ELSEVIER

European Journal of Pharmacology 308 (1996) 125–133

ejp

Effect of pertussis toxin on morphine, diphenhydramine, baclofen, clomipramine and physostigmine antinociception

Nicoletta Galeotti, Carla Ghelardini, Alessandro Bartolini *

Department of Preclinical and Clinical Pharmacology, University of Florence, Viale G.B. Morgagni 65, I-50134 Florence, Italy

Received 13 November 1995; revised 22 February 1996; accepted 5 April 1996

Abstract

The effect of pretreatment with pertussis toxin at the doses of 0.25 and 0.50 μg per mouse i.c.v. on the analgesic effect produced by morphine (7 mg kg^{-1} s.c.), baclofen (4 mg kg^{-1} s.c.), diphenhydramine (20 mg kg^{-1} s.c.), clomipramine (25 mg kg^{-1} s.c.) and physostigmine (0.1–0.2 mg kg^{-1} s.c.) was investigated in the mouse hot-plate test. Seven days after a single injection of pertussis toxin, inhibition of morphine and diphenhydramine analgesia was observed, whereas 11 days after pertussis toxin pretreatment, baclofen- and clomipramine-induced antinociception was also reduced. By contrast, pertussis toxin had no effect on physostigmine-induced antinociception. The present results indicate that the activation of pertussis toxin-sensitive G-proteins represents an important transduction step in the central analgesia induced by opioids, antihistaminics, $GABA_B$ (γ-aminobutyric acid B) agonists and tricyclic antidepressants, but not by cholinomimetics.

Keywords: Pertussis toxin; Analgesia; Morphine; Diphenhydramine; Baclofen; Clomipramine; Physostigmine

1. Introduction

Numerous neurotransmitters, such as opioids, acetylcholine, GABA (γ-aminobutyric acid), cathecolamines, serotonin, purines, histamine, somatostatin, neuropeptide Y, calcitonin, are involved in pain inhibition. All these molecules have been demonstrated to interact with metabotropic receptors coupled to G-proteins (Graziano and Gilman, 1987; Birnbaumer, 1990), leading to the modulation of intracellular effectors including adenylate cyclase, phospholipase C and ion channels (Bourne et al., 1990, 1991; Simon et al., 1991). 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 (G_s), pertussis toxin (G_i and G_o) or neither (G_q and G_{12}) (Simon et al., 1991; Hepler and Gilman, 1992). Pertussis toxin-sensitive G-proteins represent the most widespread modulatory signaling pathway in neurones (Holz et al., 1986) and are responsible for inhibition of adenylate cyclase activity and modulation of several K^+ and Ca^{2+} channels (Brown and Birnbaumer, 1990; Hepler and Gilman, 1992; Hille, 1994).

Activation of opioid and α_2-adrenoceptors causes inhibition of adenylate cyclase activity and regulates ionic conductance, as documented by biochemical and electrophysiological studies (Sharma et al., 1975; Sabol and Nirenberg, 1979; Brown and Birnbaumer, 1990), via pertussis toxin-sensitive G-proteins (Childers, 1991; Aghajanian and Wang, 1986, Dunwiddie and Su, 1988). Similarly, the stimulation of $GABA_B$ and adenosine A_1 receptors provokes inhibition of adenylate cyclase in rat brain slices (Hill, 1985) and in cultured brain cells (Van Calker et al., 1979), the opening of several K^+ channels in central neurones (Gähwiler and Brown, 1985; Trussel and Jackson, 1987) and a reduction of Ca^{2+} currents in dorsal root ganglion cells (Dolphin and Scott, 1987; McDonald et al., 1986) by a pertussis toxin-inhibitable mechanism.

With regard to in vivo studies, it has been reported that injection of pertussis toxin in mice and rats blocks both spinal and supraspinal morphine-, noradrenaline- and clonidine-induced antinociception (Parenti et al., 1986; Hoehn et al., 1988; Sanchez-Blazquez and Garzon, 1991) regardless of the route of administration or the analgesic test used (Parolaro et al., 1990). Furthermore, intrathecally administered pertussis toxin has been shown to be able to prevent antinociception induced by intrathecal injection of baclofen (Hoehn et al., 1988) and purines (Sawynok and Reid, 1988) in rats.

* Corresponding author. Tel.: +39 55 4237432; fax: +39 55 4361613.

PII S0014-2999(96)00299-3

Since it has been demonstrated that opioid, α-adrenergic, GABAergic and purinergic analgesic effects are mediated by pertussis toxin-sensitive G-proteins, we thought it worthwhile to extend the investigation to the biochemical mechanisms of other pain inhibitory systems to provide some indication of whether a $G_{i/o}$ protein is involved in central antinociception. To this end, drugs modulating cholinergic, serotoninergic and histaminergic systems were investigated since they not only represent central analgesic compounds effective in both animals (Harris et al., 1969; Sacerdote et al., 1978; Rumore and Schlichting, 1985) and humans (Hood et al., 1995; Langohr et al., 1982; Campos and Solis, 1980), but also they interact with G-protein-coupled receptors (Birnbaumer, 1990). The effect of intracerebroventricular injection of pertussis toxin on the antinociception induced by administration of the μ-δ opioid receptor agonist morphine, the $GABA_B$ agonist baclofen, the tricyclic antidepressant clomipramine, the cholinesterase inhibitor physostigmine and the histamine H_1 receptor antagonist diphenhydramine was, therefore, evaluated in mice.

2. Materials and methods

2.1. Animals

Male Swiss albino mice (23–30 g) were used. Fifteen mice were housed per cage. The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were fed on a standard laboratory diet and tap water ad libitum. Mice were randomly assigned to a vehicle (water solution containing 0.01 M sodium phosphate buffer, pH 7.0, with 0.05 sodium chloride) or a pertussis toxin group (0.25 and 0.50 μg per mouse). Naive animals did not receive any pretreatment whereas vehicle and pertussis toxin groups received a single intracerebroventricular injection on day 0.

2.2. Hot-plate test

Mice were placed inside a stainless steel container, thermostatically set at 52.5 ± 0.1°C in a precision waterbath from CW Mechanical Workshop, Siena, Italy. Reaction times (s) were measured with a stopwatch and each animal was tested before and 15, 30, 45 and 60 min after treatment. The endpoint used was the licking of the fore or hind paws. Those mice scoring below 12 and over 18 s in the pretest were rejected. An arbitrary cut-off time of 45 s was adopted. Following a single pretreatment with vehicle or pertussis toxin, the antinociceptive effect of morphine and diphenhydramine was tested 2 and 7 days later, the antinociceptive effect of baclofen and clomipramine 2, 7 and 11 days later and the antinociceptive effect of physostigmine 2, 7, 11 and 14 days later.

2.3. Rota-rod test

The apparatus consisted of a base platform and a rotating rod of 3 cm diameter with 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, at 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). The performance time was measured before and 15, 30 and 45 min after subcutaneous administration of saline. The test was performed 8 and 12 days after pretreatment with vehicle or pertussis toxin. Naive animals were used as unpretreated controls.

2.4. Drugs

The following drugs were used: (±) baclofen (β-*p*-chlorophenyl GABA), physostigmine hemisulphate (Sigma), morphine hydrochloride (U.S.L. 10/D, Florence), clomipramine hydrochloride (anafranil, Ciba-Geigy), diphenhydramine hydrochloride (De Angeli) and pertussis toxin (RBI).

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 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 subcutaneous (s.c.) injection.

2.5. Intracerebroventricular injections

Intracerebroventricular (i.c.v.) administration was performed under ether anaesthesia using isotonic saline as a solvent, 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 were injected with 5 μl of Indian ink diluted 1 : 10 and their brains were examined macroscopically after sectioning.

2.6. Statistical analysis

Results are given as the means ± S.E.M.; an analysis of variance (ANOVA) was used to verify significance be-

tween two means. P values of less than 0.05 were considered significant. Data were analysed with computer program (StatView for the Macintosh, 1992).

3. Results

3.1. Effect of pertussis toxin on mouse behaviour

Table 1 shows the effects on mouse body weight produced by the i.c.v. injection of pertussis toxin at the doses of 0.25 and 0.50 μg per mouse in comparison with vehicle-treated and naive mice used as control animals. Both of the doses of pertussis toxin tested produced a loss of weight 2 days after injection after which body weight slowly increased. In the pertussis toxin 0.50-treated group, a consistent number of deaths occurred (Table 1). When the dose of pertussis toxin was diminished to 0.25 μg per mouse, the number of deaths was greatly reduced (Table 1) and the gross behaviour of pertussis toxin 0.25-treated mice was comparable to that of the vehicle-treated group. The body weight of naive and vehicle-treated mice progressively increased and no deaths occurred (Table 1).

The motor coordination of mice pretreated with vehicle or pertussis toxin (0.25 and 0.50 μg per mouse i.c.v.), and of naive mice was evaluated by using the rota-rod test (Fig. 1a,b). Rota-rod performance, tested as number of falls in 30 s, of animals pretreated 8 (Fig. 1a) and 12 days (Fig. 1b) before the test with the doses of pertussis toxin investigated was not significantly impaired in comparison with that of naive and vehicle-treated mice as revealed by pretest values (Fig. 1a,b). Furthermore, the successive s.c. injections did not elicit any behavioural side effects since each group progressively reduced the number of falls (Fig. 1a,b). No difference was observed between vehicle-treated and naive mice (Fig. 1a,b).

3.2. Effect of pertussis toxin on the mouse pain threshold

Treatment of animals with pertussis toxin (0.25 and 0.50 μg per mouse i.c.v.) did not cause any modification of the pain threshold in comparison with controls 2 days after injection as revealed by the hot-plate test (Fig. 2). Mice receiving pertussis toxin at the dose of 0.50 μg per mouse, but not at the dose of 0.25 μg per mouse, showed, 7 days after injection, a statistically significant reduction of licking latency (14.0 ± 0.5; $n = 75$) in comparison with the vehicle-treated group (15.3 ± 0.3; $n = 130$). A decrease of the pain threshold was observed at both of the doses used 11 days after administration, showing a weak pertussis toxin-induced hyperalgesic effect (Fig. 2).

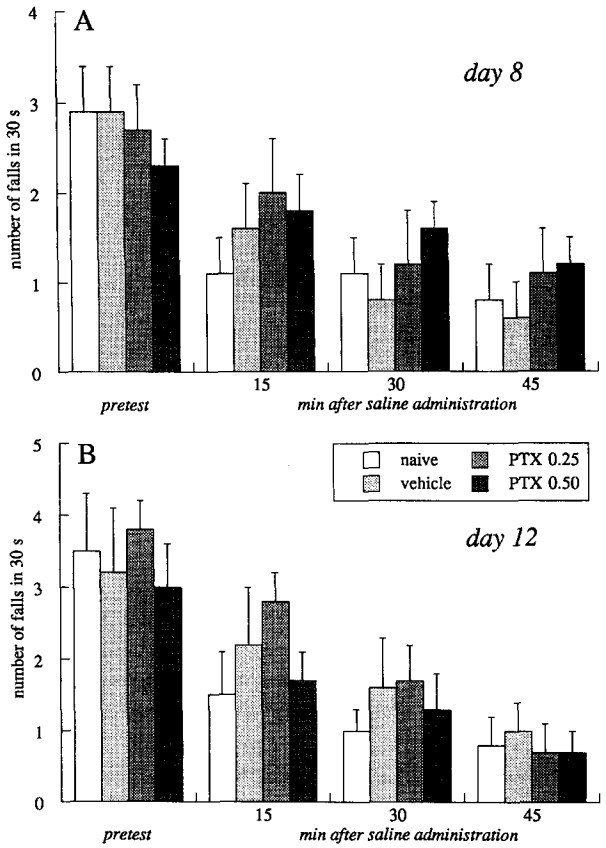

Fig. 1. Effect of pertussis toxin (PTX) in the mouse rota-rod test. Vehicle and pertussis toxin (0.25 and 0.50 μg per mouse) were injected i.c.v. 8 days (panel A) or 12 days (panel B) before the test. Vertical lines give S.E.M.; the number of mice was 14 per group.

Table 1
Effects of pertussis toxin (PTX) after i.c.v. injection in mice on the body weight

Treatment i.c.v.	n	Body weight (g)				Deaths (%)		
		Before treatment	After treatment			After treatment		
			2 days	7 days	11 days	2 days	7 days	11 days
Naive	34	25.8 ± 0.6	26.8 ± 0.5	28.8 ± 0.9	29.6 ± 0.9	0	0	0
Vehicle 5 μl	132	26.0 ± 0.2	26.7 ± 0.3	28.8 ± 0.4	30.5 ± 0.5	0	0.7	0.7
PTX 0.25 μg	77	28.0 ± 0.4	25.6 ± 0.5 [b]	26.9 ± 0.7 [a]	29.1 ± 0.8	0	6.5	23.4
PTX 0.50 μg	142	25.3 ± 0.2	21.5 ± 0.3 [b]	23.6 ± 0.5 [b]	24.3 ± 0.5 [b]	37.3	47.2	60.5

Naive animals did not receive any treatment. The before treatment body weight was measured immediately before the i.c.v. injection. [a] $P < 0.05$, [b] $P < 0.001$ in comparison with the vehicle-treated mice.

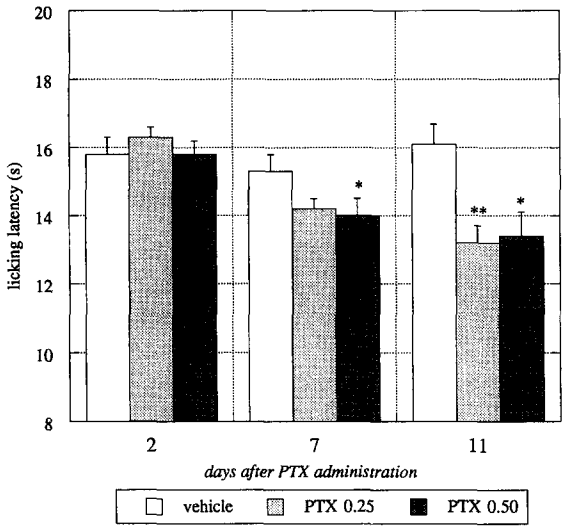

Fig. 2. Effect of pertussis toxin (PTX) on pain threshold in the mouse hot-plate test 2, 7 and 11 days after a single pertussis toxin injection. Vehicle and pertussis toxin (0.25–0.50 μg per mouse) were administered i.c.v.; vertical lines give S.E.M.; the number of mice was between 49 and 130. * $P < 0.05$, ** $P < 0.01$ in comparison with vehicle-treated mice.

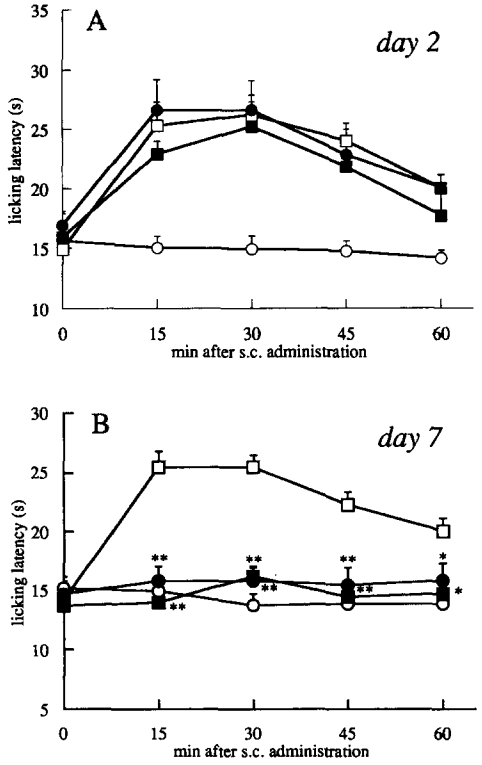

Fig. 3. Effect of pertussis toxin (PTX) pretreatment on morphine (7 mg kg^{-1} s.c.)-induced antinociception in the mouse hot-plate test. The test was performed 2 (panel A) and 7 (panel B) days after a single i.c.v. injection of vehicle or pertussis toxin (0.25 and 0.50 μg per mouse). Vertical lines represent S.E.M.; the number of mice was between 11 and 22. * $P < 0.001$, ** $P < 0.001$ in comparison with vehicle + morphine-treated mice.

3.3. Effect of pertussis toxin on morphine-, diphenhydramine-, baclofen-, clomipramine- and physostigmine-induced antinociception

The effect of pretreatment of mice with pertussis toxin (0.25 and 0.50 μg per mouse i.c.v.) on morphine (Fig. 3a,b)-, diphenhydramine (Fig. 4a,b)-, baclofen (Fig. 5a,b)-, clomipramine (Fig. 6a,b)- and physostigmine (Fig. 7a,b)-induced antinociception was evaluated in the mouse hot-plate test.

Pretreatment with pertussis toxin (0.25 and 0.50 μg per mouse i.c.v.) 2 days before the test did not produce any significant effect on morphine (7 mg kg^{-1} s.c.)-induced analgesia (Fig. 3a) whereas, 7 days after pertussis toxin administration, a complete prevention of morphine-induced antinociception in both pertussis toxin-treated groups was observed (Fig. 3b).

Pertussis toxin (0.25 and 0.50 μg per mouse i.c.v.), when injected 2 days prior to the test, was ineffective in preventing diphenhydramine (20 mg kg^{-1} s.c.)-induced antinociception (Fig. 4a). By contrast, when the toxin was

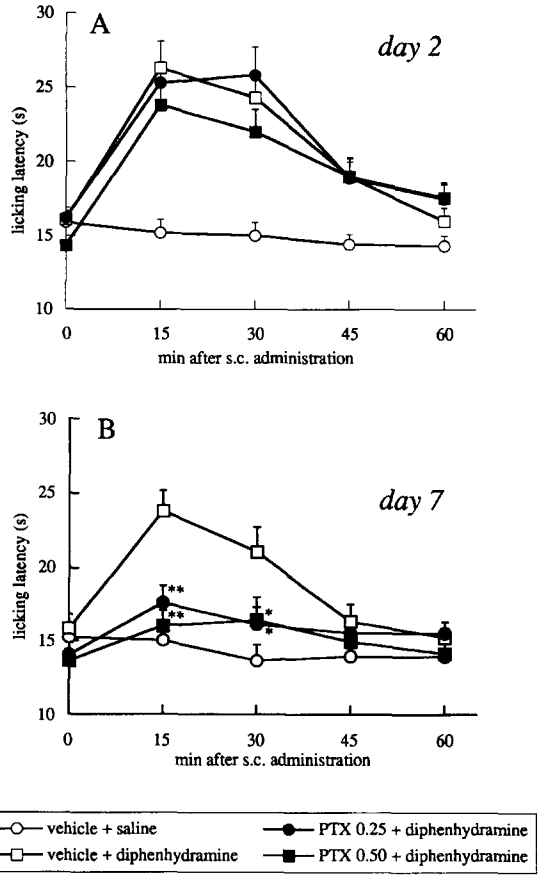

Fig. 4. Effect of pertussis toxin (PTX) pretreatment on diphenhydramine (20 mg kg^{-1} s.c.)-induced antinociception in the mouse hot-plate test. The test was performed 2 (panel A) and 7 (panel B) days after a single i.c.v. injection of vehicle or pertussis toxin (0.25 and 0.50 μg per mouse). Vertical lines represent S.E.M.; the number of mice was between 11 and 21. * $P < 0.01$, ** $P < 0.001$ on comparison with vehicle + dipenhydramine-treated mice.

injected 7 days before the test, it greatly reduced the antinociceptive effect of diphenhydramine (Fig. 4b). The pertussis toxin-induced prevention of the diphenhydramine-induced analgesia was not dose-dependent, being complete at both of the doses of pertussis toxin used (Fig. 4a,b).

Mice pretreated with pertussis toxin (0.25 and 0.50 μg per mouse i.c.v.) 2 and 7 days before the hot-plate test did not show any modified response to baclofen (4 mg kg^{-1} s.c.) as compared with the vehicle-treated group (Fig. 5a,b). Conversely, pertussis toxin pretreatment 11 days prior to the test was able to reduce baclofen-induced antinociception in a dose-dependent manner (Fig. 5c).

The effect of i.c.v. injection of pertussis toxin (0.25 and 0.50 μg per mouse) 2, 7 and 11 days before evaluation of the antinociception induced by clomipramine (25 mg kg^{-1} s.c.) is reported in Fig. 6. Pertussis toxin pretreatment led to a loss of the antinociceptive effect of clomipramine. This reduction was obtained 11 days after pertussis toxin pretreatment and it was dose-dependent, 0.50 μg being more effective than 0.25 μg (Fig. 6c). No prevention of clomipramine-evoked antinociception was obtained on days 2 and 7 (Fig. 6a,b).

The effect of pertussis toxin (0.25 and 0.50 μg per mouse) pretreatment on physostigmine-induced (0.1–0.2 mg kg^{-1} s.c.) antinociception was evaluated 2, 7, 11 and 14 days after pertussis toxin injection. Pertussis toxin, at both of the doses used, was never able to modify the antinociceptive effect of physostigmine (Fig. 7a,b). The licking latency values were recorded in correspondence

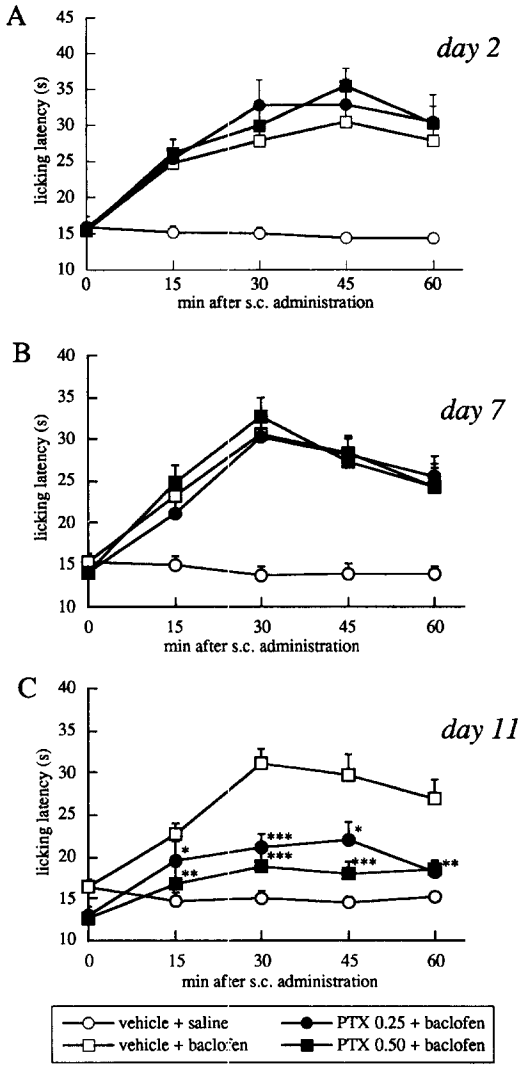

Fig. 5. Effect of pertussis toxin (PTX) pretreatment on baclofen (4 mg kg^{-1} s.c.)-induced antinociception in the mouse hot-plate test. The test was performed 2 (panel A), 7 (panel B) and 11 (panel C) days after a single i.c.v. injection of vehicle or pertussis toxin (0.25 and 0.50 μg per mouse). Vertical lines represent S.E.M.; the number of mice was between 12 and 22. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ in comparison with vehicle + baclofen-treated mice.

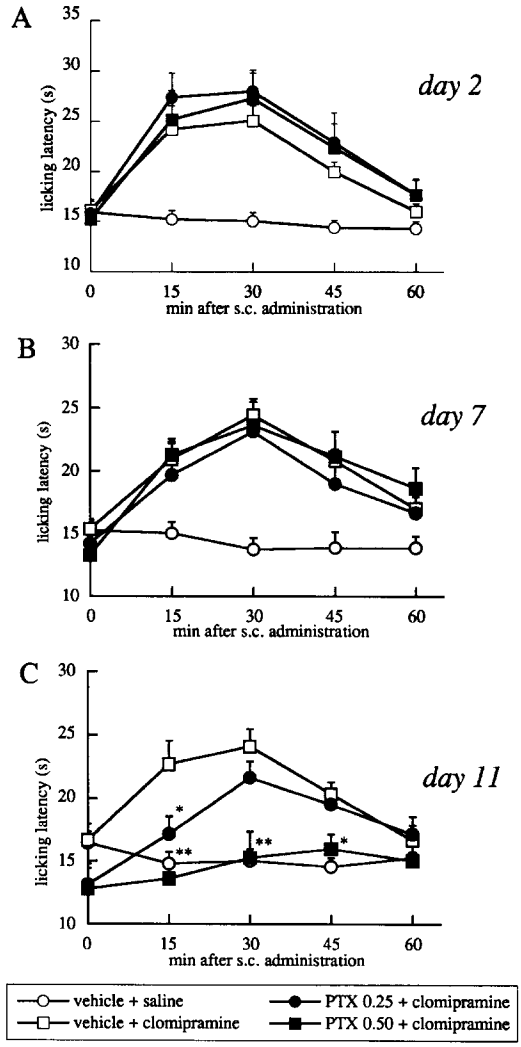

Fig. 6. Effect of pertussis toxin (PTX) pretreatment on clomipramine (25 mg kg^{-1} s.c.)-induced antinociception in the mouse hot-plate test. The test was performed 2 (panel A), 7 (panel B) and 11 (panel C) days after a single i.c.v. injection of vehicle or pertussis toxin (0.25 and 0.50 μg per mouse). Vertical lines represent S.E.M.; the number of mice between 12 and 22. * $P < 0.01$, ** $P < 0.001$ in comparison with vehicle + clomipramine-treated mice.

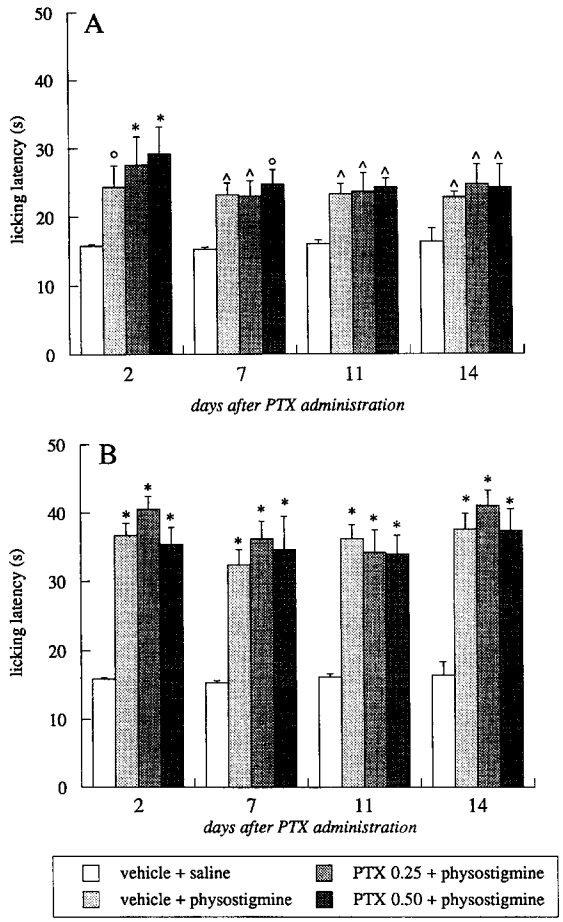

Fig. 7. Lack of effect of pertussis toxin (PTX) pretreatment on antinociception induced by physostigmine at the doses of 0.1 mg kg^{-1} s.c. (panel A) and 0.2 mg kg^{-1} s.c. (panel B) in the mouse hot-plate test. The test was performed 2, 7, 11 and 14 days after a single i.c.v. injection of vehicle or pertussis toxin (0.25 and 0.50 μg per mouse). The licking latency was measured 30 min after physostigmine administration. Vertical lines represent S.E.M.; the number of mice was between 6 and 22. $^{\wedge}P < 0.05$, $^{\circ}P < 0.01$, $^{*}P < 0.001$ in comparison with vehicle + saline-treated mice.

with the maximum effect of physostigmine that occurred 30 min after administration.

The i.c.v. injection of vehicle did not produce any modification of the sensitivity of animals to the analgesic treatments in comparison with naive mice (data not shown).

4. Discussion

The present study provides evidence for the involvement of pertussis toxin-sensitive G-proteins in central antinociception. Pretreatment with i.c.v. administration of pertussis toxin produced an inhibition of morphine-, diphenhydramine-, baclofen- and clomipramine-induced analgesia, providing evidence for the important role of pertussis toxin-sensitive G-proteins as a transduction step in central antinociception.

Pertussis toxin penetrates brain tissue slowly and incompletely. When the toxin is administered into the cerebral ventricles, its distribution is limited to a narrow zone close to the ventricles (Van der Ploeg et al., 1991). This restricted distribution of pertussis toxin suggests, therefore, that the effects observed in behavioural studies after i.c.v. injection are attributable only to a central action.

Pertussis toxin inactivates several G-proteins ($G_{i/o}$ proteins) by ADP-ribosilation of a specific C-terminal cystein (Katada and Ui, 1982; Katada et al., 1984). The degree of ADP-ribosilation of $G_{i/o}$ proteins was not evaluated by biochemical techniques. However, in confirmation of previous studies in mice and rats (Parenti et al., 1986; Parolaro et al., 1990; Chang et al., 1991; Chung et al., 1994), the morphine-induced analgesia was prevented by pertussis toxin pretreatment 7 days before the hot-plate test, making us certain that a sufficient level of inactivation of $G_{i/o}$ proteins was reached. Furthermore, the i.c.v. injection of antisense oligodeoxynucleotides against G_i and G_s proteins confirm the involvement of G_i proteins in morphine-induced antinociception (Raffa et al., 1994) as previously postulated on the basis of the effects of PTX pretreatment. It is, therefore, supposed that PTX is a reliable tool to investigate the molecular mechanism of analgesic drugs.

The antinociception induced by the histamine H_1 receptor antagonist diphenhydramine was prevented by pretreating mice with pertussis toxin 7 days before the test. The administration of histamine H_1 receptor antagonists produces various inhibitory effects including analgesia (Rumore and Schlichting, 1985; Oluyomi and Hart, 1991), but their mechanism of action has not been elucidated. Histamine exerts its numerous actions through interaction with three pharmacologically distinct receptor subtypes, H_1, H_2 and H_3, which all belong to the superfamily of G-protein-coupled receptors (Arrang, 1994). Since it has been reported that histamine can modulate the release of several neurotransmitters (Hill, 1990), an involvement of neurotransmitters different from histamine, activating $G_{i/o}$ proteins as a signal transduction mechanism, downstream from the initial receptor interaction cannot be excluded. Pertussis toxin, therefore, in preventing diphenhydramine-induced antinociception, could be working at histaminergic or non-histaminergic receptor-linked $G_{i/o}$ proteins

Injection of mice with pertussis toxin inhibited the antinociception induced by the $GABA_B$ receptor agonist baclofen. $GABA_B$ receptors are located both spinally and supraspinally and they are coupled to pertussis toxin-sensitive G-proteins which inhibit adenylate cyclase activity (Wojcik and Neff, 1984; Hill, 1985) and modulate several ion channels (Houston et al., 1990; Gage, 1992). It was not unexpected, therefore, that pertussis toxin prevented baclofen-induced analgesia. Since it has been observed that baclofen-induced spinal antinociception is abolished by intrathecal pretreatment with pertussis toxin (Hoehn et al., 1988), suggesting the involvement of a $G_{i/o}$ protein in the baclofen spinal effect, it is now possible to extend to the

baclofen supraspinal antinociceptive effect the presence of pertussis toxin-sensitive signal transduction mechanisms.

Tricyclic antidepressants have long been known to produce antinociception. The present results show that clomipramine-induced antinociception utilized a pertussis toxin-sensitive transduction mechanism. Clomipramine, as well as other tricyclic antidepressants, is an inhibitor of serotonin reuptake. The increase of endogenous serotonin in the synaptic cleft produces directly (Galeotti et al., 1995), or through the activation of endogenous opioid systems (Sacerdote et al., 1978), an increase in the pain threshold. Some serotonin (Lucas and Hen, 1995) and opioid (Loh and Smith, 1990) receptor subtypes have been reported to be coupled to pertussis toxin-sensitive G-proteins in the central nervous system. For this reason it is suggested that clomipramine, by activating the serotoninergic system, induces an analgesic effect underlying the activation of a signal transduction mechanism operated by $G_{i/o}$ proteins. Even though we cannot exclude the involvement of the opioid system in clomipramine-induced antinociception, it seems to be unlikely since clomipramine was sensitive to pertussis toxin pretreatment only on day 11 while morphine was sensitive on day 7.

The analgesic effect of diphenhydramine, as well as that of morphine, was inhibited by pretreating animals with pertussis toxin 7 days before the test, whereas baclofen- and clomipramine-induced antinociception was prevented by pertussis toxin 11 days after the injection of the toxin. To ensure a good ADP-ribosilation of the pertussis toxin-sensitive G-proteins, a latency of at least 48–72 h is necessary (Van der Ploeg et al., 1991). The 11-day latency necessary to block baclofen- and clomipramine-induced analgesia, compared with the 7-day latency required for inhibiting morphine- and diphenhydramine-induced antinociception, might be due to the difficulty of pertussis toxin of reaching the zone where $GABA_B$ and serotonin receptors are located in the highest concentration.

Physostigmine is a cholinesterase inhibitor that enhances the level of synaptic acetylcholine by inhibiting its degradation. Some muscarinic receptor subtypes have been reported to be coupled to pertussis toxin-sensitive G-proteins (the M_2 and M_4 subtypes) modulating adenylate cyclase and several ion channels, whereas others, such as M_1, M_3 and M_5, are linked to G-proteins that activate phospholipase C (Caulfield, 1993). The agonist regulation of phospholipase C appears to involve at least two separate mechanisms: the activation of a pertussis toxin-sensitive G-protein or, more commonly, the activation of pertussis toxin-insensitive G-proteins (Park et al., 1993). Even though the most widespread muscarinic receptor subtypes in the central nervous system are M_1, M_2 and M_4, pertussis toxin pretreatment did not affect physostigmine antinociception. The lack of effect of pertussis toxin could be due to the fact that the receptor subtype mainly involved in physostigmine-induced antinociception is the M_1 subtype. In support of this hypothesis, it has been reported that muscarinic M_1 receptor agonists produce antinociception in mice and rats that is prevented by muscarinic M_1 receptor selective antagonists (Bartolini et al., 1992). Furthermore, it has been observed that the human M_1 receptor, expressed in Chinese hamster oocytes (CHO), selectively activates G_q and G_{11} proteins (Mullaney et al., 1993). It seems, therefore, reasonable to suppose that physostigmine, by enhancing acetylcholine levels, induces antinociception through a molecular mechanism that does not involve $G_{i/o}$ proteins.

The doses of the investigated analgesic compounds used were chosen on the basis of the rota-rod test (data not shown). Higher doses were not used since they impaired the rota-rod performance and could lead to a misinterpretation of the results obtained. As pertussis toxin prevented the antinociception induced by all the compounds used, with the exception of physostigmine, lower doses were not investigated. The effect of physostigmine, at both of the doses used (0.1–0.2 mg kg^{-1} s.c.), was never prevented by pertussis toxin pretreatment. Doses of physostigmine lower than 0.1 mg kg^{-1} s.c. could not be investigated since they were not endowed with analgesic properties.

Pertussis toxin prevents the activation, by receptor stimulation, of $G_{i/o}$ proteins which are involved in the actions of several inhibitory neurotransmitters. For this reason i.c.v. injection of the toxin has been reported to produce widespread side effects which can make interpretation of results from behavioural studies difficult (Bodnar et al., 1990). It has been previously reported that doses of pertussis toxin of 0.25 and 0.50 μg per mouse i.c.v. (Damaj et al., 1994; Suzuki et al., 1991) do not produce any significant reduction in mouse body weight. By contrast, in our conditions mice treated with a dose of pertussis toxin of 0.50 and 0.25 μg per mouse i.c.v. weighed approximately 20% and 10%, respectively, less than controls. Even though a loss of body weight occurred, the motor coordination of mice pretreated with both of the doses of pertussis toxin used was not impaired, making the results obtained in the hot-plate test reliable.

Pertussis toxin, starting from 7 days after administration, produced a statistically significant reduction of the pain threshold, showing a weak hyperalgesic effect as previously reported (Ohnishi et al., 1990). However, the prevention of morphine, diphenhydramine-, baclofen- and clomipramine-induced antinociception cannot be attributable to pertussis toxin-induced hyperalgesia. The reduction of the pain threshold produced by pertussis toxin was of the same intensity at both of the doses used, whereas the toxin inhibited the effects of the above-mentioned analgesic drugs dose dependently. Furthermore, the effect of pertussis toxin on baclofen- and clomipramine-induced antinociception was not detectable 7 days after the administration of the toxin while the pertussis toxin-induced reduction of the pain threshold was already present. Finally, pertussis toxin, even though it is endowed with hyperalgesic properties, was never able to modify

physostigmine-induced antinociception, supporting the hypothesis that the reduction of the pain threshold produced by the toxin is not responsible for the neutralization of the antinociception induced by the investigated compounds.

In conclusion, the present data demonstrated that pertussis toxin-sensitive G-proteins represent an essential step in the transduction mechanism underlying central antinociception. An increase in pain threshold can be induced by activating different neurotransmitter systems, such as opioid, GABAergic, serotoninergic and histaminergic, that, however, produce the activation of $G_{i/o}$ proteins as a common molecular event.

Acknowledgements

This study was supported by grants from MURST and CNR.

References

Aghajanian, G.K. and Y.-Y. Wang, 1986, Pertussis toxin blocks the outward currents evoked by opiate and a_2-agonists in locus coeruleus neurons, Brain Res. 371, 390.

Arrang, J.M., 1994, Pharmacological properties of histamine receptor subtypes, Cell. Mol. Biol. 40, 275.

Bartolini, A., C. Ghelardini, L. Fantetti, M. Malcangio, P. Malmberg-Aiello and A. Giotti, 1992, Role of muscarinic receptor subtypes in central antinociception, Br. J. Pharmacol. 105, 77.

Birnbaumer, L., 1990, G proteins in signal transduction, Annu. Rev. Pharmacol. Toxicol. 30, 675.

Bodnar, R.J., D. Paul, M. Rosenblum, L. Liu and G.W. Pasternak, 1990, Blockade of morphine analgesia by both pertussis toxin and cholera toxin in the periaqueductal gray and locus coeruleus, Brain Res. 529, 324.

Bourne, H.R., D.A. Sanders and F. McCormick, 1990, The GTPase superfamily: a conserved switch for diverse cell function, Nature 348, 125.

Bourne, H.R., D.A. Sanders and F. McCormick, 1991, The GTPase superfamily: conserved structure and molecular mechanism, Nature 349, 117.

Brown, A.M. and L. Birnbaumer, 1990, Ionic channels and their regulation by G protein subunits, Annu. Rev. Physiol. 52, 197.

Campos, V.M. and E.L. Solis, 1980, The analgesic and hypothermic effects of nefopam, morphine, aspirin, diphenhydramine and placebo, J. Clin. Pharmacol. 20, 42.

Caulfield, M.P., 1993, Muscarinic receptors-Characterization, coupling and function, Pharmacol. Ther. 58, 319.

Chang, S.C., K. Lufty, V. Sierra and B.C. Yoburn, 1991, Dissociation of opioid receptor upregulation and functional supersensitivity, Pharmacol. Biochem. Behav. 38, 853.

Childers, S.R., 1991, Opioid receptor-coupled second messenger systems, Life Sci. 48, 1991.

Chung, K.M., D.K. Song, H.W. Suh, M.H. Lee and Y.H. Kim, 1994, Effect of intrathecal or intracerebroventricular pretreatment with pertussis toxin on antinociception induced by β-endorphin or morphine administered intracerebroventricularly in mice, Naunyn-Schmied. Arch. Pharmacol. 349, 588.

Damaj, M.I., S.P. Welch and B.R. Martin, 1994, Nicotine-induced antinociception in mice: role of G-proteins and adenylate cyclase, Pharmacol. Biochem. Behav. 48, 37.

Dolphin, A.C. and R.H. Scott, 1987, Calcium channel currents and their inhibition by (−)-baclofen in rat sensory neurones: modulation by guanine nucleotides, J. Physiol. 386, 1.

Dunwiddie, T.V. and M.T. Su, 1988, Pertussis toxin pretreatment antagonizes the actions of mu- and delta-opiate agonists in hippocampal slices, Neurosci. Lett. 95, 329.

Gage, P.W., 1992, Activation and modulation of neuronal K^+ channels by GABA, Trends Neurosci. 15, 46.

Gähwiler, B.H. and D.A. Brown, 1985, $GABA_B$-receptor-activated K^+ current in voltage-clamped CA3 pyramidal cells in hippocampal cultures, Proc. Natl. Acad. Sci. USA 82, 1558.

Galeotti, N., C. Ghelardini and A. Bartolini, 1995, Involvement of the serotoninergic system in the analgesic effect of tricyclic antidepressants, Behav. Pharmacol. 6 (Suppl. 1), 20.

Graziano, M.P. and A.G. Gilman, 1987, Guanine nucleotide-binding regulatory proteins: mediators of transmembrane signaling, Trends Pharmacol. Sci. 8, 478.

Haley, T.J. and G.L. McCormick, 1957, Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse, Br. J. Pharmacol. Chemother. 12, 12.

Harris, L.S., W.L. Dewey, J. Howes, J.S. Kennedy and H. Pars, 1969, Narcotic antagonists analgesics: interaction with cholinergic system, J. Pharmacol. Exp. Ther. 169, 17.

Hepler, J.R. and A.G. Gilman, 1992, G proteins, Trends Biochem. Sci. 17, 383.

Hill, D.R., 1985, $GABA_B$ receptor modulation of adenylate cyclase activity in rat brain slices, Br. J. Pharmacol. 84, 249.

Hill, S.J., 1990, Distribution, properties, and functional characteristics of three classes of histamine receptor, Pharmacol. Rev. 42, 45.

Hille, B., 1994, Modulation of ion-channel function by G-protein-coupled receptors, Trends Neurosci. 17, 531.

Hoehn, K., A. Reid and J. Sawynok, 1988, Pertussis toxin inhibits antinociception produced by intrathecal injection of morphine, noradrenaline and baclofen, Eur. J. Pharmacol. 146, 65.

Holz, G.G., S.G. Rane and K. Dunlap, 1986, GTP-binding proteins mediate transmitter inhibition of voltage-dependent calcium channels, Nature 319, 670.

Hood, D.D., J.C. Eisenach and R. Tuttle, 1995, Phase I safety assessment of intrathecal neostigmine methylsulphate in humans, Anesthesiology 82, 331.

Houston, E., R.H. Scott and A.C. Dolphin, 1990, A comparison of the effect of calcium channel ligands and $GABA_B$ agonists and antagonists on transmitter release and somatic calcium channel currents in cultured neurons, Neuroscience 38, 721.

Katada, T. and M. Ui, 1982, Direct modification of the membrane adenylate cyclase system by islet-activating protein due to ADP ribosilation of a membrane protein, Proc. Natl. Acad. Sci. USA 79, 3129.

Katada, T., G.M. Bokoch, J.K. Northup, M. Ui and A.G. Gilman, 1984, The inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase. Properties and functions of the purified protein, J. Biol. Chem. 259, 3568.

Langohr, H.D., M. Stöhr and F. Petruch, 1982, An open and double-blind cross-over study on the efficacy of clomipramine (Anafranil) in patients with painful mono- and polyneuropathies, Eur. Neurol. 2, 309.

Loh, H.H. and A.P. Smith, 1990, Molecular characterization of opioid receptors, Annu. Rev. Pharmacol. Toxicol. 30, 123.

Lucas, J.J. and R. Hen, 1995, New players in the 5-HT receptor field: genes and knochout, Trends Pharmacol. Sci. 16, 246.

McDonald, R.L., J.H. Skerritt and M.A. Werz, 1986, Adenosine agonists reduce voltage-dependent calcium conductance of mouse sensory neurones in cell culture, J. Physiol. 370, 75.

Mullaney, I., F.M. Mitchell, J.F. McCallum, N.J. Buckley and G. Milligan, 1993, The human muscarinic M1 acetylcholine receptor, when expressed in CHO cells, activates and downregulates both $G_q\alpha$ and $G_{11}\alpha$ equally and non-selectively, FEBS 324 (2), 241.

Ohnishi, T., K. Saito, S. Maeda, K. Matsumoto, M. Sakuda and R. Inoki, 1990, Intracerebroventricular treatment of mice with pertussis toxin induces hyperalgesia and enhances ^{3}H-nitrendipine binding to synaptic membranes: similarity with morphine tolerance, Naunyn-Schmied. Arch. Pharmacol. 341, 123.

Oluyomi, A.O. and S.L. Hart, 1991, Involvement of histamine in naloxone-resistant and naloxone-sensitive models of swim stress-induced antinociception in the mouse, Neuropharmacology 30 (9), 1021.

Parenti, M., F. Tirone, G. Giagnoni, N. Pecora and D. Parolaro, 1986, Pertussis toxin inhibits the antinociceptive action of morphine in the rat, Eur. J. Pharmacol. 124, 357.

Park, D., D.Y. Jhon, C.W. Lee, K.H. Lee and S.G. Rhee, 1993, Activation of phospholipase C isozymes by G protein beta gamma subunits, J. Biol. Chem. 268, 4573.

Parolaro, D., G. Patrini, G. Giagnoni, P. Massi, A. Groppetti and M. Parenti, 1990, Pertussis toxin inhibits morphine analgesia and prevents opiate dependence, Pharmacol. Biochem. Behav. 35, 137.

Raffa, R.B., R.P. Martinez and C.D. Connelly, 1994, G-protein antisense oligodeoxyribonucleotides and μ-opioid supraspinal antinociception, Eur. J. Pharmacol. 258, R5.

Rumore, M.M. and D.A. Schlichting, 1985, Analgesic effects of antihistaminics, Life Sci. 36, 403.

Sabol, S.L. and M. Nirenberg, 1979, Regulation of adenylate cyclase of neuroblastoma×glioma hybrid cells by α-adrenergic receptors, J. Biol. Chem. 254, 1913.

Sacerdote, P., A. Brini, P. Mantegazza and A.E. Panerai, 1978, A role for serotonin and beta-endorphin in the analgesia induced by some tricyclic antidepressant drugs, Pharmacol. Biochem. Behav. 26,153.

Sanchez-Blazquez, P. and J. Garzon, 1991, Cholera toxin and pertussis toxin on opioid- and α_2-mediated supraspinal analgesia in mice, Life Sci. 48, 1721.

Sawynok, J. and A. Reid, 1988, Role of G-proteins and adenylate cyclase in antinociception produced by intrathecal purines, Eur. J. Pharmacol. 156, 25.

Sharma, S.K., M. Nirenberg and W.A. Klee, 1975, Morphine receptors as regulators of adenylate cyclase activity, Proc. Natl. Acad. Sci. USA 72, 590.

Simon, M.I., M.P. Strathman and N. Gautman, 1991, Diversity of G proteins in signal transduction, Science 252, 802.

Suzuki, T., M. Funada, M. Narita, M. Misawa and H. Nagase, 1991, Pertussis toxin abolishes mu- and delta-opioid agonist-induced place preference, Eur. J. Pharmacol. 205, 85.

Trussel, L.O. and M.B. Jackson, 1987, Dependence of an adenosine-activated potassium current on a GTP-binding protein in mammalian central neurons, J. Neurosci. 7, 3306.

Van Calker, D., M. Müller and B. Hamprecht, 1979, Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells, J. Neurochem. 33, 999.

Van der Ploeg, I., A. Cintra, N. Altiok, P. Askelöf, K. Fuxe and B.B. Fredholm, 1991, Limited distribution of pertussis toxin in rat brain after injection into the lateral cerebral ventricles, Neuroscience 44 (1), 205.

Vaught, J.L., K. Pelley, L.G. Costa, P. Setler and S.J. Enna, 1985, A comparison of the antinociceptive responses to GABA-receptor agonists THIP and baclofen, Neuropharmacology 24, 211.

Wojcik, W.J. and N.H. Neff, 1984, γ-Aminobutyric acid B receptors are negatively coupled to adenylate cyclase in brain, and in the cerebellum these receptors may be associated with granule cells, Mol. Pharmacol. 25, 24.