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Role of Gi Proteins in the Antidepressant-like Effect of Amitriptyline and Clomipramine

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The effect of the i.c.v. administration of pertussis toxin (PTX) and antisense oligodeoxynucleotides directed against the α subunit of different Gi-proteins (anti-Giα_1, anti-Giα_2, anti-Giα_3, anti-Goα_1, anti-Goα_2) on the antidepressant-like effect induced by amitriptyline and clomipramine, was evaluated in the mouse forced swimming test, an animal model of depression. The administration of amitriptyline (15 mg kg^{-1} s.c.) and clomipramine (25 mg kg^{-1} s.c.) produced an increase in the mobility time that was prevented by PTX (0.25 μg per mouse i.c.v.), administered 11 days before the mouse forced swimming test. Anti-Giα_1 (12.5 μg per mouse i.c.v.), anti-Giα_2 (12.5 μg per mouse i.c.v.), anti-Giα_3 (6.25 μg per mouse i.c.v.), and anti-Goα_1 (6.25 μg per mouse i.c.v.), administered 24 and 18 h before the training session, prevented the amitriptyline and clomipramine increase of the mobility time. By contrast, pretreatment with anti-Goα_2 (1.56-12.5 μg per mouse i.c.v.) never modified the antidepressant-like effect induced by the two investigated compounds. At the highest effective doses, none of the compounds used impaired motor coordination, as revealed by the rota-rod test, nor modified spontaneous motility and inspection activity, as revealed by the hole-board test. These results suggest the important role played by Gi$_1$, Gi$_2$, Gi$_3$, and Go$_1$ protein subtypes and the lack of involvement by Go$_2$ protein subtype in the transduction mechanism responsible for the antidepressant-like effect produced by amitriptyline and clomipramine.
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KEY WORDS: *Amitriptyline; Clomipramine; Tricyclic antidepressants; Pertussis toxin; Gi-proteins; Antisense oligodeoxyribonucleotides*

For the past several decades, antidepressant drugs have been widely used in the treatment of clinical depression and other psychiatric disorders. Several distinct pharmacological compounds show therapeutic efficacy. These include monoamine oxidase inhibitors, tricyclic compounds, selective serotonin and norepinephrine reuptake inhibitors, as well as some atypical drugs. Numerous studies have examined the effects of antidepressants on neurotransmitter metabolism, turnover, and receptor sensitivity and several theories on their mechanism of action have been proposed. These include the inhibition of monoamine transporter activity in presynaptic membranes (Schildkraut 1965), downregulation of β-adrenoceptors (Mazzola-Pomietto et al. 1994), alteration at cholinergic, dopaminergic or GABAergic receptors (Henringer and Charney 1987). However, the molecular mechanism of action underlying the therapeutic effect of antidepressants is still unclear.

Despite their diversity, these treatments subserve the same final clinical effect. Therefore, the possibility that these diverse agents converge on a single postreceptorial target evoked a great research interest. Since most neurotransmitter receptors and neuromodulators are coupled to intracellular effectors through G-proteins (Birnbaumer 1990), recent studies searching for a com-

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mon mechanism of antidepressant action have focused on G-proteins as a potential target of such action.

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 with α, β and γ subunits. The α subunits can be classified into families, depending on whether they are targets for cholera toxin (Gs), pertussis toxin (PTX) (Gi and Go) or neither (Gq and G_{12}) (Simon et al. 1991). A growing body of evidence suggests that G-proteins are the molecular target of many antidepressants. Menkes et al. (1983) first reported that long-term administration of various antidepressants enhanced guanylyl-5′-imidodiphosphate and fluoride-stimulated adenylyl cyclase activity in rat cortex and hypothalamus membranes suggesting Gs-protein as target for antidepressant action. These initial findings involving the stimulation of adenylyl cyclase via Gs-proteins after antidepressant treatment was further supported by later studies (Yamaoka et al. 1988; Ozawa and Rasenick 1989; Kamada et al. 1999). Furthermore, increased expression and activity of cAMP response element binding protein has been demonstrated in various rat brain regions, such as hippocampus, cerebral cortex, amygdala, and hypothalamus (Nibuya et al. 1996; Duman et al. 1997; Thome et al. 2000). Recently, it has been suggested that chronic treatment with antidepressant drugs induces modification of the coupling between Gs-coupled receptors and adenylate cyclase in C6 glioma cells (Donati et al. 2001).

Pertussis toxin-sensitive G-proteins (G_i-proteins) represent the most widespread modulatory signaling pathway in neurones (Holtz et al. 1986) and are responsible for inhibition of adenylate cyclase activity and modulation of several K^+ and Ca^{2+} channels (Hille 1994; Sprang 1997). Contrary to Gs-proteins, the role of Gi-proteins in the mechanism of action of antidepressant drugs is more controversial. Chronic treatment with antidepressant drugs has been reported to produce a reduction of Gi_α and an increase of the Go_α protein levels (Lesch et al. 1991; Lesch and Manji 1992; Raap et al. 1999) in various regions of rat brain, including neostriatum, hypothalamus, frontal cortex and midbrain. By contrast, no alteration of Gi_α and Go_α protein and mRNA levels in rat brain (cortex, hippocampus, cerebellum) was observed after chronic treatment with tricyclic antidepressants (TCA) and monoamine oxidase inhibitors (Li et al. 1993; Chen and Rasenick 1995; Emamghoreishi et al. 1996; Dwivedi and Pandey 1997).

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 mechanism of action of antidepressant drugs. In particular, we used 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 the antidepressant-like effect induced by amitriptyline and clomipramine in the mouse forced swimming test. In order to exclude that the effects produced by aODN treatments were due to the induction of side effects, some additional behavioral tests (rota-rod, hole-board) were performed.

METHODS

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. All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Intracerebroventricular Injection Technique

Intracerebroventricular (i.c.v.) administration was performed under ether anesthesia, according to the method described by Haley and McCormick (1957). Briefly, during anesthesia, 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 determined to be 95.

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, 11 days after PTX administration, 30 min after tricyclic antidepressant injection.

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. Mice were placed on the center 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 four equal quadrants, automatically signaled 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 18–24 h after the i.c.v. injections of degenerate ODN (dODN) or aODN, 11 days after administration of PTX. 12–15 mice per group were tested.

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 five equal sections by six disks. Thus, up to five 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 three and more than six 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.

Antisense Oligonucleotides

The sequences of the antisense oligonucleotides 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). 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, C, 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, C, 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 behavioral tests. All ODNs were previously characterized 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). We also confirmed the aODN effect on Gα protein levels by performing immunoblotting experiments. We observed a statistically significant reduction of the expression of $Gi\alpha_1$, $Gi\alpha_2$, $Gi\alpha_3$, $Go\alpha_1$ and $Go\alpha_2$ subunits after aODN treatment in comparison with mice treated with dODN (data not shown).

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

Table 1. Sequences of Antisense Oligonucleotides

aODN	Sequences
anti-$Gi\alpha_1$	5′- G*C*T GTC CTT CCA CAG TCT CTT TAT GAC GCC G*G*C -3′
anti-$Gi\alpha_2$	5′- A*T*G GTC AGC CCA GAG CCT CCG GAT GAC GCC C*G*A -3′
anti-$Gi\alpha_3$	5′- G*C*C ATC TCG CCA TAA ACG TTT AAT CAC GCC T*G*C -3′
anti-$Go\alpha_1$	5′- A*G*G CAG CTG CAT CTT CAT AGG TG*T *T -3′
anti-$Go\alpha_2$	5′- G*A*G CCA CAG CTT CTG TGA AGG CA*C *T -3′

be administered in a volume of 10 ml kg^{-1} by s.c. injection or 5 μl per mouse by i.c.v. injection.

Statistical Analysis

All experimental results are given as the mean ± SEM 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 analyzed with the StatView software for the Macintosh, 1992 version. p values of less than .05 were considered significant.

RESULTS

Effect of Pertussis Toxin on TCAs Antidepressant-like Effect

Amitriptyline (15 mg kg^{-1} s.c.) and clomipramine (25 mg kg^{-1} s.c.), injected 30 min before the test, induced an increase of the mobility time in the mouse forced swimming test (Figure 1). Pretreatment with pertussis toxin (PTX), injected i.c.v. at the dose of 0.25 μg per mouse 11 days before the test, completely prevented the amitriptyline and clomipramine antidepressant-like effect (Figure 1).

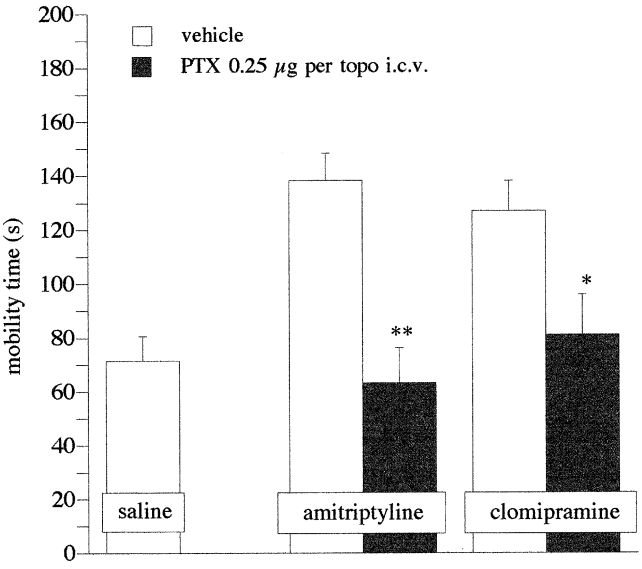

Figure 1. Prevention by pertussis toxin (PTX) of amitriptyline- and clomipramine-induced increase of the mobility time in the mouse forced swimming test. The test was performed 11 days after a single i.c.v. injection of vehicle or PTX (0.25 μg per mouse). Amitriptyline (15 mg kg^{-1} s.c.) and clomipramine (25 mg kg^{-1} s.c.), were administered 30 min before the test. Between 19 and 25 mice were tested. Vertical lines represent SEM. $^{*}p < .05$ in comparison with vehicle + clomipramine, $^{**}p < .01$ in comparison with vehicle + amitriptyline treated mice.

Effect of aODN against Giα Subunits on TCA-induced Antidepressant-like Effect

The increase of mobility time induced by amitriptyline (15 mg kg^{-1} s.c.) and clomipramine (25 mg kg^{-1} s.c.) was prevented, in the mouse forced swimming test, by pretreatment with the aODN against the α subunit of the Gi proteins (Figures 2–4). Anti-$Gi\alpha_1$ (3.12–12.5 μg per mouse i.c.v.) produced a dose-dependent antagonism of the TCA-induced antidepressant-like effect. The dose of 3.12 μg per mouse i.c.v. was completely ineffective whereas the maximum effect was obtained at 12.5 μg per mouse i.c.v. (Figure 2).

Similarly, anti-$Gi\alpha_2$ at 3.12 μg per mouse i.c.v. was devoid of any effect; at 6.25 μg per mouse i.c.v. partially prevented the TCA effect, even if the statistical significance was not reached, whereas the dose of 12.5 μg per mouse i.c.v. reduced the mobility time up to a value comparable to that produced by control animals (Figure 3).

The administration of an aODN against the α subunit of the Gi_3 proteins (1.56–6.25 μg per mouse i.c.v.) antagonized the increase of mobility time produced by amitriptyline and clomipramine reaching its maximum effect at 6.25 μg per mouse i.c.v. (Figure 4). Anti-$Gi\alpha_1$ (12.5 μg per mouse i.c.v.), anti-$Gi\alpha_2$ (12.5 μg per mouse i.c.v.), and anti-$Gi\alpha_3$ (6.25 μg per mouse i.c.v.) did not produce any modification of the mobility time in the mouse forced swimming test in comparison with i.c.v. saline- (data not shown) and dODN-treated mice when given alone (Figures 2–4).

Effect of aODN against Goα Subunits on TCA-induced Antidepressant-like Effect

Anti-$Go\alpha_1$ (1.56-6.25 μg per mouse i.c.v.) produced a dose-dependent antagonism of the increase in the mobility time of mice induced by both amitriptyline and clomipramine. The maximum effect of anti-$Go\alpha_1$ was reached at the doses of 6.25 μg per mouse i.c.v., concentration that did not modify the mobility time of animals, in comparison with control group, when administered alone (Figure 5). The administration of an aODN against the α subunit of the Go_2 proteins (1.56–12.5 μg per mouse i.c.v.), in contrast to anti-$Go\alpha_1$, was unable to prevent the TCA-induced antidepressant-like effect (Figure 6).

Effect of aODN against Giα and Goα Subunits on Mouse Rota-rod and Hole-board Tests

It should be noted that the tricyclic antidepressants (amitriptyline, clomipramine) and the aODNs under investigation elicited their modulatory effects on mobility time in the forced swimming test without changing gross behavior, motor coordination (as revealed by the rota-rod test (Figure 7), spontaneous motility, or inspection activity, as revealed by the hole-board test (Table

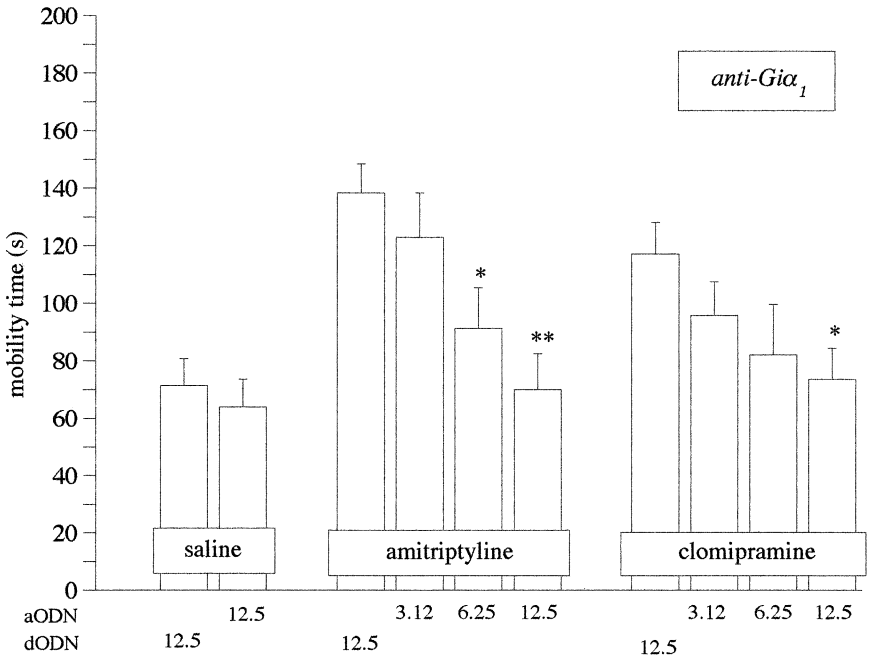

Figure 2. Prevention by pretreatment with an antisense oligonucleotide (aODN) to the α subunit of Gi_1-protein gene (3.12–12.5 μg per mouse i.c.v.) of amitriptyline (15 mg kg^{-1} s.c.)- and clomipramine (25 mg kg^{-1} s.c.)-induced antidepressant-like effect in the mouse forced swimming test. The test was performed 18–24 h after the i.c.v. injection of degenerate ODN (dODN; 12.5 μg per mouse i.c.v.) or aODN. Between 16 and 23 mice were tested. Vertical lines represent SEM; the dose administered is reported below each column. $^{*}p < .05$, $^{**}p < .01$ in comparison with the corresponding TCA.

2).] The doses of amitriptyline and clomipramine of respectively 15 and 25 mg kg^{-1} s.c. did not modify the number of falls from the rotating rod in comparison with saline-treated mice. Each group progressively reduced its number of falls because mice learned how to balance on the rotating rod. Higher doses of amitriptyline (30 mg kg^{-1} s.c.) and clomipramine (45 mg kg^{-1} s.c.) produced an increase in the number of falls indicating the presence of motor incoordination (Figure 7, panel A). The motor coordination of mice pretreated with aODN to $Gi_1\alpha$ (12.5 μg per mouse i.c.v.), $Gi_2\alpha$ (12.5 μg per mouse i.c.v.), $Gi_3\alpha$ (6.25 μg per mouse i.c.v.), $Go_1\alpha$ (6.25 μg per mouse i.c.v.), and $Go_2\alpha$ (12.5 μg per mouse i.c.v.) was evaluated by using the rota-rod test. The number of falls of aODN-treated groups was comparable to that of the dODN-treated mice (Figure 7, panel B). The spontaneous motility and exploratory activity of mice was not modified by administration of amitriptyline, clomipramine, PTX, and the above-mentioned aODNs as revealed by the hole-board test in comparison with saline-, vehicle- and dODN-treated mice (Table 2). In the same experimental conditions

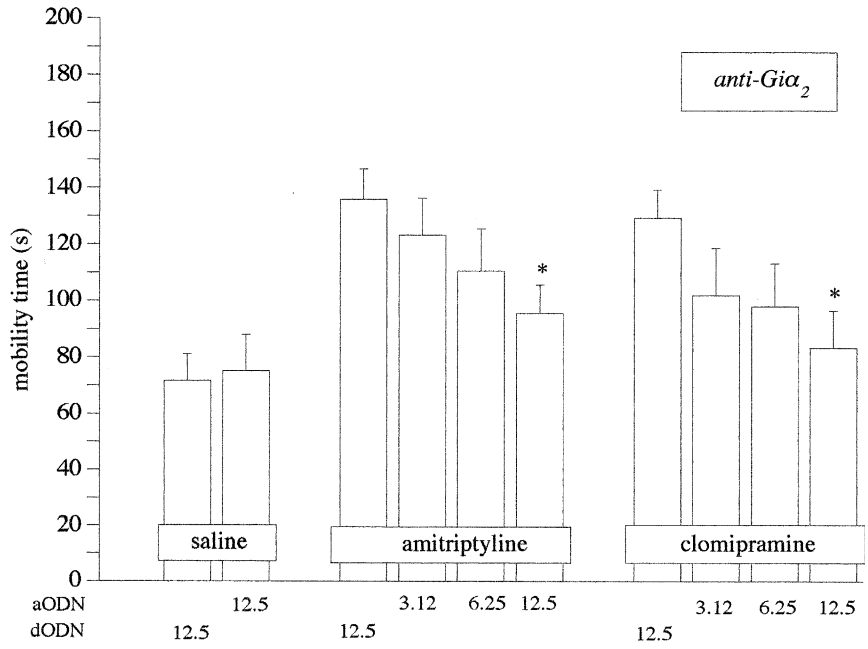

Figure 3. Prevention by pretreatment with an antisense oligonucleotide (aODN) to the α subunit of Gi_2-protein gene (3.12–12.5 μg per mouse i.c.v.) of amitriptyline (15 mg kg^{-1} s.c.)- and clomipramine (25 mg kg^{-1} s.c.)-induced antidepressant-like effect in the mouse forced swimming test. The test was performed 18–24 h after the i.c.v. injection of degenerate ODN (dODN; 12.5 μg per mouse i.c.v.) or aODN. Between 18 and 23 mice were tested. Vertical lines represent SEM; the dose administered is reported below each column. $^{*}p < .05$ in comparison with the corresponding TCA.

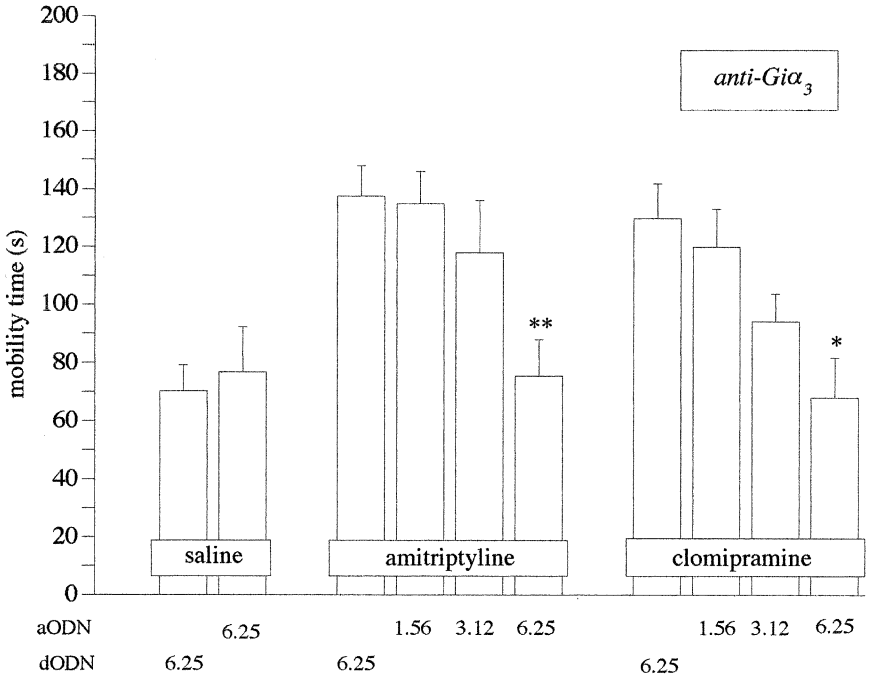

Figure 4. Prevention by pretreatment with an antisense oligonucleotide (aODN) to the α subunit of Gi_3-protein gene (1.56–6.25 μg per mouse i.c.v.) of amitriptyline (15 mg kg^{-1} s.c.)- and clomipramine (25 mg kg^{-1} s.c.)-induced antidepressant-like effect in the mouse forced swimming test. The test was performed 18–24 h after the i.c.v. injection of degenerate ODN (dODN; 12.5 μg per mouse i.c.v.) or aODN. Between 16 and 21 mice were tested. Vertical lines represent SEM; the dose administered is reported below each column. $^{*}p < .05$, $^{**}p < .001$ in comparison with the corresponding TCA.

D-amphetamine (2 mg kg^{-1} s.c.), used as the reference drug, increased both parameters evaluated.

DISCUSSION

Signal transduction mechanisms involved in the antidepressant-like effect produced by amitriptyline and clomipramine, two compounds belonging to the tricyclic antidepressant class, have been investigated in the mouse forced swimming test. 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,

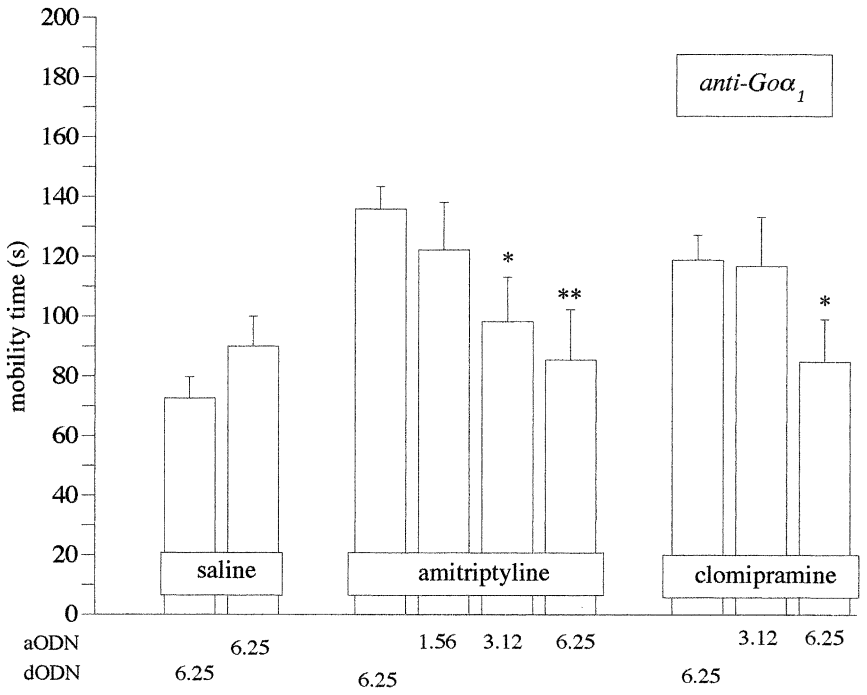

Figure 5. Prevention by pretreatment with an antisense oligonucleotide (aODN) to the α subunit of Go_1-protein gene (1.56–6.25 μg per mouse i.c.v.) of amitriptyline (15 mg kg^{-1} s.c.)- and clomipramine (25 mg kg^{-1} s.c.)-induced antidepressant-like effect in the mouse forced swimming test. The test was performed 18–24 h after the i.c.v. injection of degenerate ODN (dODN; 12.5 μg per mouse i.c.v.) or aODN. Between 15 and 22 mice were tested. Vertical lines represent SEM; the dose administered is reported below each column. $^{*}p < .05$, $^{**}p < .001$ in comparison with the corresponding TCA.

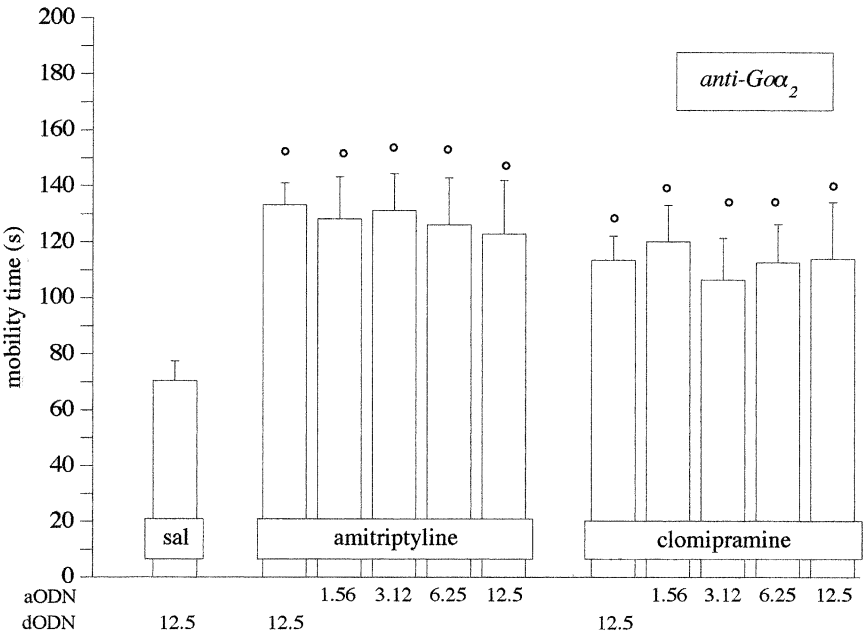

Figure 6. Lack of effect of pretreatment with an antisense oligonucleotide (aODN) to the α subunit of Go_2-protein gene (1.56–12.5 μg per mouse i.c.v.) on amitriptyline (15 mg kg^{-1} s.c.)- and clomipramine (25 mg kg^{-1} s.c.)-induced antidepressant-like effect in the mouse forced swimming test. The test was performed 18–24 h after the i.c.v. injection of degenerate ODN (dODN; 12.5 μg per mouse i.c.v.) or aODN. Between 18 and 21 mice were tested. Vertical lines represent SEM; the dose administered is reported below each column. $^{\circ}p < .01$ in comparison with control group.

this animal model has also some drawbacks represented by the possibility to obtain 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 activation of Gi proteins is required for the induction of the antidepressant-like effect produced by amitriptyline and clomipramine in the mouse forced swimming test. The administration of the investigated TCAs produced an increase of the mobility time that was prevented by pretreatment with pertussis toxin (PTX), a bacterial toxin produced by *Bordetella pertussis* that ADP-ribosylates and inactivates the α subunit of Gi-protein family (Katada and Ui 1982). These data confirm the important role played by Gi proteins in the signal transduction mechanism activated by amitriptyline and clomipramine. Gi-proteins represent the most widespread modulatory signaling pathway in neurones (Holtz et al. 1986) and one of their principal effect is the inhibition of adenylate cyclase activity (Sprang 1997). It has been reported that chronic treatment with several antidepressant drugs reduced the cAMP production stimulated by forskolin or noradrenaline (Mishra et al. 1980; Okada et al. 1986; Newman et al. 1993), further supporting the hypothesis of the involvement of this transduction system in the mechanism of action of amitriptyline and clomipramine.

The Gi protein subfamily is composed of several 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 $Gi\alpha_1$, $Gi\alpha_2$, $Gi\alpha_3$ and $Go\alpha_1$ produced a dose-dependent prevention of TCA-induced increase of mobility time, whereas the administration of an aODN against $Go\alpha_2$ never exerted any modification of amitriptyline and clomipramine activity. These results indicate a differential involvement of the Gi protein subtypes in the mechanism of action of the investigated tricyclic antidepressants. In particular, the integrity and functionality of Gi_1, Gi_2, Gi_3, and Go_1 proteins appears essential to produce the antidepressant-like effect observed. The Gi_3 and Go_1 subtypes appear to be endowed with a prominent role since the anti-Gi_3 and anti-Go_1 prevent the increase of mobility time induce by both investigated TCAs at doses lower than anti-Gi_1 and anti-Gi_2. By contrast, the Go_2 subtype, in these experimental conditions, appears not to be involved implying that this subunit is not a major component of transduction mechanisms leading to the amitriptyline and clomipramine antidepressant effect.

Chronic treatment with TCAs induces an increase in the Go-protein levels in different rat brain regions, such as frontal cortex, midbrain and hypothalamus (Lesch et al. 1991; Lesch and Manji 1992). Moreover, Yamamoto et al. (1992) reported that several TCAs, including clomipramine, were able to increase, in a PTX-dependent manner, the GTPase activity of Go proteins, purified from bovine brain membranes, indicating the ability of these compounds to directly stimulate Go proteins. Therefore, the important role played by Go_1 proteins in amitriptyline and clomipramine antidepressant-like activity could be due, at least in part, to a di-

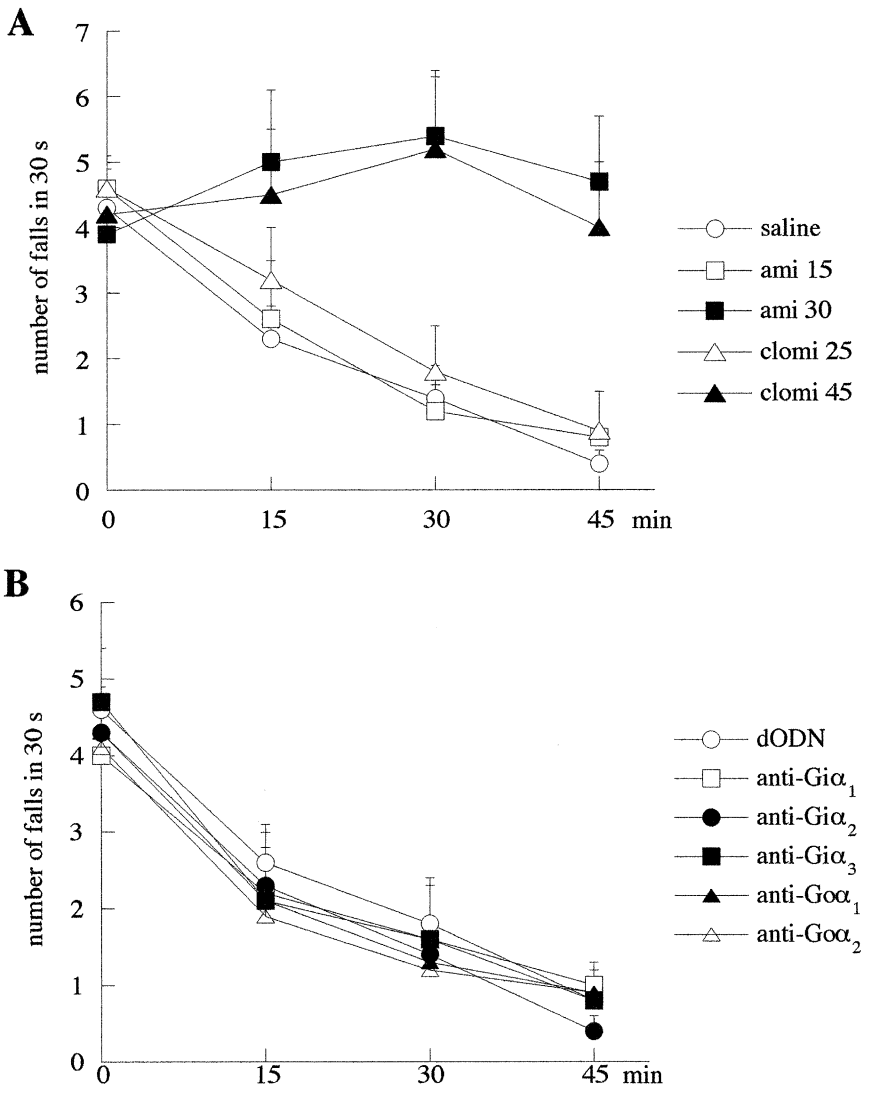

Figure 7. A. effect of amitriptyline and clomipramine on motor coordination in the mouse rota-rod test. Amitriptyline (15–30 mg kg^{-1} s.c.) and clomipramine (25–45 mg kg^{-1} s.c.) were administered 30 min before the test. B. effect of pretreatment with an aODN to the α subunit of Gi_1- (12.5 μg per mouse i.c.v.), Gi_2- (12.5 μg per mouse i.c.v.), Gi_3- (6.25 μg per mouse i.c.v.), Go_1- (6.25 μg per mouse i.c.v.), Go_2- (12.5 μg per mouse i.c.v.) protein gene on motor coordination in the mouse rota-rod test. The test was performed 18–24 h after the i.c.v. injection of dODN (12.5 μg per mouse i.c.v.) or aODN. Vertical lines represent SEM.

rect activation of the Go-protein subtype. Nevertheless, it has been observed that chronic treatment with the antidepressant drug imipramine decreases Goα mRNA levels in the rat hippocampus without modifying Giα mRNA levels (Lason and Przewlocki 1993). This discrepancy may stem from the heterogeneity of biological sample used (rat frontal cortex, midbrain and hypothalamus, bovine brain membranes, rat hippocampus) suggesting not only a differential effect of TCAs on different cerebral areas, but also a different involvement of the various brain regions in the induction of a depressive state condition. However, a change in the mRNA amount does not always imply defective protein function, but rather often appears to reflect compensatory consequences. As matter of fact, even if no clear alteration at mRNA Go protein level emerged, an increased functionality of this transduction mechanism after antidepressant treatment has been evidenced. Concerning Gi_3, a reduction of this Gi-protein subtype has been observed in the platelets from patients suffering from major depression (Garcia-Sevilla et al. 1997). On these bases we can hypothesize that amitriptyline and clomipramine might exert their antidepressant effect by stimulating Gi_3 subtypes in order to balance its deficiency.

A role in the mechanism of action of TCAs played by Gi_2 proteins has been reported in humans. A reduced coupling capability of α_2-adrenoceptors, a receptor subtype involved in the induction of depression (Garcia-Sevilla et al. 1999), with Gi_2 proteins has been observed in platelets from patients suffering from major depression. In consequence, an increase of Gi_2 proteins has been produced as a compensation mechanism (Karege et al. 1996). In these patients, repeated treatment with TCAs was reported to normalize the coupling of α_2-adrenoceptors with Gi_2 proteins, inducing, consequently, a reduction of $Gi\alpha_2$ levels (Garcia-Sevilla et al. 1997; Karege et al. 1998). However, in our experimental conditions, the involvement of the Gi_1 and Gi_2 subtypes in the mechanism of action of amitriptyline and clomipramine has been observed after administration of a dose of aODN

Table 2. Lack of Effect on Spontaneous Motility and Inspection Activity by Amitriptyline, Clomipramine, PTX, dODN and aODNs in the Mouse Hole-board Test.

		Counts in 5 min	
Pre-Treatment (i.c.v.)	**Treatment (s.c.)**	**spontaneous motility**	**inspection activity**
Vehicle	Saline	37.8 ± 5.9	63.4 ± 6.7
PTX 0.25 μg	Saline	35.1 ± 4.8	65.3 ± 7.2
Saline	Saline	36.8 ± 4.3	63.1 ± 5.1
DOTAP 13 μM	Saline	37.8 ± 3.1	61.9 ± 6.1
dODN 12.5 μg	Saline	38.7 ± 3.6	61.7 ± 5.6
Anti-$Gi_{1\alpha}$ 12.5 μg	Saline	45.2 ± 4.2	65.7 ± 3.3
Anti-$Gi_{2\alpha}$ 12.5 μg	Saline	37.9 ± 3.8	61.4 ± 4.4
Anti-$Gi_{3\alpha}$ 6.25 μg	Saline	41.9 ± 4.7	63.3 ± 7.8
Anti-$Go_{1\alpha}$ 6.25 μg	Saline	39.4 ± 4.5	62.9 ± 6.5
Anti-$Go_{2\alpha}$ 12.5 μg	Saline	34.1 ± 2.8	59.7 ± 6.0
Saline	Amitriptyline 15 mg kg^{-1}	36.0 ± 4.5	62.0 ± 6.1
Saline	Clomipramine 25 mg kg^{-1}	36.0 ± 4.5	62.0 ± 6.1
Saline	Amphetamine 2 mg kg^{-1}	73.2 ± 7.1**	104.8 ± 12.0**

Pertussis toxin (PTX) and ODNs were injected respectively 11 days and 24-18 h before the test. **$p < 0.01$ in comparison with saline-saline treated mice.

two times higher than that necessary to produce a comparable effect with the anti-Gi_3 and the anti-Go_2. These results, nevertheless, confirm the importance of the involvement of these two Gi-protein subtypes in the antidepressant-like effect induced by the two TCAs, and they also indicate a secondary role in comparison with Gi_3 and Go_2. Moreover, chronic and subchronic treatment with TCAs did not produce significant differences on Gi_1 and Gi_2 protein and mRNA levels in rat brain (Li et al. 1993; Lason and Przewlocki 1993; Emamghoreishi et al. 1996; Dwivedi and Pandey 1997), further supporting the hypothesis of a minor role for these two Gi-protein subtypes.

The two investigated TCAs induced their antidepressant-like effect by acting centrally since pertussis toxin and aODNs exerted their antagonistic effect after i.c.v. administration. However, substances injected i.c.v. can diffuse from the cerebral ventricles to the whole brain. It is, therefore, difficult to define potential site of actions of aODNs or pertussis toxin within the CNS.

Amitriptyline and clomipramine are inhibitors of the reuptake of serotonin and noradrenaline, two neurotransmitters involved in the modulation of mood. Some serotonin receptor subtypes as well as α_2 adrenoceptors (Birnbaumer 1990) are Gi-protein coupled receptors within the central nervous system. We cannot, therefore, exclude the involvement of both neurotransmission systems in the induction of the antidepressant-like effects by the two investigated TCAs.

Pretreatment with aODN, at the highest effective doses, did not produce any modification of mobility time in the mouse forced swimming test. We can, therefore, conclude that the prevention of amitriptyline and clomipramine antidepressant-like effect is not due to a depressant-like effect exerted by these treatments. Furthermore, pretreatment with dODN, used as the reference ODN, never modified the increase of mobility time induced by the two investigated TCAs in comparison with saline-treated animals (data not shown), which excludes the possibility of a sequence-independent effect induced 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 difficult. 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 the maximum that did not produce behavioral side effects. The tricyclic antidepressants amitriptyline and clomipramine, as well as ODNs, at the highest doses used, did not modify animal's gross behavior. All the substances used 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 thus 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 psychostim-

ulant 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 the important role played by $Gi\alpha_1$, $Gi\alpha_2$, $Gi\alpha_3$, and $Go\alpha_1$, but not by $Go\alpha_2$, in the anti-immobility effect induced by amitriptyline and clomipramine.

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