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Blockade of intracellular calcium release induces an antidepressant-like effect in the mouse forced swimming test

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Abstract

The role of intracellular calcium in the modulation of a depressant-like condition was investigated in the mouse forced swimming test. I.c.v. administration of TMB-8 (0.23–46.3 nmol per mouse), a blocker of Ca^{2+} release from intracellular stores, decreased the mouse immobility time. I.c.v. injection of thapsigargin (0.003–3 nmol per mouse), compound which selectively inhibits Ca^{2+} uptake into the endoplasmic reticulum, produced, 60 min after administration, a depressant-like condition. Xestospongin C (1–100 pmol per mouse i.c.v.), an $InsP_3$-receptor antagonist, decreased the mouse immobility time. By contrast, D-myo-inositol (5.4–540 pmol per mouse i.c.v.), compound which produces $InsP_3$, resulted in a depressant-like effect. Similarly, ryanodine (0.1–600 pmol per mouse i.c.v.), an RyR antagonist, decreased the immobility time values whereas the administration of 4-chloro-*m*-cresol (0.1–100 pmol per mouse i.c.v.), an RyR agonist, showed an opposite effect. The antidepressant-like effects observed with TMB-8, xestospongin C and ryanodine were comparable to that produced by the antidepressant drugs amitriptyline and clomipramine. The treatments employed did not produce any behavioural impairment of mice as revealed by the rota-rod and hole board tests indicating that the antidepressant- and depressant-like effects were not due to a compromised locomotor activity and spontaneous motility of the treated animals. These results indicate that a central variation in intracellular calcium contents is involved in the modulation of a depressive-like condition in the mouse forced swimming test. In particular, the blockade of both $InsP_3$Rs and RyRs appears to play an important role in the induction of an antidepressant-like effect, whereas the stimulation of these receptors is involved in a depressant-like response of mice.
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1. Introduction

Calcium ions have been shown to regulate the synthesis and release of neurotransmitters, neuronal excitability, cytoskeletal remodelling, and long-term neuroplastic events. Evidence has accumulated for the involvement of intracellular Ca^{2+} also in the pathophysiology of mood disorders. Platelet intracellular calcium mobilization stimulated by serotonin is enhanced in depressed patients (bipolar, major, melancholic) in comparison with normal subjects (Eckert et al., 1993; Yamawaki et al., 1998; Tomiyoshi et al., 1999). Furthermore, enhanced neutrophil response to chemotactic peptide and enhanced platelet thrombin response are also observed in some studies (Dubovsky et al., 1991; Kusumi et al., 1992; Bohus et al., 1996). Some investigations also suggested that baseline intracellular calcium levels are elevated in platelets or lymphoblasts of patients with bipolar affective disorders (Emamghoreishi et al., 1997). Other reports showed that dihydropyridine channel blockers displayed antidepressant-like activity in mice (Cohen et al., 1997; Biala, 1998) as well as some effectiveness in the treatment of patients with affective disorders (Dubovsky, 1993; Wisner et al., 2002). Acute application of antidepressant drugs inhibited intracellular calcium

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signalling and Ca^{2+}-related signalling in cultured neuronal cells and glioma cells (Yamawaki et al., 1998). Taken together, these results suggest the presence of an altered intracellular calcium homeostasis in depressed patients that is neither receptor- nor cell-specific, indicating that a component in the calcium pathway other than the receptor itself may be abnormal.

The regulation of free intracellular Ca^{2+} is a complex, multifaceted process regulated by various mechanisms related to physiological functions. One mechanism is the influx of Ca^{2+} via Ca^{2+} channels through the plasma membrane. Another is the release of Ca^{2+} from intracellular stores via intracellular Ca^{2+}-release channels, the inositol-1,4,5-trisphosphate receptor ($InsP_3R$) and the ryanodine receptor (RyR): $InsP_3R$ is a key molecule for $InsP_3$-induced Ca^{2+} release, whereas RyR is important for Ca^{2+}-induced Ca^{2+} release (Mikoshiba, 1997; Fill and Copello, 2002).

Even if elevations in both resting and stimulated intracellular Ca^{2+} levels in patients with affective disorders have been reported, little is known on the involvement of the intracellular Ca^{2+}-release channels in mood disorders. The aim of this study was, therefore, to determine the role of $InsP_3$- and Ry-receptors in the induction of an antidepressant-like condition by using the mouse forced swimming test. To elucidate the role played by $InsP_3$- and Ry-receptors at the central nervous system level, we administered the pharmacological modulators used directly into the mouse cerebral ventricles. Finally, in order to exclude that the effects produced by the intracellular calcium receptor modulators were due to the induction of side effects, some additional behavioural tests (rota-rod, hole board) were performed.

2. Methods

2.1. Animals

Male Swiss albino mice (23–25 g) from the Morini (San Polo d'Enza, Italy) breeding farm were used. Fifteen mice were housed per cage (26 × 41 cm). The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum and kept at 23 ± 1 °C with a 12-h light/dark cycle, light on at 7 a.m. Animals were naïve and used only once. All experiments were carried out in accordance with the Animal Protection Law of the Republic of Italy, DL n. 116/1992, based on the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Intracerebroventricular injection technique

Intracerebroventricular (i.c.v.) administration was performed under ether anaesthesia. Briefly, during anaesthesia, mice were grasped firmly by the loose skin behind the head. A 0.4-mm external diameter hypodermic needle attached to a 10-μl syringe was inserted perpendicularly through the skull and no more than 2 mm into the brain of the mouse, where 5 μl were then administered. The injection site was 1 mm to the right or left from the mid-point on a line drawn through to the anterior base of the ears. Injections were performed into the right or left ventricle randomly. To ascertain that the drugs were administered exactly into the cerebral ventricle, some mice (20%) were injected with 5 μl of diluted 1:10 India ink and their brains were examined macroscopically after sectioning. The accuracy of the injection technique was evaluated and the percentage of correct injections was determined to be 95%.

2.3. Forced swimming test

The forced swimming test used was the same as described by Porsolt et al. (1977). Briefly, mice were dropped individually into glass cylinders (height: 25 cm, diameter: 10 cm) containing 6 cm of water maintained at 22–23 °C and left there for 6 min. A mouse was judged to be immobile when it floated in the water, in an upright position, and made only small movements to keep its head above water. The duration of immobility was recorded during the last 4 min of the 6-min test. A decrease in the duration of immobility is indicative of an antidepressant-like effect. Fifteen to 25 mice per group were tested. The experiments reported in each figure were performed on the same day. Each treated group was compared to the corresponding control group. The reference drugs were tested concomitantly with the various compounds. The observers were unaware of the treatments.

2.4. Hole board test

The hole board test consisted of a 40-cm square plane with 16 flush mounted cylindrical holes (3 cm diameter) distributed 4 by 4 in an equidistant, grid-like manner. Mice were placed on the centre of the board one by one and allowed to move about freely for a period of 10 min each. Two electric eyes, crossing the plane from mid-point to mid-point of opposite sides, thus dividing the plane into 4 equal quadrants, automatically signalled the movement of the animal (counts in 5 min) on the surface of the plane (locomotor activity). Miniature photoelectric cells, in each of the 16 holes, recorded (counts in 5 min) the exploration of the holes (exploratory activity) by the mice. Twelve to 15 mice per group were tested.

2.5. Rota-rod test

The apparatus consisted of a base platform and a rotating rod with a diameter of 3 cm and a non-slippery surface. The rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into 5 equal sections by 6 disks. Thus, up to 5 mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 rpm. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s according to Vaught et al. (1985). Those mice scoring less than 3 and more than 6 fall in the pretest and were rejected (20%). The performance time was measured before (pretest) and 15, 30 and 45 min after the beginning of the test. Twelve to 15 mice per group were tested.

2.6. Drugs

The following drugs were used: TMB-8 (8-(*N*,*N*-diethylamino)-octyl-3,4,5-trimethoxybenzoate)-hydrochloride, clomipramine hydrochloride, amitriptyline hydrochloride (Sigma, Milan, Italy); ryanodine, 4-chloro-*m*-cresol, thapsigargin, D-myo-inositol-1,4,5-trisphosphate hexasodium salt, L-myo-inositol-1,4,5-trisphosphate hexapotassium salt, xestospongin C (Calbiochem, Milan, Italy). Other chemicals were of the highest quality commercially available.

Thapsigargin was dissolved in 10% DMSO, 4-chloro-*m*-cresol (4-Cmc) was dissolved in 0.5% ethanol, xestospongin C was dissolved in 0.01% ethanol whereas all other drugs were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use. Drug concentrations were prepared so that the necessary dose could be administered in a volume of 5 μl per mouse by intracerebroventricular (i.c.v.).

The tests were performed 15 min after i.c.v. injections of TMB-8, xestospongin C, D-myo-inositol, L-myo-inositol, ryanodine, 60 min after administration of thapsigargin, 105 min after administration of 4-chloro-*m*-cresol, 30 min after tricyclic antidepressants injection. Doses and administration schedule were chosen on the basis of time-course and dose–response experiments previously performed in our laboratory. Furthermore, literature data confirm the selectivity and efficacy of the above-mentioned treatments at time and concentration used.

2.7. Statistical analysis

All experimental results are given as the mean ± s.e.m. An analysis of variance ANOVA, followed by Fisher's Protected Least Significant Difference procedure for post hoc comparison, were used to verify the significance between the two means of behavioural results. Data were analyzed with the StatView software for the Macintosh (1992). P values of less than 0.05 were considered significant.

3. Results

3.1. Effect of TMB-8 and thapsigargin on mouse forced swimming test

TMB-8, a blocker of Ca^{2+} release from intracellular stores, induced an antidepressant-like effect. The doses of 0.23, 2.31, 4.6 and 11.6 nmol per mouse i.c.v. were devoid of any effect. The antidepressant-like effect was obtained at 23.1 nmol per mouse i.c.v. ($F(6,163)3.163$; $P < 0.01$) (Fig. 1). Doses higher than 46.3 nmol per mouse i.c.v. were not investigated since mild side effects (motor incoordination and in some cases tremors) were induced. The decrease in the immobility time induced by TMB-8 was comparable to that induced by the antidepressant drugs amitriptyline (15 μg per mouse i.c.v.) and clomipramine (25 μg per mouse i.c.v.), used as reference compounds (Fig. 1).

Time-course experiments evidenced a depressant-like effect induced by thapsigargin, an inhibitor of Ca^{2+} uptake into the endoplasmic reticulum by inhibiting the sarco-endoplasmatic reticulum Ca^{2+}-ATPases. A progressive increase in the immobility time was observed 15 and 30 min after administration of the dose of 0.1 nmol per mouse i.c.v. that reached the statistical significance 60 min after administration ($F(5,105)$ 3.950; $P < 0.05$). The depressant-like effect observed disappeared 90 min after injection. The doses of 0.003, 0.01, 1 and 3 nmol did not produce any statistical significant effect on the immobility time in comparison with control animals (Fig. 2). TMB-8 (11.6 nmol per mouse i.c.v.) reversed the depressant-like effect of 0.1 nmol per mouse i.c.v. thapsigargin ($F(3,38)3.256$; $P < 0.05$) as illustrated in Table 1.

No difference in the immobility time was observed between control animals treated with saline or vehicle (DMSO 10%).

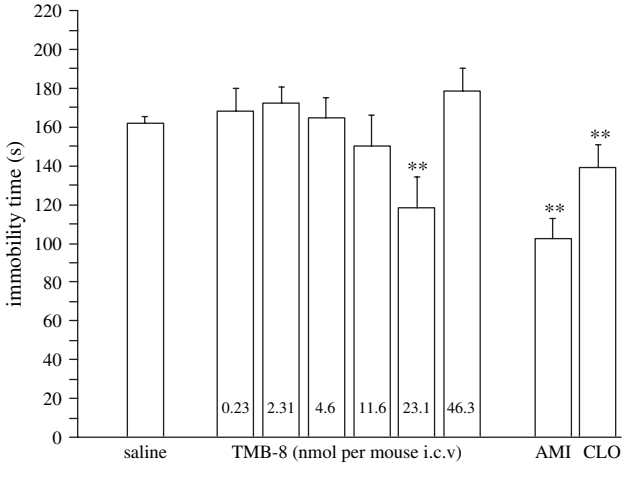

Fig. 1. Decrease by TMB-8 of the immobility time in the mouse forced swimming test. The immobility time values were recorded 15 min after TMB-8 administration. Amitriptyline (15 μg per mouse i.c.v.) and clomipramine (25 μg per mouse i.c.v.) were administered 30 min before the test. Doses administered were reported in each column. Vertical lines represent s.e.m.; between 12 and 15 mice were tested. **$P < 0.01$ in comparison with saline-treated mice.

3.2. Effect of $InsP_3R$ modulators on mouse forced swimming test

The administration of xestospongin C (1−100 pmol per mouse i.c.v.), an antagonist of $InsP_3$ receptors, produced a dose-dependent antidepressant-like effect, reaching its maximum effect at 30 pmol per mouse i.c.v. ($F(6,159)3.426$; $P < 0.01$) (Fig. 3A). The antidepressant-like effect produced by xestospongin C was comparable to that exerted by clomipramine (25 μg per mouse i.c.v.), used as reference drug.

D-myo-Inositol produced a dose-dependent increase in the immobility time, which reached the statistical significance at 18 pmol per mouse i.c.v. and peaked at 180 pmol per mouse i.c.v. ($F(5,119)3.211$; $P < 0.05$). Higher doses produced a progressive decrease in the immobility time, which returned to control values at 540 pmol per mouse i.c.v. The dose of 5.4 pmol per mouse i.c.v. was devoid of any effect (Fig. 3B). The administration of L-myo-inositol (150 pmol per mouse i.c.v.), used as negative control, did not modify the mobility time of treated animals in comparison with the control group (Fig. 3B). A reversal of the depressant-like effect induced by D-myo-inositol was observed when TMB-8 was administered at 11.6 nmol per mouse i.c.v., a dose unable to induce an antidepressant-like effect ($F(3,49)3.652$; $P < 0.05$) (Table 1).

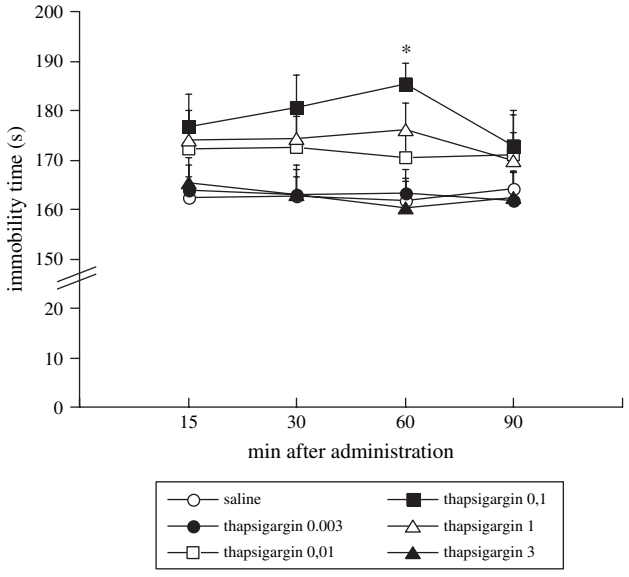

Fig. 2. Increase by thapsigargin (0.003−3 nmol per mouse i.c.v.) of the immobility time in the mouse forced swimming test. Vehicle: 10% DMSO. Vertical lines represent s.e.m.; between 12 and 15 mice were tested. *$P < 0.05$ in comparison with vehicle-treated mice.

Table 1
Reversal by TMB-8 of the increase in immobility time induced by thapsigargin, D-myo-inositol-1,4,5-trisphosphate, and 4-chloro-*m*-cresol

Pre-treatment (i.c.v.)	Treatment (i.c.v.)	Immobility time (s)
Saline	Saline	159.6 ± 3.9
Saline	TMB-8 (11.6 pmol)	152.4 ± 10.7
Thapsigargin (0.1 nmol)	Saline	185.5 ± 4.0*
Thapsigargin (0.1 nmol)	TMB-8 (11.6 pmol)	155.8 ± 9.1
D-myo-Inositol (180 pmol)	Saline	189.3 ± 6.1*
D-myo-Inositol (180 pmol)	TMB-8 (11.6 pmol)	153.2 ± 5.4
4-Cmc (10 pmol)	Saline	185.7 ± 7.3*
4-Cmc (10 pmol)	TMB-8 (11.6 pmol)	150.9 ± 7.8

4-Cmc: 4-Chloro-*m*-cresol, *$P < 0.05$ in comparison with saline-treated group.

3.3. Effect of RyR modulators on mouse forced swimming test

The i.c.v. administration of ryanodine, a selective antagonist of RyR, dose-dependently decreased the immobility time values. Ryanodine, at 0.1 pmol per mouse i.c.v., was devoid of any effect. The doses of ryanodine of 60 and 100 pmol per mouse induced a statistically significant antidepressant-like effect ($F(7,119)3.805$; $P < 0.001$). At the dose of 300 pmol per mouse i.c.v. the immobility time values were increased returning to levels comparable with the control values at 600 pmol per mouse i.c.v. The antidepressant-like effect of ryanodine was comparable to that produced by amitriptyiline (15 μg per mouse i.c.v.) (Fig. 4A).

The administration of 4-Cmc, an agonist of RyR, produced an increase in the immobility time. The depressant-like effect was observed at 10 pmol per mouse i.c.v. ($F(6,136)3.048$; $P < 0.05$) whereas at 30 and 100 pmol per mouse the mobility time values returned comparable to the control values (Fig. 4B). The depressant-like effect of 4-Cmc was prevented by TMB-8 11.6 nmol per mouse i.c.v. ($F(3,41)3.993$; $P < 0.05$) (Table 1).

No difference in the immobility time was observed between control animals treated with saline or vehicle (EtOH 0.5%).

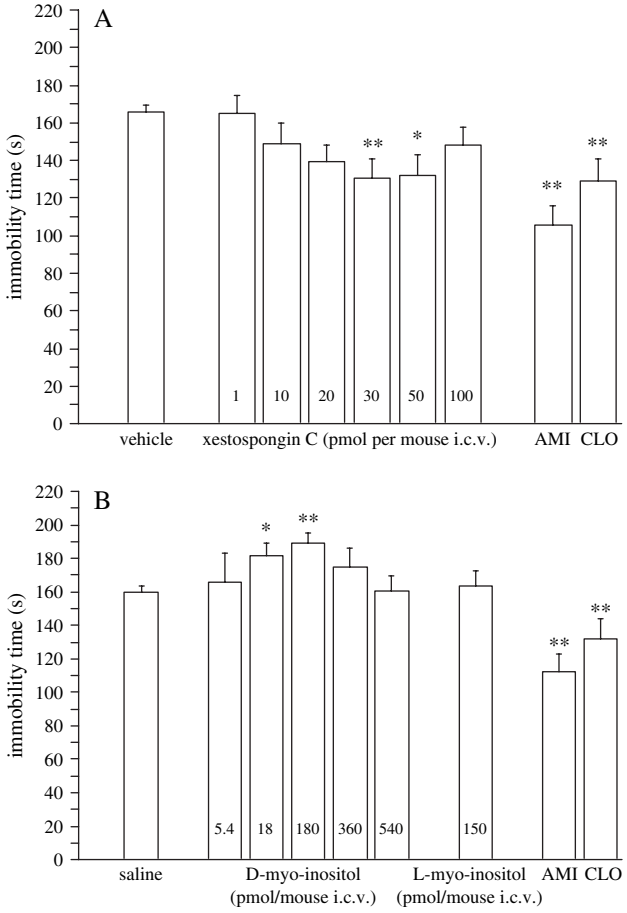

Fig. 3. Panel A: Decrease by xestospongin C of the immobility time in the mouse forced swimming test. *$P < 0.05$, **$P < 0.01$ in comparison with saline-treated mice. Panel B: Increase by D-myo-inositol of the immobility time in the mouse forced swimming test. *$P < 0.05$ in comparison with saline-treated mice. The immobility time values were recorded 15 min after xestospongin C, D-myo-inositol and L-myo-inositol administration, 30 min after amitriptyline (15 μg per mouse i.c.v.) and clomipramine (25 μg per mouse i.c.v.) injection. Doses administered were reported in each column. Vertical lines represent s.e.m.; between 12 and 15 mice were tested.

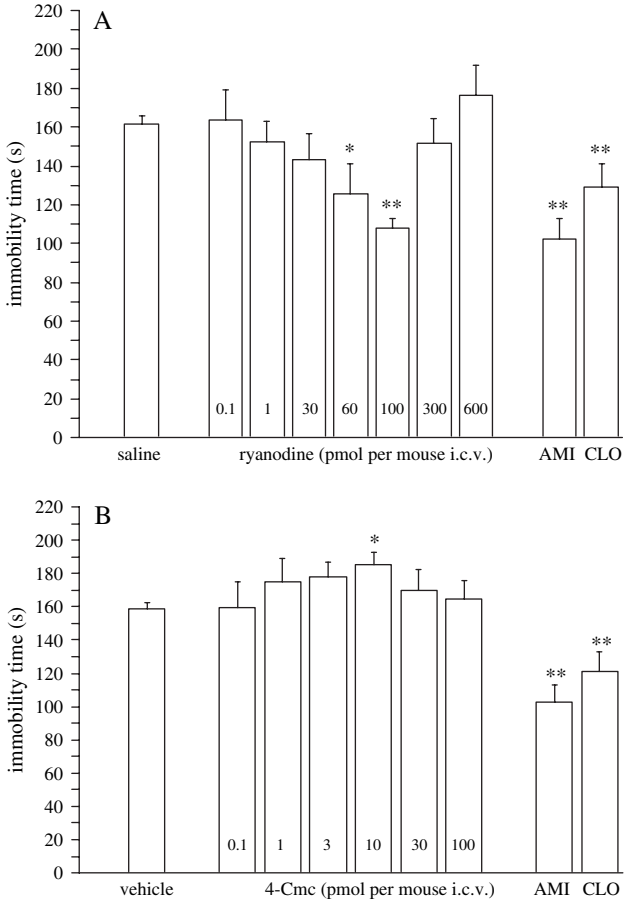

Fig. 4. Panel A: Decrease by ryanodine of the immobility time in the mouse forced swimming test. *$P < 0.05$, **$P < 0.01$ in comparison with saline-treated mice. Panel B: Increase by 4-Cmc of the immobility time in the mouse forced swimming test. Vehicle: 0.5% ethanol. *$P < 0.05$, **$P < 0.01$ in comparison with vehicle-treated mice. The immobility time values were recorded 15 min after ryanodine administration, 105 min after 4-Cmc administration and 30 min after amitriptyline (15 μg per mouse i.c.v.) and clomipramine (25 μg per mouse i.c.v.) injection. Doses administered were reported in each column. Vertical lines represent s.e.m.; between 12 and 15 mice were tested.

3.4. Effect of intracellular calcium modulators on mouse motor and exploratory behaviour

The compounds investigated in the present study were tested in order to assess their effect on mouse motor and exploratory behaviour. Mice pretreated with the highest effective doses employed of TMB-8 (23.1 nmol per mouse i.c.v.), xestospongin C (30 pmol per mouse i.c.v.), ryanodine (0.1 nmol per mouse i.c.v.), 4-Cmc C (0.01 nmol per mouse i.c.v.), thapsigargin (0.1 nmol per mouse i.c.v.) and D-myo-inositol (0.18 nmol per mouse i.c.v.) were evaluated for motor coordination by the use of rota-rod test, and for spontaneous motility and inspection activity, by the use of hole board test.

The number of falls from the rotating rod showed the lack of any impairment in the motor coordination of animals treated with all pharmacological modulators in comparison with the control group (Fig. 5).

The spontaneous motility as well as the exploratory activity of mice was unmodified by treatment with the above-mentioned compounds in comparison with control groups (Fig. 6).

Pharmacological modulators, as well as amitriptyline and clomipramine, did not produce any alteration in motor coordination, spontaneous motility and exploratory activity at all doses tested (data not shown).

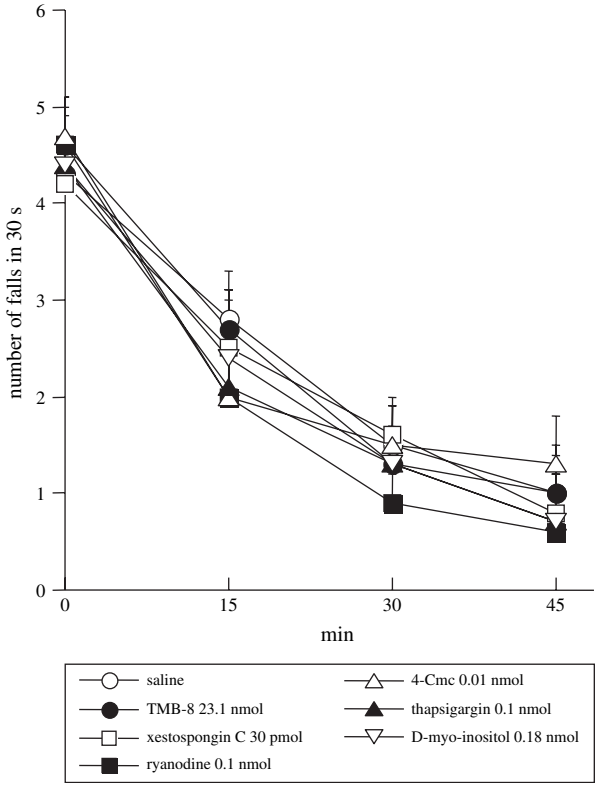

Fig. 5. Lack of effect by TMB-8 (23.1 nmol per mouse i.c.v.), xestospongin C (30 pmol per mouse i.c.v.), ryanodine (0.1 nmol per mouse i.c.v.), 4-Cmc C (0.01 nmol per mouse i.c.v.), thapsigargin (0.1 nmol per mouse i.c.v.) and D-myo-inositol (0.18 nmol per mouse i.c.v.) on motor coordination in the mouse rota-rod test. Vertical lines represent s.e.m.

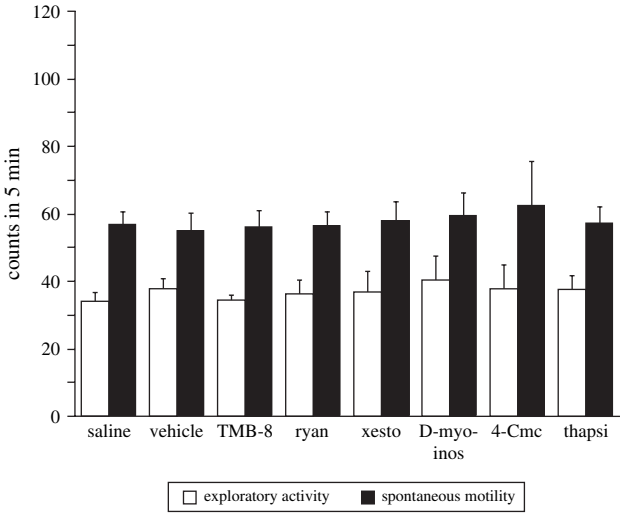

Fig. 6. Lack of effect by TMB-8 (23.1 nmol per mouse i.c.v.), xestospongin C (30 pmol per mouse i.c.v.), ryanodine (0.1 nmol per mouse i.c.v.), 4-Cmc C (0.01 nmol per mouse i.c.v.), thapsigargin (0.1 nmol per mouse i.c.v.) and D-myo-inositol (0.18 nmol per mouse i.c.v.) on spontaneous motility and exploratory activity in the mouse hole board test in comparison with saline or vehicle. Vertical lines represent s.e.m. Vehicle: 10% DMSO.

Neither alteration of animals' gross behaviour nor signs of toxicity/death of mice treated with all the compounds investigated were observed up to 7 days after the treatment (data not shown).

4. Discussion

The present study investigated the role of intracellular Ca^{2+} in the modulation of depressive states by using the forced swimming test in mice. Ca^{2+} plays an important role in a variety of central and peripheral physiological processes. To avoid the possible appearance of peripheral effects that could lead to a misinterpretation of the results obtained, the Ca^{2+} modulators used were administered directly into the cerebral ventricles.

Present results evidenced that the treatment with pharmacological compounds able to block Ca^{2+} release from intracellular stores provoked a decrease in mouse immobility time inducing an antidepressant-like effect with an intensity comparable to that produced by the tricyclic antidepressants amitriptyline and clomipramine, used as reference drugs. By contrast, administration of compounds able to increase intracellular Ca^{2+} levels produced an increase in mouse immobility time.

The i.c.v. administration of TMB-8, an agent that antagonizes the mobilization of Ca^{2+} from intracellular stores (Malagodi and Chiou, 1974), decreased the mouse immobility time indicating the induction of an antidepressant-like effect of intensity comparable to that produced by amitriptyline and clomipramine. The i.c.v. administration of thapsigargin, a compound which selectively inhibits Ca^{2+} uptake into the endoplasmic reticulum by inhibiting the sarco-endoplasmic reticulum ATPases (SERCAs) and thus increasing the intracellular Ca^{2+} concentration (Treiman et al., 1998), increased the

mouse immobility time evidencing the induction of a depressant-like effect. These observations suggest that a supraspinal increase in intracellular calcium contents is involved in the modulation of mood leading to a depressant-like effect in laboratory animals further complementing and extending studies performed on peripheral blood cells from depressed subjects indicating that patients with affective disorders have an enhanced intracellular Ca^{2+} response (Emamghoreishi et al., 1997; Yamawaki et al., 1998; Manji and Lenox, 2000). Furthermore, these data indicate that an antidepressant-like effect can be obtained by blocking the release of Ca^{2+} from endoplasmic reticulum. It should be taken into account that the administration of antidepressant drugs, such as imipramine, desipramine and mianserin, reduced depolarization-induced Ca^{2+} rise and inhibited spontaneous oscillation in cultured cortical neurones (Yamawaki et al., 1998).

TMB-8 can also produce some additional effects. Among them, it has been reported that this compound is able to inhibit K_{ATP} potassium channels (Szewczyk et al., 1992). Since the blockade of K_{ATP} channels can induce an antidepressant-like effect (Galeotti et al., 1999) to further validate the hypothesis of an involvement of a variation of the intracellular calcium levels in the antidepressant-like effect observed after TMB-8 administration in the forced swimming test, we investigated the effects produced by direct modulation of $InsP_3R$ and RyR. The release of Ca^{2+} from intracellular stores is mediated via intracellular Ca^{2+}-release channels, the inositol-1,4,5-trisphosphate receptor ($InsP_3R$) and the ryanodine receptor (RyR): $InsP_3R$ is a key molecule for $InsP_3$-induced Ca^{2+} release, whereas RyR is important for Ca^{2+}-induced Ca^{2+} release.

The i.c.v. administration of xestospongin C, an $InsP_3$-receptor antagonist (Gafni et al., 1997), produced a decrease in the immobility time and, therefore, counteracted the depressant-like condition induced in the test. Conversely, the i.c.v. injection of D-myo-inositol, compound which generates $InsP_3$, produced an increase in the mouse immobility time indicating the presence of a depressant-like condition. The administration of L-myo-inositol, used as negative control, was unable to modify the mobility time in comparison with the control group, confirming that the D-myo-inositol effect observed in the present study was due to the production of $InsP_3$ which, in turn, stimulates $InsP_3$ receptors. These results support the importance of the modulation of $InsP_3R$ to induce an antidepressant-like effect in the mouse forced swimming test. Several studies have shown an overstimulated phosphoinositide signalling system in affective disorders. Results from depressed patients evidenced the existence of a greater sensitivity of inositol–phospholipid second messenger system (Bohus et al., 1996) and an altered PIP_2 hydrolysis (Karege et al., 1996; Shimon et al., 1997; Soares et al., 2001) in blood cells and post mortem brains. Furthermore, elevated IP_3 binding sites and expressed IP_3 receptor protein levels have been noted in platelets from depressed patients (Dwivedi et al., 1998; Rosel et al., 2000). More recently, the complete disappearance of depressive symptoms after addition of subcutaneous calcium heparin to fluoxetine was observed in a patient suffering from a recurrent depression resistant to conventional antidepressant therapy (Maluquer et al., 2002).

RyRs represent the second class of Ca^{2+}-release channels present on the endoplasmic reticulum. To evaluate the role of RyRs in the mouse forced swimming test, the effect produced by 4-chloro-*m*-cresol (4-Cmc), an agonist of RyR (Herrmann-Frank et al., 1996), was tested. 4-Cmc produced an increase in the immobility time evidencing the induction of a depressant-like effect. By contrast, the i.c.v. administration of ryanodine, an RyR antagonist, produced a decrease in the immobility time in mice inducing an antidepressant-like effect of intensity comparable to that exerted by amitriptyline and clomipramine, used as reference drugs. It has been reported that ryanodine blocks Ca^{2+} release from Ca^{2+}/caffeine-sensitive microsomal pools, which are involved in the phenomenon of Ca^{2+}-induced Ca^{2+} release (McPherson et al., 1991). Ryanodine reduces the rate at which $[Ca^{2+}]_i$ increases with Ca^{2+} entry (Friel and Tsien, 1992). Thus, we can hypothesize that the antidepressant-like response produced by i.c.v. ryanodine may be due to a decrease in $[Ca^{2+}]_i$ at supraspinal level. Abnormalities of RyRs have been related to the induction of several skeletal–muscle pathologies such as malignant hyperthermia, porcine stress syndrome, central-core disease (Missiaen et al., 2000). The etiopathological role of RyRs has also been postulated at the central nervous system level. Deletion of RyR3 results in specific changes in intracellular processes underlying spatial learning and hippocampal synaptic plasticity (Balschun et al., 1999). RyR3 knockout mice have impairments of performance in the contextual fear conditioning test, passive avoidance test and Y-maze learning test (Kouzu et al., 2000) indicating the importance of RyR3 in the memory and learning processes. Present results suggest the possible involvement of RyR also in the induction of depressive conditions.

Urani et al. (2002) did not evidence any effect on mouse immobility time when xestospongin C and ryanodine were administered alone. In this study both compounds were used at concentrations at least 10 times higher than those resulted effective in the present study. This discrepancy could be explained by taking into account that calcium channel modulators produced a bell-shaped curve of modulation of the mobility time. This biphasic effect was similar to the effect produced by the physiological modulators of $InsP_3R$ and RyR (Finch et al., 1991; Copello et al., 1997).

The rodent forced swimming test is widely used to predict the antidepressant action of drugs in humans. The mobility time of mice in this test is increased by the majority of antidepressants and their effectiveness correlates significantly with clinical potency (Petit-Demouliere et al., 2005; Cryan et al., 2002). However, this animal model also has some drawbacks represented by the possibility to obtain some false positives or negatives. Drugs enhancing motor activity, such as anticholinergics and antihistamines, may give a "false" positive effect in the forced swimming test and antidepressants such as bupropion, nomifensine and amineptine would then be rejected since they increase motor activity (Borsini and Meli, 1988). Cytosolic Ca^{2+} regulates numerous neuronal functions

(Berridge, 1998) and, therefore, a variation of intracellular Ca^{2+} contents can induce behavioural side effects. Furthermore, since drugs that modify motor activity may give false positive or negative effect, it is suitable to carry out a test to check this aspect, in parallel with forced swimming test. The highest doses of the drugs used in the present work were devoid of effects on locomotor and exploratory activity as well as on spontaneous motility. All the Ca^{2+} modulators were tested before the forced swimming test was performed, to make sure that they did not alter the normal locomotor activity of the mice. An influence of the substances used on spontaneous motility has, therefore, been excluded by using the hole board test. Not only a modified spontaneous motility but also altered motor coordination could lead to a misinterpretation of the results obtained in the forced swimming test. A rota-rod test was, therefore, performed and an altered motor activity induced by substances at the doses used was excluded. The results of the rota-rod and hole board tests were of particular relevance since it has been observed that mice lacking type 1 inositol-1,4,5-trisphosphate receptor showed severe ataxia (Matsumoto et al., 1996) and motor discoordination (Ogura et al., 2001) and that deletion of the ryanodine receptor type 3 induced an increased speed of locomotion in the open-field (Balschun et al., 1999). Finally, it should be mentioned that thapsigargin is a widely used pharmacological tool to induce apoptosis. However, the apoptosis usually appears after 16–25 h of treatment whereas the forced swimming test was performed 60 min after thapsigargin. On these bases we can exclude that the effects produced on the immobility time were related to the induction of apoptosis.

Seen as a whole, present results indicate that the variation in cytosolic Ca^{2+} contents is involved in the induction of an antidepressant-like condition in the mouse forced swimming test. In particular, compounds able to decrease cytosolic Ca^{2+} concentration induced an anti-immobility effect indicating the induction of an antidepressant-like effect of intensity comparable to that obtained with the antidepressant drugs amitriptyline and clomipramine. By contrast, the administration of compounds able to increase intracellular Ca^{2+} levels increased the immobility time of mice indicating the induction of a depressant-like condition. Furthermore, both $InsP_3Rs$ and RyRs appear to participate in the modulation of depressive states.

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