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Inactivation of Gi proteins induces an antidepressant-like effect in the mouse forced-swimming test

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Abstract

The effect of Gi protein inactivation was evaluated in an animal model of depression, the mouse forced swimming test. Animals were i.c.v. injected with pertussis toxin (PTX) or with antisense oligodeoxynucleotides directed against the α subunit of each Gi-protein subtype (anti-Giα_1, anti-Giα_2, anti-Giα_3, anti-Goα_1, anti-Goα_2). The administration of PTX (0.25 μg per mouse i.c.v.) produced an increase in the mobility time. Similarly, anti-Giα_2 (25 μg per mouse i.c.v.), anti-Giα_3 (25 μg per mouse i.c.v.), anti-Goα_1 (12.5–25 μg per mouse i.c.v.) and anti-Goα_2 (12.5–25 μg per mouse i.c.v.) increased the mobility time. The antidepressant-like effect obtained was similar to that produced by amitriptyline and clomipramine. By contrast, pretreatment with anti-Giα_1 (3.12–25 μg per mouse i.c.v.) never modified the mobility time in comparison with control animals. At the highest effective doses, none of the compounds used impaired motor coordination (rota rod test), nor modified spontaneous motility and inspection activity, (hole board test). These results indicate the involvement of Gi_2, Gi_3, Go_1, and Go_2, but not Gi_1, protein subtypes in the transduction mechanism responsible for the induction of an antidepressant-like effect in the mouse forced swimming test.

Keywords: Antidepressant-like effect; Gi-proteins; Pertussis toxin; Antisense oligodeoxyribonucleotides; Forced swimming test

1. Introduction

Biochemical research in affective disorders has focused on the cascade of events involved in signal transduction: from the level of primary messenger to the level of neurotransmitter receptor, and, lately, to information transduction mechanisms beyond the receptors, involving G-proteins. Interest in G-proteins comes from the fact that abnormalities in their function and expression have been associated with various human diseases and pathophysiological states (Spiegel, 1995). G-proteins are a ubiquitous family of proteins that play a crucial role in transducing extracellular signals to cellular targets, thus transmitting messages from cell surface receptors to cellular effectors including adenylate cyclase, phospholipase C and ion channels (Sprang, 1997). G-proteins are heterotrimeric molecules composed of three different subunits termed α, β and γ. The α subunits can be classified into families, depending on whether they are targets for cholera toxin (Gs), pertussis toxin (Gi and Go) or neither (Gq and G_{12}) (Simon et al., 1991).

There is now increasing evidence for the involvement of G-proteins in the pathophysiology of a number of psychiatric diseases such as major depression and bipolar disorders, and in the biochemical mechanisms underlying the treatment of these disorders (Manji, 1992; Hudson et al., 1993). Abnormalities in the signal-transducing G-proteins have been demonstrated by several studies. Hyperfunctional Gs proteins were detected in mononuclear leukocytes of patients with mania (Schreiber et al., 1991) and in cerebral cortex of patients with bipolar disorders (Young et al., 1993). High Gsα immunoreactivity was found in post-mortem cerebral cortex, mononuclear leukocytes, platelets of patients with bipolar disorders (Young et al., 1991, 1994; Mitchell et al., 1997). Furthermore, increased levels of Gsα mRNA were observed in granulocytes from patients with bipolar disorders (Spleiss et al., 1998) even if no alteration was

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detected in cerebral cortex of post-mortem brain from subjects with ante-mortem diagnosis of bipolar disorder (Young et al., 1996a).

Contrary to Gs-proteins, the role of Gi-proteins in the pathogenesis of depressive disorders is more controversial. Higher levels of Giα were found in leukocytes and platelets from bipolar and major depressed patients (Young et al., 1994; Garcia-Sevilla et al., 1997). Conversely, lower levels of Giα were found in leukocytes and post-mortem brain of patients with major depression (Pacheco et al., 1996; Avissar et al., 1997). No significant change in Giα levels was observed in mononuclear leukocytes from patients with major depressive disorder (Young et al., 1994) or bipolar disorders (Manji et al., 1995). Furthermore, chronic treatment with antidepressant drugs has been reported to produce either a reduction or an increase of Giα and Goα protein and mRNA levels in various regions of rat brain (Lesch et al., 1991; Raap et al., 1999) and in depressed patients (Avissar et al., 1997; Karege et al., 1998). By contrast, no alteration in Giα and Goα protein and mRNA levels was observed after chronic treatment with tricyclic antidepressants and monoamine oxidase inhibitors in rat brain (Chen and Rasenick, 1995; Emamghoreishi et al., 1996; Dwivedi and Pandey, 1997) and in mononuclear leukocytes from depressed subjects (Young et al., 1996b).

In light of these controversial data, the aim of the present study was to further elucidate the role of the Gi-protein family in the induction of a depressant-like condition by using the mouse forced swimming test, an animal model of depression. In particular, we injected mice with antisense oligonucleotides (aODN) against the α subunits of the Gi_1, Gi_2, Gi_3, Go_1 and Go_2 proteins in order to determine the role of each subtype in a depressant-like condition. In order to exclude that the effects produced by aODN treatments 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 acclimatisation. 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. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) for experimental animal care. All efforts were made to minimise animal suffering, and to reduce the number of animals used.

2.2. Intracerebroventricular injection technique

Intracerebroventricular (i.c.v.) administration was performed under ether anaesthesia, according to the method described by Haley and McCormick (1957). 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 midpoint 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 examined macroscopically after sectioning. The accuracy of the injection technique was evaluated and the percentage of correct injections was 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 mobility was recorded during the last 4 min of the 6-min test. An increase in the duration of mobility is indicative of an antidepressant-like effect. The test was performed 24–18 h after i.c.v. injections of ODNs, 4–11 days after PTX administration, 30 min after tricyclic antidepressants injection.

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. The test was performed 24–18 h after the i.c.v. injections of degenerate ODN (dODN; 12.5 μg per mouse) or aODN (6.25–12.5 μg per mouse), 11 days after administration of PTX. 12–15 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 r.p.m. 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 falls in the pretest were rejected (20%). The performance time was measured before (pretest) and 15, 30 and 45 min after s.c. treatment. Animals were i.c.v. pretreated 24–18 h prior to the test with degenerate ODN (dODN) or aODN or 11 days before the test with PTX. 12–15 mice per group were tested.

2.6. Antisense oligonucleotides

The sequences of the antisense oligonucleotides (ODNs) used in the present study are shown in Table 1.

Phosphodiester ODNs protected from terminal phosphorothioate double substitution (capped ODNs) against possible exonuclease-mediated degradation were used (Tib Molbiol, Genova, Italy). All ODNs were previously characterised by in vitro (immunoblotting) and in vivo (tail flick) experiments (Kleuss et al., 1991; Raffa et al., 1994; Sanchez-Blazquez et al., 1995; Sanchez-Blazquez and Garzon, 1998). A 33-mer fully degenerated ODN (dODN) 5′-N∗N∗N NNN NNN NNN NNN NNN NNN NNN NNN N∗N∗N -3′ (where N is G, or C, or A, or T) and a 25-mer fully degenerated ODN (dODN) 5′-N∗N∗N NNN NNN NNN NNN NNN NNN NN∗N ∗N -3′ (where N is G, or C, or A, or T) were used as a control respectively for anti-Giα and anti-Goα. ODNs were vehiculated intracellularly by an artificial cationic lipid (DOTAP, Sigma) to enhance both uptake and stability, as described previously (Capaccioli et al., 1993). aODN or dODN were preincubated at 37°C for 30 min with 13 μM DOTAP and supplied to mice by i.c.v. injection of 5 μl solution 18 and 24 h prior to the behavioural tests.

2.7. Drugs

The following drugs were used: pertussis toxin (RBI); clomipramine hydrochloride amitriptyline hydrochloride, DOTAP (Sigma); D-amphetamine hydrochloride (De Angeli). All drugs were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use, except for pertussis toxin which was dissolved in a water solution containing 0.01 M sodium phosphate buffer, pH=7.0, with 0.05 M sodium chloride. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 ml kg^{-1} by s.c. injection or 5 μl per mouse by i.c.v. injection.

All animals received an i.c.v. injection of dODN or aODN 18 and 24 h prior to the test; 30 min before the test mice received a s.c. injection: the group pretreated with dODN received saline (control group), amitriptyline, or clomipramine whereas the group pretreated with aODN received saline.

2.8. Statistical analysis

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

3. Results

3.1. Effect of pertussis toxin

Pretreatment with pertussis toxin (PTX) produced an antidepressant-like effect in the mouse forced swimming test. PTX, injected i.c.v. at the dose of 0.25 μg per mouse 11 days before the test, increased the mobility time, whereas 4 days after injection was devoid of any effect (Fig. 1). The vehicle used to dissolve PTX did not modify the animals' mobility time in comparison with untreated (naive) or saline-treated groups (Fig. 1).

Table 1
Sequences of the antisense oligonucleotides

aODN	sequences
anti-Giα$_1$	5′- G∗C∗T GTC CTT CCA CAG TCT CTT TAT GAC GCC G∗G∗C -3′
anti-Giα$_2$	5′- A∗T∗G GTC AGC CCA GAG CCT CCG GAT GAC GCC C∗G∗A -3′
anti-Giα$_3$	5′- G∗C∗C ATC TCG CCA TAA ACG TTT AAT CAC GCC T∗G∗C -3′
anti-Goα$_1$	5′- A∗G∗G CAG CTG CAT CTT CAT AGG TG∗T ∗T -3′
anti-Goα$_2$	5′- G∗A∗G CCA CAG CTT CTG TGA AGG CA∗C ∗T -3′

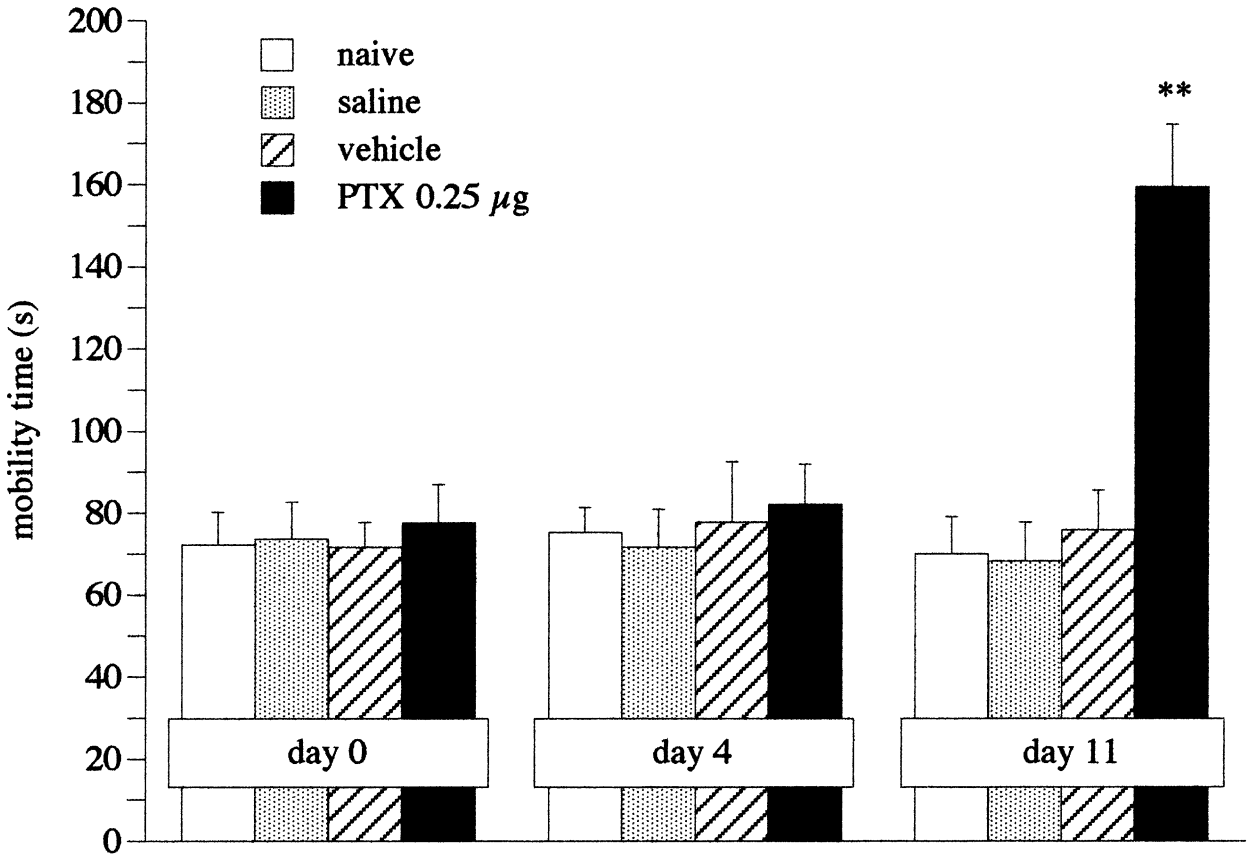

Fig. 1. Increase by pertussis toxin (PTX) of the mobility time in the mouse forced swimming test. The test was performed before, 4 and 11 days after a single i.c.v. injection of saline, vehicle or PTX (0.25 μg per mouse). Vertical lines represent s.e.m.; between 12 and 15 mice were tested. $**P<0.01$ in comparison with vehicle-treated mice.

3.2. Effect of aODN against Giα and Goα subunits

The administration of an aODN against the α subunit of the Gi_2 proteins (25 μg per mouse i.c.v.) increased the mobility time in the mouse forced swimming test up to a value comparable to that produced by amitriptyline (15 mg kg^{-1} s.c.) and clomipramine (25 mg kg^{-1} s.c.), used as reference drugs. Lower doses were ineffective (Fig. 2). Anti-$Gi\alpha_3$ at 3.12 μg per mouse i.c.v. was devoid of any effect. At 6.25 and 12.5 μg per mouse i.c.v., the mobility time was slightly increased, even if the statistical significance was not reached. The doses of 25 μg per mouse i.c.v. showed a significant antidepressant-like effect (Fig. 2). Anti-$Gi\alpha_1$ (3.12–25 μg per mouse i.c.v.), contrary to anti-$Gi\alpha_2$ and anti-$Gi\alpha_3$, was unable to modify the mobility time at all doses tested (Fig. 2).

Anti-$Go\alpha_1$ (1.56–25 μg per mouse i.c.v.) produced a dose-dependent increase of the mobility time of mice. The doses of 1.56 and 3.12 μg per mouse i.c.v. were ineffective, the dose of 6.25 increased the mobility time even if the statistical significance was not reached. At 12.5 μg per mouse a significant antidepressant-like effect was produced. The highest effect of anti-$Go\alpha_1$ was reached at the doses of 25 μg per mouse i.c.v. (Fig. 3). The administration of an aODN against the α subunit of the Go_2 proteins (1.56–25 μg per mouse i.c.v.), similarly to anti-$Go\alpha_1$, increased the mobility time in a dose-dependent manner reaching the highest effect at 25 μg per mouse i.c.v. (Fig. 3). The anti-depressant like effect produced by anti-Go treatment was comparable to that exerted by amitriptyline (15 mg kg^{-1} s.c.) and clomipramine (25 mg kg^{-1} s.c.), used as reference drugs (Fig. 3).

The antidepressant-like effect produced by aODNs, at the highest doses tested, disappeared 7 days after the end of treatment. The mobility time recorded in each group was comparable to that of dODN-treated mice (Fig. 4).

3.3. Effect of PTX and aODN against Giα and Goα subunits on mouse behaviour

It should be noted that PTX and the aODNs under investigation elicited their modulatory effects on mobility time in the forced swimming test without altering motor coordination, as revealed by the rota rod test (Fig. 5). In this test, each group progressively reduced its number of falls because mice learned how to balance on the rotating rod. PTX (0.25 μg per mouse i.c.v.) did not modify the number of falls from the rotating rod

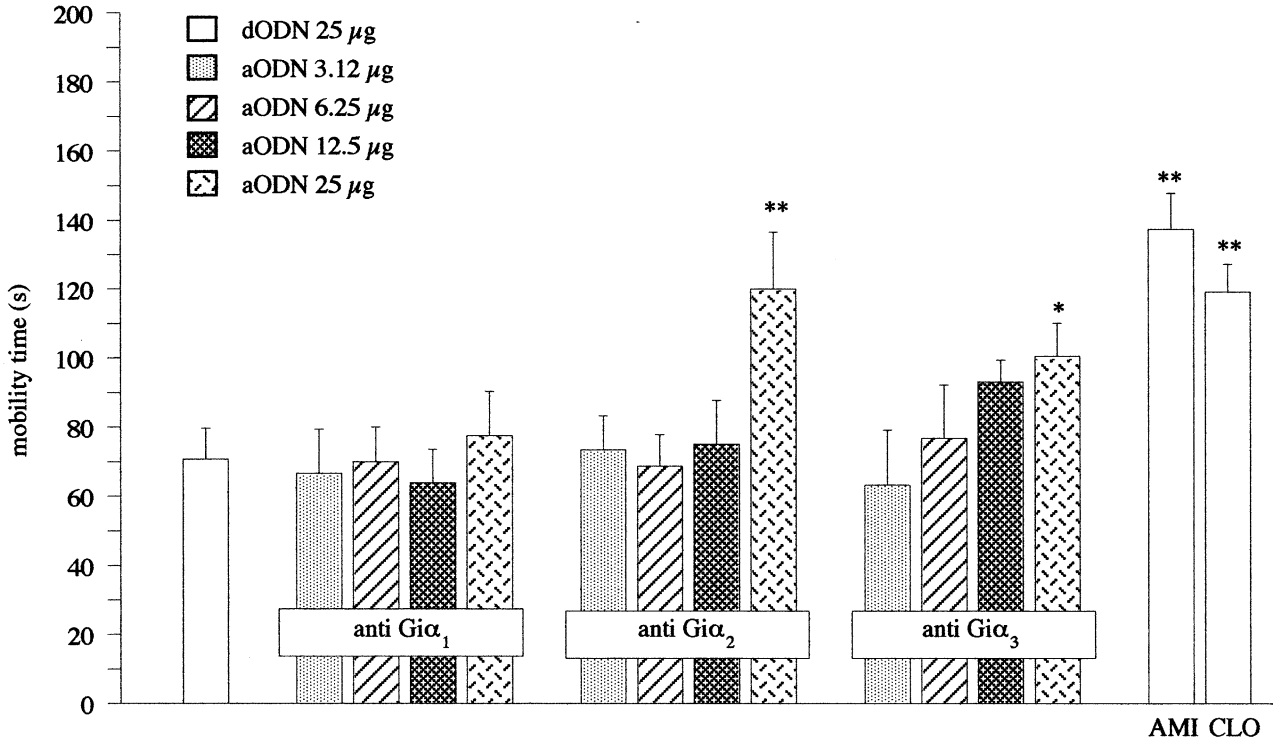

Fig. 2. Effect of pretreatment with an antisense oligonucleotide (aODN) to the α subunit of Gi_1 (3.12–25 μg per mouse i.c.v.), Gi_2 (3.12–25 μg per mouse i.c.v.), Gi_3 (3.12–25 μg per mouse i.c.v.) protein gene in the mouse forced swimming test. The test was performed 18–24 h after the i.c.v. injections of degenerate ODN (dODN; 12.5 μg per mouse i.c.v.) or aODN. Amitriptyline (AMI, 15 mg kg^{-1} s.c.) and clomipramine (CLO, 25 mg kg^{-1} s.c.) were administered 30 min before the test. Vertical lines represent s.e.m.; between 16 and 23 mice were tested. $*P<0.05$, $**P<0.01$ in comparison with dODN.

in comparison with untreated (naive), saline- or vehicle-treated mice (Fig. 5(A)). The motor coordination of mice pretreated with aODN to $Gi\alpha_1$ (25 μg per mouse i.c.v.), $Gi\alpha_2$ (25 μg per mouse i.c.v.), $Gi\alpha_3$ (25 μg per mouse i.c.v.), $Go\alpha_1$ (25 μg per mouse i.c.v.), and $Go\alpha_2$ (25 μg per mouse i.c.v.) was unaltered in the rota rod test. The number of falls of aODN-treated groups was comparable to that of the dODN-treated mice (Fig. 5(B)B).

The spontaneous motility and exploratory activity of mice was not modified by administration of PTX (0.25 μg per mouse i.c.v.), and the above-mentioned aODNs (25 μg per mouse i.c.v.) as revealed by the hole-board test in comparison with untreated (naive), saline-, and dODN (25 μg per mouse i.c.v.)-treated mice (Fig. 6). In the same experimental conditions D-amphetamine (2 mg kg^{-1} s.c.), used as the reference drug, increased both parameters evaluated.

4. Discussion

Signal transduction mechanisms mediated by Gi proteins appear to be involved in the modulation of depressive states as evidenced by results from the forced swimming test in mice. Inactivation as well as inhibition of expression of Gi proteins provoke an increase of mouse mobility time inducing an antidepressant-like effect of an intensity comparable to that produced by the tricyclic antidepressants amitriptyline and clomipramine, used as reference drugs.

The 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 including tricyclic and atypical antidepressants, MAO inhibitors and 5-HT uptake inhibitors (Porsolt et al., 1977; Bourin et al., 1991), and their effectiveness correlates significantly with clinical potency (Willner, 1984). However, this animal model has also some drawbacks represented by the possibility of obtaining some false positives or negatives (Borsini and Meli, 1988; Detke and Lucki, 1996). 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).

Present results indicate that the inactivation of Gi proteins is required for the induction of an antidepressant-like effect in the mouse forced swimming test. The administration of pertussis toxin (PTX), a bacterial toxin produced by *Bordetella pertussis* that ADP-ribosylates

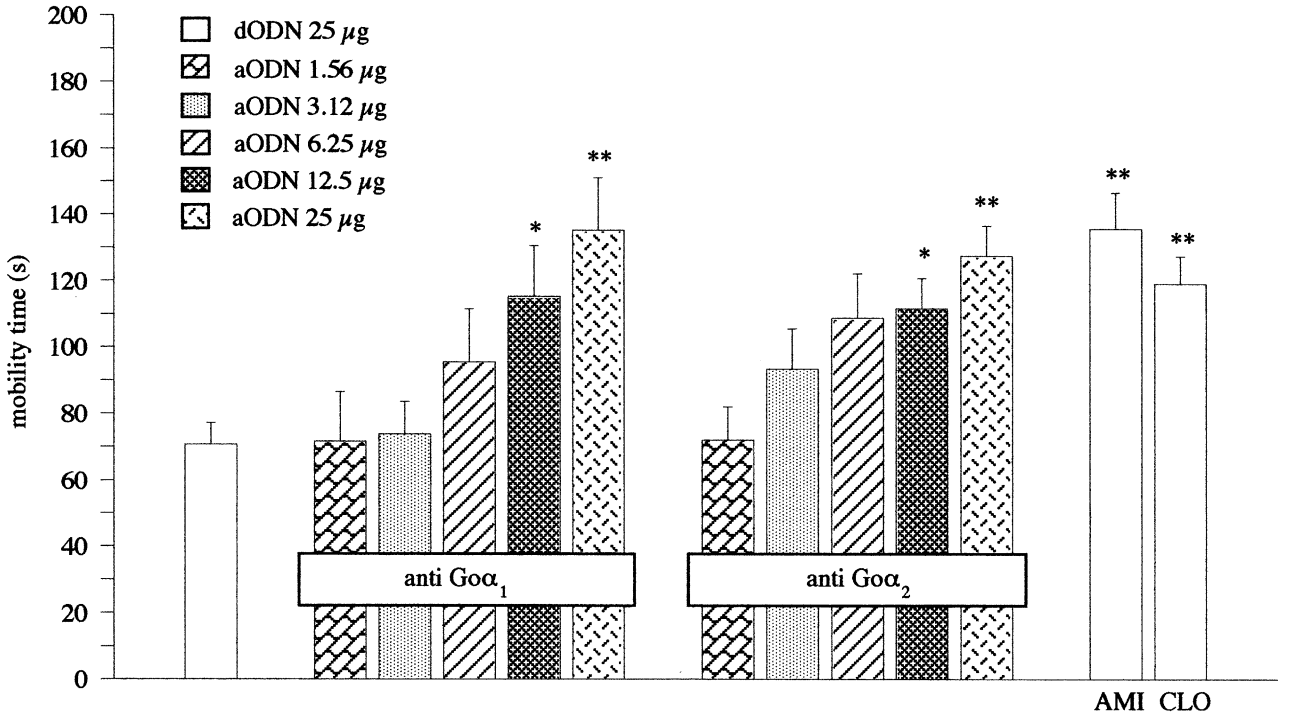

Fig. 3. Effect of pretreatment with an antisense oligonucleotide (aODN) to the α subunit of Go_1 (1.56–25 μg per mouse i.c.v.) and Go_2 (1.56–25 μg per mouse i.c.v.) protein gene in the mouse forced swimming test. The test was performed 18–24 h after the i.c.v. injections of degenerate ODN (dODN; 12.5 μg per mouse i.c.v.) or aODN. Amitriptyline (15 mg kg^{-1} s.c.) and clomipramine (25 mg kg^{-1} s.c.) were injected 30 min before the test. Vertical lines represent s.e.m.; between 18 and 21 mice were tested. $*P<0.05$, $**P<0.01$ in comparison with dODN.

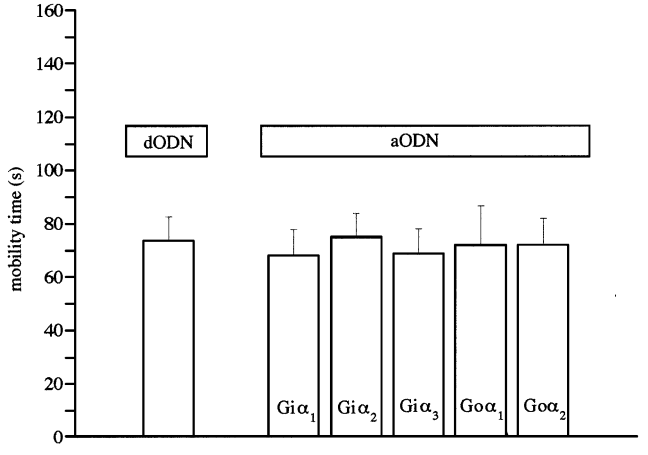

Fig. 4. Lack of effect by pretreatment with an aODN to the α subunit of Gi_1 (25 μg per mouse i.c.v.), Gi_2 (25 μg per mouse i.c.v.), Gi_3 (25 μg per mouse i.c.v.), Go_1 (25 μg per mouse i.c.v.), and Go_2 (25 μg per mouse i.c.v.) protein gene in the mouse forced swimming test 7 days after the i.c.v. injection of dODN (25 μg per mouse i.c.v.) or aODNs. Vertical lines represent s.e.m.; 15 mice per group were tested.

and inactivates the α subunit of Gi-protein family (Katada and Ui, 1982), enhanced the mobility time of mice and, therefore, counteracted the depressant-like condition induced in the test. These data evidence the important role played by Gi proteins in the signal transduction mechanism responsible for the modulation of depressive states. The Gi protein subfamily is composed by different members, Gi_1, Gi_2, Gi_3, Go_1, and Go_2 (Simon et al., 1991). Since PTX inactivates all members of the Gi-protein family, the role of each subtype was investigated by pretreating animals with aODNs against the α subunits of the above-mentioned Gi and Go protein subtypes. The inhibition of the expression of $Gi\alpha_2$, $Gi\alpha_3$, $Go\alpha_1$, and $Go\alpha_2$ produced a dose-dependent increase of mobility time, whereas the administration of an aODN against $Gi\alpha_1$ never exerted any effect in comparison with control animals. These results indicate a differential involvement of the Gi protein subtypes in the modulation of depressive states in mice. In particular, the inhibition of the expression of Gi_2, Gi_3, Go_1, and Go_2 proteins appears essential to produce an antidepressant-like effect. In particular, Go_1 and Go_2 subtypes appear to be endowed with a prominent role since the anti-Go_1 and anti-Go_2 increased the mobility time at lower doses than anti-Gi_2 and anti-Gi_3. The observation that chronic treatment with the antidepressant drug imipramine decreases $Go\alpha$ mRNA levels in the rat hippocampus without modifying $Gi\alpha$ mRNA levels (Lason and Przewlocki, 1993) further support this hypothesis. Contrary to Gi_2 and Gi_3, the Gi_1 subtype appears not to be involved in the modulation of mobility time implying that this subunit is not a major component in the transduction mechanisms involved in the induction of a depressive condition.

From these data we can hypothesise that activation of the transduction system mediated by Gi proteins may

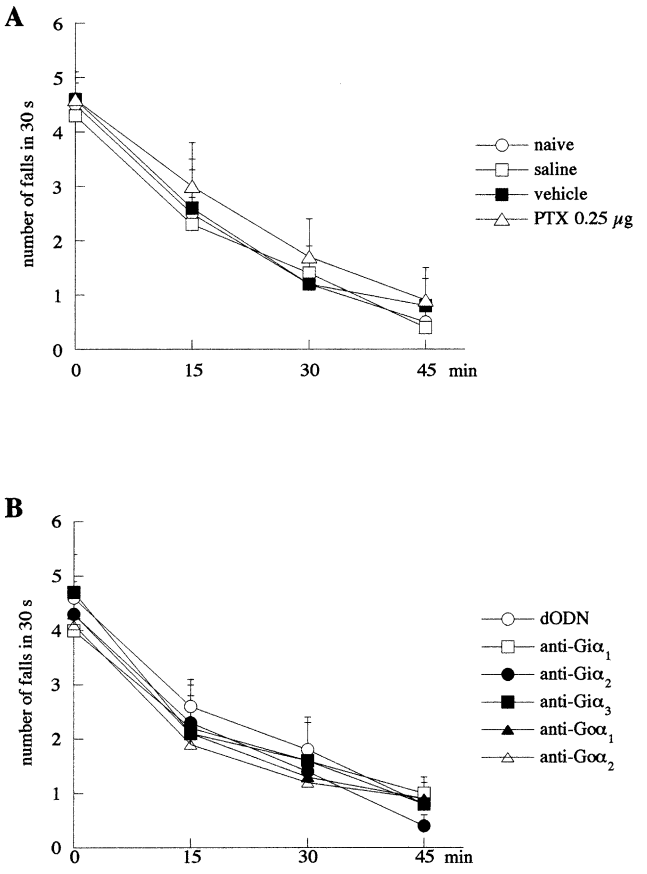

Fig. 5. Effect of PTX (panel A) and aODN (panel B) to the α subunit of Gi_1 (25 μg per mouse i.c.v.), Gi_2 (25 μg per mouse i.c.v.), Gi_3 (25 μg per mouse i.c.v.), Go_1 (25 μg per mouse i.c.v.), Go_2 (25 μg per mouse i.c.v.) protein gene on motor coordination in the mouse rota rod test. The test was performed 24–18 h after the i.c.v. injection of dODN (25 μg per mouse i.c.v.) or aODN. Vertical lines represent s.e.m.

result in the induction of a depressive state. Nevertheless, studies on the quantification of Gi protein levels in depressed patients are controversial (see introduction). These discrepancies may stem from the severity of illness in the patients included in each study and from the heterogeneity of biological sample used (post-mortem brain, peripheral blood cells etc.). However, a change in the protein or mRNA amount does not always imply defective protein function, but rather often appears to reflect compensatory consequences. As a matter of fact, even if no clear alteration at protein or mRNA Gi protein level emerged, an hyperfunctionality of this transduction mechanism has been evidenced. Gi-proteins represent the most widespread modulatory signalling pathway in neurones (Holtz et al., 1986) and one of their principal effects is the inhibition of adenylate cyclase activity (Sprang, 1997). A significant elevation in hydrolysis-resistant photoaffinity GTP analog (AAGTP) binding to Gi/o and a decrease in the ratio Gs/Gi AAGTP incorporation was seen in parietal and temporal cortices of unipolar depressive patients (Ozawa et al., 1993). It has also been reported that basal and stimulated adenylate cyclase activity, as well as cAMP response element binding protein (CREB) levels, were reduced in post-mortem brain of subjects with mood disorders (Cowburn et al., 1994; Young et al., 1996b; Dowlatshahi et al., 1999). Moreover, in response to antidepressant treatment, increased cAMP activity (Perez et al., 1991) and enhanced expression and activity of CREB (Nibuya et al., 1996; Thome et al., 2000) has been demonstrated in rat brain. Since adenylate cyclase is regulated by the balance between Gs and Gi functions, an imbalance in this messenger system evidenced by these data further suggests that depression may be a state occurring during hypofunction of the adenylate cyclase pathway.

Pretreatment with dODN, used as the reference ODN, never modified the increase of mobility time in the mouse forced swimming test in comparison with untreated (naive) or saline-treated animals, excluding the possibility of a sequence-independent effect induced by the aODNs. Moreover, the antidepressant-like effect induced by anti-Giα and anti-Goα disappeared 7 days after administration, indicating an absence of irreversible damage or toxicity on cerebral structures caused by the aODNs.

Numerous hormones and neurotransmitters activate the Gi-protein system. The administration of PTX and aODN against the α subunits of Gi proteins could induce side effects that make the interpretation of the results obtained difficult. Furthermore, since drugs that modify motor activity may give false positive or negative effects, 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 behavioural side effects. PTX and ODNs were tested on the rota-rod test before the forced swimming test was performed, to make sure that they did not influence the normal motor coordination of the mice. Since ataxic mice are not able to coordinate movements and fall from the rotating rod, while excited animals tend to jump off the rod, the good performance on the rod by mice in the present study indicates the results obtained with the forced swimming test are not due to altered motor activity induced by substances at the doses used. Furthermore, not only altered motor coordination but also a modified spontaneous motility could lead to a misinterpretation of the results obtained in the forced swimming test. An influence of the substances used on spontaneous motility has, therefore, been excluded by using the hole board test. Moreover, drugs which have a known psychostimulant effect, like (+)-amphetamine and caffeine, at the same doses at which they are able to increase the time of mobility in the rat forced swimming test, also show a significant increased motor activity in an open field (Porsolt et al., 1978).

In conclusion, our results evidence that knockdown of $Gi\alpha_2$, $Gi\alpha_3$, $Go\alpha_1$ and $Go\alpha_2$, but not $Gi\alpha_1$ induces an

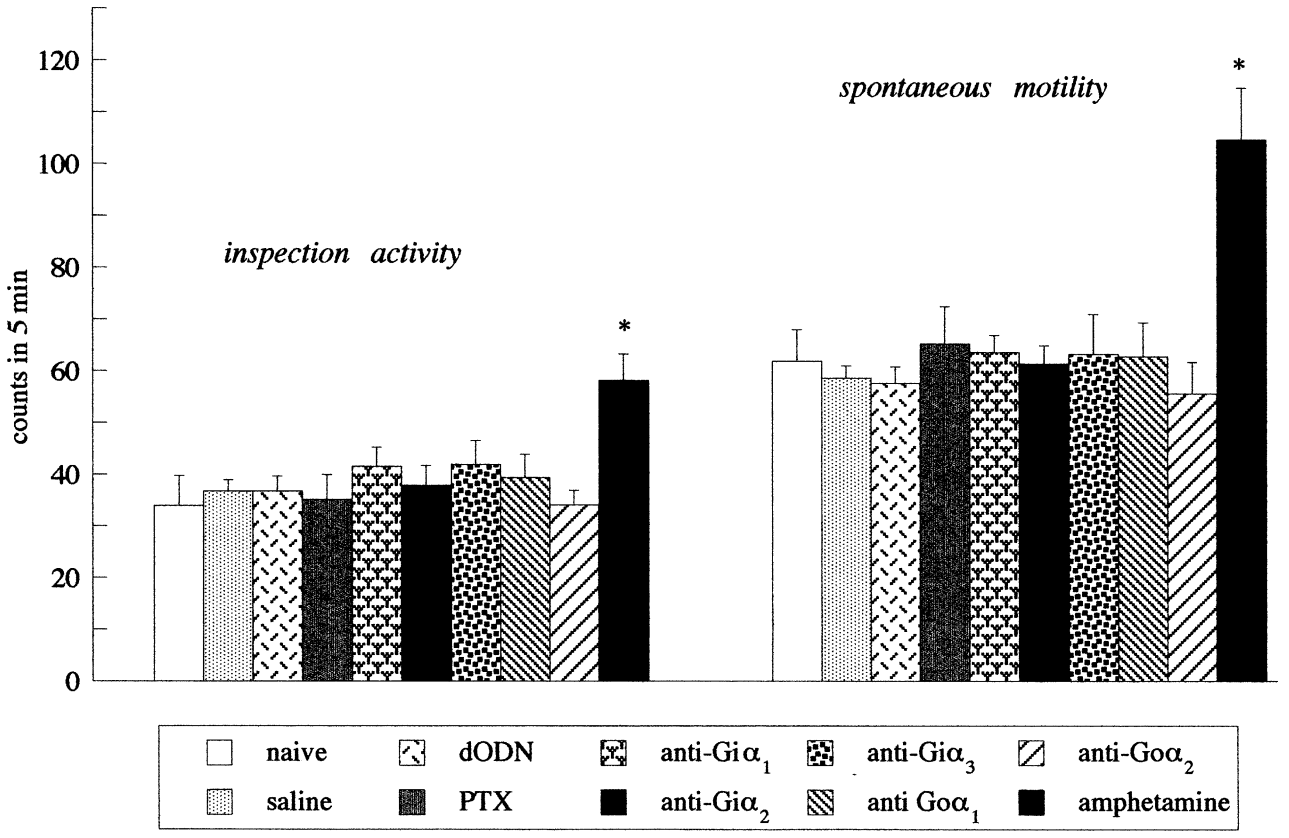

Fig. 6. Lack of effect of pretreatment with an antisense oligonucleotide (aODN) to the α subunit of Gi_1 (25 μg per mouse i.c.v.), Gi_2 (25 μg per mouse i.c.v.), Gi_3 (25 μg per mouse i.c.v.), Go_1 (25 μg per mouse i.c.v.), Go_2 (25 μg per mouse i.c.v.) protein gene on spontaneous motility and inspection activity in the mouse hole board test. The test was performed 18–24 h after the i.c.v. injections of degenerate ODN (dODN; 25 μg per mouse) or aODN. Vertical lines represent s.e.m. D-amphetamine was administered at the dose of 1 mg kg^{-1} s.c. $*P<0.05$ in comparison with naive group.

anti-immobility effect comparable to that produced by the antidepressants in the mouse forced swimming test, indicating the involvement of this transduction system in the induction of a depressive-like condition.

Acknowledgements

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