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Effect of Nonsedative Doses of Propofol on an Innate Anxiogenic Situation in Rats

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Background: **The effect of propofol on anxiety has not been well studied. In humans, such investigations are confused by the fact that sedation often coexists with anxiolysis. Therefore, the authors evaluated the effects of minimal sedation with propofol in rats placed in an innate anxiogenic situation, the elevated plus-shaped maze.**

Methods: **In experiment 1, spontaneous locomotor activity was determined in rats as a measure of sedative effect induced by propofol (0–9 mg/kg administered intraperitoneally). In experiment 2, groups of rats received propofol (0–9 mg/kg) or diazepam (0–2 mg/kg) and then were placed on a plus-shaped maze elevated above the ground that was composed of two opposite closed arms and two opposite open arms. On an initial exposure to the maze, undrugged rats avoid the open arms, with the number of entries into and time spent within the open arms constituting approximately 20% of their total activity. This reflects normal anxiety in a rodent for any elevated open platform.**

Results: **In experiment 1, 0–9 mg/kg propofol did not alter spontaneous activity in rats. In experiment 2, propofol and diazepam significantly increased the number of entries into and the time spent within the open arms. Propofol at a dose of 9 mg/kg significantly increased the rats' level of exploration of the open arms to about 50% of all exploratory activity, and a similar observation was made with 2 mg/kg diazepam.**

Conclusions: **In a standard animal model, propofol has anxiolytic properties at doses that do not produce sedation. (Key words: Anxiety; behavior; diazepam; elevated plus-shaped maze; $GABA_A$.)**

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PROPOFOL can be used at subanesthetic doses as a sedative agent.[1–5] It was initially thought that propofol had no anxiolytic effects. This position was challenged, however, by the finding that sedative doses[6–10] seemed to produce some anxiolytic effects. But this reduction in anxiety could also be attributed to sedation. As acknowledged by Curran,[11] sedation that reduces the perception of any anxiogenic situation could account for the observed anxiolysis. To what extent propofol has anxiolytic properties *per se* that can be dissociated from its sedative properties has not been determined. The aim of the current study was to determine whether propofol, in doses that do not produce sedation, can decrease anxiety.

We used a standard rodent model of anxiety—the elevated plus-shaped maze. This model is sensitive to both anxiolytic and anxiogenic compounds, whatever their pharmacologic classes,[12,13] and is used extensively to screen putative new anxiolytic agents and to investigate the psychologic and neurochemical bases of anxiety.[14–16] The elevated plus-shaped maze[17–19] presents an innate anxiogenic situation for the rat that allows us to determine the anxiolytic effect, if any, of a drug on spontaneous anxiety, in contrast to learned anxiety (that is, without interference with any memory processes). Briefly, a rat is allowed to explore a right-angle cross maze elevated 50 cm above the ground. Two opposite arms of the maze are enclosed with high walls; the remaining two are open elevated platforms. On an initial exposure to the maze, a rat spontaneously avoids the open arms, restricting most of its activity to the closed ones. The low level of exploration of the animal in the open arms (*e.g.*, 10–20%) is a behavioral reflection of

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anxiety in this paradigm. In addition, exposure to the elevated plus maze is associated with increases in plasma corticosterone concentrations,[13,20] heart rate, blood pressure, and plasma norepinephrine concentrations, compared with these parameters noted in rats left in their home cages.

In the first experiment, we searched for a dose-dependent activity impairment induced by propofol that would be indicative of sedation. In the second experiment, we examined the effects of propofol on the elevated plus maze, at doses that previously were found not to induce sedation. The effects of propofol were compared with those of the prototypical anxiolytic diazepam.

Materials and Methods

Animals

Our subjects were 72 naive male Long Evans rats (Janvier, France) that weighed 300 to 370 g. They were housed two per cage in a colony room maintained on a 14-h light, 10-h dark cycle (light on at 7:00 AM) with food and water provided *ad libitum*.

Drugs

Propofol (5 mg/ml, Diprivan; Zeneca, Paris, France) was dissolved in 5% intralipid. Diazepam (5 mg/ml, Valium; Roche, Basel, Switzerland) was dissolved in 0.9% sodium chloride. All drugs were prepared immediately before use and injected intraperitoneally in a volume of 2 ml/kg.

Apparatus

Spontaneous Activity. Eight identical copies of an activity cage (size, 45 cm × 30 cm × 30 cm) were used. The activity cage had an infrared detector (IRP124; Talco, Paris, France) placed behind a Fresnel lens and located at the roof of the cage, which allows us to monitor the movements of the animals in eight different sections of the cage. The signal was fed into a computer that totaled all the horizontal movements (one unit represented one crossing from one section to an other) in a period of 5 min.

Elevated Plus-shaped Maze. The apparatus was the same as the one described initially by Pellow and File.[13] It consisted of a wooden elevated maze placed 50 cm above the ground and in the shape of a right-angle cross with two open arms (50 cm × 10 cm) and two enclosed arms (50 cm × 10 cm; height of the walls, 40 cm; fig. 1).

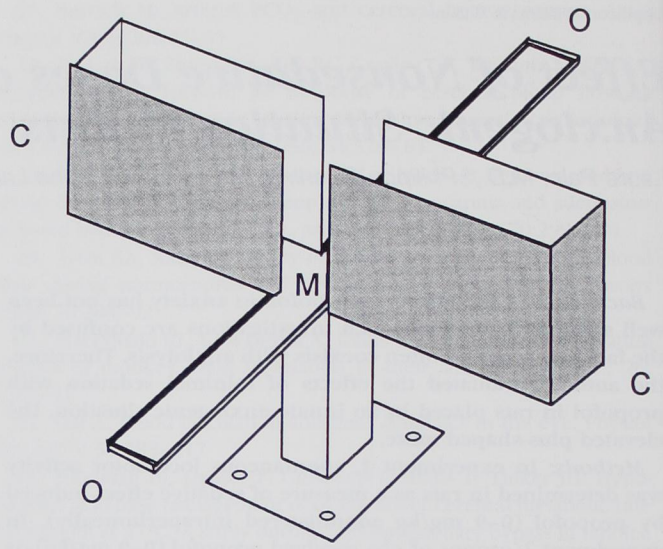

Fig. 1. The elevated plus-shaped maze is a wooden elevated maze that has two opposite open arms (O), two opposite closed arms (C), and a central platform (M). At an initial exposure, an animal placed on the central platform explores the maze but avoids the two open arms that elicit anxiety.

A spotlight (40 Watts) illuminated the room and was placed 180 cm above the central area of the maze. A video camera allowed the test sessions to be recorded and facilitated off-line behavioral analyses.

Procedure

Behavioral testing started after three daily handling sessions. All experiments were conducted between 10:00 AM and 4:00 AM.

Experiment 1: Effect of Propofol (1–9 mg/kg) on Spontaneous Activity. Twenty-four rats were randomly assigned to four groups according to the dose of propofol they received: 0, 1, 3, or 9 mg/kg propofol (n = 6 each). Each rat was injected intraperitoneally and returned to its home cage. Five minutes later, each rat was moved to a plastic cage identical to its home cage but located in the experiment room. Five minutes later it was placed in the experimental cage and tested for 5 min. Activity counts expressed as units per 5 min were used as spontaneous activity scores.

Experiment 2: Effect of Propofol (1–9 mg/kg) and Diazepam (2 mg/kg) in the Elevated Plus-shaped Maze. Forty-eight rats were randomly assigned to six groups according to the treatment they received: propofol 0 (intralipid 5%), 1, 3, or 9 mg/kg propofol; or 0 (0.9 % sodium chloride) or 2 mg/kg diazepam. Each rat was injected and then returned to its home cage. Five minutes later, the rat was moved in a plastic cage identical to

its home cage but located in the experiment room. Five minutes later, it was placed at the center of the maze, facing one of the open arms. Behavior was recorded during a 5-min test period.

For behavioral measures, entry into an arm or the distal part of an arm (delimited by a line half-way along each of the arm) was occurred when the animal placed all four paws over the limit of the area. The number of entries into and the cumulative time spent within the open arms, the closed arms, and the distal part of open arms, and the total number of arm entries were evaluated by an investigator who ignored the treatment given to the rat by using a homemade behavior analysis software. The ratios of open to total arm entries and open to total time were calculated individually for each rat. The ratios of open to total entries and open to total time, the number of entries into the distal part of open arms, and the time spent in the distal part of open arms were used as indices of anxiety. These indices should increase in the event of an anxiolytic effect of the drug or decrease in the case of an anxiogenic effect of the drug.

Statistics

Results were expressed as mean and standard deviation (SD). One-way analyses of variance were performed on the spontaneous activity score, the ratio of open to total arm entries, the ratio of open to total time, the number of entries into the distal part of open arms, and the time spent within the distal part of open arms. The multiple-comparisons test between treatment groups and their controls was performed using Dunnett's *t* test. A one-way analysis of variance was also performed on the total number of arm entries. When a drug altered the total number of arm entries and the ratio of open to total arm entries, we could not exclude that a significant modification of the ratio of open to total was not biased by a modification of the closed arms entries. Therefore, an analysis of variance with covariates was performed to determine the extent to which the alteration in open arm entries depended on any effect of the drug on closed arm entries (dependent = number of open arm entries; covariate = number of closed arm entries).[21]

Results

During preliminary studies (unpublished data), we never observed a locomotor or exploratory impairment effect for diazepam at doses less than 2 mg/kg or for propofol at doses less than 10 mg/kg intraperitoneally in Long Evans male rats that weighed 280 to 400 g.

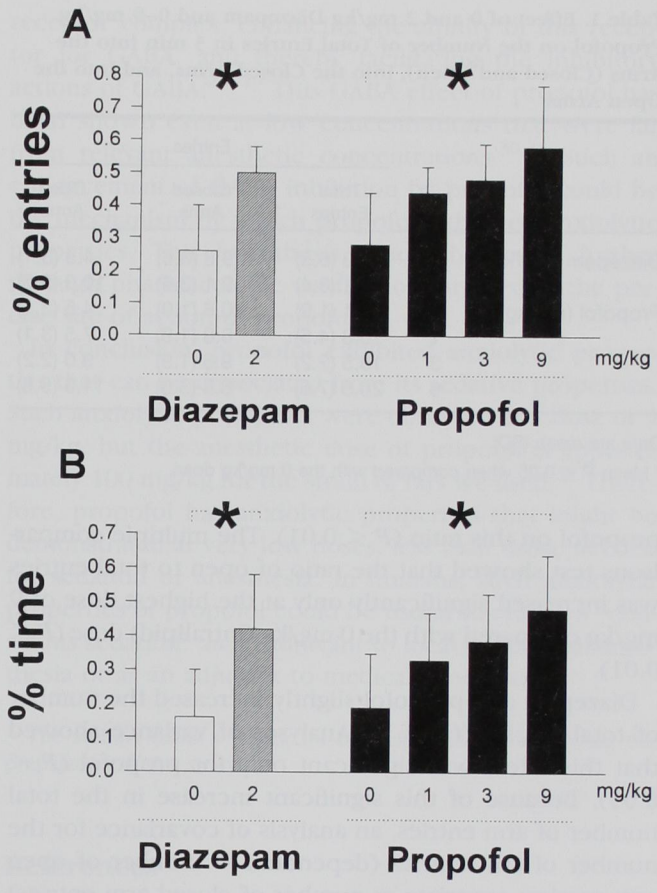

Fig. 2. Effect of 0 and 2 mg/kg diazepam (left) and 0–9 mg/kg propofol (right) on the percentage of entries in 5 min (*A*) into the open arms and on the percentage of time (*B*) spent within the open arms. Results are expressed as the mean and SD. *Means $P < 0.05$ when compared with the 0-mg/kg dose.

Experiment 1: Effect of Propofol (1–9 mg/kg) on Spontaneous Locomotor Activity

The mean activity scores did not differ according to the dose of propofol used. Mean activity scores were 289 units (SD = 58), 307 units (SD = 39), 314 units (SD = 29), and 292 units (SD = 27) for, respectively, 0, 1, 3, and 9 mg/kg propofol (not significant).

Experiment 2: Effect of Propofol (1–9 mg/kg) and Diazepam (2 mg/kg) in the Elevated Plus-shaped Maze

Exploration of the Open Arms. Figure 2A depicts the effect of diazepam (left side) and propofol (right side) on the ratio of open to total entries. Diazepam (2 mg/kg) significantly increased the ratio of open to total entries ($P < 0.01$). For propofol, the ratio of open to total entries increased with the dose of propofol. Analysis of variance showed a significant effect of the dose of

Table 1. Effect of 0 and 2 mg/kg Diazepam and 0–9 mg/kg Propofol on the Number of Total Entries in 5 min into the Arms (Closed and Open), into the Closed Arms, and into the Open Arms

		Entries		
		Total Entries	Closed Arms	Open Arms
Diazepam (mg/kg)	0	14.3 (6.5)	9.8 (3.6)	4.5 (3.1)
	2	19.1 (8.4)	9.1 (3.6)	10.0 (5.6)
Propofol (mg/kg)	0	15.1 (1.9)	10.6 (1.9)	4.5 (3.1)
	1	16.3 (4.5)	8.8 (1.9)	7.5 (3.1)
	3	18.5 (2.2)	9.6 (1.9)	9.0 (2.2)
	9	20.5 (7.6)	8.8 (3.6)	11.6 (5.6)

Data are mean (SD).

* Mean $P < 0.05$ when compared with the 0 mg/kg dose.

propofol on this ratio ($P < 0.01$). The multiple comparisons test showed that the ratio of open to total entries was increased significantly only at the highest dose of 9 mg/kg compared with the 0-mg/kg (intralipid) dose ($P < 0.01$).

Diazepam and propofol slightly increased the number of total entries (table 1). Analyses of variance showed that this effect was significant only for propofol ($P < 0.05$). Because of this significant increase in the total number of arm entries, an analysis of covariance for the number of arm entries (dependent = number of open arm entries; covariate = number of closed arm entries) was performed and confirmed a significant increase in the entries into the open arm independent of any modification of the total number of entries ($P < 0.05$).

Figure 2B depicts the effect of diazepam (left) and propofol (right) on the ratio of open to total time. Diazepam (2 mg/kg) significantly increased the ratio of open to total time ($P = 0.01$). For propofol, the ratio of open to total time increased with the dose of propofol. Analysis of variance showed a significant effect of the dose of propofol on this ratio ($P = 0.01$). The multiple comparisons test showed that the ratio of open to total time was significantly increased only at the highest dose of 9 mg/kg compared with the 0-mg/kg (intralipid) dose ($P < 0.01$).

Exploration of the Distal Part of the Open Arms

Figures 3A and B depict the effect of diazepam (left) and propofol (right), respectively, on the number of entries into the distal part of open arms and on the time spent within this distal part of open arms. Diazepam (2 mg/kg) significantly increased the number of entries into and the time spent within the distal part of open arms (entries: $P = 0.01$; time, $P = 0.01$). The number of entries into and the time spent within the distal part of open arms increased with the dose of propofol. Analyses of variance showed a significant effect of the dose of propofol on the number of entries and the time (entries, $P < 0.05$; time, $P < 0.05$). The multiple comparisons test showed that the number of entries into and the time spent within the distal part of open arms was significant at the 9-mg/kg dose compared with the 0-mg/kg control dose (all, $P < 0.01$).

Discussion

Propofol increased the number of entries into the open arms and the time spent within these arms. For the largest dose of propofol studied (9 mg/kg), the level of exploration of the open arms was approximately 50% of all the exploratory activity of the rat. This increase in exploratory activity paralleled the increase in the num-

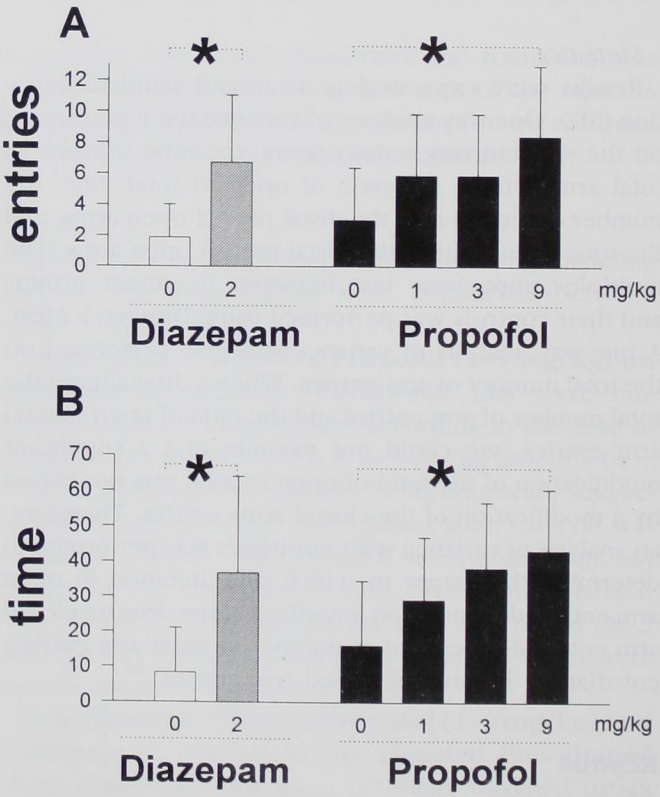

Fig. 3. Effect of 0 and 2 mg/kg diazepam (left) and 0–9 mg/kg propofol (right) on the number of entries in 5 min (*A*) into the distal part of open arms and on the time (*B*) spent within this distal part of the open arms. Results are expressed as the mean and SD. *Means $P < 0.05$ when compared with the 0-mg/kg dose.

ber of entries into the distal part of the open arms and the time spent within this distal part. These results indicate that propofol suppressed the anxiety elicited by the open arms of the elevated plus-shaped maze. Many studies that used a elevated plus-shaped maze have shown that clinically effective anxiolytic agents (such as diazepam, chlordiazepoxide, and midazolam) increase the number of entries into the open arms and the amount of time spent within the open arms.[12,13,22] In contrast, nonanxiolytic major tranquilizers, such as haloperidol,[12] decrease the overall activity but have no specific effect on open arms compared with closed arms. Anxiogenic agents such as yohimbine suppress open-arm exploration below baseline control levels.[12,13] Our results show clearly that propofol has an anxiolytic effect in rats when they are placed in an innate anxiogenic situation. The magnitude of the anxiolytic effect of propofol was comparable to that observed with the prototypical anxiolytic benzodiazepine diazepam during the same experiment. Therefore, propofol displays anxiolytic properties of the same magnitude as those of a standard anxiolytic dose of benzodiazepines (namely diazepam) in a standard animal model of anxiety.

The anxiolytic effect was independent of any sedative effect of propofol. We did not observe an impairment of the locomotor activity either in the activity cage or in the elevated plus-shaped maze itself for the range of doses (1–9 mg/kg) used. Rather, we observed a slight increase in the total number of entries at the highest dose of propofol (9 mg/kg) in the elevated plus-shaped maze. At first glance, the hyperlocomotion in the elevated plus-shaped maze may appear to contradict the absence of any increase in spontaneous locomotor activity in experiment 1. In fact, propofol-induced hyperactivity in the elevated plus-shaped maze might reflect the disinhibitory effect on locomotion that usually accompanies the anxiolytic effect.[23] We also observed an increase of the total number of entries with diazepam, even if this effect was not statistically significant.

The GABA inhibitory transmission plays an important role in anxiety.[24] The anxiolytic effect of benzodiazepines is thought to result from an allosteric modulation of gamma-aminobutyric acid$_A$ ($GABA_A$) receptors. Shekhar *et al.*[25] showed recently that directly enhancing the GABA inhibition with muscimol, a $GABA_A$ agonist, reduced the anxiety observed in the elevated plus-shaped maze, whereas a GABA blockade with bicuculline methionid potentiated the anxiety. Propofol is thought to act on GABA neurons by facilitating the interaction of the inhibitory neurotransmitter GABA with its $GABA_A$ receptor complex, enhancing the affinity of this receptor for GABA, and thereby facilitating the inhibitory actions of GABA.[26–29] This GABA effect of propofol has been shown even at low concentrations that were far from relevant anesthetic concentrations.[30,31] Such an enhancement of GABA inhibition by propofol could be the mechanism by which propofol exhibited anxiolytic properties. This hypothesis should be tested further through pharmacologic verification targeted at the precise site of action of propofol.

In conclusion, propofol exhibited anxiolytic properties that can be dissociated from its sedative properties. Such anxiolytic properties were observed at a dose of 9 mg/kg, but the anesthetic dose of propofol is approximately 100 mg/kg for the strain of rats we used.[32] Therefore, propofol has anxiolytic properties that might be demonstrated at very low doses, less than those needed for sedation or anesthesia. In humans, such anxiolytic properties of propofol could be useful to realize a "conscious sedation" as an adjuvant to local or regional anesthesia or as an adjuvant to medical procedures.

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