BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther 20(2), 234-238 (2012)

Dependence Potential of Propofol: Behavioral Pharmacology in Rodents

Hye Jin Cha[1], Ji-Hun Cha[1], Hea-Young Cho[2], Eun-Yong Chung[2], Kyoung-Jin Kwon[1], Jun Yeon Lee[3], Ho-Sang Jeong[1], Hye-Soo Kim[2], Hye-Joo Chung[2] and Eun Jung Kim[1,*]

[1]National Institute of Food and Drug Safety Evaluation, [2]Korea Food and Drug Administration, Cheongwon 363-951, [3]Department of Physiology, College of Oriental Medicine, Daegu Haany University, Daegu 706-060, Republic of Korea

Abstract

Propofol is an anesthetic commonly used to provide sedation or to induce and maintain an anesthetic stated. However, there are reports which indicate propofol may cause psychological dependence or be abused. In the present study, we used various behavioral tests including climbing test, jumping test, conditioned place preference, and self-administration test to assess the dependence potential and abuse liability of propofol compared to a positive control (methamphetamine) or a negative control (saline or intralipid). Among the tests, the conditioned place preference test was conducted with a biased method, and the self-administration test was performed under a fixed ratio (FR) 1 schedule, 1 h per session. No difference was found in the climbing test and jumping test, but propofol (30 mg/kg, i.p.) increased the rewarding effect in the conditioned place preference test, and it showed a positive reinforcing effect compared to the vehicle. These results indicate that propofol tends to show psychological dependence rather than physical dependence, and it seems not to be related with dopaminergic system.

Key Words: Propofol, Psychological dependence, Physical dependence, Animal behavioral test

INTRODUCTION

Drug dependence is defined as the loss of control over drug use, or the compulsive seeking and taking of drugs despite adverse consequences (Koob, 1996). It is caused by drug activity in the brain, but relates to physiologic and social factors. Drug dependence can show a life-long effect. Animal experiments can measure two types of drug dependence: physical dependence and psychological dependence. Physical dependence refers to the state resulting from chronic use of a drug, to the point of tolerance, in which negative physical symptoms or withdrawal result from abrupt drug discontinuation or dosage reduction. The jumping behavior test is used to determine a drug's potential to lead to physical dependence. Psychological dependence indicates non-self restraint of drug use, and involves reinforcement and reward. Reinforcement is an event that increases the probability of a response. Reward has a similar meaning but it is usually related to positive sensations such as pleasure (Koob, 1992). The conditioned place preference test and self-administration test are valid models for investigating the reward effect and reinforcing effect, respectively, of drugs (Mucha *et al*., 1982; Gorelick *et al*., 2004). The climbing behavior has been used in many studies as pre-evaluation test to evaluate a drug's dopaminergic effect.

Propofol is a common anesthetic for conscious sedation or to induce and maintain general anesthesia (Pain *et al*., 1996; LeSage *et al*., 2000). Its pharmacological action sites are gamma-aminobutyric acid (GABA) receptors, N-methyl-D-aspartate (NMDA) receptors, and glycine receptors (Iwersen-Bergmann *et al*., 2001; Nguyen *et al*., 2009). Propofol shows rapid anesthesia induction and rapid recovery after medical processes (Roussin *et al*., 2007). However, there are several reports on its dependence potential (LeSage *et al*., 2000; Pain *et al*., 2002), and further studies are needed to evaluate the dependence potential and abuse liability of propofol. We therefore performed several animal behavioral tests including climbing behavior, jumping behavior, conditioned place preference test, and self-administration test using experimental mice or rats to assess the dependency of propofol.

MATERIALS AND METHODS

Animals and drugs

Male Sprague-Dawley rats (180-220 g) and ICR mice (15-20 g) were obtained from Korea Food and Drug Administration

www.biomolther.org

Open Access http://dx.doi.org/10.4062/biomolther.2012.20.2.234

pISSN: 1976-9148 eISSN: 2005-4483

Received Jul 19, 2011 **Revised** Feb 2, 2012 **Accepted** Feb 7, 2012

***Corresponding Author**
E-mail: lucia@kfda.go.kr
Tel: +82-43-719-5201, Fax: +82-43-719-5200

(AAALAC member, Seoul, Korea) and they were housed in groups, or adequate size, in a temperature-controlled 23 ± 2°C room with a 12 hour light/dark cycle (lights on 08:00 to 20:00). The animals received a solid diet and tap water *ad libitum*, and their treatment conformed to the *Guide for the Care and Use of Laboratory Animals* (NRC 1996). We performed all experiments between 09:00 and 18:00. Methamphetamine HCl and propofol were obtained from Sigma (St. Louis, MO, USA).

Apparatus

The climbing behavior test apparatus was a stainless steel cylinder with many vertical bars, which an experimental mouse could climb. Its floor diameter was 12 cm, and each vertical bar's length was 24 cm. To evaluate jumping behavior test, a transparent box sans ceiling, measuring 30×30×40 cm was used.

The conditioned place preference test chamber had three distinct compartments (white, black, and grey) separated by automatic guillotine doors. To automate data collection, 15 infrared photo-beam detectors were added. The overall inside dimensions were 21×21×68 cm, and the unit's base measured 86.4×25.4 cm. The manufacturer provided the mounting holes for the ENV-013 IR Infrared Sensor Package (Med Inc., USA), which places six photo-beams across the white and black zones, 1.25 cm from each end wall, with 5 cm intervals between the beams. The choice compartments were 28 cm long. One choice compartment was all black, with a stainless steel grid rod floor consisting of 4.8 mm rods on 16 mm centers. The other compartment was all white, with a 1.25×1.25 cm stainless steel mesh floor.

The self-administration test chamber was from Med Inc. (USA) and measured 29×21×24 cm. The chambers contained two levers, an active lever to deliver a drug dose, via the jugular vein, through a connected catheter and an inactive lever, not connected to the experimental animal. Infusion pumps were placed outside the chamber and connected to a 10 ml syringe. We connected the chamber to a computer, to record test data and control the experimental processes.

Methods

Climbing behavior test: One group of mice was administered with the negative control (saline, 1 mg/kg, i.p.) or one of the three doses of propofol (30, 60, or 90 mg/kg, i.p.) for 40 min. Then for 1 min, their climbing duration was checked, using a stopwatch. The other group of mice was pre-treated with the negative control (saline, 1 mg/kg, i.p.) or one of the three doses of propofol (30, 60, or 90 mg/kg, i.p.) for 40 min before the test. Then just before testing, apomorphine (2 mg/kg, i.p.) was administered to each subject and timed their climbing duration as above. The tests were repeated three times, with a time-out period of 10 min.

Jumping test: One group of mice was administered the negative control (saline, 1 mg/kg, i.p.), or one of the three doses of propofol (30, 60, or 90 mg/kg, i.p.) for 40 min and followed by naloxone (10 mg/kg, i.p.). Then for 15 min, the jumping numbers of the animals were counted. The other group of mice was pre-treated with the negative control (saline, 1 mg/kg, i.p.) or one of the three doses of propofol (30, 60, or 90 mg/kg, i.p.) for 40 min before the test. Next, morphine (150 mg/kg, s.c.) was administered and followed by naloxone administration (10 mg/kg, i.p.) 4 hrs after the morphine treatment. The jumping number was counted for 15 min. The experiment was repeated three times.

Conditioned place preference test: Before starting the experiment, the rats were acclimated to the experimental apparatus and handled for 6 days. The procedure was similar to that described previously (Bardo *et al*., 1995; Narita *et al*., 2004).

Each experiment consisted of three phases, as follows.

Pre-conditioning: For 2 days (days 1 and 2) the rats were allowed free access to both compartments of the apparatus for 15 min (900 s) each day. One day 2, the time spent by the rats in each compartment was recorded and served as a baseline. The rats showed preference for the black compartment was selected for further experiments and divided into two groups.

Conditioning: Conditioning was conducted for 8 days (days 3 to 10), for one session per day. On day 3, one group of the selected rats was treated with drugs (methamphetamine, 1 mg/kg, i.p., one of the three doses of propofol, 30, 60, and 90 mg/kg, i.p.), and placed in the non-preferred compartment (white) for 30 min. The other group of rats was treated with saline, and placed in the preferred compartment (black) for 30 min. The groups were switched everyday and the same procedure was conducted.

Post-conditioning: On day 11, the rats were allowed to access freely both compartments of the apparatus for 15 min (900 s). The time spent by the rats in each compartment was recorded, with these values serving as a test line.

Self-administration test: Surgical procedures were as follows. The rats were anesthetized with pentobarbital sodium (Entobar®, Hanlim pharmaceuticals). The surgical procedures adhered to aseptic conditions described previously (Weeks, 1972; Mucha *et al*., 1982). Briefly, a catheter was inserted into each rat's right jugular vein. The catheter exited on the rat's shoulder. The rats received heparin everyday of the experimental periods. After surgery, each rat recovered for at least 14 days in a controlled cage, receiving a solid diet and tap water *ad libitum*.

The testing procedures were as follows. The rats self-administered 2 mg/kg of propofol for 3 days to stabilize the response (Picetti *et al*., 2011). Then the experiment was continued for more than 30 days at 1 mg/kg of propofol in the rats that showed stabilized response. The self-administration test was performed for 6 s followed by 20 s of time-out, during daily 1 h session on a fixed-ratio 1 (FR1) reinforcement schedule. With this schedule, when a rat presses the active lever, it receives a certain drug dose injected into the jugular vein through the catheter. The self-administration chamber contains two levers linked to a computer program which records the experimental data. The vehicle substance (intralipid) was used as a negative control.

Statistics: The data are expressed as the mean ± S.E. The climbing and jumping data were analyzed via paired *t*-tests. Likewise, paired *t*-tests were used to compare time spent in the drug- and saline-paired compartments in the CPP test. To analyze the self-administration test data, a two-way ANOVA was employed ($p<0.05$).

RESULTS

Climbing behavior test

We measured climbing behavior in experimental mice with

or without pre-treatment with the negative control (saline, 1 mg/kg, i.p.) or propofol (30, 60, or 90 mg/kg, i.p.). In the group without apomorphine treatment, there was statistically significant decrease of climbing duration in the propofol treated group (90 mg/kg, i.p.). In the apomorphine-treated group, on the other hand, 2 of the propofol treated groups (60 and 90 mg/kg, i.p.) tended to spend less time for climbing as compared to the saline treated group. However, the difference between these two groups was neither statistically significant nor dose dependent (Fig. 1).

Jumping behavior test

In the jumping behavior test, we administered the negative control (saline, 1 mg/kg, i.p.) or one of the three doses of propofol (30, 60, or 90 mg/kg, i.p.) prior to administrating morphine. The mice received morphine (150 mg/kg, s.c.) 4 hrs before naloxone (10 mg/kg, i.p.) administration. As shown in Fig. 2, there was no significant different response between the rats in the saline and propofol treated groups without morphine administration. Interestingly, however, animals in the propofol treated groups which were treated with morphine showed a tendency of decreasing number of jumps compared with the corresponding saline treated animals, though it was not statistically significant.

Conditioned place preference

The conditioned place preference test was conducted with 8 SD rats in each group. This experiment was performed for 11 days and comprised 3 phases: pre-conditioning (2 days) where rats were acclimated to the CPP apparatus, conditioning (8 days) where the rats were administered the drug (methamphetamine or 3 doses of propofol) or saline, and post-conditioning (1 day). The CPP was assessed 2 times on the second day of pre-conditioning and post-conditioning day. Methamphetamine (1 mg/kg) increased the place preference more than 200 sec compared to the negative control (saline) group. Only one dose of propofol (30 mg/kg) increased the place preference compared to saline treatment, but did not show dose dependency (Fig. 3).

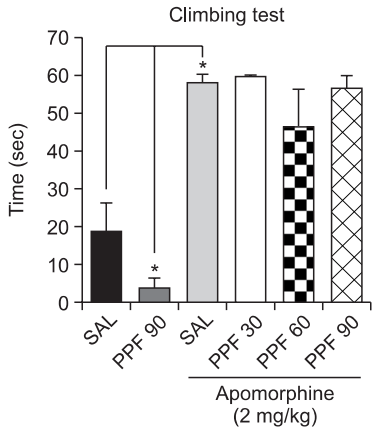

Fig. 1. Climbing behaviors were measured after injection of apomorphine to each subject (2 mg/kg,subcutaneously). The pre-treatments were propofol (30, 60, or 90 mg/kg, intraperitoneally), administeredbefore the apomorphine treatment. Data are expressed as mean ± S.E. (n=5). The experiment was repeated 3 times. *p< 0.05, compared with saline treated group (t-test).

Self-administration

The self-administration test was maintained on a fixed-ratio (FR) 1 schedule for more than 30 days, and the responses on the active lever were checked on a daily basis. The experimental rats treated with propofol (1 mg/kg) demonstrated relatively higher active responses than the negative control (intralipid) group. The difference of the responses on the active lever between the two groups was more distinctive as the time passes. The results are shown in Fig. 4.

DISCUSSION

There are many case reports and surveys warning abuse possibility of propofol (Wischmeyer *et al*., 2007; Wilson *et al*., 2010; Kim, 2011), and studies on dependence possibility of propofol (Pain *et al*., 1996; Weerts *et al*., 1999; Pain *et al*., 2002) as well. However previous studies dealt with only one aspect of dependency such as rewarding effect or reinforcing effect represented by conditioned place preference and self-administration respectively. In our study, we covered animal behavioral tests related with drug dependence including psychological and physical dependence, and presented comprehensive data from conditioned place preference and self-administration data in a single setting.

Climbing behavior was performed as pre-evaluating experiment to see if propofol has any relation with dopaminergic system. In this experiment, there was statistically significant decrease of climbing period in the propofol (90 mg/kg) treated group without apomorphine. The result indicated that propofol might have a relation with dopaminergic system. However there was no difference between the negative control group and propofol treated group with apomorphine. Also relationship of a drug with dopaminergic system can be inferred out of other animal behavioral experiment such as locomotor activity. Pain *et al*. had showed that high doses of propofol (60, 90 mg/kg) decreased locomotor activity in rats, whereas our

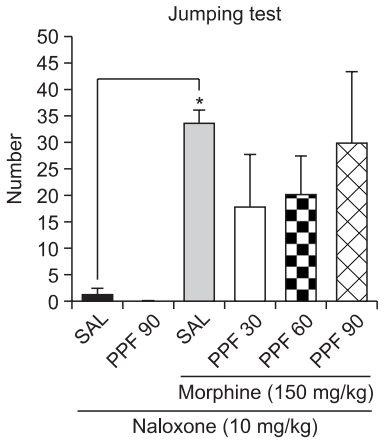

Fig. 2. Propofol (30, 60, or 90 mg/kg, intraperitoneally) was administered 40 min prior to the administrationof morphine. And then morphine (150 mg/kg, subcutaneously) was administered 4 hrs prior to naloxonetreatment. The jumping score was measured for 15 min immediately after the injection of naloxone (10 mg/kg, intraperitoneally). Each value was the mean ± S.E. (n=5). The experiment was repeated 3 times. *p<0.05, compared with saline treated group (t-test).

locomotor activity data using rats showed increases in 30 mg/kg and 60 mg/kg administered group (data not shown). Pain *et al*. inferred about the reason that the doses might induce sedation to the experimental rats. However, it might be caused by a pharmacological effect of propofol, since our locomotor activity experiment showed the contrary result. Therefore, further studies are needed such as proopofol induced locomotor activity using mice with these doses to investigate the relationship between propofol and dopaminergic system.

The jumping behavior test was conducted to check if propofol is potential to have physical dependence. In our result, there was a tendency for decrease in the number of jumping in the propofol treated animals (30, 60 and 90 mg/kg) with morphine, compared with saline treated animals. Studies have commonly noted that withdrawal jumping behavior is the most reliable and generally useful for measuring physical dependence in rodents, especially with regard to opioids (Way *et al*., 1969; Saelens *et al*., 1971; Smits, 1975; Ritzmann, 1981; el-Kadi, 1994; Kest *et al*., 2001). However, there is a report that has indicated that different neural substrates may contribute to the various signs and symptoms of withdrawal syndrome (Koob *et al*., 1992). Therefore, additional research is needed to confirm propofol's tendency to cause physical dependence.

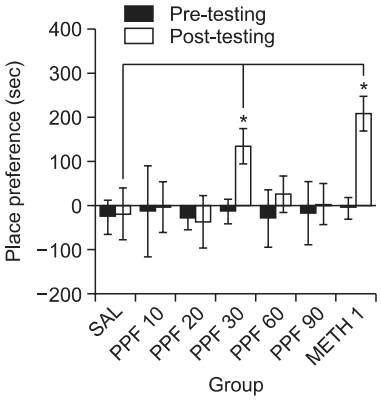

Fig. 3. Rats were pre-tested for 2 days without drug treatment. Propofol (30, 60, or 90 mg/kg,intraperitoneally) and Methamphetamine (1 mg/kg, intraperitoneally) were administered to the rats once aday for 8 days. Place preference was measured on the next day after the 8 days' drug administration. Thetime spent in the black chamber was counted as minus figures, and the time spent in the white chamber wascounted as plus figures. The final score was calculated with the figures arithmetically. Data were expressedas the mean ± S.E. (n=5). The experiment was repeated 3 times. **p*<0.05, compared with saline treatedgroup.

In the conditioned place preference test, we selected rats that spent more time in the black chamber through pre-conditioning test. This is called "biased" procedure. The biased-unbiased distinction is used to describe the experimental procedure for assigning the drug-paired conditioned stimulus. It means that if naïve untrained animals show a significant preference during the pre-test for one side of the apparatus, the apparatus is described as "biased". It is described as "unbiased" when the animals show no preference for one compartment specifically (LeFoll and Goldberg, 2005). The "biased" experimental design was used in the present study to reduce bias from individual difference of experimental animals.

As shown in the Fig. 3, propofol (30 mg/kg) increased time spent in the white chamber, suggesting that propofol might have psychological dependence. The decline in preference at higher doses (60 and 90 mg/kg) may result from sedation in that those doses might have been too high for the experimental animals. This result is consistent with previous work (Pain *et al*., 1996; Iwersen-Bergmann *et al*., 2001).

Self-administration is an accepted model in animals and humans (Sneyd, 1994; Kim *et al*., 1998) to investigate psychological dependence of drugs with the conditioned place preference test. There are two ways of setting self-administration experimental design: fixed-ratio schedule and progressive-ratio schedule. It is noted that the fixed-ratio schedule is suitable for determining reinforcing effects of a particular drug whereas the progressive-ratio schedule is proper detecting relative magnitude of reinforcing effects of different drugs (Ward *et al*., 1996). The fixed-ratio schedule was applied in the present study because it is necessary to find out whether propofol induced self-administration or not.

In our research, the experimental rats that propofol was treated acquired self-administration overall, which means the rats treated with propofol demonstrated statistically significant active responses compared with that of the negative control (intralipid) group. The differences between the negative control group and the propofol treated group were more distinctive as the time passed.

However, there has been a controversy that the results from conditioned place preference and self-administration are

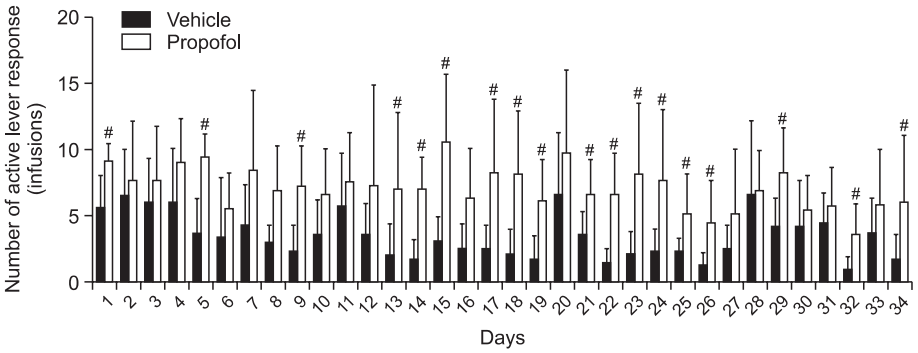

Fig. 4. The rats were administered propofol (1 mg/kg per infusion) in the way of self-administration for 1 hsession each day. Lever responses were checked everyday for 34 days. Intralipid was used as a negativecontrol. Data are expressed as mean ± S.E. (n=7). $^{\#}p$<0.05, compared with the negative control group.

sometimes different for some animal strains. Especially, in tests with conditioned place preference, animals do not voluntarily self-administer drugs, so whether subjects actually will differ with regard to drug taking is unclear (Ward *et al*., 1996). Thus, it would be reasonable to check both experimental methods in order to determine whether a drug has psychological dependence or not. For this reason, both methods were applied in the present study, and it could be concluded that propofol certainly has the potential for psychological dependence in rats.

The results from the present research suggest that it would be worthwhile monitoring usage of propofol with precaution to prevent possible drug abuse in the future.

ACKNOWLEDGMENTS

This work was supported by the National Institute of Food and Drug Safety Evaluation (08171KFDA461).

REFERENCES

Bardo, M. T., Rowlett, J. K. and Harris, M. J. (1995) Conditioned place preference using opiate and stimulant drugs: a meta-analysis. *Neurosci. Biobehav. Rev.* **19**, 39-51.

el-Kadi, A. O. and Sharif, S. I. (1994) The influence of various experimental conditions on the expression of naloxone-induced withdrawal symptoms in mice. *Gen. Pharmacol.* **25**, 1505-1510.

Gorelick, D. A., Gardner, E. L. and Xi, Z. X. (2004) Agents in development for the management of cocaine abuse. *Drugs.* **24**, 1547-1573.

Iwersen-Bergmann, S., Rösner, P., Kühnau, H. C., Junge, M. and Schmoldt, A. (2001) Death after excessive propofol abuse. *Int. J. Legal. Med.* **114**, 248-251.

Kest, B., Palmese, C. A., Hopkins, E., Adler, M. and Juni, A. (2001) Assessment of acute and chronic morphine dependence in male and female mice. *Pharmacol. Biochem. Behav.* **70**, 149-156.

Kim, D. K. (2011) Propofol use for sedation or sedation for propofol use? *J. Anesth.* (Epub ahead of print)

Kim, H. S., Jang, C. G., Oh, K. W., Oh, S., Rheu, H. M., Rhee, G. S., Seong, Y. H. and Park, W. K. (1998) Effects of ginseng total saponin on morphine-induced hyperactivity and conditioned place preference in mice. *J. Ethnopharmacol.* **60**, 33-42.

Koob, G. F. (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends. Pharmacol. Sci.* **13**, 177-184.

Koob, G. F. (1996) Drug addiction: the yin and yang of hedonic homeostasis. *Neuron.* **16**, 893-896.

Le Foll, B. and Goldberg, S. R. (2005) Nicotine induces conditioned place preferences over a large range of doses in rats. *Psychopharmacology (Berl).* **178**, 481-492.

LeSage, M. G., Stafford, D. and Glowa, J. R. (2000) Abuse liability of the anesthetic propofol: self-administration of propofol in rats under fixed-ratio schedules of drug delivery. *Psychopharmacology (Berl).* **153**, 148-154.

Mucha, R. F., van der Kooy, D., O'Shaughnessy, M. and Bucenieks, P. (1982) Drug reinforcement studied by the use of place conditioning in rat. *Brain Res.* **243**, 91-105.

Narita, M., Akai, H., Nagumo, Y., Sunagawa, N., Hasebe, K., Nagase, H., Kita, T., Hara, C. and Suzuki, T. (2004) Implications of protein kinase C in the nucleus accumbens in the development of sensitization to methamphetamine in rats. *Neuroscience* **127**, 941-948.

Nguyen, H. T., Li, K. Y., daGraca, R. L., Delphin, E., Xiong, M. and Ye, J. H. (2009) Behavior and cellular evidence for propofol-induced hypnosis involving brain glycine receptors. *Anesthesiology* **110**, 326-332.

Pain, L., Gobaille, S., Schleef, C., Aunis, D. and Oberling, P. (2002) In vivo dopamine measurements in the nucleus accumbens after nonanesthetic and anesthetic doses of propofol in rats. *Anesth. Analg.* **95**, 915-919.

Pain, L., Oberling, P., Sandner, G. and Di Scala, G. (1996) Effect of propofol on affective state as assessed by place conditioning paradigm in rats. *Anesthesiology* **85**, 121-128.

Picetti, R., Caccavo, J. A., Ho, A. and Kreek, M. J. (2011) Dose escalation and dose preference in extended-access heroin self-administration in Lewis and Fischer rats. *Psychopharmacology (Berl).* (Epub ahead of print)

Ritzmann, R. F. (1981) Opiate dependence following acute injections of morphine and naloxone: the assessment of various withdrawal signs. *Pharmacol. Biochem. Behav.* **14**, 575-577.

Roussin, A., Montastruc, J. L. and Lapeyre-Mestre, M. (2007) Pharmacological and clinical evidences on the potential for abuse and dependence of propofol: a review of the literature. *Fundam. Clin. Pharmacol.* **21**, 459-466.

Saelens, J. K., Granat, F. R. and Sawyer, W. K. (1971) The mouse jumping test--a simple screening method to estimate the physical dependence capacity of analgesics. *Arch. Int. Pharmacodyn. Ther.* **190**, 213-218.

Smits, S. E. (1975) Quantitation of physical dependence in mice by naloxone-precipitated jumping after a single dose of morphine. *Res. Commun. Chem. Pathol. Pharmacol.* **10**, 651-661.

Sneyd, J. R. (1994) Propofol at a subanesthetic dose may have abuse potential in healthy volunteers. *Anesth. Analg.* **78**, 1032-1033.

Ward, A. S., Li, D. H., Luedtke, R. R. and Emmett-Oglesby, M. W. (1996) Variations in cocaine self-administration by inbred rat strains under a progressive-ratio schedule. *Psychopharmacology (Berl)*. **127**, 204-212.

Way, E. L., Loh, H. H. and Shen, F. H. (1969) Simultaneous quantitative assessment of morphine tolerance and physical dependence. *J. Pharmacol. Exp. Ther.* **167**, 1-8.

Weeks, J. R. (1972). Long term intraveneous infusion. (R. D. Myers, Ed.), pp. 155-168. Academic Press, New York.

Weerts, E. M., Ator, N. A. and Griffiths, R. R. (1999) Comparison of the intravenous reinforcing effects of propofol and methohexital in baboons. *Drug. Alcohol. Depend.* **57**, 51-60.

Wilson, C., Canning, P. and Caravati, E. M. (2010) The abuse potential of propofol. *Clin. Toxicol (Phila)*. **48**, 165-170.

Wischmeyer, P. E., Johnson, B. R., Wilson, J. E., Dingmann, C., Bachman, H. M., Roller, E., Tran, Z. V. and Henthorn, T. K. (2007) A survey of propofol abuse in academic anesthesia programs. *Anesth. Analg.* **105**, 1066-1071.