ (Wilkinson and Allerd, 1994; Wilkinson, 1995). One such drug is zolpidem, which is a BZ\textsubscript{1} GABA\textsubscript{A} agonist (Depoortere et al., 1986; Langer and Arbilla, 1988; Benavides et al., 1988; Schmid et al., 1995). Because the results of some experiments suggested that chronic alcohol administration had different effects on different subunits of the GABA receptor (Thielen et al., 1993; Khan et al., 1994; Mhatre and Ticku, 1994), it may be important that zolpidem exhibits strong binding capacity towards GABA\textsubscript{A} receptor subunits (Duncan et al., 1995; Schmid et al., 1995; Itier et al., 1996), mostly in the brain areas where ethanol particularly enhances GABA action (Criswell et al., 1993, 1995; Devaud and Morrow, 1994; Chen et al., 1997). Although a relatively low incidence of zolpidem adverse events has been reported (Allain and Monti, 1997), the possible occurrence of amnestic episodes or transient memory disturbances after zolpidem treatment has been observed (Plaminteri and Narbonne, 1988; Evans et al., 1990; Berline et al., 1993; Wesensten et al., 1995; Patat et al., 1996). Because there are only few data concerning zolpidem and alcohol-induced behavioural effects on learning and memory...
(Wilkinson, 1995), it was of interest to investigate the effect of multiple administration caused by zolpidem on performance of passive avoidance tasks and the ethanol-induced hypnotic action in chronically ethanol-treated rats.

The present experiments were designed to use our chronically ethanol-treated animals known to be alcohol non-prefering (under the two-bottle, free-choice paradigm), because of their stronger behavioural responses in different pharmacodynamic tests, compared to preferring or control Wistar rats, in particular their stronger response to the hypnotic action of ethanol and their more anxiogenic behaviour (Mikolajczak et al., 1995, 1997). This behaviour of non-prefering rats was in agreement with the data of others (Moller et al., 1997; Overstreet et al., 1997; McMillen et al., 1998). Because there are some suggestions that preferring animals represent a genetic model of alcoholism (Colombo, 1997; Hammoumi et al., 1997) and correspond to Type 2 alcoholism (Cloninger, 1987) it is tempting to speculate that non-prefering rats may represent the other type of alcoholism and would be more sensitive [contrary to preferring AA rats (Wegelius et al., 1994)] to the anxiolytic effects of BZ-like agonists.

**MATERIALS AND METHODS**

**Animals**

The experiments were conducted on male Wistar rats housed individually in their home cages under standard ambient conditions (at 20 ± 2°C and a relative humidity of 65%) with a 12 h light–12 h dark cycle (lights on at 07:00–19:00). Rats were fed on a standard laboratory diet (pellets) and had tap water freely available in their home cages (control rats). Animals weighed 232 ± 19 g at the start of experiments (n = 100).

**Alcohol**

The rats were given ethanol (12% solution) in an average dose of 9 g/kg/day during the first 8 weeks of the experiment as the only source of drinking fluids (Okulicz-Kozaryn et al., 1992). After this period, the animals could drink ethanol on a free-choice basis for 2 weeks and the preference was measured as the percentage of ethanol to the total volume of liquids drunk by the rats (Daoust et al., 1987). Only animals for whom alcohol solution constituted <50% of all liquids were selected for the experiment (non-prefering rats) (Daoust et al., 1987; Lin and Hubbard, 1994). During behavioural experiments, the animals had free access to water and ethanol, and their ethanol consumption was 1.2 ± 0.2 g/kg/24 h, therefore the possible effects of withdrawal could be excluded. Rats of the same age which were not given ethanol formed the control groups.

**Zolpidem**

The animals were given one (1×) or multiple (10×) doses of zolpidem tartrate orally (Stilnox, 10 mg tablets, Synthelabo, Le Plessis Robinson, France) in doses of 1.0 mg/kg and 2.0 mg/kg; tablets were ground and suspended in 1% solution of methylcellulose (MC). The control groups were given the corresponding volume of 1% MC.

**Effects on spontaneous locomotor activity: the actimeter**

The test was conducted using ‘PAN’ (Polska Akademia Nauk), a licensed activity meter, by placing animals in the centre of the apparatus and recording activity of rats by electromechanical counters. The data were expressed as signals corresponding to spontaneous locomotor movements during 5 min (Chodera et al., 1994). The locomotor activity was measured in control animals 15 min after a single zolpidem (1.0, 2.0 and 3.5 mg/kg, p.o.) or MC (control 1% MC) treatment.

**The effect of zolpidem on hypnotic action of ethanol**

The effects of zolpidem on the hypnotic action of ethanol were assessed after administering one dose or 10 doses (for 10 consecutive days) of zolpidem. At 15 min after drug administration (Thenot et al., 1988; Garrigou-Gadenne et al., 1989; Rowlett and Woolverton, 1997), ethanol was administered intraperitoneally (20% v/v solution) in a dose of 3.0 g/kg (Okulicz-Kozaryn et al., 1992) and the duration of sleep time was measured (in minutes); it being expressed as the time from the loss until the first return of the righting reflex, as described in our earlier report (Okulicz-Kozaryn et al., 1992).

**Passive avoidance test**

Passive avoidance behaviour was studied in a learning step-through situation which relies on the natural preference of rats for darkness. After 2 min
of habituation to a dark compartment (425×404×456 mm) the rat was placed on an illuminated (100 W) platform and allowed to enter the dark compartment (Ader et al., 1972). Two more approach trials were given on the following day with a 2 min interval between them. At the end of the second trial, an unavoidable scrambled electric footshock [500 μA AC (alternating current), 3 s] was delivered through the grid floor of the dark compartment (learning trial). Retention of the passive avoidance response (task) was tested 48 h later by placing the animal on the platform and measuring the latency in re-entering the dark compartment to maximum time of 180 s (Mikolajczak et al., 1994). A preliminary selection of the rats was made before the experiments (pretest procedure without any acoustic or pain stimulus); in order to select arbitrarily those rats where latency values were <180 s.

The assessment of the effect of a single administration of zolpidem on latency was done 48 h after the electric footshock and 24 h after zolpidem was administered orally. Multiple administration of zolpidem was carried out for 9 consecutive days. On the 10th day, the test was carried out, the tenth drug administration took place on the 11th day of the experiment (Mikolajczak et al., 1994) and latency was measured 48 h after electric footshock was applied.

Statistical analysis

The results obtained were expressed as the arithmetic means ± SEM. Statistical analyses were carried out using one-way analysis of variance (ANOVA) and Newman–Keuls post-hoc test or unpaired t-test for analysis of data on locomotor activity or hypnotic effects of ethanol. Kruskal–Wallis and Mann–Whitney tests were used for memory task data analysis.

RESULTS AND DISCUSSION

In a pilot study, it was found that a single administration of zolpidem (p.o.) produced a dose-dependent lowering of locomotor activity in control rats (Fig. 1) (one-way ANOVA $[F(3,23) = 14.24, P < 0.01]$). The single oral treatment with a 1.0 mg/kg dose of the drug significantly increased the activity of rats ($P \leq 0.01$, Newman–Keuls test), whereas activity after a 2.0 mg/kg dose was not different from that of the control group receiving 1% MC. However, after the highest dose of zolpidem (3.5 mg/kg, p.o.) a lowering of locomotor activity was observed ($P \leq 0.01$, Newman–Keuls test). Accordingly, for the further investigations, doses of 1.0 and 2.0 mg/kg of the drug were chosen due to their lack of sedative properties.

We found that zolpidem altered the duration of ethanol-induced sleep time in control rats (Fig. 2)

![Fig. 1. The dose-dependent effect of single zolpidem treatment on locomotor activity in control rats.](image1)

- Data are expressed as the mean (± SEM) of six animals, one-way ANOVA $[F(3,23) = 14.24, P < 0.01]$. $^xP \leq 0.01$ significantly different from control 1×MC (MC = 1% methylcellulose) (Newman–Keuls post-hoc test).

![Fig. 2. The effect of single and multiple administration of zolpidem on the duration of ethanol-induced sleep in control (ethanol-naïve) rats.](image2)

- Ethanol was given i.p. in a dose of 3 g/kg. Other details are as in Materials and methods. Data are expressed as the mean (± SEM) of six animals, one-way ANOVA $[F(5,35) = 10.25, P < 0.01]$. $^xP \leq 0.05$ significantly different from control 1×MC (MC = 1% methylcellulose) and control 10×MC or proper dose of single zolpidem administration, respectively (Newman–Keuls post-hoc test).
Ethanol was given i.p. in a dose of 3 g/kg. Other details are as in Materials and methods. Data are expressed as the mean (± SEM) of seven animals, one-way ANOVA \([F(5.41) = 8.17, P < 0.01]\), \(\*P \leq 0.05\) significantly different from control 1xMC (MC = 1% methylcellulose) or control 10xMC, \(\*P \leq 0.05\) significantly different from proper dose of single zolpidem administration, \(\*P \leq 0.05\) significantly different compared with the dose of 1.0 mg/kg of zolpidem (Newman–Keuls post-hoc test).

as well in chronically ethanol-treated animals (Fig. 3) \([\text{one-way ANOVA } F(5,35) = 10.25, P < 0.01]\) and \([F(5.41) = 8.17, P < 0.01]\), respectively. Thus, single oral administration of the drug, in doses of 1.0 and 2.0 mg/kg (p.o.), resulted in a significant prolongation of ethanol-induced sleep in the control group \((P < 0.01, \text{ Newman–Keuls test})\) (Fig. 2), although the higher dose produced a weaker effect than the lower dose of zolpidem. Multiple \((10\times)\) administration of either dose of zolpidem resulted in a weaker, compared to single administration of zolpidem, effect on the hypnotic response to ethanol \((P < 0.01, \text{ Newman–Keuls test})\). This effect may be the result of tolerance developing towards the inhibitory effect of zolpidem. A similar effect of tolerance towards that action of the drug was observed after multiple administration of zolpidem in a similar dose \((1.5 \text{ mg/kg})\) in the experiment where rats’ locomotor activity was examined; the highest locomotor activity paradigm being observed in the 2nd and 3rd weeks of the experiment (Chodera et al., 1994). However, other researchers noticed very weak tolerance or no tolerance at all; these differences may have been connected with the fact that higher drug doses (up to 20–30 mg/kg) were used in some experiments with rodents (Perrault et al., 1992; Sanger and Zivkovic, 1992). Moreover, although generally no tolerance to the hypnotic action of zolpidem has been observed in humans (Allain and Monti, 1997; Monti et al., 1997), there are some reports of tolerance and withdrawal effects after treatments with high doses of zolpidem for 3 months or longer in patients treated for sleep disorders (Cavallaro et al., 1993; Watsky, 1996; Bottlender et al., 1997). Also it is known that sometimes zolpidem functions as a reinforcer in baboons under conditions of continuous and long-term availability, and the development of behavioural signs associated with physical dependence was noticed (Weerts and Griffiths, 1998).

A single administration of zolpidem to chronically ethanol-treated rats led to a significant prolongation of the duration of ethanol-induced sleep when the dose of 1.0 mg/kg was used \((P < 0.01, \text{ Newman–Keuls test})\), whereas there was no significant prolongation when a dose of 2.0 mg/kg was used (Fig. 3). The enhancement of the hypnotic effect of ethanol was greater after the serial administration of zolpidem at the dose of 2.0 mg/kg \((P < 0.01, \text{ Newman–Keuls test})\) but there was also a significant but lower effect after administering a lower dose of the drug \((P < 0.01, \text{ Newman–Keuls test})\), compared with the control group receiving 10x 1% MC (Fig. 3).

Moreover, comparing the duration of ethanol-induced sleep after multiple administration of zolpidem with the duration after a single dose, it was observed that the effect of the drug administered in a dose of 1.0 mg/kg was significantly weaker, whereas a dose of 2.0 mg/kg produced a statistically significantly stronger effect (Fig. 3).

Chronic administration of ethanol to rats results in developing tolerance towards the duration of ethanol-induced sleep \((P < 0.01, \text{ unpaired } t\text{-test, both for } 1\times \text{ and } 10\times 1\% \text{ MC})\) (Figs 2 and 3). We have observed this phenomenon earlier (Okulicz-Kozaryn et al., 1992; Mikolajczak et al., 1995) and it corresponds with the results of research on tolerance development towards the sedative, hypothermic and anxiolytic effects of chronic administration of ethanol to animals (Samson and Harris, 1992).

This tolerance seems to be responsible for the potentiation of the previously observed, weaker effect of ethanol action after single administration of 2.0 mg/kg of zolpidem to chronically ethanol-treated rats, when compared with the 1.0 mg/kg
dose (Fig. 3). It is possible that, at this point, the interaction between zolpidem, the selective agonist of BZ1 GABA_A receptors, with ethanol, at the level of GABA_A receptor subunits has some significance (Thielen et al., 1993; Khan et al., 1994; Mhatre and Ticku, 1994; Sanger, 1997). However, the mechanism of zolpidem-binding to different subunits of GABA_A in chronically ethanol-treated animals is not fully clear (Mhatre and Ticku, 1994; Devaud and Morrow, 1994; Criswell et al., 1995; Chen et al., 1997). Therefore the prolongation of ethanol-induced sleep, observed after multiple administration of zolpidem, particularly at the dose of 2.0 mg/kg in chronically ethanol-treated rats, might be interpreted either as the effect of interaction of other neurotransmitting systems which may condition the development of ethanol tolerance (Khanna et al., 1991; Samson and Harris, 1992; Morrisett and Swartzwelder, 1993; Freund and Anderson, 1996; Hoffman and Tabakoff, 1996) or as a result of changes in cross-tolerance between GABA_A/BZ/chloride-channel complex function and ethanol activity (Toki et al., 1996). So it is possible that, in the course of longer administration of the higher dose of zolpidem, the symptoms of alcohol tolerance are suppressed to the extent that the duration of alcohol-induced sleep in chronically ethanol-treated rats was similar to values observed in the control animals which did not receive zolpidem (Figs 2 and 3). One might suggest that this effect of zolpidem could also be explained in terms of the partial substitution of the ethanol cue produced by this drug in ethanol discrimination tests (Shelton and Balster, 1994; Sanger, 1997).

Figures 4 and 5 depict the effects of zolpidem upon the change of latency to the dark compartment, 48 h after the moment the negative stimulus was applied, in the passive avoidance test, both for control (ethanol-naïve) and chronically ethanol-treated rats respectively. The results show that zolpidem alters the latency observed both in control (ethanol-naïve) (Fig. 4) and in chronically ethanol-treated animals (Fig. 5) (Kruskal–Wallis test \(H(5,35) = 22.12, P < 0.01\) and \(H(5,35) = 10.57, P < 0.07\), respectively).

Thus, in the control (ethanol-naïve) groups the single administration of zolpidem in both the 1.0 and 2.0 mg/kg doses (24 h before latency was measured) significantly prolonged the time during which the animals remained on the platform, in comparison to 1×1% MC-treated control animals \((P < 0.05\), for both single zolpidem-treated groups,
Mann–Whitney test) (Fig. 4). Repeated (10×) 1% MC treatment led by itself to increased latency (P < 0.05, Mann–Whitney test). Furthermore, after multiple administration of zolpidem at both dose levels, the latency values were significantly lower when compared to the animals that were given a single dose of MC (P < 0.01, Mann–Whitney test), or to the groups that were given only a single dose of zolpidem (P < 0.01, for comparisons of both doses, Mann–Whitney test). Longer times remaining on the platform might indicate long-term memory consolidation and unwillingness to return to the dark compartment (Ader et al., 1972) and/or the result of the sedative or anxiolytic-like effect of zolpidem action (Griebel et al., 1996). However, these effects of zolpidem 24 h after its single administration are likely, because of its short half-life (Thenot et al., 1988; Garrigou-Gadenne et al., 1989). The low latency values after the 10× administration of the drug may indicate the appearance of memory consolidation disorders being the result of a negative effect of multiple administration of GABAergic drugs (McNamara and Skelton, 1993; Kalueff and Nutt, 1996–1997) or the development of tolerance towards the sedative and/or anxiolytic-like action of zolpidem, as shown in the experiments examining the duration of ethanol-induced sleep (Fig. 2). Thus, these results confirm the suggestions of others (Wesensten et al., 1995) that amnestic and hypnotic and/or anxiolytic effects of zolpidem in our investigated conditions are functionally coupled.

In chronically ethanol-treated and ethanol-naïve (control) rats, the multiple 1% MC treatment led to prolongation of latency in comparison to its single administration (P < 0.05, Mann–Whitney test) (Fig. 5). After multiple administration of zolpidem at a 2.0 mg/kg dose to chronically ethanol-treated rats, a higher latency value was obtained, in comparison with the 10× 1% methylcellulose administration (P < 0.05, Mann–Whitney test) (Fig. 5). This significantly prolonged latency time during which the animals remained on the platform could be the result of increased sedation, which was also observed in the experiments examining the hypnotic effect of ethanol in an analogous group of animals (Fig. 3).

Therefore it seems that the passive avoidance test used in this work to study the effects of zolpidem on memory in chronically ethanol-treated animals may give misleading results due to the impossibility of ruling out the sedative or anxiolytic component (Okulicz-Kozaryn et al., 1995; Griebel et al., 1996).

In conclusion, because multiple zolpidem treatment of chronically ethanol-treated rats resulted in similar values of sleep-time to those observed in zolpidem-non-treated control animals, it is possible that multiple administration of zolpidem partially inhibits the tolerance resulting from chronic ethanol treatment.

REFERENCES


