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Piracetam and Aniracetam Antagonism of Centrally Active Drug-Induced Antinociception

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GALEOTTI, N., C. GHELARDINI AND A. BARTOLINI. *Piracetam and aniracetam antagonism of centrally active drug-induced antinociception.* PHARMACOL BIOCHEM BEHAV **53**(4) 943–950, 1996.—The effects of the nootropic drugs piracetam and aniracetam on antinociception induced by baclofen, bicuculline, and picrotoxin and on baclofen-induced muscle relaxation were studied in mice. Antinociception was investigated using both the hot plate (thermal stimulus) and abdominal constriction (chemical stimulus) tests. Both behaviour inhibition and muscle relaxation were observed by using the rota-rod test. Piracetam (30 mg/kg, IP) and aniracetam (10 mg/kg, PO) reduced baclofen, bicuculline, and picrotoxin antinociception without modifying analgesia induced by non-GABAergic drugs such as morphine, physostigmine, clomipramine, and diphenhydramine. In this concentration range, piracetam, and aniracetam were also able to reduce the inhibition of rota-rod performance. At higher doses piracetam (100 mg/kg, IP) and aniracetam (100 mg/kg, PO) were able to completely prevent baclofen antinociception. However, when prevention of GABAergic antinociception was complete, piracetam and aniracetam were able to block non-GABAergic antinociception also. Comparing the effects of piracetam and aniracetam with those exerted by the $GABA_B$ antagonist CGP 35348, a reduction of non-GABAergic analgesia was also observed using higher doses of CGP 35348 (2.5 μg per mouse ICV). The present results indicate that piracetam and aniracetam, by preventing both of the investigated effects of baclofen, have some selectivity against $GABA_B$-mediated inhibition. The well-known activity of piracetam and aniracetam on learning and memory might, therefore, depend, at least in part, on the removal of inhibitory $GABA_B$ mechanisms that impair attention and cognitive functions.

Learning and memory Nootropic Piracetam Aniracetam CGP 35348 GABA $GABA_B$ receptor Analgesia Antinociception

NOOTROPICS (noos = mind, tropein = towards) represent a heterogeneous compound group that facilitates learning and memory or overcomes natural or induced cognitive impairments in laboratory animals (10,23). Nevertheless, results from controlled clinical trials have questioned the usefulness of nootropic compounds for treatment of cognitive disorder in humans (31). They typically have low toxicity, no sedative or stimulatory effects, and variously affect flow and metabolism in the brain (13). The nootropic drugs include the 2-pyrrolidinone derivatives piracetam, oxiracetam, pramiracetam, etiracetam, aniracetam, rolziracetam, and tenilsetam (23). Although no commonly accepted mechanism of action has been established (10), it has been shown that these compounds facilitate the transcallosal, interhemispheric transfer of information (25) and enhance long-term potentiation (LTP) in guinea pig hippocampal slices (28,32). Piracetam might alter presynaptic cholinergic functions, possibly by enhancing high-affinity neuronal uptake of choline (26,36), but these data are still a matter of controversy (8,34). Pilch and Müller showed that piracetam elevated muscarinic receptor density in the frontal cortex of aged but not of young mice (27). Aniracetam was found to be able to increase the acetylcholine content in the hippocampus but not in the corpus striatum (35). The possible involvement of steroids in the piracetam-like nootropics mechanism of action has also been examined (19,20). Copani et al. reported that piracetam, aniracetam, and oxiracetam may act as positive modulators of AMPA-sensitive glutamate receptors in neurons (5).

The numerous mechanisms so far suggested to explain the nootropic effect of the 2-pyrrolidinone compounds are a clear indication that the exact mechanism of action of these drugs is still unknown.

Recently, Mondadori et al. (21), Carletti et al. (4), and Bianchi and Panerai (2) reported that the $GABA_B$ receptor antagonists CGP 36742 and CGP 35348 are able to ameliorate cognitive processes including memory and learning in mice, rats, and rhesus monkeys. Numerous hormonal, behavioural, and cellular effects have been attributed to the activation of

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$GABA_B$ receptors (3). Baclofen, a $GABA_B$ agonist, is able, for instance, to impair learning and memory (33) and to induce antinociception and muscle relaxation (7,16). Malcangio et al. (17,18) have shown that, by blocking $GABA_B$ receptors with the selective antagonist CGP 35348 (24), it is possible to prevent the analgesic effect consequent to direct (baclofen) and indirect (bicuculline and picrotoxin) $GABA_B$ activation. Furthermore, CGP 35348 was also able to antagonize the motor incoordination consequent to the muscle-relaxant effect of baclofen (17,24).

Because the effects of $GABA_B$ antagonists (CGP 36742 and CGP 35348) on ameliorating learning and memory and on preventing antinociception could be attributed to the removal of the GABAergic tonic inhibition, we considered it worthwhile to investigate whether the 2-pyrrolidinone compounds could act through the same mechanism. This hypothesis was also supported by the structural similarity between the GABA moiety and the moieties of the 2-pyrrolidinone compounds that are all cyclic derivatives of GABA itself (Fig. 1). To this end, because the $GABA_B$ antagonist CGP 35348 was not only able to improve learning and memory but also to prevent baclofen-induced antinociception and muscle relaxation, effects of piracetam and aniracetam on analgesia produced by baclofen, bicuculline, and picrotoxin in the mouse hot plate and abdominal constriction tests were examined. Effects of piracetam and aniracetam on baclofen-induced impairment of rota-rod performance in mice were also investigated. Morphine, physostigmine (12), clomipramine (30), and diphenhydramine (29) were used as non-GABAergic reference drugs.

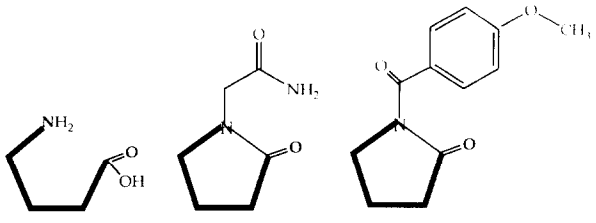
METHODS

Animals

Male Swiss albino mice (23–30 g) were used. Fifteen mice were housed per cage. The cages were placed into the experimental room 24 h before the test for acclimatization. The animals were fed a standard laboratory diet and tap water ad lib.

Hot Plate Test

Mice were placed inside a stainless container, thermostatically set at 52.5 ± 0.1°C in a precision water bath from CW Mechanical Workshop (Siena, Italy). Reaction times (seconds), 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 cutoff time of 45 s was adopted.



GABA *Piracetam* *Aniracetam*

FIG. 1. GABA, piracetam, and aniracteam chemical structure.

Abdominal Constriction Test

Mice were injected IP with a 0.6% solution of acetic acid (10 ml/kg). The number of stretching movements was counted for 10 min, starting 5 min after acetic acid injection.

Rota-Rod Test

The apparatus consisted of a base platform and a rotating rod of 3 cm diameter with a nonslippery surface. This rod was placed at height of 15 cm from the base. The rod, 30 cm in length, was divided into five equal sections by six disks. Thus, up to five mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 rpm. The integrity of motor coordination was assessed on the basis of endurance time of the animals on the rotating rod. One day before the test, the animals were trained twice. On the day of the test only the mice that were able to stay balanced on the rotating rod between 70 and 120 s (cutoff time) were selected for testing. The performance time was measured before and at various times after treatment.

Drugs

The following drugs were used: (±)-baclofen (β-*p*-chlorophenyl GABA), picrotoxin, (+)-bicuculline, physostigmine hemisulphate, piracetam (Sigma), morphine hydrocloride (U.S.L. 10/D, Florence), aniracetam (A. Menarini Industrie Farmaceutiche Riunite S.r.l.), CGP 35348 (3-aminopropyl-diethoxymethyl-phosphinic acid, Ciba-Geigy), clomipramine hydrocloride (anafranil, Ciba-Geigy), and diphenhydramine hydrocloride (De Angeli).

All drugs were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use, except aniracetam, which was dispersed in sodium carboxymethyl cellulose 1%. Solutions of bicuculline were prepared immediately before the experiments by dissolving 10 mg of bicuculline base in 400 μl HCl (0.1 N) and then adding saline up to 10 ml. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 ml/kg by SC, IP, and esophageal (PO) injections.

The ICV administration was performed under ether anaesthesia using isotonic saline as solvent, according to the method described by Haley and McCormick (11). 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 with no more than 2 mm into the brain of the mouse, where 5 μl was then administered. The injection site was 1 mm on the right or on the left from the midline along a line drawn through to the anterior base of the ears. Injection was performed into the right or into the left ventricle randomly. To ascertain that the drugs were administered exactly into the cerebral ventricle, some mice were injected with 5 μl of diluited 1 : 10 Indian ink and their brains examined macroscopically after sectioning.

Statistical Analysis

Results are given as the mean ± SEM; ANOVA test was used to verify significance between two means. Values of $p < 0.05$ were considered significant. Data were analyzed with computer program (StatView for the Macintosh, 1992).

TABLE 1

EFFECT OF PIRACETAM ON BACLOFEN-, BICUCULLINE-, AND PICROTOXIN-INDUCED ANTINOCICEPTION IN THE MOUSE HOT PLATE TEST (52.5°C)

Pretreatment (mg/kg, IP)	Treatment (mg/kg, SC)	Number of Mice	Licking Latency in Mice (s)				
			Before Treatment	After Treatment			
				15 min	30 min	45 min	60 min
Saline	saline	25	15.0 ± 0.7	14.0 ± 0.7	14.4 ± 0.8	14.8 ± 0.7	14.0 ± 0.6
Piracetam 1	saline	8	15.6 ± 0.8	14.4 ± 1.1	15.2 ± 0.9	16.0 ± 0.9	14.0 ± 0.6
Piracetam 5	saline	8	13.4 ± 0.6	15.2 ± 0.7	16.4 ± 1.0	15.0 ± 1.0	14.9 ± 0.9
Piracetam 10	saline	9	14.4 ± 0.8	16.1 ± 0.7	14.7 ± 0.9	14.3 ± 1.0	15.4 ± 0.9
Piracetam 30	saline	10	15.2 ± 0.8	15.0 ± 1.8	13.8 ± 1.4	13.0 ± 1.0	15.6 ± 1.4
Piracetam 100	saline	8	14.7 ± 0.9	14.2 ± 0.4	11.9 ± 0.7	13.5 ± 1.0	12.4 ± 1.0
Piracetam 300	saline	15	15.4 ± 0.5	14.6 ± 1.2	13.7 ± 1.1	14.5 ± 1.1	15.7 ± 1.2
Saline	baclofen 4	25	14.4 ± 0.5	24.4 ± 1.8	33.4 ± 1.9	30.4 ± 2.2	26.9 ± 2.2
Piracetam 1	baclofen 4	17	15.1 ± 0.6	22.8 ± 1.3	29.7 ± 2.2	28.8 ± 1.6	24.6 ± 2.1
Piracetam 5	baclofen 4	7	14.0 ± 0.6	24.0 ± 2.6	22.7 ± 3.6*	26.6 ± 4.1	22.8 ± 2.7
Piracetam 10	baclofen 4	17	15.0 ± 0.6	20.4 ± 2.4	23.4 ± 2.0†	28.0 ± 2.1	24.5 ± 2.6
Piracetam 30	baclofen 4	20	14.2 ± 0.5	18.9 ± 1.4*	24.4 ± 2.0†	34.1 ± 2.4	25.5 ± 2.6
Piracetam 100	baclofen 4	12	14.4 ± 0.8	14.0 ± 0.7‡	17.2 ± 0.8‡	21.2 ± 1.8†	23.7 ± 2.1
Piracetam 300	baclofen 4	15	13.8 ± 0.4	14.4 ± 0.9‡	15.7 ± 1.1‡	17.3 ± 0.8‡	18.1 ± 1.1‡
Saline	bicuculline 1.5	14	13.9 ± 0.6	39.1 ± 2.5	24.6 ± 2.2	17.2 ± 2.2	15.3 ± 0.9
Piracetam 30	bicuculline 1.5	14	13.7 ± 0.6	24.0 ± 2.8‡	15.3 ± 1.9†	13.1 ± 1.9	11.8 ± 1.1
Saline	picrotoxin 1	13	14.8 ± 0.7	33.9 ± 2.4	30.3 ± 3.0	17.5 ± 1.2	13.8 ± 0.7
Piracetam 30	picrotoxin 1	13	15.4 ± 0.7	22.0 ± 2.2†	20.8 ± 2.4*	15.2 ± 1.0	14.8 ± 0.8

Piracetam was administered 15 min before bicuculline and simultaneously injected with baclofen and picrotoxin.
*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ vs. saline/corresponding analgesic drug-treated mice.

TABLE 2

EFFECT OF ANIRACETAM ON BACLOFEN-, BICUCULLINE-, AND PICROTOXIN-INDUCED ANTINOCICEPTION IN THE MOUSE HOT PLATE TEST (52.5°C)

Pretreatment (mg/kg, PO)	Treatment (mg/kg, SC)	Number of Mice	Licking Latency in Mice (s)				
			Before Treatment	After Treatment			
				15 min	30 min	45 min	60 min
CMC	saline	19	14.1 ± 0.4	14.5 ± 0.6	14.4 ± 0.6	14.1 ± 0.4	15.0 ± 0.6
Aniracetam 1	saline	8	15.2 ± 0.7	14.4 ± 0.7	14.2 ± 0.9	16.5 ± 1.1	14.2 ± 0.7
Aniracetam 3	saline	8	14.1 ± 0.8	14.3 ± 0.9	14.1 ± 1.1	15.6 ± 1.3	14.7 ± 1.2
Aniracetam 10	saline	8	15.1 ± 0.5	15.7 ± 0.8	16.4 ± 0.7	16.6 ± 0.9	16.2 ± 1.2
Aniracetam 30	saline	10	13.5 ± 0.7	13.1 ± 1.2	14.6 ± 0.5	12.4 ± 0.9	13.6 ± 0.6
Aniracetam 100	saline	8	15.2 ± 0.7	15.9 ± 0.6	15.1 ± 0.5	16.4 ± 0.8	17.0 ± 0.8
CMC	baclofen 4	19	14.1 ± 0.5	32.3 ± 2.2	37.6 ± 2.2	32.9 ± 2.4	28.8 ± 2.6
Aniracetam 1	baclofen 4	10	14.8 ± 0.6	20.6 ± 2.0‡	24.8 ± 2.7†	27.0 ± 2.9	26.7 ± 3.3
Aniracetam 3	baclofen 4	8	13.0 ± 0.7	20.4 ± 1.3‡	23.2 ± 2.0†	24.0 ± 2.2	21.0 ± 1.9
Aniracetam 10	baclofen 4	10	14.1 ± 0.7	21.4 ± 1.3‡	30.3 ± 2.4*	25.8 ± 2.7	22.8 ± 2.2
Aniracetam 30	baclofen 4	14	14.1 ± 0.5	22.9 ± 2.0†	28.0 ± 3.0*	27.3 ± 2.5	27.6 ± 3.0
Aniracetam 100	baclofen 4	11	13.3 ± 0.4	14.1 ± 1.8‡	20.3 ± 1.8‡	17.4 ± 1.5‡	16.9 ± 0.8‡
CMC	bicuculline 1.5	8	13.4 ± 0.7	42.4 ± 1.9	29.0 ± 2.1	14.6 ± 1.0	15.6 ± 1.1
Aniracetam 10	bicuculline 1.5	16	13.9 ± 0.4	29.9 ± 1.9‡	22.7 ± 1.7*	17.0 ± 0.7	16.3 ± 0.9
CMC	picrotoxin 1	9	15.5 ± 1.0	32.4 ± 2.9	31.9 ± 3.7	17.4 ± 2.0	15.4 ± 0.9
Aniracetam 10	picrotoxin 1	10	13.4 ± 0.6	25.1 ± 2.6†	19.4 ± 2.4*	17.6 ± 1.4	18.7 ± 1.6

Aniracetam was administered 30 min before bicuculline and 15 min before baclofen and picrotoxin.
*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ vs. mice treated with carboxymethylcellulose (CMC)/corresponding analgesic drug.

RESULTS

Effect of Piracetam and Aniracetam on Antinociception Induced by Direct and Indirect $GABA_B$ Receptor Activation

As shown in Tables 1 and 2 and in Fig. 2, piracetam (1-300 mg/kg, IP, and 10 μg per mouse, ICV), and aniracetam (1-100 mg/kg, PO) did not modify the pain threshold in the mouse hot plate test. Similarly, piracetam (30 mg/kg, IP) and aniracetam (10 mg/kg, PO) did not modify the number of abdominal constrictions in the mouse writhing test (Fig. 3).

Piracetam, starting from 5 mg/kg up to 100 mg/kg, reduced baclofen analgesia in a dose-dependent manner. The dose of 100 mg/kg was able to completely prevent this effect up to 30 min after administration, whereas the dose of 300 mg/kg exerted its antagonism at all times that it was tested (Table 1).

Subconvulsant doses of bicuculline (1.5 mg/kg, SC) and picrotoxin (1 mg/kg, SC) showed analgesic properties with an efficacy similar to that of baclofen. Their latency and duration of action, however, were shorter than those of baclofen whose antinociceptive action reaches its maximum after 30 min and persists up to 60 min. Bicuculline and picrotoxin reached their maximum effect 15 min after administration and the antinociceptive effect completely disappeared after 45 min. Piracetam (30 mg/kg, IP), administered simultaneously with picrotoxin and 15 min before bicuculline, showed the capability to prevent the increase of pain threshold at both 15 and 30 min after analgesic drugs administration (Table 1). A statistically significant reduction of baclofen antinociception was also obtained with aniracetam, at doses ranging between 1 and 30 mg/kg, whereas a full antagonism was reached when 100 mg/kg PO of aniracetam were administered. Aniracetam, moreover, reduced bicuculline and picrotoxin analgesia when administered at the dose of 10 mg/kg PO 30 and 15 min, respectively, before the two analgesic compounds (Table 2). ICV injection of piracetam at the dose of 1 μg per mouse did not antagonize baclofen-induced antinociception whereas 10 μg per mouse significantly reduced it (Fig. 2).

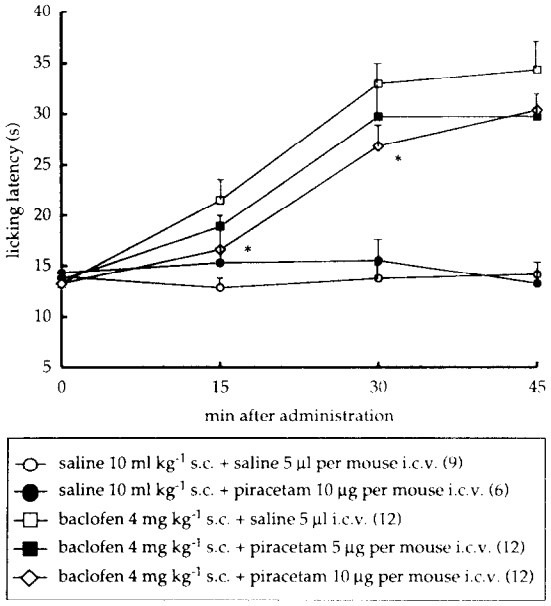


FIG. 2. Effect of piracetam on baclofen-induced analgesia after ICV administration. Piracetam was injected 5 min before analgesic treatment. Vertical lines give SEM; the number of mice is shown in parentheses. *$p < 0.05$ compared with baclofen-treated mice.

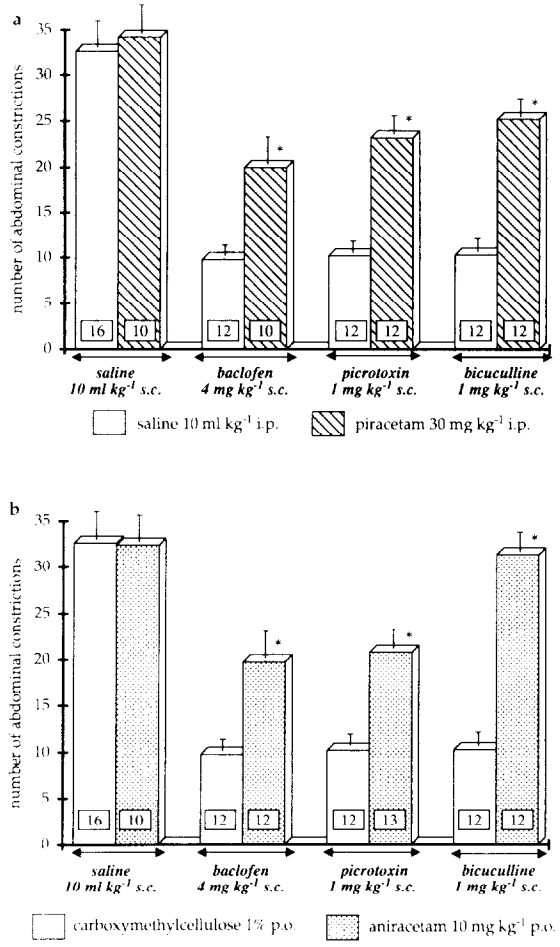


FIG. 3. Effect of (a) piracetam and (b) aniracetam on baclofen-, picrotoxin-, and bicuculline-induced antinociception in the mouse acetic acid abdominal constriction test. Piracetam was simultaneously administered with baclofen and picrotoxin whereas bicuculline was injected 15 min after piracetam. Aniracetam was administered 15 min before picrotoxin and baclofen. Bicuculline was injected 30 min after aniracetam. The number of writhes was counted between 15 and 25 min after bicuculline and between 30 and 40 min after baclofen and picrotoxin administration. Vertical lines represent SEM; the number of mice is shown in parentheses. *$p < 0.001$ vs. corresponding analgesic drug-treated mice.

The effect of piracetam and aniracetam on antinociception induced by baclofen, bicuculline, and picrotoxin was also tested using the abdominal constriction test in mice. Both nootropic drugs were able to antagonize the analgesic effect produced by SC injection of all three analgesic compounds, as illustrated in Fig. 3.

Effect of Piracetam, Aniracetam, and CGP 35348 on Pain Threshold Increased by non-GABAergic Drugs

Piracetam, up to the dose of 30 mg/kg IP, did not modify the enhanced paw licking latency in the mouse hot plate

test induced by analgesic compounds such as morphine (7 mg/kg, SC), physostigmine (0.2 mg/kg, SC), clomipramine (25 mg/kg, SC), and diphenhydramine (20 mg/kg, SC), which are considered as nonacting through the GABA system. However, the dose of 50 mg/kg IP antagonized morphine-induced antinociception and diminished the clomipramine analgesic effect. The enhanced pain threshold induced by physostigmine and diphenhydramine was significantly reduced only when piracetam was given at the dose of 100 mg/kg IP (Table 3). Aniracetam, at the doses of 10 and 30 mg/kg PO, was uneffective in modifying non-GABAergic analgesia. The dose of 100 mg/kg PO showed the capability of preventing clomipramine- and diphenhydramine-induced antinociception, but did not affect morphine and physostigmine analgesic effect (Table 3).

Figure 4 shows the results obtained with the abdominal constriction test in mice showing the piracetam and aniracetam uneffectiveness in preventing morphine (1 mg/kg, SC), physostigmine (0.2 mg/kg, SC), clomipramine (25 mg/kg, SC), and diphenhydramine (20 mg/kg, SC) induced analgesia at the doses used (10 and 100 mg/kg).

The increased paw licking latency due to the abovementioned analgesic drugs was not affected by pretreating mice with CGP 35348 up to the dose of 0.5 μg per mouse ICV, as reported in Table 4. A progressive increase of the dose of CGP 35348 (from 0.5 to 5 μg per mouse) prevented antinociception induced by non-GABAergic drugs in the following order: diphenhydramine, clomipramine, physostigmine, and morphine. Table 4 shows that CGP 35348, at the dose of 0.5 μg per mouse, was able to reduce diphenhydramine (20 mg/kg, SC) analgesia. At the dose of 2.5 μg per mouse, CGP 35348 completely prevented diphenhydramine effect and also reduced the enhanced pain threshold induced by clomipramine (25 mg/kg, SC) administration. At higher doses (5 μg per mouse), CGP 35348 completely antagonized physostigmine (0.2 mg/kg) analgesic effect and significantly prevented morphine-induced (7 mg/kg) antinociception.

TABLE 3

EFFECT OF PIRACETAM AND ANIRACETAM ON DIPHENHYDRAMINE-, CLOMIPRAMINE-, PHYSOSTIGMINE-, AND MORPHINE-INDUCED ANTINOCICEPTION IN THE MOUSE HOT PLATE TEST (52.5°C)

Pretreatment (mg/kg)	Treatment (mg/kg SC)	n	Licking Latency in Mice (s)		
			Before Treatment	After Treatment	
				15 min	30 min
Saline IP	saline	25	15.0 ± 0.7	14.0 ± 0.7	14.4 ± 0.8
Saline IP	diphenhydramine 20	12	15.4 ± 0.6	24.5 ± 1.3	22.2 ± 1.4
Piracetam 30 IP	diphenhydramine 20	12	16.0 ± 0.5	22.9 ± 1.2	20.5 ± 1.2
Piracetam 50 IP	diphenhydramine 20	13	15.7 ± 0.7	25.8 ± 1.5	24.8 ± 2.0
Piracetam 100 IP	diphenhydramine 20	13	14.5 ± 0.6	20.5 ± 1.0*	20.1 ± 1.1
Saline IP	clomipramine 25	10	14.8 ± 0.9	22.0 ± 1.8	25.0 ± 2.3
Piracetam 30 IP	clomipramine 25	10	14.6 ± 0.7	20.3 ± 2.0	22.9 ± 2.4
Piracetam 50 IP	clomipramine 25	10	13.4 ± 0.6	19.8 ± 1.7	18.0 ± 1.3*
Saline IP	physostigmine 0.2	12	14.7 ± 0.9	34.0 ± 2.3	32.5 ± 3.2
Piracetam 30 IP	physostigmine 0.2	12	13.5 ± 0.6	33.2 ± 2.7	33.7 ± 3.0
Piracetam 50 IP	physostigmine 0.2	13	15.6 ± 0.7	36.5 ± 2.3	40.0 ± 2.1
Piracetam 100 IP	physostigmine 0.2	13	13.3 + 0.6	23.5 + 2.8*	26.5 ± 2.8
Saline IP	morphine 7	13	15.4 ± 0.6	26.6 ± 1.6	26.9 ± 2.2
Piracetam 30 IP	morphine 7	10	15.9 ± 0.7	25.0 ± 2.1	26.6 ± 2.5
Piracetam 50 IP	morphine 7	10	13.4 ± 0.6	16.0 ± 1.2‡	17.1 ± 1.5‡
CMC PO	saline	10	14.5 ± 0.6	15.0 ± 0.7	15.2 ± 0.8
CMC PO	diphenhydramine 20	10	13.7 ± 0.4	25.4 ± 1.1	23.6 ± 1.4
Aniracetam 10 PO	diphenhydramine 20	10	14.7 ± 0.5	24.8 ± 0.8	22.1 ± 0.5
Aniracetam 30 PO	diphenhydramine 20	9	14.4 ± 0.7	26.7 ± 2.0	24.0 ± 1.8
Aniracetam 100 PO	diphenhydramine 20	9	14.3 ± 0.6	18.5 ± 2.5*	16.4 ± 1.2†
CMC PO	clomipramine 25	9	15.5 ± 0.5	24.3 ± 0.9	32.7 ± 1.8
Aniracetam 10 PO	clomipramine 25	10	14.6 ± 0.7	23.1 ± 1.1	32.5 ± 2.4
Aniracetam 30 PO	clomipramine 25	10	15.9 ± 0.7	25.6 ± 1.9	31.1 ± 2.6
Aniracetam 100 PO	clomipramine 25	9	14.4 ± 0.7	18.1 ± 1.9†	21.1 ± 2.7‡
CMC PO	physostigmine 0.2	12	13.5 ± 0.4	34.5 ± 2.7	36.3 ± 3.1
Aniracetam 10 PO	physostigmine 0.2	13	14.7 ± 0.6	31.1 ± 3.2	36.4 ± 2.6
Aniracetam 30 PO	physostigmine 0.2	9	15.1 ± 0.8	38.3 ± 2.5	37.4 ± 3.2
Aniracetam 100 PO	physostigmine 0.2	10	16.7 ± 0.6	36.0 ± 2.4	39.0 ± 2.0
CMC PO	morphine 7	12	13.8 ± 0.7	28.1 ± 3.0	29.8 ± 3.1
Aniracetam 10 PO	morphine 7	12	14.9 ± 0.6	29.0 ± 2.9	34.3 ± 2.7
Aniracetam 30 PO	morphine 7	12	14.3 ± 0.5	22.0 ± 2.4	28.6 ± 2.6
Aniracetam 100 PO	morphine 7	10	14.5 ± 0.6	27.3 ± 1.9	27.8 ± 1.8

*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ vs. corresponding analgesic drug-treated mice.

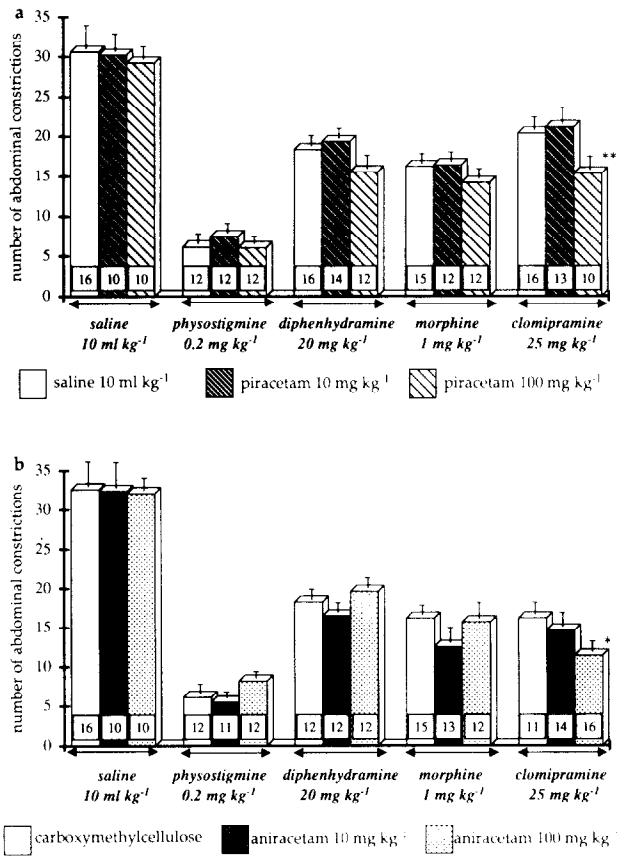


FIG. 4. Effect of (a) piracetam and (b) aniracetam on physostigmine, diphenhydramine, morphine, and clomipramine-induced analgesia in the mouse abdominal constriction text. Piracetam (IP), physostigmine (SC), morphine (SC), and clomipramine (SC) were administered 30 min before the test. Diphenhydramine (SC) was administered 15 min after piracetam administration. Aniracetam (PO) was administered 15 min before physostigmine, morphine, and clomipramine. Diphenhydramine was administered 30 min after aniracetam administration. The number of writhes was counted between 30 and 40 min after piracetam injection and between 45 and 55 min after aniracetam administration. The number of mice is shown in parentheses. Vertical lines represent the SEM. $*p < 0.05$, $**p < 0.01$ compared with clomipramine-treated mice.

Antagonism by Piracetam and Aniracetam of Baclofen-Induced Motor Incoordination

Baclofen (6 mg/kg, SC) produced impairment of rota-rod performance in mice (Fig. 5). The motor incoordination reached its maximum 45 min after baclofen administration and then this effect was slowly reduced. Piracetam, at the dose of 30 mg/kg IP, simultaneously administered with baclofen, showed the ability to significantly reduce the impairment of rota-rod performance, as shown in Fig. 5. Aniracetam, at the dose of 10 mg/kg PO injected 15 min before baclofen, completely antagonized the decrease of the endurance time on the rotating treadmill (Fig. 5).

DISCUSSION

The results obtained have shown a very similar pharmacological behaviour between the effect of CGP 35348 and the two nootropic drugs piracetam and aniracetam. Piracetam and aniracetam, like CGP 35348, were able to reduce analgesia mediated via $GABA_B$ receptor activation. Such reduction was obtained not only on antinociception induced by baclofen (a direct $GABA_B$ agonist), but also on antinociception induced by subconvulsant doses of bicuculline and picrotoxin (indirect $GABA_B$ agonists). Malcangio et al. (18) have shown that bicuculline and picrotoxin, acting as $GABA_A$ antagonists, might be able to release GABA and thus induce antinociception. The reduction of GABAergic analgesia observed after piracetam, aniracetam, and CGP 35348 administration was detected in both of the analgesic tests used (hot plate and abdominal constriction). The two nootropic drugs as well as CGP 35348 were also able to antagonize morphine, physostigmine, clomipramine, and diphenhydramine antinociception. However, the antagonism towards the above mentioned non-GABAergic drugs was observed only in the hot plate test. Moreover, doses of CGP 35348, piracetam, and aniracetam higher than those able to interfere with baclofen, bicuculline, and picrotoxin antinociception were necessary. No interference with non-GABAergic antinociception was observed in the abdominal constriction test also following high doses of piracetam and aniracetam. Similar results concerning the lack of antagonism in the abdominal constriction test on non-GABAergic analgesia using CGP 35348 have been previously reported (17). In the abdominal constriction test, however, piracetam and aniracetam, unlike CGP 35348, never were able to completely prevent baclofen antinociception, showing, therefore, a lower potency than CGP 35348.

The pharmacological analogy among the three compounds (piracetam, aniracetam, and CGP 35348) is validated by the fact that, in both of the analgesic tests, they were able to selectively reduce GABAergic antinociception when administered at the minimal effective doses. At the moment, we do not have any convincing explanation about the lack of antagonism in the abdominal constriction test of non-GABAergic analgesia by high doses of piracetam, aniracetam, and CGP 35348. It should be taken into account, however, that the abdominal constriction test induces, unlike the hot plate test, a prolonged inflammatory pain that activates endogenous antinociceptive mechanisms that are different from those involved in acute pain.

Piracetam action on analgesia appears centrally mediated because it reduced baclofen antinociception also when injected ICV. Aniracetam, because of its poor water solubility, was not tested after ICV administration and only the oral route of administration was used.

The $GABA_B$ antagonist CGP 35348 was found to be able to selectively prevent baclofen-induced motor incoordination (17). A similar effect has now also been obtained with piracetam and aniracetam, which respectively reduced and completely antagonized the impairment of rota-rod performance induced by baclofen administration, without modifying the endurance time on the rota-rod treadmill when given alone. Therefore, because the two nootropic drugs, like CGP 35348, were able to selectively antagonize both baclofen-induced motor incoordination and analgesia, we could hypothesize that the nootropic activity of piracetam and aniracetam might be due to a reduction of the inhibitory effects of endogenous GABA. Piracetam and aniracetam might, therefore, interfere either directly with $GABA_B$ receptors sharing the same mechanism as CGP 35348, or indirectly with some other $GABA_B$ postreceptorial events.

Binding studies have demonstrated that only high pira-

TABLE 4

EFFECT OF CGP 35348 ON DIPHENHYDRAMINE-, CLOMIPRAMINE-, PHYSOSTIGMINE-, AND MORPHINE-INDUCED ANTINOCICEPTION IN THE MOUSE HOT PLATE TEST (52.5°C)

Pretreatment (μg per Mouse, ICV)	Treatment (mg/kg, SC)	Number of Mice	Licking Latency in Mice (s)		
			Before Treatment	After Treatment	
				15 min	30 min
Saline	saline	10	14.8 ± 0.9	13.4 ± 0.7	14.8 ± 1.3
CGP-35348 5	saline	10	13.5 ± 0.7	13.1 ± 1.2	14.6 ± 0.5
Saline	diphenhydramine 20	10	15.8 ± 0.6	24.0 ± 1.0	24.1 ± 0.7
CGP-35348 0.5	diphenhydramine 20	11	15.4 ± 1.0	19.5 ± 1.7*	24.0 ± 1.6
CGP-35348 2.5	diphenhydramine 20	7	14.0 ± 0.9	17.7 ± 1.8‡	14.7 ± 1.3‡
Saline	clomipramine 25	7	16.8 ± 0.9	20.1 ± 0.7	23.0 ± 1.5
CGP-35348 0.5	clomipramine 25	10	14.6 ± 0.6	17.6 ± 1.1	21.6 ± 0.9
CGP-35348 2.5	clomipramine 25	10	15.3 ± 0.7	15.5 ± 1.2†	17.3 ± 1.0†
Saline	physostigmine 0.2	8	15.5 ± 0.8	37.2 ± 3.8	37.6 ± 1.9
CGP-35348 2.5	physostigmine 0.2	8	16.0 ± 0.9	38.7 ± 2.3	39.8 ± 2.0
CGP-35348 5	physostigmine 0.2	13	15.1 ± 0.8	16.8 ± 1.9‡	16.3 ± 1.9‡
Saline	morphine 7	9	14.9 ± 0.8	35.0 ± 3.1	37.8 ± 2.4
CGP-35348 5	morphine 7	14	13.6 ± 1.1	20.3 ± 1.9‡	20.6 ± 2.8†

All pretreatment drugs were administered 5 min before treatment injection.
*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ vs. saline/corresponding analgesic drug-treated mice.

cetam and aniracetam concentrations (IC_{50} ranging between 0.2 and 50 mM) inhibited GABA, $GABA_A$, opiate, serotonin, and benzodiazepine receptor binding sites in the central nervous system (1,22). Such concentrations were much higher than those able to exert a nootropic effect in vivo; therefore, in spite of the fact that piracetam and aniracetam are cyclic derivatives of GABA, their action cannot be explained by a direct GABA receptor antagonism. In fact, although no literature on the affinity of piracetam and aniracetam for $GABA_B$ receptor is available, the very high concentrations necessary to inhibit GABA binding seem to exclude, for these two drugs, an action mechanism similar to that described for CGP 35348 that directly blocks $GABA_B$ receptors. Because the inhibitory activity of $GABA_B$ receptors is mediated by pertussis toxin-sensitive G-proteins (9,15) and baclofen-induced antinociception is prevented by pretreatment with pertussis toxin (14), we can hypothesize an interference of 2-pyrrolidinone compounds directly on G-proteins or on G-protein-mediated effects.

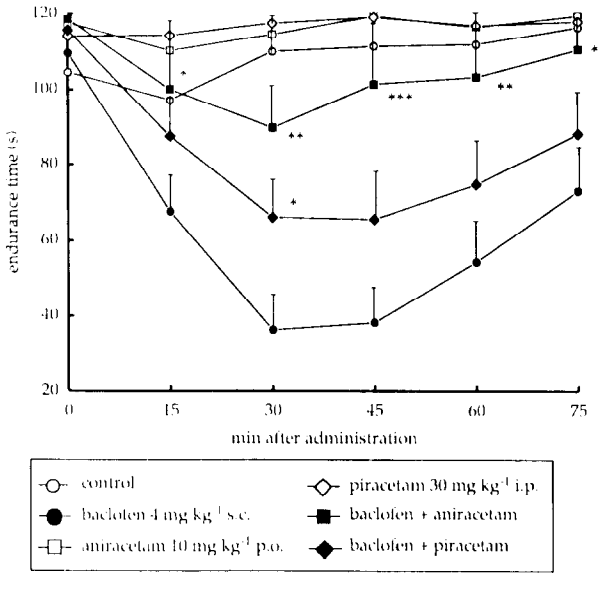


FIG. 5. Effect of piracetam and aniracetam on baclofen-induced impairment of rota-rod performance in the mouse rota-rod test. Piracetam was simultaneously injected with baclofen while aniracetam was administered 15 min before baclofen. Endurance time of mice on the treadmill was recorded before treatment and then starting 15 min after baclofen treatment up to 75 min. The number of mice ranging was between 8 and 16. Vertical lines represent SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. baclofen-treated mice.

An interaction of piracetam and aniracetam metabolites with GABA receptors seems very unlikely. It has been reported, in fact, that piracetam is excreted pratically unchanged in the urine (10). Moreover, although aniracetam is transformed in vivo to amide and acid (10), there is no evidence of a possible interaction of these metabolites with GABA receptors.

We can exclude that the antagonism of baclofen analgesia produced by piracetam and aniracetam may be due to some behavioral side effects elicited by these two drugs. At all doses used of piracetam and aniracetam, the normal behavior of mice appeared on observation to be wholly comparable to that of the controls and the animals did not exhibit any impairment on the rota-rod test.

Because it is necessary for the reversal of cognitive impairment to use doses of the three nootropic drugs that are higher than those we found selective for GABAergic analgesia, we cannot exclude that the nootropic effect might be mediated by inhibition not only of GABAergic system, but also of other inhibitory mechanisms such as those involved in opioid, cholinergic, and serotoninergic analgesias. To ameliorate cognitive impairment in mice it is, in fact, necessary to use doses

equal to or higher than 30 mg/kg IP for piracetam (8), 10 mg/kg PO for aniracetam (6), and 75 mg/kg IP for CGP-35348 (2). There are no data regarding the nootropic effect of CGP 35348 after ICV administration, but it has been previously reported that the dose of 100 mg/kg IP is approximately corresponding to 2.5 μg per mouse ICV (17). By using these nootropic doses, we obtained a complete block of the baclofen antinociception in the hot plate test as well as a reduction of non-GABAergic antinociception.

In conclusion, these findings may suggest a $GABA_B$ receptor-mediated component in piracetam and aniracetam nootropic effect. This is supported by the fact that, like $GABA_B$ antagonists, they can prevent baclofen-induced antinociception and muscle relaxation as well as improve learning in mice.

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