Pharmacology, Biochemistry and Behavior 112 (2013) 42–48

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Anxiolytic-like effects of carvacryl acetate, a derivative of carvacrol, in mice

Lúcio Fernandes Pires [a], Luciana Muratori Costa [b], Oskar Almeida Silva [c], Antonia Amanda Cardoso de Almeida [d], Gilberto Santos Cerqueira [e], Damião Pergentino de Sousa [f], Rivelilson Mendes de Freitas [a,b,c,*]

[a] Postgraduate Program of Pharmacology, Federal University of Piauí, Teresina, Piauí, Brazil
[b] Department of Biochemistry and Pharmacology, Laboratory of Experimental Neurochemistry Research, Center of Pharmaceutical Technology, Federal University of Piauí, Teresina, Piauí, Brazil
[c] Postgraduate Program in Pharmaceutical Sciences, Federal University of Piauí, Teresina, Piauí, Brazil
[d] Postgraduate Program in Biotechnology (RENORBIO), Federal University of Piauí, Teresina, Piauí, Brazil
[e] Department of Physiology and Pharmacology, Faculty of Medicine, Federal University of Ceará, Rua Coronel Nunes de Melo, 1127 Ceará, Brazil
[f] Department of Physiology, Federal University of Paraíba (DFS/UFPB), João Pessoa, Paraíba, Brazil

ARTICLE INFO

Article history:
Received 8 May 2013
Received in revised form 25 August 2013
Accepted 5 September 2013
Available online 12 September 2013

Keywords:
Carvacryl acetate
Light–dark test
Marble burying test
Mouse
Plus maze test

ABSTRACT

Studies showing anxiolytic-like properties of natural products have grown. This paper evaluated if carvacryl acetate (CA) could be studied as an alternative drug to treat anxiety disorders. Elevated plus maze (EPM) tests , light-dark box (LDB) tests, and marble-burying tests (MBTs) were performed on mice. In the first protocol, the anxiolytic-like activities of CA 25, 50, 75 and 100 mg/kg at single doses were compared to those of the vehicle, buspirone 5 mg/kg (BUSP) and diazepam 1 mg/kg (DZP). In the second protocol, the anxiolytic-like actions of CA were tested for GABAergic and serotonergic systems. The time spent in the open arms (TSOA) and the number of open arms entries (NOAE) were measured in EPM; the time spent in the light box (TSLB) and the number of entries to light box (NELB) were measured in LDB; and the number of marbles buried (NMB) were measured in MBT. CA increased TSOA and NOAE in the EPM, as well as TSLB and NELB in the LDB and the NMB in the MBT. The anxiolytic-like activity of CA 25; 50; 75 and 100 mg/kg was not associated with psychomotor retardation in the open field test and in the Rota rod test, contrarily with what happened with DZP. In the second protocol, to suggest the mechanism of action of CA, flumazenil 25 mg/kg ip (FLU) and WAY 100,635 10 mg/kg ip (WAY–5-HT1A antagonist) were also used. FLU + CA100 reduced TSOA in the EPM when compared to CA100 but WAY + CA100 did not. In LDB, FLU + CA100 reduced the TSLB when compared to CA100 but WAY + CA100 did not. In the MBT, FLU + CA100 inhibited the effect of CA100 on the NMB but WAY + CA100 did not. In conclusion, CA seems to have an anxiolytic-like effect, probably due to GABAergic agonist action, without psychomotor side effects.

1. Introduction

Anxiety is a behavioral mechanism in animals to cope with difficult situations. Fear and anxiety share the same physical and mental symptoms, like avoidance, hypervigilance and an increased alert level to avoid damage (Bernick, 2010; Higgins and George, 2010).

Anxiety disorders, which are among the most prevalent psychiatric conditions in most populations, produce morbidity, frequent use of health services, impair the functional performance of the individual and comorbidities with chronic medical conditions (Campbell-Sills et al., 2013; Johansson et al., 2013).

However, most patients with anxiety disorders experience a progressive reduction of their symptoms with continuous use of selective serotonin reuptake inhibitors (SSRIs) and immediate symptom relief with benzodiazepines (BDZ), as diazepam (DZP) (Ravindran and Stein, 2010). Buspirone (BUSP) is another drug used to treat anxiety symptoms (Celada et al., 2013). These drugs which reduce anxiety are described as anxiolytic drugs and act by altering the chemical synaptic transmission in the brain.

Several studies have shown that certain substances derived from plants may have central nervous system (CNS) effects, such as the ethanolic extract from *Platonia insignis* (Costa Júnior et al., 2010), the *Citrus limon* essential oil (Campêlo et al., 2011), the *Bellis perennis* (Marques et al., 2011), the essential oil from *Eupatorium triplinerve*

Abbreviations: BDZ, benzodiazepinic; BUSP, buspirone; CA, carvacryl acetate; DZP, diazepam; EPM, elevated plus-maze test; EL, epoxy-limonene; FLU, flumazenil; GABA, gamma-aminobutyric acid; LDB, light–dark box test; MBT, marble-burying test; NF, number of falls; NELB, number of entries to the light box; NMB, number of marbles buried; NOAE, number of open arms entries; NSC, number of squares crossed; OFT, open-field test; SSRI, selective serotonin reuptake reuptake inhibitor; TSLB, time spent in light box; TSOA, time spent in the open arms; TSRB, time spent on the rotating bar; WAY, WAY 100635.

* Corresponding author at: Laboratório de Pesquisa em Neuroquímica Experimental, Universidade Federal do Piauí-UFPI, Campus Universitário Ministro Petrônio Portella, Curso de Farmácia, Bairro Ininga, Teresina, Piauí, Cep: 64.049-550, Brazil. Tel.: +55 86 3215 5870.
E-mail addresses: lucio-pires@uol.com.br (L.F. Pires), rivelilson@pq.cnpq.br (R.M. de Freitas).

0091-3057/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.pbb.2013.09.001

Vahl (Melo et al., 2013), the monoterpenes isopulegol (Silva et al., 2007), 1,4 cineole (Gomes et al., 2010), linalool (Souto-Maior et al., 2011), carvone (Costa et al., 2012) and epoxy-limonene (Almeida et al., 2012).

Accordingly, this study evaluated if carvacryl acetate (CA) has potential to be studied as an alternative drug to treat anxiety disorders. CA (Fig. 1), a semisynthetic monoterpenic ester, is derived from carvacrol, a component of oregano (*Origanum vulgare* L.) oil (Manou et al., 1998). Carvacrol has also been recognized to have anxiolytic-like potential in animal models of anxiety (Melo et al., 2010).

The elevated plus-maze (EPM) test, the light-dark box (LDB) test and the marble-burying test (MBT) were applied in mice treated with a single dose of CA to identify its anxiolytic-like potential (Badgujar and Surana, 2010; Deacon, 2013).

Flumazenil (FLU), a GABAergic antagonist, and N-{2-[4-(2-metho xyphenyl)-1-piperazinyl] ethyl}-N-(2-pyridyl) cyclohexanecarboxamide – WAY 100635 (WAY), a selective antagonist of 5-HT_{1A} receptor – were applied previously to CA to suggest its mechanism of action (Dalvi and Rodgers, 1999; Castro et al., 2008).

An open-field test (OFT) and a Rota-rod test were used to evaluate if the probable action of CA on the CNS is related to sedative and muscular relaxation effects. The effect of a single dose of CA on the CNS was compared with single doses of DZP and BUSP, which are standard drugs to treat anxiety disorders (Celada et al., 2013; Ravindran and Stein, 2010).

2. Material and methods

2.1. Reagents and drugs

Polyoxyethylene sorbitan monooleate (Tween 80) (Eg Sigma Chem. Co., St. Louis, Missouri, USA) was used as the vehicle in the groups. DZP and BUSP (Union Chemical, Brazil) were used as standard anxiolytic drugs. FLU and WAY were obtained from Eg Sigma Chem. Co., St. Louis, Missouri, USA. All the administrations were in acute form – single doses – intraperitoneally (ip).

2.2. Substance preparation

DZP, BUSP, WAY and FLU were emulsified with 0.2% Tween 80 and dissolved in distilled water. DZP was administered at a dose of 1 mg/kg, which is considered an anxiolytic dose on rodents (Bert et al., 2001). BUSP was used at 5 mg/kg, which is considered an anxiolytic dose on rodents (López-Rubalcava et al., 1999). FLU was used at 25 mg/kg and WAY at 10 mg/kg. Negative controls received only 0.2% of Tween 80 dissolved in distilled water (10 ml/kg).

CA was obtained by the carvacrol acetylation procedure, which used the acylating agent acetic anhydride as catalyst. There were carvacrol (5 g, 0.033 mol), pyridine (7.5 ml) and acetic anhydride (12.5 ml) in a flask (50 ml) equipped with magnetic stirrer, coupled to a Friedrich condenser and with an inert atmosphere. Then the solution was subjected to a constant magnetic stirring and reflux for about 24 h.

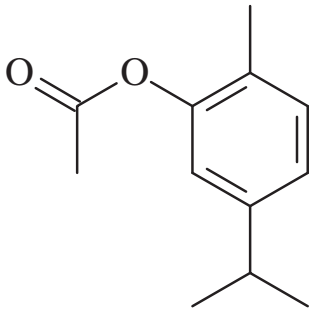

Fig. 1. Chemical structure of carvacryl acetate (5-isopropyl-2-methyl-phenyl).

The reaction mixture was poured into ice water (60 ml), and the extraction of the reaction product was done into a decantation funnel, using chloroform as a solvent extractor (3 × 60 ml). Chloroform phases were combined and washed with saturated copper sulfate (3 × 60 ml). The chloroform phases were also washed with water (3 × 60 ml) and dried with anhydrous Na_2SO_4. Subsequently, the solvent was evaporated on a rotary evaporator. The reaction product was subjected to column chromatography, using silica gel as the stationary phase and a mixture of hexane, CA (95:5), as the mobile phase.

With a 76% yield, approximately, 4779 g (0.025 mol) of CA was obtained, chemically defined as 5-isopropyl-2-methyl-phenyl (Fig. 1), having a purity of 98%, molecular weight of 192.26 g/mol, refractive index of 1497, boiling point of 94.56 °C at 760 mm Hg, enthalpy of vaporization of 48,414 kJ/mol and density 0.994 g/cm^3 (Vogel et al., 1996). Its color is yellow-green. It has an astringent and pungent taste and the characteristic odor of oregano (*Origanum vulgare* L.). CA is found in a liquid state at room temperature, with a density of 0.994 ± 0.06 g/cm^3.

The confirmation of the chemical structure of CA was performed by infrared (IR) spectroscopic data, ^{1}H NMR and ^{13}C NMR DEPT: IR (4000–400 cm^{-1}): 3050; 2950; 2850; 1750; 1500; 850. ^{1}H NMR (200 MHz, CDCl3): 7.20 (d, J = 7,80 Hz, 1H); 7,00 (d, J = 7,80 Hz, 1H); 6,90 (s, 1H); 2,95–2,75 (m, 1H); 2,30 (s, 3H); 2,15 (s, 3H); 1,26 (d, J = 6,80 Hz, 6H); ^{13}C NMR DEPT (50 MHz, $CDCl_3$): 169,1; 149,1; 147,9; 130,7; 127,0; 124,0; 119,6; 67,3; 33,4; 23,7; 20,6; 15.6.

Subsequently, CA was emulsified with 0.05% Tween 80 (Sigma Chem. Co., St. Louis, Missouri, USA) dissolved in distilled water (vehicle) and administered intraperitoneally at doses of 25, 50, 75 and 100 mg/kg for the behavioral tests in order to determine its anxiolytic-like effect.

2.3. Animals

Male *Swiss* mice (25–30 g), 2 months of age, were used throughout this study. All the animals were maintained at a controlled temperature (26 ± 1 °C) on a 12 h light/dark cycle (lights on 06:00 am–18:00 pm) with free access to water and food (Purina®). Different groups of mice were used for each test.

All the experiments were previously submitted for the approval of the Ethics Committee on Animal Experimentation of the Federal University of Piauí (UFPI) (# 013/2011).

2.4. Experimental protocols

Initially, the animals were acclimatized in a site 24 h before the behavioral experiments. In the next step, the mice were randomly divided into thirteen groups (10 mice per group), for the two protocol analysis.

In the first protocol, there were a control group, treated with the vehicle (negative control), four CA groups, with doses of 25, 50, 75 and 100 mg/kg and two groups of reference drugs (positive controls): DZP (1 mg/kg) group and BUSP (5 mg/kg) group. After 30 min from each administration, the mice were individually placed on each apparatus and observed for 5 min (EPM; LDB; MBT of OFT) or 3 min for a Rota-rod test. After each test, the equipment was washed with soap and water, cleaned with ethanol 70% and dried for the subsequent mouse test. For all the experiments, each mouse was tested only once. This protocol was designed to test the anxiolytic-like potential and the influence on the psychomotor activity of CA on the mice.

Once the CA anxiolytic-like potential was observed in the first protocol, the second protocol was performed. One group of mice was treated with FLU 25 mg/kg and another group with WAY 10 mg/kg. A third group was formed with the administration of FLU 25 mg/kg, 15 min before DZP 1 mg/kg administration, and a fourth group received FLU 25 mg/kg, 15 min before CA 100 mg/kg. This was the CA dose with the best anxiolytic-like potential. The prior administration of FLU and then DZP was aimed at confirming that the FLU antagonized the GABAergic effect of DZP (Almeida et al., 2012; Silva et al., 2011a). Furthermore, the