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Silexan, an essential oil from flowers of *Lavandula angustifolia*, is not recognized as benzodiazepine-like in rats trained to discriminate a diazepam cue

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ABSTRACT

Recently, an essential oil of selected quality produced from the flowering tops of *Lavandula angustifolia* Mill. by steam distillation (Silexan) has been approved in Germany for the treatment of restlessness in case of anxious mood. Based on the observed clinical effects, it has been speculated that lavender oil may exert benzodiazepine-like action including the known dependence and abuse potential of this class of drugs. Although no evidence for such an activity was generated during the long-standing medicinal use of lavender oil, further preclinical investigations were now conducted to evaluate this potential side effect in more detail.

Twelve adult, male, Sprague-Dawley rats were trained to discriminate the benzodiazepine drug diazepam (2 mg/kg i.p.) from saline using a two-lever operant procedure. After approximately 40 training sessions the majority of rats learned the discrimination and pre-treatment with ascending doses of diazepam (0.3–2 mg/kg i.p.) produced a dose related generalization to the diazepam cue. In these same animals Silexan was administered to see if animals recognized the drug as "diazepam-like" i.e. generalized to diazepam or "saline-like". Silexan tested at doses 3–30 mg/kg i.p. produced almost exclusively (>90%) saline-like responding. Also there was no effect of Silexan on response rate, i.e. rate of lever pressing, at any dose suggesting that the test article is well tolerated and does not exert a sedating effect.

In sum, Silexan has no diazepam-like interoceptive property in adult, male rats. This suggests that Silexan does not share the potential of benzodiazepines to induce the development of tolerance, dependence and addiction.

Introduction

The use of lavender oil by oral intake, inhalation or topical application for the treatment of anxiety and improvement of sleep quality as well as an agent with calming, mood enhancing, spasmolytic, antihypertensive, antimicrobial, analgesic and wound healing effects has a long tradition (Sasannejad et al. 2012). Recently, an essential oil of selected quality produced from the flowering tops of *Lavandula angustifolia* Mill. (syn. *Lavandula officinalis* Chaix ex Vill., *Lavandula vera* DC.) by steam distillation (Silexan[1]) has been approved in Germany for the treatment of restlessness in case of anxious mood. The efficacy and safety of Silexan in anxiety disorders has been demonstrated in several randomized, controlled clinical trials as well as an open pilot study (Kasper et al. 2010).

Although the medical use of lavender oil is well established and steadily growing, knowledge on its pharmacological mechanism of action is still limited. Based on the observed clinical effects, it has been speculated that lavender oil may exert benzodiazepine-like action on $GABA_A$ receptors (Huang et al. 2008). $GABA_A$ receptors are pentameric ligand-gated chloride ion channels that are activated by binding gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the central nervous system (Sieghart 2006). Benzodiazepines bind to a specific site present on most isoforms of $GABA_A$ receptors and thereby positively modulate the ability of GABA to increase chloride conductance resulting in hyperpolarization and transmission of inhibitory signals. Benzodiazepines have a significant medical value for conditions such as anxiety, insomnia, seizure disorders or spasticity. Although they are generally safe during short-time application, long-term use of benzodiazepines may lead to the development of tolerance, dependence, withdrawal symptoms, and addiction (Tan et al. 2011).

The evaluation of dependence potential generally needs to be considered for all new CNS-active medicinal products (Anonymous 2006). In clinical studies Silexan was devoid of sedative effects and

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[1] Silexan® is the active pharmaceutical ingredient of Lasea® (W. Spitzner Arzneimittelfabrik GmbH, Ettlingen, Germany).

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did not display any potential for drug abuse (Woelk & Schlaefke 2010). Similarly, no withdrawal symptoms were observed after discontinuation of treatment (Kasper et al. 2011). However, detailed studies on abuse liability in human volunteers are often limited by the constraints that participants are willing to commit for the conduct of a comprehensive assessment. For a thorough evaluation of a potential abuse liability of drugs, animal models offer advantages and generally provide tools with a high predictive value (Carter & Griffiths 2009). In particular, drug discrimination studies are considered to be most powerful methods for assessing similarities between a test compound and a prototypic drug of abuse (Moser et al. 2011).

Drug discrimination procedures are based on the inherent property of a number of drugs including a variety of abused substances such as opioids (e.g. morphine, heroin) and benzodiazepines (e.g. diazepam, midazolam) to induce a discriminative cue to which animals may be trained. Typically this is achieved using a two lever operant test chamber where rats are trained to press either lever for food reward. Over a period of weeks the rats are trained to associate one lever with vehicle or placebo treatment, and the other level with drug administration. For example, benzodiazepine drug discrimination has been extensively studied and characterized using training drugs such as diazepam (e.g. Haug and Götestam 1982; Michelsen et al. 2007). Once successfully trained, tests of drug substitution can be conducted subsequently. A wide number of benzodiazepine derivatives, e.g. midazolam or chlordiazepoxide will generalize to a diazepam cue. Drugs from other classes will tend not to be generalized to the drug cue, i.e. seen as placebo-like, demonstrating that the cue is pharmacologically specific. Hence, it was the purpose of the present study to evaluate the effect of Silexan in rats trained to discriminate diazepam from saline, with a view to establish whether the essential oil would be recognized as diazepam-like and by extension may possess a similar abuse liability.

Materials and methods

Experimental materials

Silexan (WS® 1265, lot FSa 3/112) is an essential oil of selected quality produced from the flowering tops of *Lavandula angustifolia* by steam distillation. It was prepared as a liquid injectable solution at three different concentrations (1.5, 5 and 15 mg/ml) in a mixture of medium chain triglycerides (Myritol 318 PH, Cognis, Monheim, Germany) and filled into clear glass septa vials. Vials were stored at room temperature away from light until use. The test article was administered intraperitoneally (i.p.) at a dose volume of 2 ml/kg body weight. In deviation from the oral route of administration in humans, i.p. injection was deliberately chosen to avoid any possible interference or preference due to the characteristic lavender odour.

Diazepam (5 mg/ml Injection USP, Sandoz) was diluted for i.p. injection in 5% Tween in saline. Solutions were prepared freshly every 2–3 days and were stored at room temperature in glass ampoules. Diazepam was tested as a positive control at doses of 0.3, 1 and 2 mg/kg. Dose volume was 1 ml/kg.

Apparatus

The operant test system consisted of twelve test chambers each measuring 28 cm long, 21 cm wide and 21 cm high (Med. Associates Inc., St. Albans, VT). Each chamber contained a food pellet dispenser, 2 response levers 4.5 cm wide and located 7 cm above the floor of the chamber, and a stimulus light located 6 cm above each lever. Each chamber was illuminated by a house light and housed in a sound-attenuating box equipped with a ventilating fan. The apparatus was controlled by Med-PC software (Med. Associates Ins., St. Albans, VT).

Experimental animals

The animal experiments were conducted in accordance with internationally accepted principles for the care and use of laboratory animal (Anonymous 2011). The investigations were performed with 12 male Sprague-Dawley rats purchased from Charles River Laboratories (St. Constant, Quebec, Canada). Their initial body weights on arrival were approximately 200 g. Animals were housed singly, in solid bottom plastic cages, (approx. 42 cm × 22 cm × 20 cm) with corn cob bedding and environmental enrichment. Cages were changed once per week or as needed.

Animals were provided with automated 12 h light/12 h dark cycle, which could be interrupted for study-related activities. Heating and cooling was electronically controlled and was set to maintain the animal room in a temperature range from 19 to 23 °C with a relative humidity range of 30–70%. The test room ventilation was designed to provide approximately 10–20 cf. filtered air changes per hour.

Animals had restricted food access, sufficient to maintain body weight and encourage motivation to respond for food reinforcement. The restricted amount of standard rodent chow (approximately 18 g) was weighed daily and provided on stainless steel feeders at the end of the day. Tap water was provided *ad libitum* in water bottles made of polysulfone with stainless steel sipper tubes.

All animals were given a brief acclimatization period to the testing facility prior to commencement of training in the operant test system. The entire training was divided in two phases:

Phase 1

Animals were trained to press on each lever for food reinforcement (45 mg precision pellets, Bio Serve, Frenchtown, NJ, USA). The schedule requirements gradually increased to a final fixed ratio of 10, i.e. 10 responses on the designated lever required for delivery of a single pellet, whereas responses on the second lever was recorded but not reinforced. At this point, drug discrimination training began. Rats were treated with either diazepam (2 mg/kg) or saline (1 ml/kg) by the i.p. route 30 min before each operant session. The animals were trained to press the left lever after saline injection and the right lever after drug injection, i.e. if the rats received drug only the right lever was associated with food reward, and if the rats received saline only the left lever was associated with food reward. Training sessions were either of 20 min duration or until the delivery of 50 pellets. Care was taken to randomize treatment sequence over consecutive sessions. Training sessions were typically run 5 days/week with a single session conducted per day. Training continued until animals attained appropriate stimulus control that was defined as six consecutive sessions where animals made no more than 18 lever presses before the delivery of the first reward, and at least 95% total responses on the appropriate lever. These training criteria were based on previously published work (Joharchi et al. 1993; Recker and Higgins 2004).

Phase 2

Phase 2 commenced after successful outcome from Phase 1, i.e. robust discrimination training to diazepam in at least half of the study animals, i.e. 6 rats. Other rats entered Phase 2 testing as they acquired adequate stimulus control.

Substitution testing was conducted no more than twice per week, subject to appropriate performance on intervening days. On test days, both levers were designated active, i.e. every 10th response on either lever resulted in the delivery of a food pellet. Test