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Antidepressant-like effect of lectin from *Canavalia brasiliensis* (ConBr) administered centrally in mice

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Abstract

This study investigates the action of the central administration of the lectins isolated from *Canavalia brasiliensis* seeds (ConBr) and from *Canavalia ensiformes* seeds, (Concanavalin A, ConA) in the forced swimming test (FST) in mice. ConBr (1–10 μg/site, i.c.v.), but not ConA, produced a decrease in the immobility time in the FST (observed at the time points 15, 30, 60 and 120 min after the injection), without changing the locomotor activity in the open-field test. The effect of ConBr in the FST was dependent on its protein structure integrity. ConBr (0.1 μg/site, i.c.v.) caused a potentiation of the action of fluoxetine, a selective 5-HT reuptake inhibitor. The anti-immobility effect elicited by ConBr (10 μg/site, i.c.v.) in the FST was prevented by the pretreatment of mice with pindolol (32 mg/kg, a 5-$HT_{1A/1B}$ receptor/β-adrenoceptor antagonist), NAN-190 (0.5 mg/kg, a 5-HT_{1A} receptor antagonist), ketanserin (5 mg/kg, a 5-$HT_{2A/2C}$ receptor antagonist), sulpiride (50 mg/kg, a D_2 receptor antagonist) or yohimbine (1 mg/kg, an α_2-adrenoceptor antagonist), but not with SCH 23390 (0.05 mg/kg, a D_1 receptor antagonist) or prazosin (1 mg/kg, an α_1-adrenoceptor antagonist). These results indicate that the antidepressant-like effect of ConBr in the FST is dependent on its interaction with the serotoninergic (via 5-HT_{1A} and 5-HT_2), noradrenergic (via α_2-adrenoceptors) and dopaminergic (via D_2 receptors) systems. Considering the presence of lectins in the brain and based on the results, it will be important to determine a possible role of endogenous lectins in the modulation of the central nervous system function.

Keywords: Lectin; ConBr; ConA; Forced swimming test; Antidepressant; Monoaminergic system; 5-HT; Dopamine; Noradrenaline

1. Introduction

Recent advances in glycobiology have revealed the importance of sugar chains as biosignals in cell–cell communication and control of protein function within the cells (Varki 1993; Sharon and Lis, 1993; Dube and Bertozzi, 2005; Endo, 2005). Sugar chains bearing the glycoproteins, glycolipids or proteoglycans are found at the cell surface (i.e., membrane receptors and neurotransmitter transporters) and in extracellular compartments, playing pivotal roles in the regulation of nervous system development, as well as synaptic activity and neuroplasticity (Endo, 2005). Glycan binding proteins (lectins) are a structurally heterogeneous group of reversible carbohydrate-binding proteins, ubiquitous in animals, plants and microorganisms (Van Damme et al., 1998; Gabius and Gabius, 1997; Cavada et al., 2001). The information contained in the enormous variety of oligosaccharide surfaces (glycocodes) may be recognized and deciphered by lectins with considerable specificity (Drickamer, 1995; Rini, 1995; Cebo et al., 2002; Dube and Bertozzi, 2005; Liu and Rabinovich, 2005). Therefore, lectins may regulate physiological and pathological events of cell function (Dube and Bertozzi, 2005; Liu and Rabinovich, 2005). The glycans from brain are extremely complex, and although animals have a large variety of lectins, only a few of them have been isolated

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from brain and characterized concerning their endogenous roles (Marschal et al., 1989; Endo, 2005).

Lectins from plants have been used as a tool to study molecular mechanisms that modulate animal cell physiology and pathology. For example, some lectins from Leguminosae family have been used to study immunological responses in peripheral cells as well as glutamate and odorant receptors activity in the nervous system (Cavada et al., 2001; Dai et al., 2004; Kirner et al., 2003; Fay and Bowie, 2006). Moreover, mistletoe lectins are useful to study the mechanisms that cause apoptosis in human hepatocarcinoma (Kim et al., 2004) and either lectin-rich mistletoe extracts or purified mistletoe lectins have been considered as possible treatments in some types of cancer in humans (Kim et al., 2004; Rostock et al., 2005).

Legume lectins are a large group of structurally similar proteins with distinct carbohydrate specificities (Cavada et al., 2001). Concanavalin A (ConA), from *Canavalia ensiformes* seeds, was the first lectin to be isolated (Summer and Howell, 1936), sequenced (Cunningham et al., 1975; Wang et al., 1975) and to have its three-dimensional structure determined by X-ray crystallography (Hardman and Ainsworth, 1972; Becker et al., 1975). ConA has been used to study the mammalian nervous system function and neuroplasticity (Lin and Levitan, 1991; Scherer and Udin, 1994; Kirner et al., 2003) and to isolate synaptic glycoproteins such as glutamate receptors (Suzuki and Okomura-Noji, 1995; Clark et al., 1998; Partin et al., 1993). ConA can bind to and modulate ionotropic glutamate receptors such as the kainate-type, inhibiting its desensitization (Dai et al., 2004; Fay and Bowie, 2006) or the AMPA-type, increasing [^{3}H] AMPA binding to it (Hoffman et al., 1998). Additionally, it may cause presynaptic inhibition in sympathetic neurons (Boehm and Huck, 1998) and can inhibit dopamine transporter desensitization in PC12 cell lines (Huang et al., 2003). Moreover, in studies that address the role of serotonin (5-HT) in the modulation of immune system, ConA (used as a mitogenic factor to stimulate lymphocytes) may increase the binding sites of 5-HT on the $5HT_{1A}$ receptor (Sempere et al., 2003) and may reduce the binding of [^{3}H]paroxetine to 5-HT transporter (Cedeño et al., 2005).

ConBr, a lectin isolated from *Canavalia brasiliensis* seeds, presents similar homology (99%) and physical properties to ConA (Cavada et al., 2001; Sanz-Aparicio et al., 1997). The biological effects of ConBr include stimulation of histamine release from mast cells (Ferreira et al., 1996), stimulation of nitric oxide (NO) production by murine macrophages (Andrade et al., 1999), and cell activation or induction of apoptosis in lymphocytes (Barbosa et al., 2001). In spite of all these effects and the structural similarity between ConBr and ConA, the biological effects of ConBr on neural cells or central nervous system function are largely unknown.

The forced swimming test (FST) is a behavioral model widely used for screening compounds or drugs with an antidepressant-like action. It also provides a useful model to study neurobiological mechanisms underlying antidepressant responses. Briefly, when exposed to a cylinder filled with water and without the possibility of escape mice exhibit "immobility", in fact, making just those movements necessary to maintain the head above water (Porsolt et al., 1977). This behavior has been related to failure to persevere in escape directed movements, which would reflect a state of despair (as the animal gives up the hope of escaping). Several studies have shown that the modulation of serotoninergic, noradrenergic and dopaminergic systems, among others, underlie the behavioral effects of antidepressants in the FST (Cryan et al., 2002). In fact, the FST shows a strong sensitivity to monoamine alterations (Porsolt et al., 1977) and neurobiological basic research as well as clinical studies have revealed that the monoamines (5-HT, noradrenaline and dopamine) have a crucial role in the development of the depression syndrome (Millan, 2004).

Therefore, the present work was designed to investigate the possible action of the central administration of ConA and ConBr in the FST. Additionally, we aimed at investigating, by the use of pharmacological procedures, some of the possible mechanisms underlying their effects in the FST.

2. Materials and methods

2.1. Animals

Male Swiss mice weighing 30–40 g were maintained at constant room temperature (22–27 °C) with free access to water and food, under a 12:12-h light–dark cycle (lights on at 0700 h). All manipulations were conducted in the light phase between 0900 and 1700 h, with each animal used only once. The procedures in this study were performed according to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 80–23, revised 1985) and were approved by the local Ethics Committee for Animal Research. All efforts were made to minimize animal suffering.

2.2. Purification of lectins and production of FITC-labeled ConBr

The *C. brasiliensis* lectin (ConBr) (Moreira and Cavada, 1984) and *Canavalia ensiformis* lectin (ConA) (Summer and Howell, 1936) were isolated by affinity chromatography.

In order to produce FITC-labeled ConBr, the lectin was dissolved separately in a 0.1 M solution of its specific sugar (α-methyl-D-mannoside) and then mixed with 2 ml of a conjugation solution (1.5 ml of 0.2 M sodium carbonate/bicarbonate buffer, pH 9.3 with 0.5 ml ethylene glycol). After quick agitation in a vortex mixer, 500 µl of a fluorescein isothiocyanate (FITC) (Sigma, TX, USA) solution (0.05 mg dissolved in ethylene glycol) was added and the material was submitted to constant agitation for 5 h at 4 °C in the dark. After incubation, the complex lectin–FITC was separated from non-complexed FITC by molecular exclusion chromatography using a P10 column (Amersham-Biosciences, USA) previously equilibrated with Milli-Q water, saturated with 5% *n*-butanol and at constant flow rate kept by gravity. Right after the chromatography, 450 µl of the equilibrium solution was added to the fraction FITC–lectin and released from the column. The complex was eluted at 3.5 ml of 5% *n*-butanol, whereas the non-conjugated FITC was eluted with 10 ml.

2.3. Histology

In order to verify the site of injection and the diffusion of the ConBr in the ventricles, a ConBr conjugated to FITC was carried out.

Fifteen minutes following the injection of FITC-conjugated ConBr, mice were anesthetized (chloral hydrate 15%, 20 μl/g, i.p.) and perfused intracardially with saline solution followed by paraformaldehyde (PFA) 4% in phosphate-buffered saline solution (PBS). Brains were dissected, postfixed in PFA 4% overnight, cryoprotected in sucrose 30% and frozen in dry ice. Slices of 40 μm were obtained with a cryostat, mounted on gelatinized slides, covered with PBS/glycerol 1:1 and observed with an epifluorescence microscope. Fifteen minutes subsequent to the i.c.v. injection of FITC-conjugated ConBr, a strong fluorescence was observed in the ventricles at the level of the injection site but also more caudally at the level of the dorsal hippocampus.

2.4. Drugs and treatment

ConBr and ConA were diluted with HEPES–saline buffer without glucose (NaCl 124 mM, KCl 4 mM, $MgSO_4$ 1.2 mM, HEPES 25 mM, $CaCl_2$ 1 mM, pH 7.4). The lectins were administered to mice by the intracerebroventricular (i.c.v.) route (0.5–50 μg/site) in a constant volume of 5 μl/site 15 min before the FST or the open-field test. Intracerebroventricular injections were administered under light ether anesthesia, directly into the lateral ventricle as described previously (Zomkowski et al., 2002), using the bregma fissure as a reference. The control group was treated with vehicle (HEPES–saline buffer without glucose). To determine if the ConBr action in the FST depends on the protein structure integrity, ConBr (10 μg/site) was denatured by boiling for 5 min (90 °C). In the experiments designed to study the time-course effect of ConBr, each independent group of mice was used for different time points (15, 30, 60 and 120 min). The animals were submitted to the FST or open-field test only once.

To assess the possible interaction of ConBr with a selective 5-HT reuptake inhibitor (fluoxetine), mice were co-administered with subeffective doses of both fluoxetine (0.1 nmol/site, i.c.v.) and ConBr (0.1 μg/site, i.c.v.). HEPES–saline buffer (vehicle) was used as a control. The FST was performed 15 min after the treatment.

In order to investigate the possible involvement of monoaminergic systems in the action of ConBr in the FST, the following antagonists were administered: pindolol, 1-(2-methoxyphenyl)-4[-(2-phthalimido) butyl]piperazine) (NAN-190), ketanserin tartarate, sulpiride, yohimbine, SCH 23390 or prazosin (all from Sigma Chemical Company, St. Louis, MO, USA). The drugs were dissolved in saline, except for NAN-190 and pindolol, which were diluted in saline with 1% Tween 80. Appropriate vehicle-treated groups were also assessed simultaneously. The drugs or vehicle were administered by the intraperitoneal (i.p.) route in a constant volume of 10 ml/kg body weight, except for SCH 23390, which was administered by the subcutaneous (s.c.) route. The drugs were administered 30 min before the mice received ConBr (10 μg/site, i.c.v.) and 15 min later the FST was carried out.

The involvement of the 5-HT receptor subtypes in the effect of ConBr in the FST was studied by the pretreatment of mice with either pindolol (32 mg/kg, a $5\text{-HT}_{1A/1B}$ receptor/β-adrenoceptor antagonist), or NAN-190 (0.5 mg/kg, a 5-HT_{1A} receptor antagonist), or ketanserin (5 mg/kg, a $5\text{-HT}_{2A/2C}$ receptor antagonist), or vehicle.

The possible involvement of D_1 and D_2 receptors in the effect of ConBr in the FST was investigated by the pretreatment of mice with either SCH 23390 (0.05 mg/kg, a D_1 receptor antagonist), sulpiride (50 mg/kg, a D_2 receptor antagonist), or vehicle.

In another set of experiments, the involvement of adrenoceptor subtypes in the antidepressant-like effect of ConBr in the FST was evaluated by pretreatment with either prazosin (1 mg/kg, an α_1-adrenoceptor antagonist), yohimbine (1 mg/kg, an α_2-adrenoceptor antagonist), or vehicle.

The doses of the drugs used in this study were selected on the basis of literature data, in which the antagonists were effective in blocking the receptors without causing changes in the locomotor activity of mice (O'Neill and Conway, 2001; Redrobe and Bourin, 1997; Redrobe et al., 1996; Rojas-Corrales et al., 1998; Zomkowski et al., 2002, 2004).

2.5. Forced Swimming Test (FST)

The test was conducted using the method of Porsolt et al. (1977) with minor modifications. Briefly, mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm) containing 19 cm of water at 25±1 °C. The duration of immobility, defined as the absence of escape-oriented behaviors, such as swimming, was scored during 6 min, as described previously (Zomkowski et al., 2002, 2004; Kaster et al., 2005).

2.6. Open-field behavior

Ambulatory behavior was assessed in an open-field test as described previously (Rodrigues et al., 1996). The apparatus consisted of a wooden box measuring 40×60×50 cm. The floor of the arena was divided into 12 equal squares. The number of squares crossed with all paws (crossing) was counted in a 6-min session.

2.7. Statistical analysis

Comparisons between experimental and control groups were performed by one-way and two-way ANOVA followed by the Newman-Keul's test when appropriate. A value of $p<0.05$ was considered significant. All statistical procedures were carried out using Statistic software version 6.0 for the IBM-compatible microcomputer.

3. Results

The results depicted in Fig. 1A show the effect of the treatment of mice with ConBr (1–50 μg/site) in the immobility

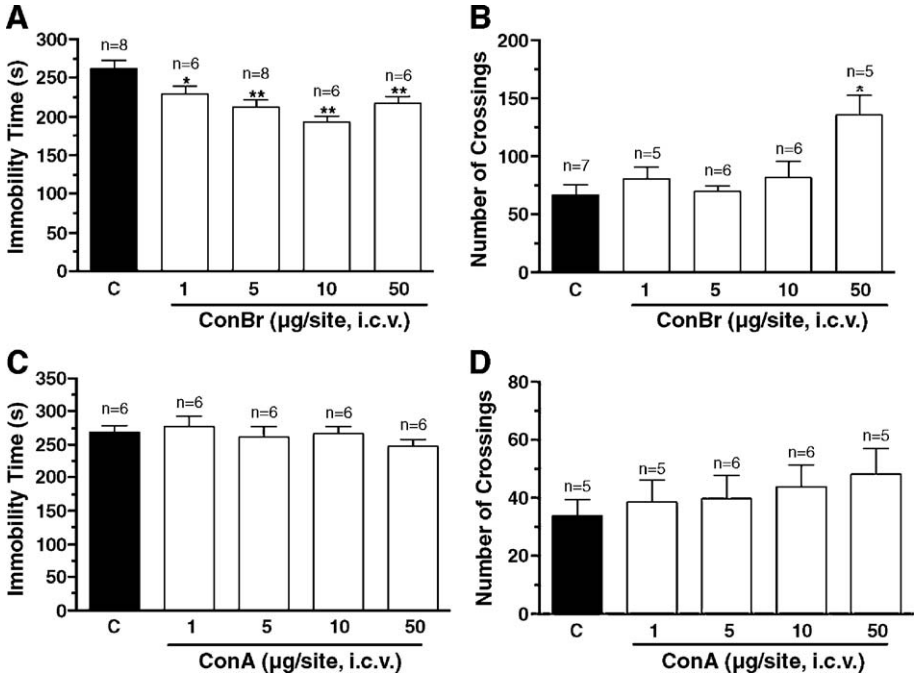

Fig. 1. Effect of administration of ConBr (1–50 µg/site) and ConA (1–50 µg/site) in the FST (A, C) and in the open-field test (B, D). ConBr and ConA were administered 15 min before the tests by the i.c.v. route. Values are expressed as mean ± S.E.M., $*p<0.05$; $**p<0.01$ compared with vehicle treated group (Control; C); ($n=5-8$).

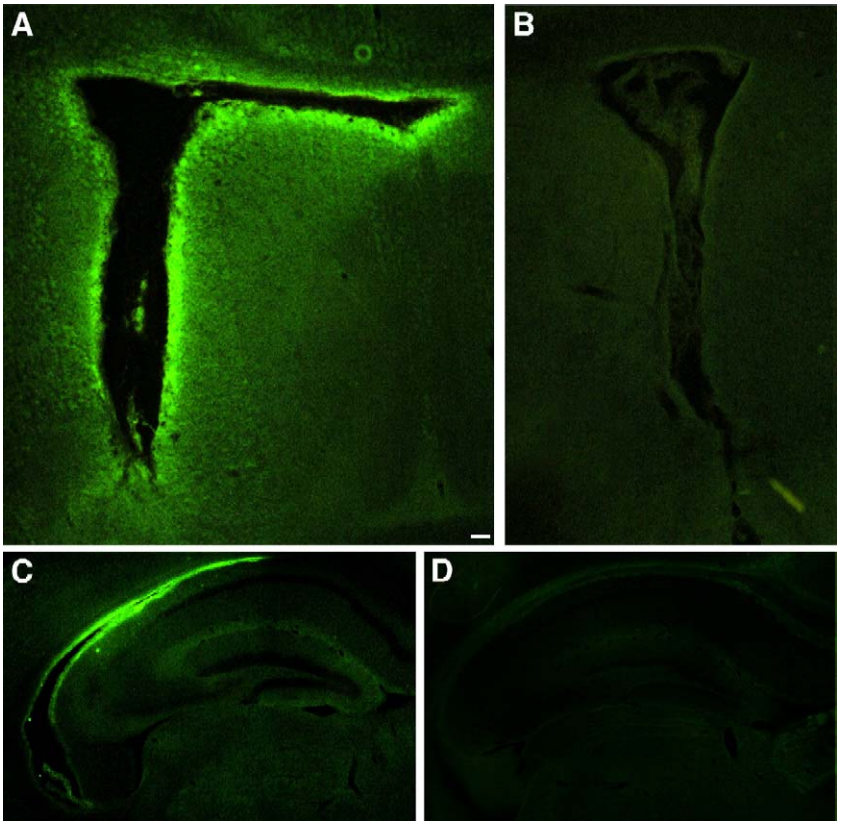

Fig. 2. Representative images of ConBr–FITC distribution into the mice brain. The lectin ConBr FITC-conjugated or saline (control) was injected by i.c.v. route. Mice were perfused 15 min following the injection. Slices of 40 µm were obtained with a cryostat, mounted on gelatinized slides and observed with an epifluorescence microscope. Brain sections at the level of the lateral ventricle (A–B; scale 40 µm) from mice injected with ConBr–FITC (A) or with saline (control; B), and sections at the level of the hippocampus (C–D; scale 100 µm) from mice treated with ConBr–FITC (C) or saline (control; D); ($n=3$).

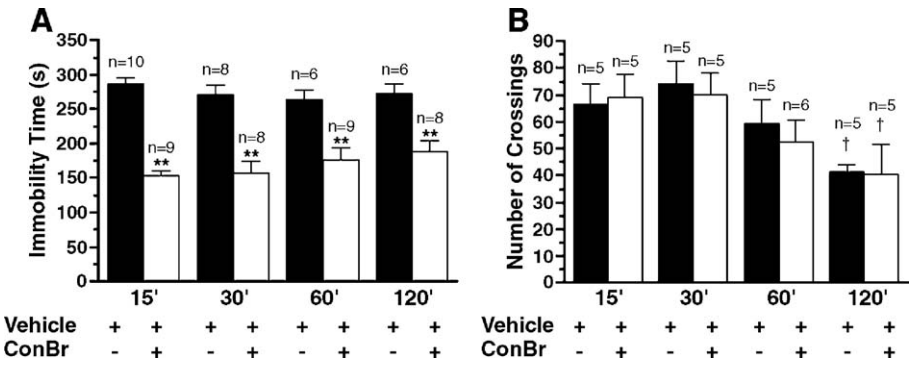

Fig. 3. Time course of antidepressant-like effect of ConBr in the FST (A) and open-field test (B). ConBr (10 μg/site, i.c.v.) was administered 15, 30, 60 and 120 min before the FST. Values are expressed as mean±S.E.M., $^{**}p<0.01$ compared with the vehicle-treated group (Control; C). $^{\dagger}p<0.05$ compared with the time points 15 and 30 min; ($n=5$–10).

time in the FST. One-way ANOVA revealed a main effect of the treatment $F(4,27)=7.34$, $p<0.01$. Post hoc analyses indicated that the treatment of mice with ConBr produced a significant reduction in the immobility time in the FST. Fig. 1B shows the effect of ConBr (1–50 μg/site) in the open-field test. One-way ANOVA revealed a main effect of the treatment $F(4,24)=5.81$, $p<0.01$. The post hoc analysis indicated that ConBr at lower doses (1–10 μg/site) did not cause a significant change in ambulation scores measured by the open-field test. However, the highest dose (50 μg/site) caused an increase in ambulation scores when compared to the control group. The administration of ConBr did not produce overt signs of toxicity in mice, like convulsion, stereotyped behavior or ataxia. The results depicted in Fig. 1C show the effect of ConA (1–50 μg/site) in the FST. One-way ANOVA did not reveal significant differences of the treatment $F(4,25)=1.42$, $p=0.25$. Similarly, the treatment with ConA (1–50 μg/site) did not cause a significant change in ambulation scores in open-field, as revealed by one-way ANOVA, $F(4,22)=0.48$, $p=0.75$ (Fig. 1D).

In order to determine the accuracy of the injection and the distribution of ConBr into the lateral ventricle, ConBr labeled with FITC was administered by the i.c.v. route. Fig. 2A shows that ConBr–FITC was distributed into the lateral ventricle reaching more caudally at the level of the dorsal hippocampus (Fig. 2C).

A time-course analysis of the effect of ConBr (10 μg/site) in the FST is shown in Fig. 3A. The groups were independent, since the animals were analyzed once, at different time points after the injection (15–120 min). A two-way ANOVA revealed a significant effect of treatment, $F(1,56)=108.76$, $p<0.01$, but not of time, $F(3,56)=0.46$, $p=0.71$ and of treatment × time interaction, $F(3,56)=1.41$, $p=0.25$. Post-hoc comparisons indicated that ConBr treatment produced a marked effect in the FST as early as 15 min after i.c.v. administration, an action that remains significant up to 120 min after administration. Additionally, Fig. 3B shows the ambulation scores assessed by the open-field. A two-way ANOVA showed a significant effect of time on the crossing responses, $F(3,33)=5.56$, $p<0.05$, but not of treatment, $F(1,33)=0.14$, $p=0.71$, nor of treatment × time interaction, $F(3,33)=0.12$, $p=0.94$. Post hoc comparisons only indicated a decrease in the ambulation scores at 120 min as compared to 15 and 30 min after administration of either vehicle or ConBr. Thus, the time point 15 min was chosen for all further studies, since at this time point, a maximum effect of ConBr in the FST was observed without producing changes in the ambulation scores.

Another important question to address is whether the ConBr action in the FST depends on the integrity of the tertiary/quaternary protein structure. Fig. 4A shows that denaturation of ConBr (10 μg/site) blocked its effect in the FST. One-way

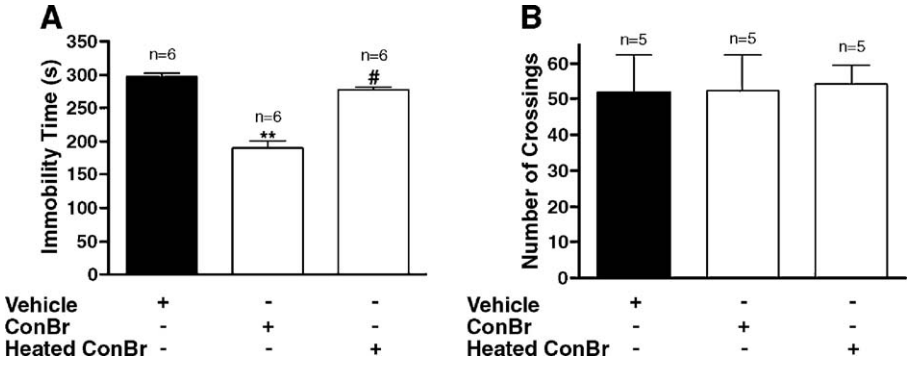

Fig. 4. Effect of treatment of mice with ConBr (10 μg/site) or with heated-ConBr in FST (A) and open-field test (B). ConBr was denatured by boiling. Either heated or not heated ConBr was injected by the i.c.v. route 15 min before the tests. Values are expressed as mean±S.E.M., $^{**}p<0.01$ when compared to vehicle-treated group. $^{\#}$When compared with ConBr-treated-group (control; C); ($n=5$–6).

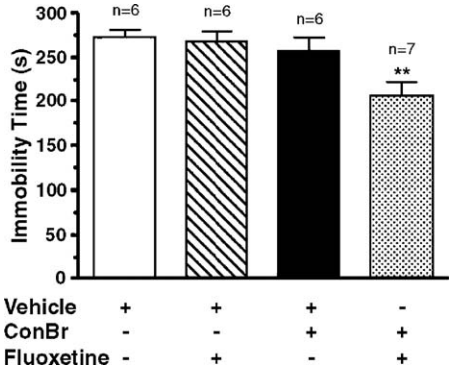

Fig. 5. Effects of ConBr (0.1 μg/site) in potentiating the action of a subeffective dose of fluoxetine (0.1 nmol/site) in the FST. Mice were co-administered with ConBr plus fluoxetine 15 min before the test. Values are expressed as mean±S.E.M., $^{**}p<0.01$ compared with the vehicle-treated group (Control; C); (n=6–7).

ANOVA revealed a main effect of the treatment, $F(2,15)=58.38$, $p<0.01$. Post hoc analyses indicated that denaturation of ConBr prevented its effect in the FST. The ambulation scores were also analyzed in the open-field test (Fig. 4B). One-way ANOVA did not reveal a main effect of the treatment, $F(2,12)=0.17$, $p=0.98$.

In order to investigate if the antidepressant-like effect of ConBr in the FST is associated with the serotoninergic system, mice were treated with ConBr (0.1 μg/site) plus fluoxetine (0.1 nmol/site), both at subeffective doses. One-way ANOVA revealed a significant effect of treatment $F(3,21)=7.08$, $p<0.01$. Post hoc analyses indicated that ConBr significantly enhanced the antidepressant-like effect of a subeffective dose of fluoxetine (Fig. 5).

The involvement of monoaminergic systems in the antidepressant-like effect of ConBr was analyzed by using specific receptor antagonists. Fig. 6A shows the influence of NAN-190 (0.5 mg/kg, i.p., a 5-HT_{1A} receptor antagonist) in the anti-immobility effect of ConBr in the FST. A two-way ANOVA revealed a significant effect of treatment, $F(1,20)=24.81$, $p<0.01$, pretreatment, $F(1,20)=30.27$, $p<0.01$ and of treatment×pretreatment interaction, $F(1,20)=21.79$, $p<0.01$. Fig. 6B shows the results of pretreatment of mice with pindolol (32 mg/kg, a 5-$HT_{1A/1B}$ receptor/β-adrenoceptor antagonist) on the reduction in immobility time elicited by ConBr in the FST. There was a significant effect of pretreatment, $F(1,20)=15.66$, $p<0.01$, treatment $F(1,20)=31.43$, $p<0.01$ and of treatment×pretreatment interaction $F(1,20)=26.16$, $p<0.01$, as revealed by a two-way ANOVA. Fig. 6C shows the effect of pretreatment of mice with ketanserin (5 mg/kg, a 5-HT_{2A} receptor antagonist) in the antidepressant-like effect of ConBr in the FST. A two-way ANOVA revealed a main effect of pretreatment $F(1,20)=31.56$, $p<0.01$, treatment, $F(1,20)=13.46$, $p<0.01$ and of treatment×pretreatment interaction, $F(1,20)=22.79$, $p<0.01$. Fig. 6D shows the results of pretreatment of mice with sulpiride (50 mg/kg, a D_2 receptor antagonist) on the reduction in immobility time elicited by ConBr in the FST. The two-way ANOVA revealed significant differences of pretreatment, $F(1,20)=36.78$, $p<0.01$, treatment, $F(1,20)=34.12$, $p<0.01$ and of treatment×pretreatment interaction $F(1,20)=15.10$, $p<0.01$. Fig. 6E shows the influence of pretreatment of mice with SCH 23390 (0.05 mg/kg, a D_1 receptor antagonist), on the anti-immobility effect of ConBr in the FST. There was a significant effect of treatment, $F(1,20)=16.78$, $p<0.01$, but not of pretreatment, $F(1,20)=0.37$, $p=0.55$, and treatment×pretreatment interaction, $F(1,20)=2.24$, $p=0.15$, as revealed by a two-way ANOVA. Fig. 6F shows the results of the pretreatment of mice with yohimbine (1 mg/kg, an α_2 receptor antagonist), on the reduction in immobility time elicited by ConBr in the FST. The two-way ANOVA revealed significant differences of pretreatment, $F(1,20)=29.65$, $p<0.01$, treatment, $F(1,20)=54.47$, $p<0.01$ and of treatment×pretreatment interaction $F(1,20)=12.73$ $p<0.01$. Fig. 6G shows the results of pretreatment with prazosin (1 mg/kg, a α_1 receptor antagonist) on the antidepressant-like effect of ConBr in the FST. The two-way ANOVA showed a significant effect of treatment, $F(1,20)=2.77$, $p<0.01$, but not of pretreatment, $F(1,20)=2.77$, $p=0.11$ or of treatment×pretreatment interaction, $F(1,20)=0.13$, $p=0.71$. Post hoc analyses indicated that the pretreatment of mice with NAN-190, pindolol, ketanserin, sulpiride and yohimbine blocked the decrease in immobility in the FST elicited by ConBr. On the other hand, SCH 23390 and prazosin did not prevent the effect of ConBr in the FST.

4. Discussion

The actions of ConBr described in the literature are not related to the nervous system, but mainly to the immune system, in which it may cause mitogenesis, apoptosis, induction of NO and cytokine production (Andrade et al., 1999; Barbosa et al., 2001; Cavada et al., 2001). The present study shows for the first time that ConBr, a lectin with high affinity for glycans containing mannose/glucose sugars, given centrally, produces a decrease in the immobility time in the FST (observed at time points ranging from 15 min to 120 min after the injection), an effect consistent with an antidepressant-like action (Porsolt et al., 1977; Petit-Demouliere et al., 2005). Among all animal models, the FST remains one of the most used tools for screening antidepressants (Petit-Demouliere et al., 2005). This test is quite sensitive and relatively specific to all major classes of antidepressant drugs, including tricyclics, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, and atypicals. All these classes of drugs reduce the immobility time in the FST when administered acutely (Porsolt et al., 1977; Cryan et al., 2002; Petit-Demouliere et al., 2005).

The reduction in the immobility time in the FST elicited by ConBr was not accompanied by changes in the locomotor activity assessed in the open-field test, except at a very high dose (50 μg/site) in which a psychostimulant effect was observed. This result indicates that ConBr exerts a specific antidepressant-like effect in the FST at relatively low doses (1–10 μg/site, i.c.v.). Moreover, the action of ConBr in the FST was dependent on the integrity of its protein structure, since its denaturation by the high temperature blocked its anti-immobility effect.

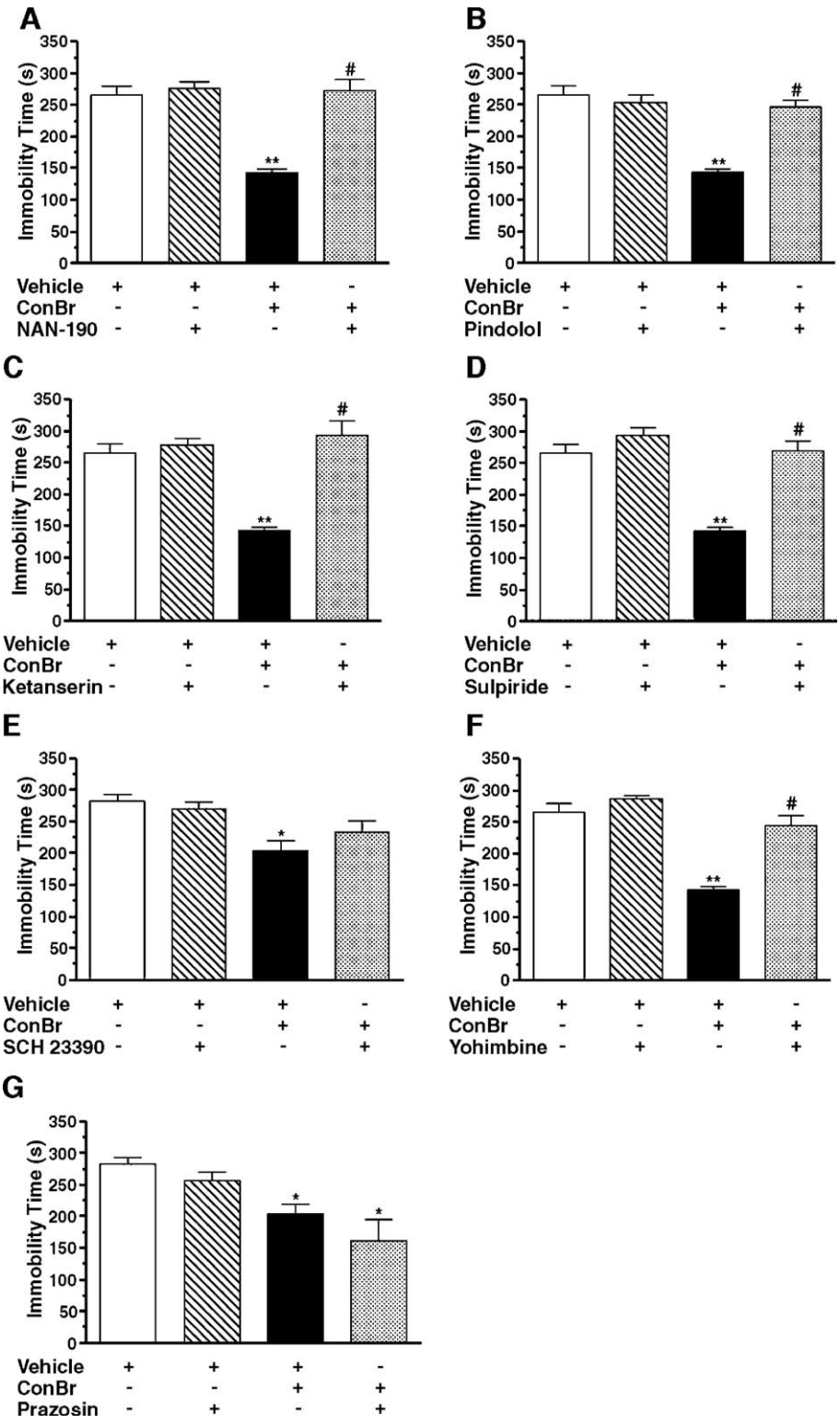

Fig. 6. Effect of pretreatment of mice with NAN-190 (0.5 mg/kg, panel A), pindolol (32 mg/kg, panel B), ketanserin (5 mg/kg, panel C), sulpiride (50 mg/kg, panel D), SCH 23390 (0.05 mg/kg, panel E), yohimbine (1 mg/kg, panel F), prazosin (1 mg/kg, panel G) or vehicle on the ConBr-induced reduction in immobility time in the FST. Values are expressed as mean ± S.E.M., $^{**}p<0.01$ as compared with vehicle-treated group (control, C); $^{\#}p<0.01$ as compared with the same group pretreated with vehicle; ($n=6$).

ConA is a well-studied lectin from Leguminosae family that can act on the nervous system or PC12 cells (Hoffman et al., 1998; Dai et al., 2004; Huang et al., 2003; Fay and Bowie, 2006). However, in contrast to ConBr, ConA was not effective in altering the immobility time of mice in the FST, in spite of the fact that ConA and ConBr present the same

sugar affinity, having 99% homology. Indeed, the amino acid at position 58 (glycine in ConBr and aspartic acid in ConA) is the only different residue between ConA and ConBr involved in tetramer formation. At this position, replacement of Asp by Gly disrupts the hydrogen bond between Asp-58 of subunit A and Ser-62 of subunit C in ConA and the 3D conformation of ConBr is more open than ConA (Sanz-Aparicio et al., 1997). However, whether these structural differences are responsible for the differences in the lectin action is still an open question.

It is important to highlight that ConBr shows different biological effects from ConA regarding other biological activities (Cavada et al., 2001) like induction of rat paw edema (Bento et al., 1993), peritoneal macrophage spreading in the mouse (Rodriguez et al., 1992), human peripheral blood mononuclear cells activation (Cavada et al., 2001), induction of histamine release (Ferreira et al., 1996; Lopes et al., 2005) and NO release (Andrade et al., 1999).

ConBr administered at a subeffective dose caused a potentiation of the action of the selective 5-HT reuptake inhibitor fluoxetine (Wong et al., 1995; Millan, 2004). This result suggests that ConBr and fluoxetine may share a similar mechanism of action to produce an antidepressant-like effect in the FST. Therefore, the action of ConBr in the FST, a model predictive of antidepressant activity, and its capacity to potentiate the fluoxetine action, a classical antidepressant used extensively in clinical treatment (Blier and Montigny, 1994; Wong et al., 1995; Malberg and Blendy, 2005; Berton and Nestler, 2006), reinforces the idea that the lectin ConBr produces an antidepressant-like effect when administered centrally.

The administration of ConBr–FITC by the i.c.v. route caused a distribution into the lateral ventricle reaching more caudally at the level of the dorsal hippocampus that is a key target structure for the action of antidepressant drugs (Malberg and Blendy, 2005; Berton and Nestler, 2006). In spite of this observation, it is not possible to establish whether the effect of ConBr in the FST is a consequence of its attachment in the walls of the ventricle or its entrance into the blood stream. Alternatively, it is possible that its effect in the FST is due to its discrete diffusion into the hippocampus, a brain region closely related to the pathophysiology of depression (Santarelli et al., 2003).

As the monoaminergic systems are important targets in the pathophysiology and treatment of depression (Elhwuegi, 2004; Millan, 2004; Berton and Nestler, 2006), we investigated the involvement of the serotoninergic, noradrenergic and dopaminergic systems in the anti-immobility effect elicited by ConBr in the FST to reveal some of the possible mechanisms involved in its effect. Thus, we have assessed herein the effects of several pharmacological antagonists on the anti-immobility action of the ConBr (10 μg/site, i.c.v.) in mice. The involvement of the serotoninergic system in the antidepressant-like action of ConBr is suggested by two sets of evidence. The first one is the synergistic effect on immobility time in mice treated with ConBr and fluoxetine. The second one is the reversal of its action in the FST by the pretreatment of mice with the 5-$HT_{1A/1B}$ receptor/β-adrenoceptor antagonist pindolol, the 5-HT_{1A} receptor antagonist NAN-190 and the 5-$HT_{2A/2C}$ receptor antagonist ketanserin. 5-$HT_{1A/1B}$ and 5-$HT_{2A/2C}$ receptors are reported to be involved in the mechanism of action of antidepressant drugs (Cryan et al., 2002; Millan, 2004).

Our study also indicates that ConBr might interact with α_2-adrenoceptors and with D_2 receptors to produce its anti-immobility effect in the FST, since its effect in the FST was reversed by the pretreatment of animals with yohimbine and sulpiride. Indeed, several studies have shown that these receptors have been implicated in the behavioral responses of drugs in the FST (Zomkowski et al., 2002; Yamada et al., 2004; Rodrigues et al., 2005). In spite of the fact that α_1-adrenoceptors and D_1 receptors have been shown to play a role in the actions of antidepressant agents (Millan, 2004; Yamada et al., 2004; Dailly et al., 2004), these two subtypes of receptors seem not to underlie the antidepressant-like action of ConBr observed in our study.

Although the importance of complex brain glycans as biosignals and the presence and functional importance of endogenous brain lectins as modulators (Dube and Bertozzi, 2005; Endo, 2005; Liu and Rabinovich, 2005) are stressed, their functional roles in the nervous system, synaptic activity and neuroplasticity are not yet clear (Marschal et al., 1989; Dube and Bertozzi, 2005; Endo, 2005). Animals have a large variety of lectins, but only a few of them have been isolated from brain and characterized regarding their possible endogenous roles (Marschal et al., 1989; Endo, 2005).

Another aspect that we have to consider is the possibility that ConBr may cause proinflamatory effects. In peripheral tissues, such effects have been described (Bento et al., 1993). In the present work, we were not able to rule out this possible action of ConBr in the central nervous system. However, it is important to note that proinflammatory compounds may cause a depressant effect rather than an antidepressant action (Raison et al., 2006). Therefore, further studies should be carried out to test the possible proinflammatory action, as well as to clarify the targets and molecular mechanisms underlying the functional effects of ConBr.

In summary, results from the present study show, for the first time, that ConBr exerts an antidepressant-like effect in the FST at doses that do not alter locomotor activity. The results also indicate that the antidepressant-like effect of ConBr in the FST is dependent upon its interaction with the serotoninergic (via 5-HT_{1A} and 5-HT_2), noradrenergic (via α_2-adrenoceptors) and dopaminergic (via D_2 receptors) systems. Finally, on the basis of the present results, and considering the presence of lectins in the brain, whose physiological roles are not established, further studies are necessary to investigate whether similar endogenous lectins exert a role in the modulation of the nervous system function.

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