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European Journal of Pharmacology 368 (1999) 205–211

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Tiagabine antinociception in rodents depends on $GABA_B$ receptor activation: parallel antinociception testing and medial thalamus GABA microdialysis

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Received 28 September 1998; revised 11 January 1999; accepted 15 January 1999

Abstract

The effects of a new antiepileptic drug, tiagabine, (*R*)-*N*-[4,4-di-(3-methylthien-2-yl)but-3-enyl] nipecotic acid hydrochloride, were studied in mice and rats in antinociceptive tests, using three kinds of noxious stimuli: mechanical (paw pressure), chemical (abdominal constriction) and thermal (hot plate). In vivo microdialysis was performed in parallel in awake, freely moving rats in order to evaluate possible alterations in extracellular γ-aminobutyric acid (GABA) levels in a pain-modulating region, the medial thalamus. Systemic administration of tiagabine, 30 mg kg^{-1} i.p., increased nearly twofold the extracellular GABA levels in rats and increased significantly the rat paw pressure nociceptive threshold in a time-correlated manner. Dose-related significant tiagabine-induced antinociception was also observed at the doses of 1 and 3 mg kg^{-1} i.p. in the mouse hot plate and abdominal constriction tests. The tiagabine antinociception was completely antagonised by pretreatment with the selective $GABA_B$ receptor antagonist, CGP 35348, (3-aminopropyl-diethoxy-methyl-phosphinic acid) (2.5 μg/mouse or 25 μg/rat i.c.v.), but not by naloxone (1 mg kg^{-1} s.c.), both administered 15 min before tiagabine. Thus, it is suggested that tiagabine causes antinociception due to raised endogenous GABA levels which in turn activate $GABA_B$ receptors.

Keywords: Tiagabine; Antinociception; Microdialysis; GABA (γ-aminobutyric acid); $GABA_B$ receptor; Medial thalamus

1. Introduction

The antinociceptive effects of directly acting γ-aminobutyric acid (GABA) receptor agonists and antagonists and indirectly acting GABAergic agents (GABA uptake and GABA transaminase inhibitors) have been widely studied in the last two decades. These drugs, which increase synaptic GABA levels, induce antinociception in laboratory animals. Many inactivators of GABA transaminase, such as amino-oxyacetic acid, γ-acetylenicGABA, γ-vinylGABA, gabaculline, valproic acid and ethanolamine-*O*-sulphate (Buckett, 1980; Kendall et al., 1982; Mesdjian et al., 1983), have an antinociceptive effect in rodents. Baclofen, a $GABA_B$ receptor agonist, increases the pain threshold in rodents (Bartolini et al., 1981; Sawynok and LaBella, 1982), and picrotoxin and bicuculline, two $GABA_A$ receptor antagonists, induce a dose-related antinociceptive effect through augmented release of GABA (Malcangio et al., 1992). CGP 35348 (3-aminopropyl-diethoxy-methyl-phosphinic acid), a selective $GABA_B$ receptor antagonist, is able to antagonise the antinociceptive effects not only of baclofen, but also of picrotoxin and bicuculline, confirming the importance of $GABA_B$ receptors in pain modulation (Malcangio et al., 1991, 1992).

The GABA uptake inhibitors, nipecotic acid, and its derivatives, SKF-100330A (*N*-(4,4-diphenyl-3-butenyl)-guvacine) and SKF-89976A (*N*-(4,4-diphenyl-3-butenyl-3-piperidine carboxylic acid hydrochloride), display significant antinociceptive activity in the mouse tail immersion assay (Kendall et al., 1982; Zorn and Enna, 1985). Tiagabine HCl, (*R*)-*N*-[4,4-di-(3-methylthien-2-yl)but-3-enyl] nipecotic acid hydrochloride, a new GABA uptake blocker, shows a higher selectivity for the GABA transporter-1 than do other previously synthesised nipecotic

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00034-5

acid derivatives. Since GABA transporter-1 is the predominant transporter in the rat brain, accounting for approximately 85% of GABA transport when assayed in vitro (Borden et al., 1994, 1995), its block induces a considerable increase in GABA extracellular levels. The tiagabine potency and specificity for the GABA uptake system have been evaluated in vitro and in vivo experiments. Tiagabine decreases [^{3}H]GABA uptake into synaptosomal membranes, neurons and glial cells with a potency greater than that of other known GABA uptake inhibitors such as nipecotic acid, NNC 05-0329 ((*S*)-*N*-(4,4-di-(3-methylthien-2-yl)but-3-enyl)nipecotic acid) and SKF 100330A (Braestrup et al., 1990). In vivo microdialysis showed tiagabine to increase extracellular GABA levels in the globus pallidus, ventral pallidum and substantia nigra in a dose-related manner after its systemic administration (Fink-Jensen et al., 1992).

Due to its potency, specificity and selectivity, tiagabine has been studied as an anticonvulsant agent in a wide range of animal seizure models (Giardina, 1994; Smith et al., 1995). The compound is now marketed in Europe and in the United States for use in epilepsy.

Since the increase in extracellular GABA levels seems to enhance anticonvulsive activity (Löscher and Schmidt, 1988; Fink-Jensen et al., 1992; Holland et al., 1992), we supposed that the same mechanism of action would provide an antinociceptive effect. The analgesic activity of tiagabine has been tested against electric shocks on a mouse grid in a new antinociceptive assay (Swedberg, 1994).

The aim of our study was to investigate if this new antiepileptic drug also has antinociceptive effects in three classical antinociceptive tests with two different species. Secondly, we thought it worthwhile to relate such effects to an eventual alteration of GABA extracellular levels in a pain-modulating brain region, the medial thalamus (Sherman et al., 1997), by means of in vivo microdialysis, using a new modification for high-performance liquid chromatography (HPLC) GABA dosing.

2. Materials and methods

2.1. Animals

Male Swiss–Webster mice, weighing 20–25 g, and Wistar rats, weighing 180–200 g, were used. Fifteen mice or four rats were housed per cage. The cages were brought into the experimental room 24 h before the experiment for adaptation. The animals were fed a standard laboratory diet and tap water ad libitum.

2.2. Hot plate test

The mice were placed in a stainless steel container ($36 \times 28 \times 30$ cm), thermostatically set at 52.5 ± 0.1°C in a precision water-bath (KW Mechanical Workshop, Siena, Italy) according to the method described by O'Callaghan and Holtzman (1976). The endpoint was licking or kicking of fore or hind paws. Mice scoring below 12 and over 18 s in the pretest (30%) were rejected. An arbitrary cut-off time of 45 s was adopted.

2.3. Abdominal constriction test

The test was performed according to Koster et al. (1959). The number of stretching movements was counted for 10 min, starting 5 min after 0.6% acetic acid i.p. injection.

2.4. Paw pressure test

The nociceptive threshold in rats was determined with an analgesimeter (Ugo Basile, Varese, Italy), according to the method described by Leighton et al. (1988). Rats scoring below 40 g or over 65 g during the test, run before drug administration, (20%) were rejected. An arbitrary cut-off value of 160 g was adopted.

2.5. Rotarod test

The integrity of motor coordination was assessed with a rotarod apparatus, (Ugo Basile, Varese, Italy) at a rotating speed of 24 r.p.m. Immediately after each hot plate trial, the number of falls from the rod was counted for 30 s (Vaught et al., 1985).

2.6. In vivo microdialysis and experimental procedures

Male Wistar rats weighing 180–200 g were anaesthetised with chloral hydrate (400 mg kg^{-1} i.p.) and placed in a stereotaxic apparatus with the upper incisor bar set to allow the bregma to be at the same height as lambda. A CMA/12 microdialysis guide cannula (CMA/Microdialysis, Stockholm, Sweden) was implanted into the medial thalamus using the following coordinates from the bregma: AP: -3.14 mm, L: ± 0.9 mm, V: 4 mm (Paxinos and Watson, 1986). After surgery, the animals were allowed to recover prior to the microdialysis experiments. From 5 to 7 days later, the stopper of the guide cannula was removed and the CMA/12 microdialysis probe (membrane 3 mm, outer diameter 0.5 mm, molecular weight cutoff 20000 D) was inserted so that the tip of the membrane arrived at V: 7 mm. The rat was placed in a CMA/120 system for freely moving animals and the probe was perfused continuously with a physiological solution by means of a CMA/102 microinfusion pump at a flow rate of 3 μl/min. The solution consisted of NaCl (154 mM), KCl (5.63 mM), $CaCl_2 \cdot 2H_2O$ (2.18 mM), $NaHCO_3$ (5.95 mM) (pH 6.0). Fractions were collected over 20 min. When stable basal levels were reached after 2 h, saline or tiagabine was

administered i.p. and sample collection was continued for another 2 h and 20 min.

At the end of the experiment, the probe was removed, the animal was killed under ether anaesthesia, the brain was removed, fixed in formaldehyde solution for 2 days and frontally cut along the mark left by the insertion of the guide cannula. The location of the tip was verified macroscopically.

2.7. GABA assay

2.7.1. Apparatus

Levels of GABA in the microdialysate samples were determined using high-performance liquid chromatography (HPLC). The chromatographic apparatus consisted of a Thermo Separation Product-Spectra System P 1500 pump, a Merck LiChrospher 100 RP-18 (5 μm) endcapped 250 × 4 mm column, a Rheodyne injector (mod. 7125) equipped with a 20-μl loop and a Croco-Cil TM column oven. A fluorescence detector, Thermo Separation Product-Spectra System SP 4600, was used. The sonication apparatus was a Julabo USR 3 (HF-Freq.: 35 kHz).

2.7.2. Reagents and materials

HPLC grade water was prepared using a 'Euro 15' purification system. Acetonitrile, methanol, acetone and glacial acetic acid (all HPLC grade) were purchased from Carlo Erba. 9-Fluorenylmethyl chloroformate and 1-aminoadamantane were obtained from Fluka and sulfanilic acid from British Drug Houses. All other reagents (analysis grade) were obtained from Merck.

9-Fluorenylmethyl chloroformate was dissolved freshly daily in acetone (0.015 mM) and stored at 4°C. A solution of sulfanilic acid (1.31 μM) in borate buffer (10 g of boric acid in 500 ml of water adjusted to pH 8.5 with 1 N sodium hydroxide) was freshly prepared and used as internal standard and buffer solution. The stopper solution was obtained by dissolving 100 mg of 1-aminoadamantane in 10 ml of methanol and was stored at 4°C. The sodium acetate buffer used for the mobile phase, contained 7 ml glacial acetic acid and 2 ml trimethylamine made up to 1 l with water, adjusted to pH 4.3 with 1 N sodium hydroxide.

2.7.3. Sample preparation

Internal standard solution, 50 μl, was added to 50 μl of biological sample. To this mixture, 20 μl of 9-fluorenylmethyl chloroformate solution was added and the sample was sonicated for 2 min at room temperature. The reaction was terminated by adding 20 μl of 1-aminoadamantane solution followed by a second 2-min sonication. Immediately after, 20 μl of each sample was injected into the column.

2.7.4. Separation and quantitation

GABA was determined by precolumn derivatization with 9-fluorenylmethyl chloroformate according to the method of Kisby et al. (1988) with some modifications, to separate the GABA peak from the other reactant products contained in the biological sample. Separation was carried out by isocratic elution. Derivative GABA was detected at a flow rate of 1 ml/min with a mobile phase of 36/64 (v/v) acetonitrile/sodium acetate buffer for 20 min. The first step was followed by a second step of 75/25 (v/v) acetonitrile/sodium acetate buffer for next 10 min to wash residual products from the column. The column oven kept the column at the same temperature (24°C) and constant retention times. The fluorescence detector was set with the following detector parameters: excitation and emission wavelengths, respectively, 260 and 314 nm; lamp flash

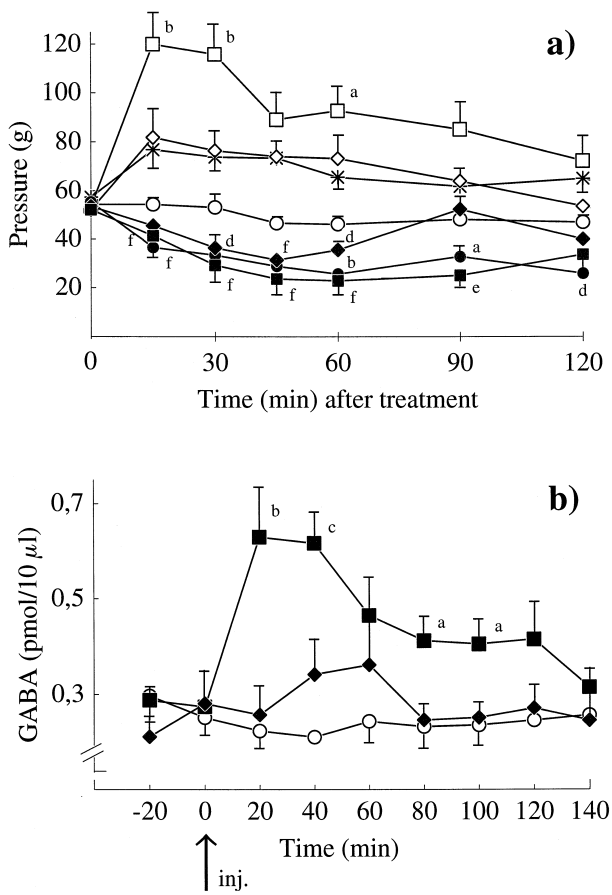

Fig. 1. (a) Antinociceptive effect of tiagabine and its antagonism by CGP35348 in the rat paw pressure test. Groups (pretreatment μg per rat i.c.v. + treatment mg kg^{-1} i.p.) were as follows: saline 5 μl + saline 10 ml kg^{-1} (○); CGP 35348 25 + saline (●); tiagabine 3 (∗); saline + tiagabine 10 (◇); saline + tiagabine 30 (□); CGP 35348 25 + tiagabine 10 (♦); CGP 35348 25 + tiagabine 30 (■). Pretreatment with saline or CGP 35348 was performed 15 min before treatment. Each point represents the mean for 7–11 rats. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ vs. control rats; $^{d}P < 0.05$, $^{e}P < 0.01$, $^{f}P < 0.001$ vs. tiagabine-treated rats. Vertical lines give the S.E.M. (b) Effect of tiagabine 10 (♦) and 30 (■) mg kg^{-1} i.p. on extracellular levels of GABA in the thalamus. The curve (○) represents the basal GABA levels before and after saline 10 ml kg^{-1} i.p. Each point represents the mean for 5–8 rats. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ vs. control rats. Vertical lines give the S.E.M.

rate 100 Hz; photomultiplier tube 600 μl; rise time 2 s; flow cell 2 × 1 mm with 3.1 μl illuminated.

The GABA levels were quantified using the ratio of GABA and sulfanilic acid peak areas.

2.8. Drugs

The following drugs were used: tiagabine HCl monohydrate (Novo Nordisk, Bagsværd, Denmark); CGP 35348 (Ciba Geigy, Basel, Switzerland); (±)-baclofen and naloxone HCl (Research Biochemicals, Natick, MA, USA). The doses given in the text are for the salts. All drugs were dissolved in isotonic saline solution (NaCl 0.9%) and all solutions were prepared immediately before the experiment.

Intracerebroventricular (i.c.v.) administration was performed in two different ways in the two animal species used. For the first method, in mice, substances were injected at the necessary dose dissolved in 5 μl of vehicle, under short ether anaesthesia (Haley and McCormick, 1957). The second approach, used in rats, consisted of injecting the substances into conscious animals implanted with permanent i.c.v. polyethylene cannulae (5 μl of drug solution + 1 μl air + 5 μl of saline). Implantation of the cannulae in the lateral ventricle (Altaffer et al., 1970) was performed under chloral hydrate anaesthesia (400 mg kg^{-1} i.p.) at least 5 days prior to the experiment. To ascertain the exact site of i.c.v. injection, some mice and all rats were deeply anaesthetised and injected i.c.v. with 5 or 10 μl of 1:10 diluted India ink and the brains were examined macroscopically after sectioning.

2.9. Statistical analysis

The results are given as the means ± S.E.M.. Statistical analysis was performed by analysis of variance (ANOVA), followed by Scheffe's multiple comparison test for the paw pressure test and hot plate tests, and by the Kruskal–Wallis test for rotarod and abdominal constriction tests. The microdialysate data were statistically analysed with Student's *t*-test. *P* values of less than 0.05 were considered significant. The data were analysed with the Number Cruncher Statistical System, Vers. 5.03 9/92 computer program.

3. Results

3.1. Tiagabine antinociceptive effect in the rat paw pressure test

Systemic administration of tiagabine, 3, 10 and 30 mg kg^{-1} i.p., caused a dose-related antinociceptive effect. While the effect of the two lowest doses did not reach statistical significance, the dose of 30 mg kg^{-1} caused a strong long-lasting antinociception (Fig. 1a). At this dose the maximum effect was detected 15 min after administration and lasted for 1 h.

3.2. Tiagabine and baclofen antinociceptive effects in the mouse hot plate test

The doses of 1, 3 and 10 mg kg^{-1} s.c. tiagabine increased the mouse pain threshold in the hot plate test in a dose-dependent manner. All doses had a statistically significant effect. The antinociceptive effect appeared 15 min after tiagabine administration. The 3-mg kg^{-1} dose raised the nociceptive threshold comparably to baclofen, 4 mg kg^{-1} s.c., and the effect lasted for 90 min (Table 1).

3.3. Tiagabine effects in the mouse rotarod test

As shown in Fig. 2, tiagabine 1 and 3 mg kg^{-1} s.c., did not affect motor coordination in the mouse rotarod test,

Table 1
Antinociceptive effect of tiagabine in the hot plate test in comparison with that of baclofen: its antagonism by CGP 35348 but not by naloxone

Pretreatment	Treatment (mg kg^{-1} s.c.)	*n*	Reaction latency (s)						
			Pretest	15 min	30 min	45 min	1 h	90 min	2 h
Saline (10 ml kg^{-1})	Saline (10 ml kg^{-1})	15	14.5 ± 0.6	14.7 ± 0.7	14.2 ± 0.8	15.1 ± 0.8	14.3 ± 1.0	15.0 ± 0.8	15.6 ± 0.8
–	Tiagabine 1	12	14.9 ± 0.5	18.9 ± 1.2	22.0 ± 1.4[a]	19.1 ± 1.3	22.6 ± 1.6	17.7 ± 1.1	19.0 ± 1.3
Saline (10 ml kg^{-1})	Tiagabine 3	18	15.2 ± 0.5	31.3 ± 1.8[c]	36.8 ± 2.2[c]	35.4 ± 2.5[c]	28.2 ± 2.3[c]	20.4 ± 1.8[a]	16.6 ± 1.1
–	Tiagabine 10	12	15.5 ± 0.5	45.0 ± 0.0[c]	45.0 ± 0.0[c]	43.6 ± 1.3[c]	43.1 ± 1.8[c]	37.1 ± 2.7[c]	27.2 ± 3.3[c]
–	Baclofen 4	14	14.5 ± 0.4	26.1 ± 2.8[c]	34.3 ± 3.2[c]	33.3 ± 3.4[c]	34.2 ± 3.0[b]	–	–
Naloxone (1 mg kg^{-1} s.c.)	Saline (10 ml kg^{-1})	11	14.2 ± 0.6	12.0 ± 0.8	12.2 ± 1.3	13.4 ± 1.4	13.4 ± 1.0	13.0 ± 1.0	14.5 ± 3.5
Naloxone (1 mg kg^{-1} s.c.)	Tiagabine 3	12	15.1 ± 0.5	31.6 ± 2.9[c]	33.2 ± 2.8[c]	32.5 ± 3.4[c]	32.6 ± 2.9[c]	27.4 ± 2.7[c]	18.3 ± 1.1
CGP 35348 (2.5 μg i.c.v.)	Saline (10 ml kg^{-1})	12	15.5 ± 0.5	11.8 ± 0.6	11.9 ± 0.6	13.2 ± 0.9	12.9 ± 0.5	14.6 ± 0.7	12.8 ± 1.0
CGP 35348 (2.5 μg i.c.v.)	Tiagabine 3	14	14.3 ± 0.5	15.4 ± 1.6[e]	12.6 ± 0.6[e]	13.0 ± 0.4[e]	15.5 ± 1.2[e]	15.9 ± 0.7	15.8 ± 0.8
CGP 35348 (2.5 μg i.c.v.)	Baclofen 4	14	14.9 ± 0.5	12.4 ± 0.9[e]	16.0 ± 1.1[e]	18.8 ± 1.2[e]	17.9 ± 1.2[d]	–	–

[a] $P < 0.05$. [b] $P < 0.01$. [c] $P < 0.001$ vs. saline-treated mice. [d] $P < 0.01$. [e] $P < 0.001$ vs. tiagabine- or baclofen-treated mice.
Pretreatment was performed 15 min before treatment.

while 10 mg kg^{-1} s.c. enhanced significantly the number of falls from the rotating rod.

3.4. Tiagabine and baclofen antinociceptive effects in the mouse abdominal constriction test

An antinociceptive effect was detected 30 min after treatment with tiagabine 0.1–3 mg kg^{-1} s.c. The doses of 0.1 and 0.3 lowered the number of abdominal constrictions but the effect was not significant (Fig. 3). The doses of 1 and 3 mg kg^{-1} s.c. caused a statistically significant effect. Tiagabine 1 mg kg^{-1} s.c. and baclofen 4 mg kg^{-1} s.c induced similar antinociception.

3.5. Antagonism of tiagabine antinociception

In all three tests CGP 35348, at the doses of 2.5 μg/mouse or 25 μg/rat i.c.v. administered 15 min before tiagabine, completely prevented the antinociception induced by the latter (Fig. 1a and Fig. 3; Table 1). The dose of CGP 35348 used was that capable of completely antagonising baclofen antinociception (4 mg kg^{-1} s.c.) in the mouse antinociceptive tests. Naloxone 1 mg kg^{-1}, when tested in the hot plate and abdominal constriction tests failed to antagonise tiagabine antinociception (Table 1; Fig. 3).

3.6. Effect of tiagabine on extracellular GABA levels in the rat thalamus

In the rat microdialysis study run in parallel the antinociceptive dose, 30 mg kg^{-1} i.p., of tiagabine enhanced significantly the GABA levels in the 0–20 and 20–40 min perfusate samples with peak values of 182% and 194%, respectively, of the corresponding basal value obtained after saline i.p. injection (Fig. 1b). After reaching these values, the extracellular GABA levels gradually declined but the difference from the controls continued to be statistically significant up to 1 h 40 min after tiagabine injection.

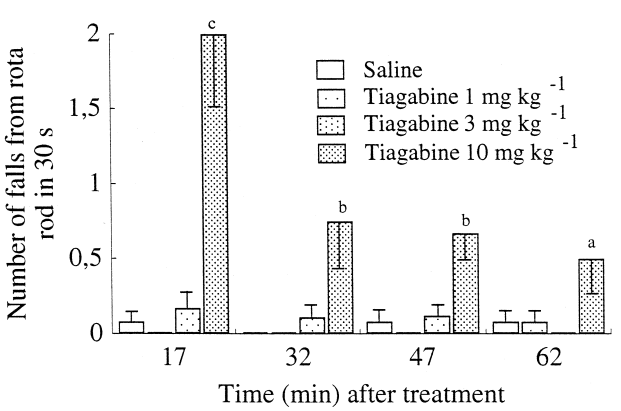

Fig. 2. Effects of tiagabine on integrity of locomotor coordination in the mouse rotarod test. The test was performed soon after each hot plate trial. Each column represents the mean ± S.E.M. for 12–18 mice. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ vs. saline-treated mice. Vertical lines give S.E.M.

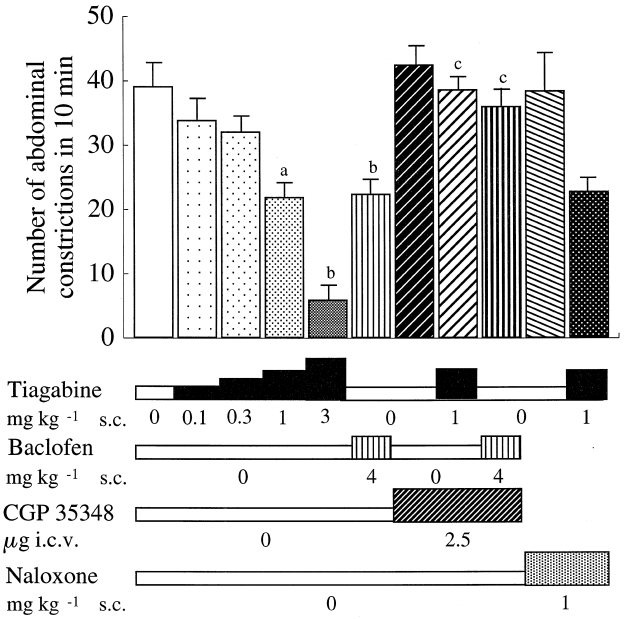

Fig. 3. Tiagabine and baclofen antinociceptive effects in the mouse acetic acid (0.6%) abdominal constriction test: antagonism by CGP 35348 but not by naloxone. Pretreatment was performed 15 min before treatment and the test 30 min thereafter. Each column represents the mean for 8–12 mice. [a]$P < 0.01$, [b]$P < 0.001$ vs. saline controls; [c]$P < 0.01$ vs. tiagabine (1 mg kg^{-1} s.c.)- and baclofen-treated mice. Vertical lines give the S.E.M.

4. Discussion

In the present work we studied the antinociceptive properties of tiagabine in tests inducing three different kinds of noxious stimuli and correlated the results with the alteration of thalamic GABA levels.

Tiagabine induced a dose-dependent, long-lasting antinociception in all three tests in both mice and rats. Firstly, we studied the tiagabine effect on rats in the paw pressure test. Tiagabine was administered systemically since it is able to cross the blood–brain barrier (Fink-Jensen et al., 1992). The doses of 3 and 10 mg kg^{-1} tended to increase the pain threshold in a dose-dependent manner with the highest dose of 30 mg kg^{-1} causing a significant antinociceptive effect that persisted for 1 h after i.p. administration. The pain threshold then returned slowly to its basal nociceptive level (Fig. 1a). The tiagabine antinociceptive and anticonvulsant effects occurred in the same dose range. The molecule has been reported to antagonise pentylenetetrazol-induced convulsions in rats with an ED_{50} and ED_{85}, 11.5 and 21 mg kg^{-1} i.p., respectively (Fink-Jensen et al., 1992), while locomotor impairment was detected at an ED_{50} of 40 mg kg^{-1} (Nielsen et al., 1991).

To assess whether the tiagabine-induced antinociception was actually related to the inhibition of GABA reuptake, extracellular GABA levels were measured in a pain-mod-

ulating brain region, the medial thalamus. [^{3}H]Tiagabine binds to the brain GABA uptake carrier with a high-affinity constant for binding of 18 nM and B_{max} of 669 pmol/g of rat forebrain tissue (Braestrup et al., 1990) and shows a high selectivity for human and rat GABA transporter-1 (human GABA transporter-1 $IC_{50} = 0.07$ μM, rat GABA transporter-1 $IC_{50} = 0.64$ μM) (Borden et al., 1994). Thus, the pharmacological effects observed in animals are thought to be due to changes in GABA levels. The results obtained with the antinociceptive tests were consistent with those of the microdialysis experiments since the dose of 30 mg kg^{-1} produced a significant, nearly twofold, increase in GABA extracellular levels in the medial thalamus in the 0–20 and 20–40 min samples (Fig. 1b). The lower rat dose of 10 mg kg^{-1} i.p. of tiagabine increased both the pain threshold and the GABA level, although not significantly (Fig. 1a,b). The ability of tiagabine to increase extracellular GABA has been studied for other brain regions. Fink-Jensen et al. (1992) demonstrated an increase in extracellular GABA levels in the globus pallidus, ventral pallidum and substantia nigra following systemic tiagabine administration at doses known to inhibit pentylenetetrazol-induced tonic seizures in rats. We measured the GABA levels, localising the probe to perfuse parts of the following thalamic nuclei: central mediodorsal, lateral mediodorsal, paracentral, central medial and ventrolateral thalamic nuclei. The choice of these nuclei was related to the importance of several thalamic nuclei for pain modulation. Ventroposterolateral and ventroposteromedial portions of the ventrobasal nucleus are thought to be relevant to pathological pain states because of a widely distributed GABAergic inhibitory system operating in the thalamus (Roberts et al., 1992; Craig et al., 1994). Richards and Bowery (1996) observed an increase of GABA in the ventrolateral thalamus of rats following local and systemic tiagabine administration. These authors found no significant increase in dialysate GABA levels following intraperitoneal injection of 10 mg kg^{-1} tiagabine, while a dose of 20 mg kg^{-1} produced a 70% increase that peaked 2 h after drug administration. At 40 mg kg^{-1}, the maximal increase was 130%, again peaking at 2 h. The results of our microdialysis study seem to support the importance of the medial thalamic nuclei since at the dose of 30 mg kg^{-1} i.p. we observed a higher GABA increase (182%), which appeared more rapidly (in 20 min) than in the experiments of Richards and Bowery (1996). This increase was time-correlated to the elevated pain threshold observed in the rat paw pressure test. Sherman et al. (1997) demonstrated that the medial thalamus contains nociceptive-specific neurons which have a significant increase in firing rate in response to pinch of the controlateral hind paw.

The advantages of the GABA analysis method we now used were indicated for similar methods described by Kisby et al. (1988) and Bank et al. (1996). We shortened the derivatization procedure to 2 min by sonication of the sample. Since only GABA was required to be detected among all the amino acids, no gradient elution was adopted but the 36/64 (v/v) acetonitrile/sodium acetate buffer mobile phase proved to be the best isocratic elution solvent for separation of the GABA peak, which had a retention time of 15 min. The GABA limit of detection was 50 fmol. The total volume of the derivative sample (140 μl) was so small that the extraction with pentane to remove excess reagent (9-fluorenylmethyl chloroformate) and its hydrolysis product with water was not possible. Therefore, we introduced a second elution step using 75/25 (v/v) acetonitrile/sodium acetate buffer in order to eliminate the reactant product remaining in the column as quickly as possible. Like the former authors, we confirmed the excellent stability and reproducibility of the method, which may permit automated analysis of multiple samples.

Antinociceptive activity was also studied in mice. Tiagabine had been reported to be much more potent to prevent pentylenetetrazol-induced convulsions in mice than in rats (ED_{50} 1.2 and 11.5 mg kg^{-1} i.p., respectively) (Nielsen et al., 1991; Fink-Jensen et al., 1992). As it was in the anticonvulsant tests, tiagabine was more potent in the two mouse antinociceptive tests than in the rat test. Tiagabine antinociception was comparable to that by baclofen. The long-lasting effect of tiagabine could be due to its long half-life, 1.8 h in the rat (Fink-Jensen et al., 1992). The highest dose used (10 mg kg^{-1}) impaired rotarod performance (Fig. 2), so that the increased pain threshold at this dose cannot be considered a true antinociceptive effect, although Nielsen et al. (1991) observed tiagabine-induced locomotor impairment in mice at ED_{50} 14 mg kg^{-1}. Morphine over 8.2 mg kg^{-1} s.c. also significantly impairs mouse coordination in the rotarod test (Malmberg-Aiello et al., 1994).

Pretreatment with CGP 35348, a selective $GABA_B$ receptor antagonist (Olpe et al., 1990), 25 μg per rat i.c.v., completely antagonised the antinociception elicited by 30 mg kg^{-1} i.p. of tiagabine in the rat paw pressure test (Fig. 1a). The antagonism was also observed with CGP35348 at 2.5 μg per mouse i.c.v. in the hot plate and abdominal constriction tests (Fig. 3; Table 1). The doses of CGP35348 used to antagonise tiagabine antinociception were those capable of preventing baclofen (4 mg kg^{-1} s.c.) antinociception in rats and mice (Malcangio et al., 1992). It seems likely that the $GABA_B$ receptor is involved in the tiagabine antinociceptive effects. To exclude a non-selective tiagabine effect, the mice were pretreated with naloxone 1 mg kg^{-1} s.c. In contrast, naloxone, administered at a dose capable of completely antagonising morphine antinociception (Lamberti et al., 1996), did not alter the tiagabine-increased pain threshold either in the hot plate or abdominal constriction tests, thus supporting the specificity of the tiagabine antinociceptive effect (Fig. 3; Table 1).

The present data demonstrate an antinociceptive effect for tiagabine and suggest that elevation of GABA levels in the medial thalamus may modulate pain through activation of $GABA_B$ receptors.

Acknowledgements

We are grateful to Novo Nordisk for the generous supply of tiagabine HCl and to Ciba-Geigy for CGP 35348. We also wish to express our gratitude to Colonel G. Santoni and to General G. Polidori from the Stabilimento Chimico Farmaceutico Militare in Florence for their help and use of HCLP. This work was partially supported by grants from Ministero dell'Università e della Ricerca Scientifica e Tecnologica (Ex MURST 60%).

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