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# ANXIOLYTIC-LIKE PROFILE OF PROPOFOL, A GENERAL ANESTHETIC, IN THE PLUS-MAZE TEST IN MICE

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The present study was performed to investigate the effect of propofol on anxiety using the elevated plus-maze test.

Groups of mice received propofol (20, 40, 60 mg/kg) or diazepam (2 mg/kg), caffeine (30 mg/kg), L-arginine (100 mg/kg), m-chlorophenylpiperazine (m-CPP, 2.5 mg/kg) and then were placed in an elevated plus-maze that was composed of two opposite closed arms and two opposite open arms.

Propofol (20, 40, 60 mg/kg) and diazepam (2 mg/kg) significantly increased the percentage of time spent in the open arms compared to control. Caffeine (30 mg/kg) and m-CPP (2.5 mg/kg) decreased the percentage of time spent in the open arms and these effects were antagonized when propofol (40 mg/kg) was administered before the test. L-arginine (100 mg/kg) has also produced anxiogenic effect and this effect was not prevented by propofol. All drugs used in this study did not significantly change locomotor activity.

These results suggest that propofol has anxiolytic effect in plus-maze test.

***Key words:*** *propofol, anxiety, elevated plus-maze test, mice*

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## INTRODUCTION

Propofol is a short-acting intravenous (*iv*) anesthetic agent. There is evidence that propofol acts at several different sites in the nervous system. It has been previously demonstrated that *iv* administration of propofol at subanesthetic doses produces anxiolytic effect [5, 22]. In addition to clinical trials, several experimental studies have demonstrated that propofol produces an anxiolytic effect in animal models [12, 16]. The elevated plus-maze test, one of the most popular animal test for research on anxiety, is based on the natural aversion of rodents for open spaces and uses an elevated plus-maze with two open and two closed arms. This test is rapid and was found to be sensitive to the effects of both anxiolytic and anxiogenic agents [15, 21].

The effects of propofol on anxiety has not been well investigated. It was recently shown that propofol produces anxiolytic effect in both Vogel type conflict test and plus-maze test [16, 20]. The aim of present study was to evaluate the effect of systemic administration of propofol on anxiety in mice using the elevated plus-maze test. We further determined the influence of propofol on anxiogenic-like effects induced by m-CPP a mixed 5-HT agonist, caffeine an adenosine receptor antagonist and L-arginine a NO precursor with the same apparatus.

## MATERIALS and METHODS

### Animals

ICR mice of either sex, weighing 25–40 g were used. Animals were obtained from our department breeding facility and housed in groups of eight mice in a temperature controlled room (20 ± 2°C) with a 12 h light 12 h dark cycle (lights on at 07.00 a.m.). The animals had a period of adaptation for three days and were allowed free access to food and water, prior the experiments. Each animal was used once. All experiments were carried out according to the guidelines of the European Community Council for Experimental Animal Care.

### Drugs

The following drugs were used; propofol (20, 40, 60 mg/kg, *ip*), caffeine (30 mg/kg, *ip*), L-arginine (100 mg/kg, *ip*), m-chlorophenylpiperazine (m-CPP, 2.5 mg/kg, *ip*) and diazepam (2 mg/kg, *ip*). All drugs were obtained from Sigma (USA) and freshly prepared in saline and given ip 30 min before testing in a volume of 10 ml/kg. When combinations of propofol and other drugs were employed, propofol was administered 15 min later than other drugs.

### Elevated plus-maze test

The method initially suggested by Pellow and File to test exploratory activity of mice [21] was employed. The used plus-maze was built of wood and consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm). The arms extended from a central platform (10 × 10 cm) and were raised 50 cm. Each mice was placed at the center of the maze facing closed arm and was allowed to explore the maze for 5 min.

The following measures were taken by an observer unaware of a the treatment: the time spent in open arms, time spent in closed arms, percentage of time spent in open arms.

### Locomotor activity

Spontaneous locomotor activity was measured in an activity cage (Ugo Basile, Varese, Italy) having dimensions of 39 × 28 × 26 cm. The values indicate pulses recorded by the apparatus as the stainless steel bars tilt in response to animal movements. The activity of each mice was automatically recorded for 10 min.

### Statistics

All data are presented as means ± SEM. The data were analyzed using two-way analysis of variance (ANOVA) followed by the post-hoc test. Data from locomotor activity testing were analyzed using two-way ANOVA for treatment as between-subject factor. Statistical analysis was performed using a software package (SPSS 10.1). The level of significance was defined as $p < 0.05$.

## RESULTS

### Elevated plus-maze test

Systemic treatment of mice with 40 and 60 mg/kg of propofol increased the percentage of time spent in open arms when compared to control mice treated with saline ($p < 0.05$). Treatment of mice with propofol at 20 mg/kg did not change percentage of time spent in open arms (Tab. 1). Diazepam significantly increased the percentage of time spent in open arms compared to saline-treated group. Treat-

*Table 1.* Effects of propofol and diazepam on mouse behavior in the plus-maze test

| Treatment | Dose (mg/kg) | n | Time spent in open arms (s) | Time spent in closed arms (s) | Percentage of time spent in open arms |
|---|---|---|---|---|---|
| Saline | | 8 | 78.4 ± 9.2 | 221.6 ± 5.3 | 25.9 ± 3.5 |
| Diazepam | 2 | 8 | 132.9 ± 12.8* | 167.1 ± 9.2 | 44.3 ± 4.8* |
| Propofol | 20 | 8 | 95.8 ± 8.1 | 204.2 ± 5.9 | 31.9 ± 2.5 |
| Propofol | 40 | 8 | 143.9 ± 13.7* | 156.1 ± 6.4 | 47.9 ± 6.1* |
| Propofol | 60 | 8 | 135.7 ± 11.8* | 164.3 ± 8.7 | 44.8 ± 5.3* |

* $p < 0.05$ compared with saline-treated group

*Table 2.* Effects of propofol, caffeine, L-arginine and m-CPP on mouse behavior in the plus-maze test

| Treatment | Dose (mg/kg) | n | Time spent in open arms (s) | Time spent in closed arms (s) | Percentage of time spent in open arms |
|---|---|---|---|---|---|
| Saline | | 8 | 81.2 ± 6.1 | 218.8 ± 11.4 | 27.0 ± 3.4 |
| Saline + saline | | 8 | 79.2 ± 5.1 | 220.8 ± 9.5 | 26.0 ± 2.9 |
| Propofol | 40 | 9 | 135.1 ± 7.4* | 164.9 ± 12.2 | 45.0 ± 4.4* |
| Caffeine | 30 | 8 | 32.9 ± 2.4* | 267.1 ± 9.4 | 10.9 ± 3.1* |
| L-arginine | 100 | 8 | 14.2 ± 2.1* | 285.8 ± 10.9 | 4.7 ± 1.9* |
| m-CPP | 2.5 | 10 | 36.4 ± 3.8* | 263.6 ± 8.4 | 12.1 ± 2.8* |
| Propofol + saline | 40 | 8 | 130.2 ± 2.8 | 169.8 ± 6.4 | 44.0 ± 2.9 |
| Propofol + caffeine | 40 + 30 | 9 | 134.5 ± 4.9$^{\#\sim}$ | 165.5 ± 6.7 | 44.8 ± 5.6$^{\#\sim}$ |
| Propofol + L-arginine | 40 + 100 | 10 | 152.4 ± 10.1$^{\#\alpha}$ | 147.6 ± 9.4 | 50.8 ± 4.7$^{\#\alpha}$ |
| Propofol + m-CPP | 40 + 2.5 | 9 | 145.0 ± 6.4$^{\#\beta}$ | 155.0 ± 5.4 | 48.3 ± 6.8$^{\#\beta}$ |

* $p < 0.05$ compared with saline-treated group, $^{\#}$ $p < 0.05$ compared with saline + saline-treated group, $^{\sim}$ $p < 0.05$ compared with caffeine (30 mg/kg)-treated group, $^{\alpha}$ $p < 0.05$ compared with L-arginine (100 mg/kg)-treated group, $^{\beta}$ $p < 0.05$ compared with m-CPP (2.5 mg/kg)-treated group

*Table 3.* Effects of propofol, caffeine, L-arginine and m-CPP on mouse behavior in the locomotor activity test

| Treatment | Dose (mg/kg) | n | Locomotor activity count (10 min) |
|---|---|---|---|
| Saline | | 8 | 122.4 ± 12.9 |
| Propofol | 20 | 8 | 133.5 ± 9.6 |
| Propofol | 40 | 8 | 136.4 ± 11.2 |
| Propofol | 60 | 10 | 118.5 ± 8.4 |
| Caffeine | 30 | 8 | 114.5 ± 5.6 |
| L-arginine | 100 | 8 | 109.5 ± 8.3 |
| m-CPP | 2.5 | 8 | 138.4.± 11.6 |
| Saline + saline | | 8 | 121.8 ± 8.6 |
| Propofol + caffeine | 40 + 30 | 8 | 126.5 ± 9.6 |
| Propofol + L-arginine | 40 + 100 | 10 | 121.4 ± 12.4 |
| Propofol + m-CPP | 40 + 2.5 | 8 | 105.4 ± 8.7 |

ment of mice with caffeine (30 mg/kg), L-arginine (100 mg/kg) and m-CPP (2.5 mg/kg) significantly decreased the percentage of time spent in open arms (Tab. 2). In mice treated with propofol before administration of caffeine, m-CPP and L-arginine the percentage of time spent in open arms was significantly higher than in saline + saline administered group ($p < 0.05$) (Tab. 2). Administration of propofol after caffeine (30 mg/kg), L-arginine (100 mg/kg) and m-CPP (2.5 mg/kg) significantly increased the percentage of time spent in open arms compared with single drug administrations ($p < 0.05$) (Tab. 2).

## Locomotor activity

All drugs did not significantly change locomotor activity ($p > 0.05$) (Tab. 3).

## DISCUSSION

We have investigated the anxiolytic-like effects of propofol in mice using elevated plus-maze test. The elevated plus-maze test has been validated by several groups, using both anxiogenic and anxiolytic agents. Untreated animals (rats or mice) usually spent more time in closed arms. As a result, the percentage of time spent in open arms have been considered as an index of anxiety in mice [15, 21]. In this study caffeine, a nonselective adenosine antagonist, L-arginine, a nitric oxide (NO) precursor and m-CPP, a 5-HT agonist, significantly decreased the percentage of time spent in the open arms in accordance with previous findings [2, 9, 13]. Propofol (40, 60 mg/kg) and diazepam (2 mg/kg) significantly increased the percentage of time spent in the open arms. Moreover, the effects of propofol on caffeine-, L-arginine- and m-CPP-induced anxiety were also examined. A dose of 40 mg/kg of propofol was used for this purpose. Propofol (40 mg/kg) significantly increased the percentage of time spent in open arms in groups treated with anxiogenic agents (caffeine, L-arginine and m-CPP). The present results suggest that propofol produced anxiolytic-like effect when administered both alone and before anxiogenic drugs. In this study, motor activity was not altered by any of the drugs. Pain et al. demonstrated that propofol did not produce locomotor impairment in rats [19]. These results suggest that the effect of drugs on anxiety has not involved the modulation of motor activity. Neurochemical investigations have linked anxiety to dysfunction in central GABAergic, serotonergic and noradrenergic systems. Because of its role in neuronal function and its wide distribution in the brain, the anxiolytic effect of propofol may result from GABA-potentiating action. The $GABA_A$ receptor complex plays a major role in the pharmacology of anxiety and propofol potentiates $GABA_A$ receptor-mediated effects [4, 5]. It was reported that propofol activated the $GABA_A$ receptor chloride ionophore conductance, which may have contributed to the anxiolytic effect [10]. Chen et al. have demonstrated that propofol activated the rat locus coeruleus neuron $GABA_A$ receptors *in vitro* in brain slice preparations [6]. There is another possible explanation of anxiolytic effects of propofol which is based on an interaction between propofol and serotonergic system in some regions of brain. It is generally accepted that increased 5-HT activity is responsible for anxiety. m-CPP a mixed 5-HT receptor agonist produces anxiogenic-like effect in some models of anxiety probably by activation $5\text{-}HT_{2c}$ receptors [1, 14]. On the other hand, 5-HT receptor agonists and antagonists showed anxiolytic-like activity and the results of this experiment indicated that some of those effects are connected with some functional changes in hippocampus [7, 11]. It is noteworthy that propofol inhibits 5-HT release in the dorsal hippocampus [16]. Inhibition of 5-HT activity in this region may participate in a mechanism underlying the anxiolytic action of propofol. Barann et al. suggest that propofol inhibits function of $5\text{-}HT_3$ receptors [3]. Moreover Grouzmann et al. reported that 5-HT concentrations decreased significantly in the plasma of the patients treated by propofol [10]. We also showed that anxiogenic effect of m-CPP was abolished by propofol in this study. All these results suggest that the effect of propofol on serotonergic system may be involved in anxiolytic-like effect. Adenosine is able to modulate 5-HT release in the hippocampus. Okada et al. have demonstrated a modulatory role of adenosine receptor in 5-HT release in the hippocampus [18]. Caffeine has been shown to have anxiogenic effect in different animal models [2, 13]. In this study, caffeine-induced anxiety-like effect was prevented by propofol (40 mg/kg). It may be assumed that modulatory effect of propofol on serotonergic neurotransmission is involved in the antagonism of the anxiogenic effect of caffeine though another mechanism cannot be excluded.

NO is involved in a large number of physiological functions in the brain [25]**.** In the central nervous system, NO synthase has been localized in such brain structures as the hippothalamus, amygdala and hippocampus [25]. Inhibition of NO synthesis induces an anxiolytic-like effect in animals [24]. Tonner and Scholz have suggested that nitrergic system is involved in anesthetic action of propofol [23]. L-arginine activated NO-cGMP pathway and increased intracellular cGMP resulting in anxiogenic-like effect. On the other hand, propofol has decreased intracellular c-GMP [17]. The inhibition of L-arginine-induced anxiety by propofol seems to be releated to opposite effects of L-arginine and propofol on intracellular cGMP level. These results may suggest that NO may be involved in the effect of propofol on anxiety.

## CONCLUSIONS

Our results clearly suggest that propofol has anxiolytic proporties at doses that do not produce locomotor impairment in the plus-maze test. The anxiolytic effect of propofol may be related to several neuromediator systems that are known to be involved in neuropharmacology of anxiety, such as serotonergic, adenosinergic and nitrergic systems.

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