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Differential prevention of morphine amnesia by antisense oligodeoxynucleotides directed against various Gi-protein α subunits

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1 The effect of the i.c.v. administration of pertussis toxin (PTX) and antisense oligodeoxynucleotide directed against the α subunit of different Gi-proteins (anti-Giα$_1$, anti-Giα$_2$, anti-Giα$_3$) on amnesia induced by morphine was evaluated in the mouse passive avoidance test.

2 The administration of morphine (6–10 mg kg^{-1} i.p.) immediately after the training session produced amnesia that was prevented by PTX (0.25 μg per mouse i.c.v.) administered 7 days before the passive avoidance test.

3 Anti-Giα$_1$ (6.25 μg per mouse i.c.v.) and anti-Giα$_3$ (12.5 μg per mouse i.c.v.), administered 18 and 24 h before the training session, prevented the morphine amnesia. By contrast, pretreatment with anti-Giα$_2$ (3.12–25 μg per mouse i.c.v.) never modified the impairment of memory processes induced by morphine.

4 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.

5 These results suggest the important role played by Gi$_1$ and Gi$_3$ protein subtypes in the transduction mechanism involved in the impairment of memory processes produced by morphine. *British Journal of Pharmacology* (2001) **133,** 267–274

Keywords: Morphine; amnesia; learning and memory; pertussis toxin; Gi-proteins; antisense oligodeoxynucleotides

Abbreviations: aODN, antisense oligodeoxyribonucleotide; dODN, degenerate oligodeoxyribonucleotide; i.c.v., intracerebroventricular; i.p. intraperitoneal; PTX, pertussis toxin

Introduction

Learning and memory in animals are well known to be affected by opioids and opioid antagonists. Generally it is reported that morphine and opioid peptides tend to interfere with these processes and to produce amnesia. The post-training administration of β-endorphin or enkephalins produces memory impairment in different behavioural tasks (Izquierdo, 1980; Castellano & Pavone, 1985; Schulteis *et al*., 1988). The retrograde amnesia induced by β-endorphin can be antagonized by pretreatment with a relatively low dose of naloxone (Introini & Baratti, 1984; Del Cerro & Borrell, 1987). Furthermore, the systemic administration of morphine, when injected shortly after training, has been reported to impair memory processes in mice and rats (Castellano, 1975; Izquierdo, 1979; Messing *et al*., 1981). The morphine-induced impairment of memory functions can be antagonized by naloxone (Izquierdo, 1979). Several studies have also reported facilitation of retention by opiate antagonists, primarily naloxone, in normal animals in a variety of tasks such as passive avoidance (Messing *et al*., 1979; Rush, 1986), active avoidance, habituation (Izquierdo, 1979) and radial arm maze (Gallagher *et al*., 1983).

Opioids produce their principal effects by binding to at least three different types of receptors, the μ, δ and κ opioid receptors (Loh & Smith, 1990; Knapp *et al*., 1995). Pharmacological studies have provided arguments for the existence of several subsites for each μ (μ1, μ2 and μ3), δ (δ1 and δ 2), or κ (κ1, κ2 and κ3) receptor class (Kieffer, 1999). At present, only three homologous genes have been cloned, and the correlation between the biological activity of their encoded proteins and the pharmacological subtypes remains to be established (Law *et al*., 2000). Morphine, in contrast to many synthetic opioids, is an opioid compound with low receptor selectivity. Binding studies performed on rodent brain membranes (Matthes *et al*., 1998) or recombinant receptor preparation (Raynor *et al*., 1994) have shown that morphine exhibits a preference for μ receptors, with K_i values in the nanomolar range, but also binds the δ and κ receptors with submicromolar affinities.

The μ, δ and κ opioid receptors have all been observed to be involved in memory processes. In laboratory animals, the administration of selective μ and δ opioid receptors tends to interfere with learning and memory and to produce retrograde amnesia (Itoh *et al*., 1994; Ukai *et al*., 1997a). Although κ-opioid receptor agonists such as dynorphine A-(1–13) and U-50,488H alone fail to influence learning behaviour in normal animals, such drugs have been reported to both ameliorate pharmacologically-induced amnesia (Ukai *et al*., 1995; 1997b) and to impair memory (Tilson *et al*., 1986; Colombo *et al*., 1992). Furthermore, selective changes in μ, δ and κ opioid receptor binding have been reported in certain limbic regions of the brain in Alzheimer's disease

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patients with memory dysfunction (Hiller *et al*., 1987). These data further support the hypothesis of a role of opioid receptors in memory processes.

By considering the regulation of receptor signalling, μ, δ and κ opioid receptors could regulate the same spectrum of effectors. All subtypes of opioid receptors are prototypical Gi-coupled receptors and they have long been known to inhibit adenylyl cyclase and Ca^{2+} channels, as well as to stimulate K^+ channels and to increase intracellular Ca^{2+} levels (Law *et al*., 2000). The post-receptorial mechanism involved in morphine-induced amnesia has not yet been established. By taking into account that opioid receptors are Gi-protein coupled receptors, we investigated the involvement of Gi proteins into the mechanism of action of morphine by means of an antisense strategy. In particular, we used antisense oligonucleotides (aODN) against the α subunits of the Gi_1, Gi_2 and Gi_3 proteins in order to determine the role of each subtype in the memory impairment induced by morphine in a mouse passive avoidance paradigm.

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.

Methods

Animals

Male Swiss albino mice (23–30 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 0700 h. All experiments were carried out according to the guidelines of the European Community Council for experimental animal care.

Intracerebroventricular injection technique

Intracerebroventricular (i.c.v.) administration was performed under ether anaesthesia, according to the method described by Haley & 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.

Passive-avoidance test

The test was performed according to the step-through method described by Jarvik & Kopp (1967). The apparatus consisted of a two-compartment acrylic box with a lighted compartment connected to a darkened one by a guillotine door. As soon as the mouse entered the dark compartment, it received a punishing electrical shock (0.5 mA, 1 s). The latency times for entering the dark compartment were measured in the training test and after 24 h in the retention test. The maximum entry latency allowed in the training and retention sessions was, respectively, 60 and 180 s. In this test, morphine (6–10 mg kg^{-1}) was i.p. injected immediately after the training session, PTX (0.25 μg per mouse) was i.c.v. injected 7 days prior to the training session whereas aODNs (1.56–25 μg per mouse) were i.c.v. injected 24 and 18 h before training. Between 11 and 25 mice were tested.

Hole-board test

The hole board test consisted of a 40 cm square plane with 16 flush mounted cylindrical holes (3 cm diameter) distributed four by four 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 four 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 18–24 h after the i.c.v. injection of degenerate ODN (dODN; 25 μg per mouse) or aODN (12.5–25 μg per mouse). Twelve 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 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 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 i.p. administration of saline solution. Animals were i.c.v. pretreated 18–24 h prior to the test with degenerate ODN (dODN; 25 μg per mouse) or aODN (12.5–25 μg per mouse). Twelve mice per group were tested.

Antisense oligonucleotides

Phosphodiester oligonucleotides (ODNs) protected from terminal phosphorothioate double substitution (capped ODNs) against possible exonuclease-mediated degradation were purchased by Tib Molbiol. The sequences of the 33-mer antisense oligonucleotides used in the present study were the following:

aODN	sequence
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′

All oligos were previously characterized by *in vitro* (immunoblotting) and *in vivo* (tail flick) experiments (Raffa *et al*., 1994; Sanchez-Blazquez *et al*., 1995; Sanchez-Blazquez & 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) was used as a control. 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.

Drugs

The following drugs were used: pertussis toxin (RBI); morphine hydrochloride (S.A.L.A.R.); D-amphetamine hydrochloride (De Angeli); scopolamine hydrobromide, DOTAP (Sigma).

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 i.p. injection or 5 μl per mouse by i.c.v. injection.

Statistical analysis

All experimental results are given as the mean ± s.e.mean. 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.

Results

Effect of pertussis toxin on morphine amnesia

Morphine, injected immediately after the training session, induced amnesia in the mouse passive avoidance test. A statistically significant amnesic effect of morphine was reached at the dose of 10 mg kg^{-1} i.p. whereas the dose of 6 mg kg^{-1} i.p. was completely ineffective (Figure 1). The amnesia induced by morphine was of the same intensity as that produced by the well known amnesic drug scopolamine (1.5 mg kg^{-1} i.p.) used as the reference drug (Figure 1).

Pretreatment with pertussis tossin (PTX), injected i.c.v. at the dose of 0.25 μg per mouse 7 days before the test, completely prevented the morphine induced amnesia (Figure 2). PTX, when injected alone, was devoid of any effect on memory processes in comparison with vehicle + saline-treated mice (Figure 2).

No difference between the entrance latencies of each group in the training session of the passive avoidance test was observed (Figures 1 and 2).

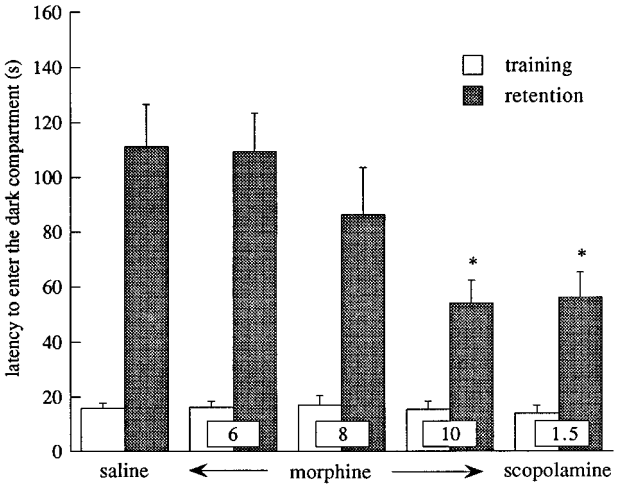

Figure 1 Dose-response curve of morphine in the mouse passive avoidance test. Morphine (6–10 mg kg^{-1} i.p.) and scopolamine (1.5 mg kg^{-1} i.p.) were administered immediately after the training session. Vertical lines represent s.e.mean; between 19 and 25 mice were tested; the dose administered is reported in each column. *$P<0.05$ in comparison with saline-treated mice.

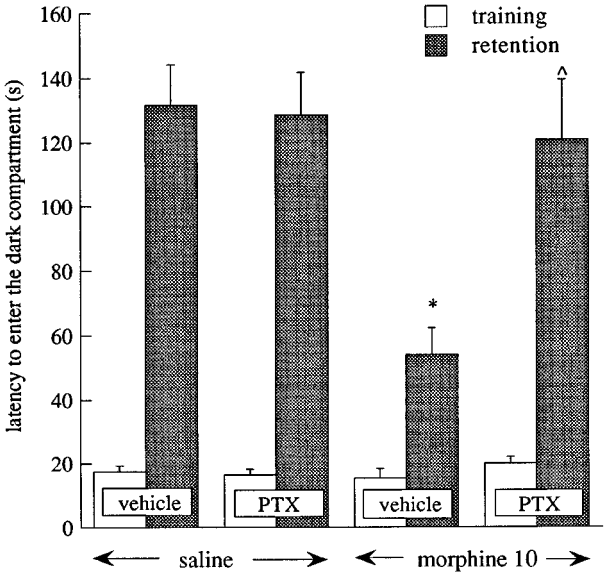

Figure 2 Prevention by pertussis toxin (PTX; 0.25 μg per mouse i.c.v.) pretreatment on morphine-induced (10 mg kg^{-1} i.p.) amnesia in the mouse passive avoidance test. The test was performed 7 days after a single i.c.v. injection of vehicle or PTX. Vertical lines represent s.e.mean; between 11 and 16 mice were tested. *$P<0.05$ in comparison with vehicle-saline treated mice; ^$P<0.05$ in comparison with vehicle-morphine treated mice.

Effect of aODN against Giα subunits on morphine amnesia

The amnesia induced by morphine (10 mg kg^{-1} i.p.) was prevented, in the mouse passive avoidance test, by pretreatment with the aODN against the α subunit of the Gi_1 proteins (Figure 3). Anti-Giα_1 (1.56–12.5 μg per mouse i.c.v.) produced a dose-dependent antagonism of the morphine-induced amnesia. The dose of 1.56 μg per mouse i.c.v. was completely ineffective; 3.12 μg per mouse i.c.v., partially

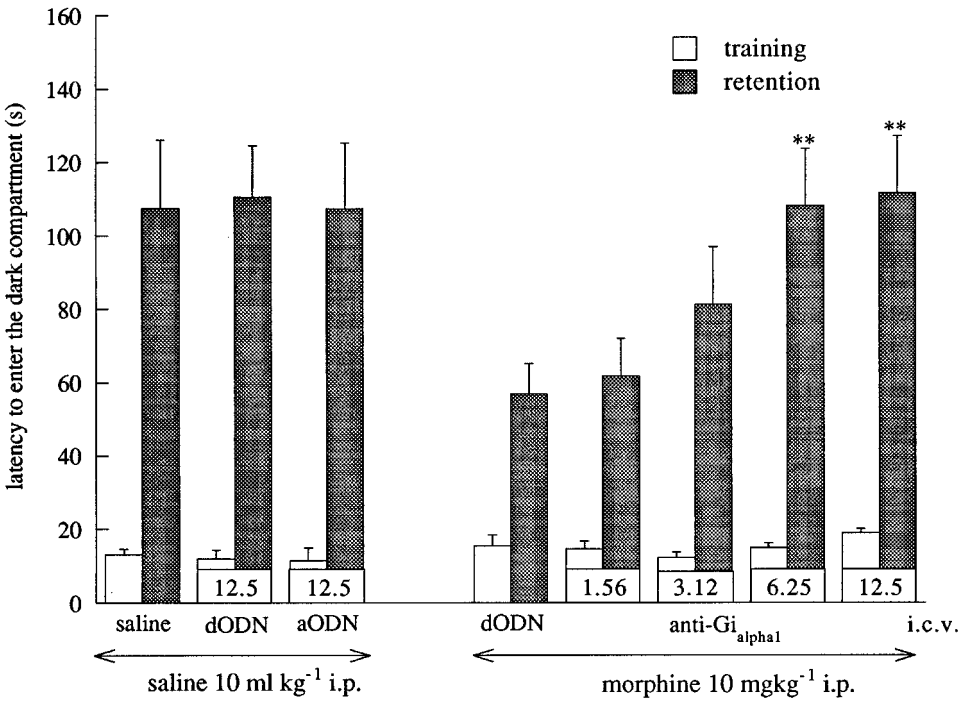

Figure 3 Prevention by pretreatment with an antisense oligonucleotide (aODN) to the α subunit of Gi_1-protein gene (1.56–12.5 μg per mouse i.c.v.) of morphine-induced (10 mg kg^{-1} i.p.) amnesia in the mouse passive avoidance test. The test was performed 18–24 h after the i.c.v. injection of degenerate ODN (dODN; 12.5 μg per mouse i.c.v.) or aODN. Vertical lines represent s.e.mean; between 12 and 16 mice were tested; the dose administered is reported in each column. $^{**}P<0.01$ in comparison with dODN + morphine group.

prevented the morphine amnesic effect, even if the statistical significance was not reached, while the doses of 6.25 and 12.5 μg per mouse i.c.v. enhanced the entrance latency in the retention session up to a value comparable to that produced by control animals (Figure 3). Anti-Giα_1 (12.5 μg per mouse i.c.v.) did not produce any effect in the mouse passive avoidance test in comparison with saline- and dODN-treated mice when given alone (Figure 3).

The administration of an aODN against the α subunit of the Gi_2 proteins (3.12–25 μg per mouse i.c.v.), in contrast to anti-Giα_1, was unable to prevent morphine-induced amnesia (Figure 4). At the highest dose employed, anti-Giα_2 did not modify the entrance latency in mice in comparison with the control groups (Figure 4).

The administration of an aODN against the α subunit of the Gi_3 proteins (12.5–25 μg per mouse i.c.v.) antagonized the memory disruption produced by morphine without showing any memory facilitating activity when given alone (Figure 5). Anti-Giα_3 was inactive in preventing morphine-induced amnesia at lower doses (3.12–6.25 μg per mouse i.c.v.) as illustrated in Figure 5.

No difference between the entrance latencies of each group in the training session of the passive avoidance test was observed (Figures 3–5).

Effect of aODN against Giα subunits on mouse rota rod and hole board tests

The administration of the aODN against the α subunits of the Gi proteins used in the present investigation elicited their effect on cognitive processes without changing either gross behaviour or motor coordination as revealed by the rota rod test (Figure 6). None of the aODNs, administered at the highest active doses, increased the number of falls from the rotating rod in comparison with dODN-treated mice (Figure 6). The number of falls in the rota rod test progressively decreased since mice learned how to balance on the rotating rod.

The spontaneous motility and exploratory activity of mice was not modified by administration of the above-mentioned aODNs as revealed by the hole-board test in comparison with saline-, vehicle- and dODN-treated mice (Figure 7). In the same experimental conditions D-amphetamine (1 mg kg^{-1} i.p.), used as the reference drug, increased both parameters evaluated.

Discussion

Present results indicate that the activation of Gi proteins is required for the induction of the amnesia produced by morphine in the mouse passive avoidance test.

In the present experimental conditions, post-training i.p. administration of morphine produced an impairment of memory functions of intensity comparable to that exerted by the amnesic drugs scopolamine. The morphine-induced amnesia was completely prevented by pretreatment with pertussis toxin (PTX), a bacterial toxin produced by *Bordetella pertussis* that ADP-ribosylates and inactivates the α subunit of Gi proteins (Katada & Ui, 1982), indicating the important role played by the Gi proteins in the signal transduction mechanism activated by morphine.

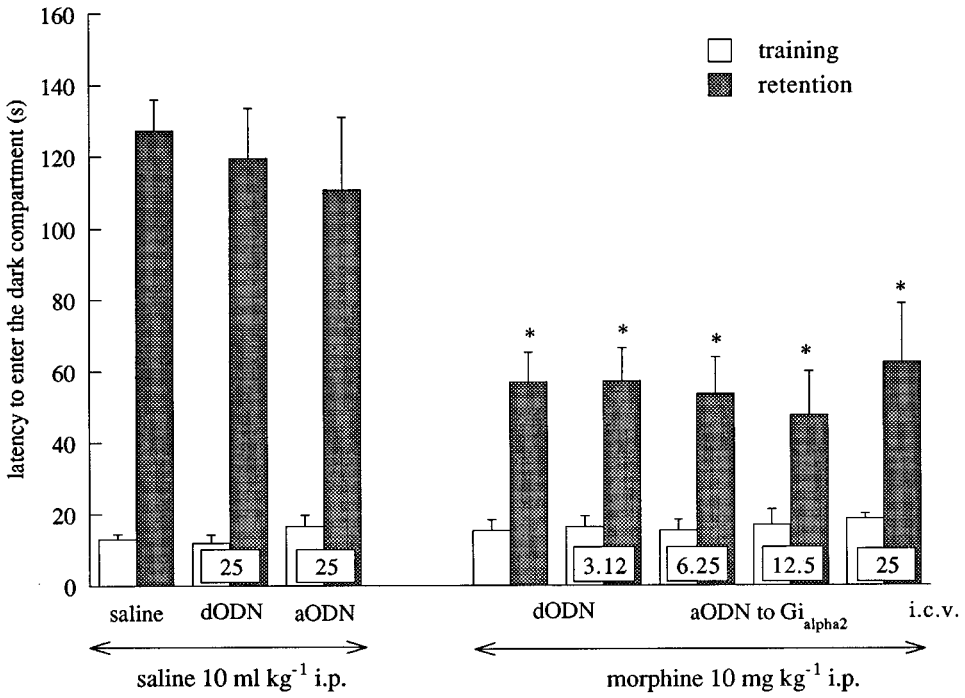

Figure 4 Lack of effect of pretreatment with an antisense oligonucleotide (aODN) to the α subunit of Gi_2-protein gene (3.12–25 μg per mouse i.c.v.) on morphine-induced (10 mg kg^{-1} i.p.) amnesia in the mouse passive avoidance test. The test was performed 18–24 h after the i.c.v. injection of degenerate ODN (dODN; 25 μg per mouse i.c.v.) or aODN. Vertical lines represent s.e.mean; between 11 and 16 mice were tested; the dose administered is reported in each column. *$P<0.05$ in comparison with dODN + saline group.

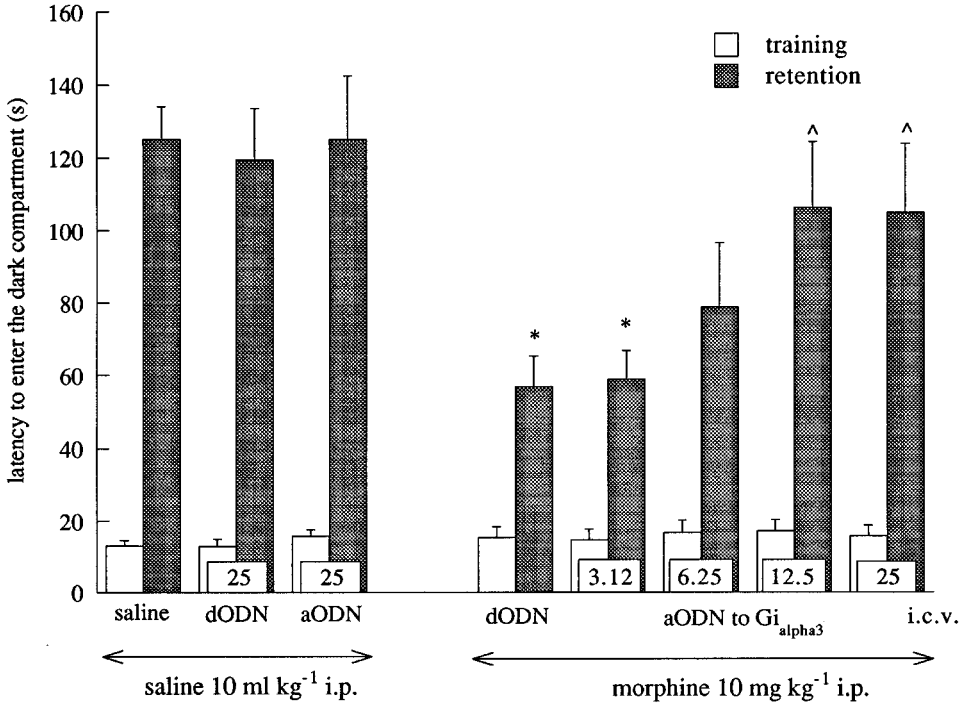

Figure 5 Prevention by pretreatment with an antisense oligonucleotide (aODN) to the α subunit of Gi_3-protein gene (3.12–25 μg per mouse i.c.v.) of morphine-induced (10 mg kg^{-1} i.p.) amnesia in the mouse passive avoidance test. The test was performed 18–24 h after the i.c.v. injection of degenerate ODN (dODN; 25 μg per mouse i.c.v.) or aODN. Vertical lines represent s.e.mean; between 13 and 18 mice were tested; the dose administered is reported in each column. *$P<0.05$ in comparison with dODN + saline group; ^$P<0.05$ in comparison with dODN + morphine-treated mice.

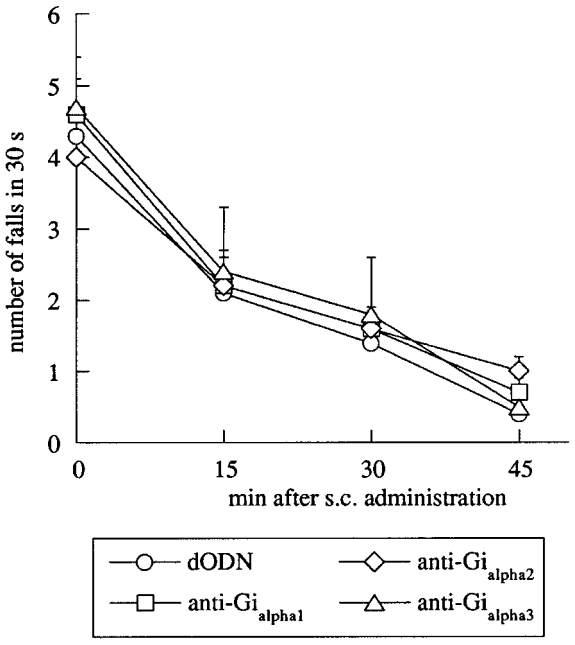

Figure 6 Lack of effect of pretreatment with an antisense oligonucleotide (aODN) to the α subunit of Gi_1- (12.5 μg per mouse i.c.v.), Gi_2- (25 μg per mouse i.c.v.) and Gi_3- (25 μg per mouse i.c.v.) protein gene on motor coordination in the mouse rota rod test. The test was performed 18–24 h after the i.c.v. injection of degenerate ODN (dODN; 25 μg per mouse) or aODN. Vertical lines represent s.e.mean; 12 mice per group were tested.

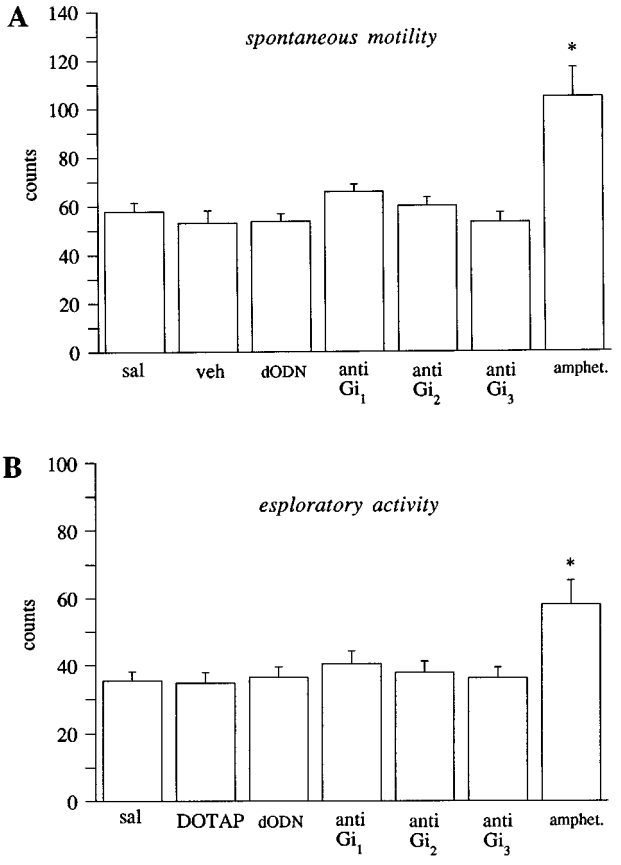

Figure 7 Lack of effect of pretreatment with an antisense oligonucleotide (aODN) to the α subunit of Gi_1- (12.5 μg per mouse i.c.v.), Gi_2- (25 μg per mouse i.c.v.) and Gi_3- (25 μg per mouse i.c.v.) protein gene on spontaneous motility (A) and inspection activity (B) in the mouse hole board test. The test was performed 18–24 h after the i.c.v. injection of degenerate ODN (dODN; 25 μg per mouse) or aODN. Vertical lines represent s.e.mean; 12 mice per group were tested. Veh: vehicle (DOTAP 13 μM); amphet: D-amphetamine 1 mg kg^{-1} i.p. $^*P<0.05$ in comparison with saline group.

The Gi protein subfamily is composed of different members, Gi_1, Gi_2 and Gi_3 (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 these Gi protein subtypes. The inhibition of the expression of $Gi\alpha_1$ and $Gi\alpha_3$ produced a dose-dependent prevention of morphine-induced amnesia whereas the administration of an aODN against $Gi\alpha_2$ never exerted any modification of morphine activity. These results indicate a differential involvement of the Gi protein subtypes in the mechanism of action of the investigated opioid agonist. In particular, the integrity and functionality of Gi_1 and Gi_3 proteins appears essential to produce memory impairment after activation of opioid receptors by morphine. By contrast, the Gi_2 subtype, in these experimental conditions, appears not to be involved.

Morphine is able to activate $Gi\alpha_1$, $Gi\alpha_2$ and $Gi\alpha_3$ to a similar extent (Burford *et al*., 1998). However, the stimulation of μ opioid receptors has been reported to produce a preferential activation of the $Gi\alpha_3$ subtypes (Chakrabarti *et al*., 1995; Connor & Christie, 1999). By taking into account that morphine has a preferential interaction with μ receptors, the above-mentioned data can, at least in part, explain the involvement of $Gi\alpha_3$ into the mechanism of amnesic action of morphine. Although μ receptors show some preference for Gi_3, the μ, δ and κ receptors appear to preferentially activate the Gi_2 over PTX-sensitive G-proteins (Connor & Christie, 1999). In the present study we observed the lack of effect of an aODN against $Gi\alpha_2$ that implies that this subunit is not a major component of transduction mechanisms leading to amnesia. The lack of involvement of $Gi\alpha_2$ has also been observed for other pharmacological activities of morphine, such as constipation or acute dependence, whereas some others, such as analgesia, are mediated only by activation of the Gi_2 protein subtype (Raffa *et al*., 1994; 1996). It is possible that multiple morphine-induced effects are mediated by different Gi-protein subunits and that morphine could have different intrinsic activity for each effect.

All three Giα subtypes were shown to inhibit the adenylyl cyclase activity with a consequent reduction of intracellular cyclic AMP levels (Wong *et al*., 1992). The role of the cyclic AMP cascade in memory processes has been recently evidenced. Genetic and pharmacological studies in mice and rats demonstrated that the cyclic AMP responsive element binding protein (CREB) is required for a variety of complex forms of memory, including spatial and social learning (Silva *et al*., 1998). The activation of the cyclic AMP-dependent protein kinase (PKA) induces long-term memory (Muller, 2000) and the inhibition of cyclic AMP phosphodiesterase reverses memory deficits in the radial arm maze task (Zhang *et al*., 2000). Several studies have also shown that the regulation of adenylyl cyclase activity is disrupted in Alzheimer's disease patients. The alteration of adenylyl cyclase activity in post-mortem brain is related to an impairment of the stimulatory G-proteins (Gs) whereas the

Gi-protein-mediated inhibition of the enzyme is unaltered (Schnecko *et al*., 1994; Fowler *et al*., 1995). Furthermore, lower Gsα levels and unmodified Giα levels were observed in fibroblasts from familial Alzheimer's disease patients (Shanahan *et al*., 1997). Recently, it has been reported that amyloid β-peptides cause toxicity through activation of Gi proteins (Soomets *et al*., 1999; Rymer & Good, 2001). We can hypothesize that an intact Gi-protein functionality is essential for the induction of amnesia. Our results on morphine amnesia confirm the hypothesis of a Gi-protein involvement in the induction of memory impairment. By considering that high cyclic AMP levels are required for mnemonic integrity, we can also suppose that morphine induces amnesia through a reduction of intracellular cyclic AMP levels *via* the activation of Gi_1 and Gi_3 proteins.

Pretreatment with PTX did not produce any ameliorative effect on memory processes in the mouse passive avoidance test, in agreement with previous results obtained in the rat (Chou & Lee, 1995). Similarly, aODN against the α subunits of Gi proteins never modified the entrance latency in comparison with control animals. We can, therefore exclude that the prevention of morphine amnesia is due to a procognitve effect exerted by these treatments. Furthermore, pretreatment with dODN, used as the reference ODN, never modified the amnesia induced by morphine in comparison with saline-treated animals, excluding the possibility of a sequence-independent effect induced by the aODNs.

These results have been obtained by using a working memory test. In future research, these data should be validated in spatial memory paradigms in order to further elucidate the intracellular processes involved in opioid-induced amnesia.

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. PTX, at the dose used, did not modify the motor coordination of the animal (Galeotti *et al*., 1996). Moreover, aODN did not impair motor coordination as revealed by the rota rod test nor modify spontaneous motility and inspection activity as indicated by the hole board test. We can, thus, suppose that the effects produced by these treatments were not due to compromised behavioural paradigms.

In conclusion, our results evidence the important role played by $Gi\alpha_1$ and $Gi\alpha_3$, but not by $Gi\alpha_2$, in morphine-induced amnesia. The elucidation of the post-receptorial mechanisms involved in memory processes can not only help unravel the complex systems mediating cognitive functions, but also the manipulation of Gi-protein activity may lead to novel treatments for pathologies characterized by memory dysfunction.

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