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Effects of K^+ channel blockers and openers on antinociception induced by agonists of 5-HT_{1A} receptors

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Abstract

The modulation by K^+ channel-acting drugs of the antinociceptive effect of several 5-HT_{1A} receptor agonists was examined with the hot plate test in mice. All the 5-HT_{1A} receptor agonists tested induced dose-dependent antinociception, the order of potency being (±)-8-hydroxy-2-(di-*n*-propyl-amino)tetralin (8-OH-DPAT) > buspirone ⩾ lesopitron ⩾ tandospirone. The blockers of ATP-sensitive K^+ channels (K_{ATP}) gliquidone and glipizide (1–4 and 16–64 μg/mouse i.c.v., respectively) reduced the antinociceptive effect of 8-OH-DPAT, whereas cromakalim (32–64 μg/mouse i.c.v.), an opener of K_{ATP} channels, enhanced the effect. In contrast, 4-aminopyridine (25–250 ng/mouse i.c.v.) and tetraethylammonium (10–20 μg/mouse i.c.v.), which antagonize several non-ATP-dependent K^+ conductances, were inactive. The same results were found with other agonists of 5-HT_{1A} receptors (lesopitron, buspirone and tandospirone): gliquidone inhibited whereas cromakalim increased their antinociceptive effects. None of the K^+ channel-acting drugs modified the binding of [^{3}H]8-OH-DPAT to hippocampal membranes, whereas all the 5-HT_{1A} receptor agonists displaced the ligand. These results suggest that ATP-sensitive K^+ conductances are involved in the antinociception induced by agonists of 5-HT_{1A} receptors.

Keywords: Antinociception; 5-HT_{1A} receptor; 8-OH-DPAT ((±)-8-hydroxy-2-(di-*n*-propylamino)tetralin); ATP-sensitive K^+ channel; Sulfonylurea; Gliquidone; Cromakalim; Receptor binding

1. Introduction

Drugs acting on K^+ channels modulate the antinociception induced by agonists of several receptors. Blockers of ATP-sensitive K^+ (K_{ATP}) channels, such as sulfonylurea derivatives (Edwards and Weston, 1993), inhibit antinociception induced by morphine or [D-Pen^{2,5}]-enkephalin through μ- or δ_1-opioid receptors respectively (Ocaña et al., 1990; Wild et al., 1991; Narita et al., 1992; Welch and Dunlow, 1993; Roane and Boyd, 1993), and also inhibit antinociception induced by the α_2-adrenoceptor agonist clonidine (Ocaña and Baeyens, 1993; Raffa and Martinez, 1995) and by the adenosine A_1 receptor agonist phenylisopropyladenosine (R-PIA) (Ocaña and Baeyens, 1994). On the other hand, K_{ATP} channel openers, such as pinacidil or cromakalim (Longman and Hamilton, 1992), enhance morphine-induced antinociception (Vergoni et al., 1992; Narita et al., 1993; Ocaña et al., 1995) and R-PIA-induced antinociception (Ocaña and Baeyens, 1994). The modulatory effect of K_{ATP} channel-acting drugs seems rather selective, since blockers of other types of K^+ channels (including Ca^{2+}- and voltage-dependent K^+ channels) such as 4-aminopyridine or tetraethylammonium (Cook and Quast, 1990), have no effect on morphine-, [D-Pen^{2,5}]-enkephalin-, R-PIA- or clonidine-induced antinociception (Ocaña et al., 1990, 1993; Wild et al., 1991; Narita et al., 1992; Welch and Dunlow, 1993; Ocaña and Baeyens, 1993, 1994). Although these agonists stimulate different receptors, electrophysiological studies have demonstrated that all of them open a very similar type of K^+ channel (see

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North, 1989 for a review), which may explain their common sensitivity to K_{ATP} channel-active drugs. These receptors also share a transduction mechanism which mediates their action on K^+ channels: a pertussis toxin-sensitive G protein (North, 1989).

In addition to K_{ATP} channels, other types of K^+ conductances are also involved in antinociception mediated by receptors coupled to G_o/G_i proteins. The antinociceptive effect of baclofen, a $GABA_B$ receptor agonist, was inhibited by 4-aminopyridine and tetraethylammonium, whereas it was not modified by sulfonylureas (Ocaña and Baeyens, 1993). Similarly, the antinociception induced by the δ_2-opioid receptor agonist [D-Ala^2]-deltorphin II is also antagonized by tetraethylammonium but not by sulfonylureas (Wild et al., 1991). In the light of these findings, it seems that different types of K^+ channels participate in the antinociception induced by drugs that activate receptors coupled to pertussis toxin-sensitive G protein: agonists of μ- or δ_1-opioid, adenosine A_1 and α_2-adrenoceptors induced a K_{ATP}-sensitive antinociception, whereas agonists of $GABA_B$ and δ_2-opioid receptors induced an antinociceptive effect modulated by non-ATP-dependent K^+ channels.

The involvement of serotonin in the control of nociception at both the spinal and supraspinal level has been widely documented (for reviews see Richardson, 1990; Eide and Hole, 1993). Among the many serotonin receptors described up to now, 5-HT_{1A} belongs to the family of receptors coupled to G_o/G_i proteins (Zifa and Fillion, 1992). Functional 5-HT_{1A} receptors have been located in nociceptive pathways in the spinal cord (Zemlan et al., 1994) and in the nucleus raphe magnus (El-Yassir and Fleetwood-Walker, 1990). Furthermore, 5-HT_{1A} receptor agonists produce antinociception when injected either intrathecally (Eide and Hole, 1991; Xu et al., 1994), or systemically (Bragin et al., 1989; Giordano and Rogers, 1989).

The activation of 5-HT_{1A} receptors produces neuronal hyperpolarization (Innis et al., 1988) by enhancement of K^+ conductances (Bobker and Williams, 1990; Penington et al., 1993), and this effect is pertussis toxin-sensitive (Andrade et al., 1986; Zgombick et al., 1989; Penington et al., 1993), suggesting that G_o/G_i protein participates in the transduction pathway. In summary, the 5-HT_{1A} receptors share transduction mechanisms with some other receptors that are involved in antinociception and are coupled to K^+ channels; however, the type(s) of K^+ channels linked to 5-HT_{1A} receptors is not still clear, and the role of these channels on 5-HT_{1A}-mediated antinociception has not been examined. The present study was designed to evaluate whether the antinociceptive effect of 5-HT_{1A} receptor agonists can be modulated by blockers and openers of K^+ channels, and to determine which type(s) of these channels control this effect.

2. Materials and methods

2.1. Animals

Female Swiss CD1 mice weighing 25–30 g (Criffa, Spain) were used in the behavioral experiments, and male Wistar rats weighing 150–300 g (Interfauna Ibérica, Spain) were used in binding assays. All animals were kept in temperature-controlled rooms at 21 ± 2°C, with 12 h dark/light cycles (lights on at 8:00 and off at 20:00) and free access to food and water. The behavioral experiments were done between 9:00 and 13:00 h.

The animals were handled according to guidelines for the care of laboratory animals and ethical guidelines for research in experimental pain with concious animals (Zimmermann, 1983), and in compliance with EEC Council Directive 86/609.

2.2. Drugs

The 5-HT_{1A} receptor agonists used were (±)-8-hydroxy-2-(di-*n*-propyl-amino)tetralin hydrobromide (8-OH-DPAT; Sigma), lesopitron hydrochloride (Laboratorios Dr. Esteve), buspirone hydrochloride (Sigma) and tandospirone hydrochloride (Laboratorios Dr. Esteve). Cromakalim (Sigma) represented the opener, and gliquidone (Europharma) and glipizide (Farmitalia Carlo Erba) the blockers of K_{ATP} channels. Finally, tetraethylammonium bromide (Sigma) and 4-aminopyridine (Sigma) were used as blockers of non-ATP-dependent K^+ channels. All K^+ channel-acting drugs were dissolved in 1% Tween 80 in deionized water. The 5-HT_{1A} receptor agonists were dissolved in saline solution.

For binding experiments, 8-OH-DPAT, [propyl-2,3-ring-1,2,3-^{3}H] (Dupont) was used as the labeled ligand, and serotonin creatinine sulfate (Merck) was used to determine non-specific binding.

2.3. Measurement of antinociception

Antinociception was assessed by the hot plate test at 50 ± 0.1°C. Mice were placed on a metal surface at this temperature, and the latency to the beginning of forepaw licking was recorded; to prevent severe lesions, the animals were removed from the plate after a 'cut-off' time of 75 s. The results were expressed as percentage of maximal possible antinociception, according to the equation: % antinociception = [(LTT − LTC)/(CT − LTC)] × 100, where LTT is the latency in treated mice; LTC is the latency in the control group; and CT is the 'cut-off' time or maximum possible time on the plate (75 s).

All 5-HT_{1A} receptor agonists were injected subcutaneously (s.c.) in a volume of 5 ml/kg, 30 min before the animals were tested. K^+ channel openers and

blockers were injected intracerebroventricularly (i.c.v., 5 μl/mouse) 30 min before the test. The i.c.v. procedure has been described previously (Ocaña et al., 1993); briefly, unanesthetized mice were gently restrained, and the drug was injected with an adapted Hamilton syringe at the location described by Haley and McCormick (1957). Control animals received the same treatment except that vehicle (1% Tween 80 in deionized water) was injected in place of drugs. At the end of the experiments, the trajectory of the injection was checked and the results from animals with misaimed injections were discarded.

2.4. 5-HT_{1A} receptor binding assays

Radioligand receptor binding studies were done according to the published procedure (Peroutka, 1986) with some modifications. The rats were killed by decapitation and the brains rapidly removed. The hippocampus was dissected and homogenized in 10 volumes of 50 mM Tris-HCl buffer (pH 7.7). The homogenate was centrifuged at $48000 \times g$ for 10 min, and the resulting pellet was resuspended in Tris buffer and incubated at 37°C for 10 min and then centrifuged at $48000 \times g$ for 10 min. The final pellet was suspended in 100 volumes of the binding buffer (50 mM Tris pH 7.7, 0.1% ascorbic acid, 4 mM $CaCl_2$ and 10 μM pargyline).

Tissue preparations (900 μl) were incubated in triplicate with 1 nM [^{3}H]8-OH-DPAT in the presence of unlabeled drugs for 15 min at 37°C. The final volume was 1 ml. Non-specific binding was determined with 10 μM 5-HT. The binding reaction was terminated by filtration under vacuum through GF/B filters. Radioactivity of separate aliquots was determined with a scintillation counter.

2.5. Statistical analysis

The doses of 5-HT_{1A} receptor agonists that produced half of the maximal antinociception (ED_{50}) were calculated from the dose-response curves estimated with the GraphPad Inplot computer program (GraphPad Software). The same program was used to calculate the doses of sulfonylureas that reduce the antinociceptive effect of 5-HT_{1A} receptor agonists by 50% (ID_{50}). The data from the control and the treated groups were compared using one-way analysis of variance (ANOVA) followed by a Newman-Keuls test. $P < 0.05$ was accepted as the level of significance.

All competition binding data were analyzed by nonlinear iterative curve fitting procedures (Munson and Rodbard, 1980) with the EBDA-LIGAND computer program (McPherson, 1983), and the affinity estimate (K_i) was calculated from IC_{50} values with the method of Cheng and Prusoff (1973).

3. Results

3.1. Effects of 5-HT_{1A} receptor agonists in the hot plate test in mice

The subcutaneous administration of the 5-HT_{1A} receptor agonists 8-OH-DPAT (0.5–4 mg/kg), lesopitron (5–40 mg/kg), buspirone (5–40 mg/kg) and tandospirone (10–80 mg/kg) produced a dose-dependent antinociceptive effect in the hot plate test (Fig. 1). The dose-response curves for all drugs were parallel and only their ED_{50} differed: 8-OH-DPAT (ED_{50}, 1.09 mg/kg; 95% confidence interval, 0.80–1.49) < buspirone (18.41 mg/kg; 13.87–24.54) ≤ lesopitron (24.68 mg/kg; 19.29–31.59) ≤ tandospirone (36.17 mg/kg; 21.92–59.69) (Fig. 1).

3.2. Effects of 4-aminopyridine and tetraethylammonium on the antinociception induced by 8-OH-DPAT

To test the possible influence of a wide range of K^+ channel types on 5-HT_{1A}-induced antinociception, 4-aminopyridine and tetraethylammonium were administered as a pretreatment to the pure 5-HT_{1A} receptor agonist 8-OH-DPAT.

The percentage of antinociception induced by 2 mg/kg of subcutaneous 8-OH-DPAT (65.00 ± 8.47; mean ± S.E.M.) was not significantly modified by the i.c.v. administration of 4-aminopyridine (25 ng/mouse: 62.12 ± 8.70; 250 ng/mouse: 71.24 ± 8.13) or tetraethylammonium (10 μg/mouse: 75.97 ± 7.89; 20 μg/mouse: 72.09 ± 7.66). None of the doses of 4-aminopyridine or tetraethylammonium changed the latency to forepaw licking in control animals.

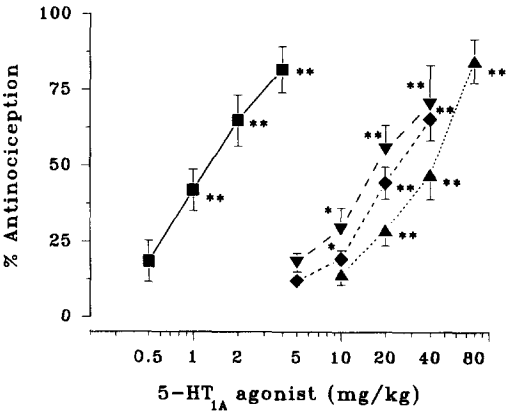

Fig. 1. Percentage of antinociception induced by s.c. administration of increasing doses of the 5-HT_{1A} receptor agonists: (■) 8-OH-DPAT (0.5–4 mg/kg); (▼) buspirone (5–40 mg/kg); (◆) lesopitron (5–40 mg/kg); and (▲) tandospirone (10–80 mg/kg) in a hot plate test in mice. Results are represented as the means ± S.E.M. of the values obtained in at least eight animals. Significant differences with respect to control (ANOVA followed by Newman Keuls test): * $P < 0.05$; ** $P < 0.01$.

3.3. Effects of K_{ATP} channel blockers and openers on the antinociception induced by 8-OH-DPAT

The possible modulation by K_{ATP} channels of 5-HT_{1A}-induced antinociception was evaluated by testing the influence of K_{ATP} channel-acting drugs on the antinociceptive effect of 8-OH-DPAT.

The i.c.v. administration of the K_{ATP} channel blockers gliquidone (1–4 μg/mouse) and glipizide (16–64 μg/mouse) produced a dose-dependent antagonism of the antinociception induced by 8-OH-DPAT (2 mg/kg s.c.) (Fig. 2). Doses of gliquidone greater than 4 μg/mouse did not produce a greater degree of antagonism (data not shown), and doses of glipizide higher than 64 μg/mouse were not tested due to solubility problems. The ID_{50} of gliquidone against 8-OH-DPAT-induced antinociception [1.69 (0.36–7.75; 95% confidence interval) μg/mouse] was lower than that of glipizide [19.20 (15.55–23.70) μg/mouse]. None of the doses of gliquidone or glipizide significantly modified the latency to forepaw licking in control animals (data not shown).

When we tested the effect of gliquidone (2 and 4 μg/mouse i.c.v.) on the antinociception produced by different doses of 8-OH-DPAT, a dose-dependent displacement to the right of the 8-OH-DPAT dose-response curve was observed (Fig. 3). The ED_{50} of 8-OH-DPAT was increased from 1.09 mg/kg (0.80–1.49; 95% confidence interval) in vehicle-pretreated animals, to 2.57 mg/kg (2.38–2.78) and 6.23 mg/kg (3.93–9.88) in the animals pretreated with 2 and 4 μg/mouse of gliquidone respectively. In contrast, the i.c.v. administration of cromakalim (32 and 64 μg/mouse), an opener of K_{ATP} channels, produced a dose-dependent displacement to the left of the 8-OH-DPAT dose-response curve, decreasing its ED_{50} from 1.09 mg/kg (0.80–1.49) in vehicle-pretreated animals, to 0.73 mg/kg (0.57–0.92) and 0.33 mg/kg (0.20–0.53) in the animals pretreated with 32 and 64 μg/mouse of cromakalim (Fig. 4). At these doses, cromakalim did not significantly modify the latency to forepaw licking in control animals (data not shown).

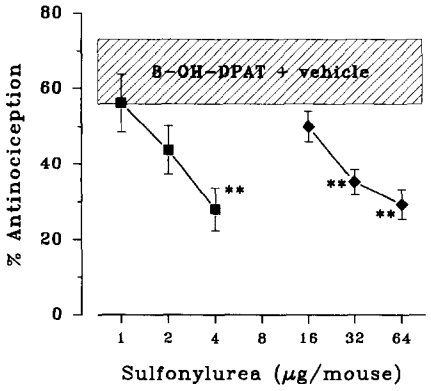

Fig. 2. Effect of i.c.v. administration of several doses of the K_{ATP} channel blockers (■) gliquidone (1–4 μg/mouse) and (◆) glipizide (16–64 μg/mouse) on antinociception induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT (2 mg/kg s.c.). The shaded area represents the effect of 8-OH-DPAT plus i.c.v. administered vehicle (1% Tween 80 in deionized water). Results are represented as the means ± S.E.M. of the values obtained in at least eight animals. Significant differences with respect to the vehicle group (ANOVA followed by Newman-Keuls test): ** $P < 0.01$.

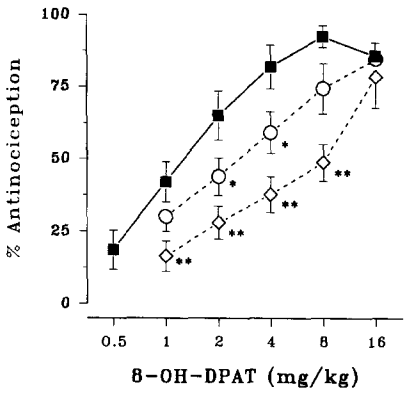

Fig. 3. Effect of the K_{ATP} channel blocker gliquidone at the doses of 2 (○) and 4 (◇) μg/mouse i.c.v. on the dose-response curve of antinociception induced by (■) 8-OH-DPAT (0.5–8 mg/kg s.c.) in the hot plate test. Results are represented as the means ± S.E.M. of the values obtained in at least eight animals. Significant differences with respect to the vehicle group (ANOVA followed by Newman-Keuls test): * $P < 0.05$; ** $P < 0.01$.

3.4. Effects of gliquidone and cromakalim on the antinociception induced by other agonists of 5-HT_{1A} receptors

To test whether the effect of other 5-HT_{1A} receptor agonists could also be mediated by K_{ATP} channels, the

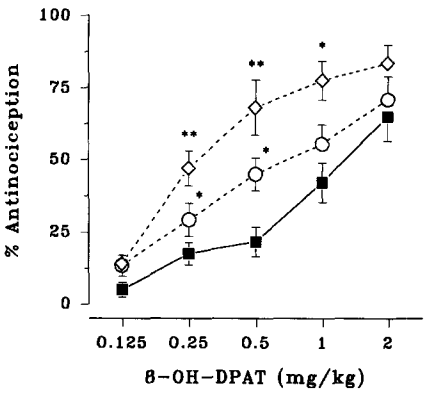

Fig. 4. Effect of the K_{ATP} channel opener cromakalim at doses of 32 (○) and 64 (◇) μg/mouse i.c.v. on the dose-response curve of antinociception of (■) 8-OH-DPAT (0.125–2 mg/kg s.c.) in the hot plate test. Results are represented as the means ± S.E.M. of the values obtained in at least eight animals. Significant differences with respect to the vehicle group (ANOVA followed by Newman-Keuls test): * $P < 0.05$; ** $P < 0.01$.

Table 1
Effect of the K_{ATP} channel blocker gliquidone on antinociception induced by the 5-HT_{1A} receptor agonists lesopitron, buspirone and tandospirone in a hot plate test

	5-HT_{1A} receptor agonist		
	Lesopitron 40 [a]	Buspirone 40 [a]	Tandospirone 80 [a]
Vehicle	65.53 ± 6.98	81.67 ± 7.68	84.85 ± 7.20
Gliquidone 1 [b]	n.d.	46.46 ± 5.04 [d]	n.d.
Gliquidone 2 [b]	46.70 ± 4.26 [c]	36.17 ± 7.06 [d]	55.77 ± 8.59 [d]
Gliquidone 4 [b]	32.16 ± 6.11 [d]	27.21 ± 3.09 [d]	33.75 ± 7.87 [d]
Gliquidone 8 [b]	30.95 ± 4.07 [d]	25.62 ± 2.36 [d]	29.08 ± 3.84 [d]

Antinociception is expressed as a percentage of the maximum possible effect (mean ± S.E.M. of the values obtained in 8–12 animals). [a] Dose expressed in mg/kg s.c., administered 30 min before the test. [b] Dose expressed in μg/mouse i.c.v., administered 30 min before the test. [c] Significantly different from vehicle-treated group (ANOVA followed by Newman Keuls test): $P < 0.05$. [d] Significantly different from vehicle-treated group (ANOVA followed by Newman Keuls test): $P < 0.01$. n.d., not determined.

Table 2
Effect of the K_{ATP} channel opener cromakalim on antinociception induced by the 5-HT_{1A} receptor agonists lesopitron, buspirone and tandospirone in a hot plate test

	5-HT_{1A} receptor agonist		
	Lesopitron 10 [a]	Buspirone 10 [a]	Tandospirone 20 [a]
Vehicle	19.47 ± 2.96	23.47 ± 4.67	28.94 ± 5.06
Cromakalim 32 [b]	30.81 ± 6.13	38.97 ± 5.54	45.44 ± 6.24 [c]
Cromakalim 64 [b]	43.83 ± 5.01 [d]	54.41 ± 6.54 [c]	72.19 ± 5.94 [d]

Antinociception is expressed as a percentage of the maximum possible effect (mean ± S.E.M. of the values obtained in 8–12 animals). [a] Dose expressed in mg/kg s.c., administered 30 min before the test. [b] Dose expressed in μg/mouse i.c.v., administered 30 min before the test. [c] Significantly different from vehicle-treated group (ANOVA followed by Newman Keuls test): $P < 0.05$. [d] Significantly different from vehicle-treated group (ANOVA followed by Newman-Keuls test): $P < 0.01$.

antinociception induced by nearly equieffective doses of several 5-HT_{1A} receptor agonists (lesopitron, buspirone and tandospirone) was evaluated after treatment with K_{ATP} channel openers and blockers.

As with 8-OH-DPAT, the i.c.v. administration of gliquidone (1–8 μg/mouse) produced a dose-dependent antagonism of the antinociception induced by lesopitron (40 mg/kg s.c.), buspirone (40 mg/kg s.c.) and tandospirone (80 mg/kg s.c.) (Table 1). The ID_{50}'s of gliquidone against the antinociception induced by each 5-HT_{1A} receptor agonist were similar: 1.92 (1.90–1.94) against lesopitron, 0.75 (0.51–2.83) against buspirone and 1.96 (1.94–1.98) against tandospirone.

Cromakalim (32–64 μg/mouse i.c.v.) produced a dose-dependent enhancement of the antinociception induced by lesopitron (10 mg/kg s.c.), buspirone (10 mg/kg s.c.) and tandospirone (20 mg/kg s.c.) (Table 2).

Table 3
Inhibitory effect of 5-HT_{1A} receptor agonists and K^+ channel-acting drugs on [^{3}H]8-OH-DPAT binding to rat hippocampal membranes

	K_i (nM)
5-HT_{1A} receptor agonists	
8-OH-DPAT	1.37 ± 0.14
Buspirone	15.70 ± 0.58
Tandospirone	28.80 ± 4.17
Lesopitron	70.50 ± 3.40
K^+ channel blockers	
Gliquidone	> 10000
4-Aminopyridine	> 10000
Tetraethylammonium	> 10000
K^+ channel opener	
Cromakalim	> 10000

Results of K_i are represented as means ± S.E.M. of at least three experiments.

3.5. Effect of 5-HT_{1A} receptor agonists and K^+ channel-acting drugs on 8-OH-DPAT binding

To rule out that the interaction between K_{ATP} channel-acting drugs and 5-HT_{1A} receptor agonists took place at the receptor level, we designed [^{3}H]8-OH-DPAT binding displacement experiments. All the 5-HT_{1A} receptor agonists at concentrations in the nanomolar range reduced the binding of [^{3}H]8-OH-DPAT (1 nM) to rat hippocampal membranes with the following order of potency: 8-OH-DPAT ≫ buspirone > tandospirone > lesopitron (Table 3).

Despite their modulatory action on antinociceptive tests, neither cromakalim nor gliquidone modified [^{3}H]8-OH-DPAT binding at concentrations higher than the micromolar range (Table 3). Similarly, 4-aminopyridine and tetraethylammonium failed to displace the 5-HT_{1A} ligand from its receptors (Table 3).

4. Discussion

Our results show that the systemic (s.c.) administration of 5-HT_{1A} receptor agonists produced dose-dependent antinociception in the hot plate test in mice. These results are in agreement with previous reports of an antinociceptive effect in the hot plate test after the i.p. or s.c injection of 8-OH-DPAT (Fasmer et al., 1986; Eide and Hole, 1993; Millan and Colpaert, 1991), buspirone (Giordano and Rogers, 1989; Bragin et al., 1989) and other partial agonists such as gepirone or ipsapirone (Millan, 1994). Nevertheless, to our knowledge no data are available on the antinociceptive effect of lesopitron and tandospirone, two 5-HT_{1A} receptor agonists with potent anxiolytic activity (Zifa and Fillion, 1992; Costall et al., 1992; Haj-Dahmane et al., 1994). Both drugs had a dose-dependent antinociceptive effect in the hot plate test, and their potency was similar to that of buspirone. The antinociceptive efficacy and the slopes of the dose-response curves were very similar for all 5-HT_{1A} receptor agonists tested here. These data are discrepant with those of Millan (1994), who found a lower efficacy with buspirone and other partial agonists than with 8-OH-DPAT. This discrepancy may reflect the difference in stimulus intensity (50°C in our experiments versus 55°C in Millan's study). Two facts support this notion: (A) lower doses of buspirone than those used in the present experiments produced a similar degree of antinociception when the intensity of the stimuli (46°C) was even lower than the one we used (Giordano and Rogers, 1989), and (B) the degree of antinociception induced by many drugs depends on the intensity of the thermal stimulus used (Luttinger, 1985). In our experiments the order of potency of 5-HT_{1A} receptor agonists as antinociceptive drugs parallelled their ability to displace [^{3}H]8-OH-DPAT from its binding sites: thus, 8-OH-DPAT was about 10-fold more potent than buspirone both in nociception and binding experiments. These results were expected, because 8-OH-DPAT has been described as a selective pure agonist which binds with higher affinity than buspirone to 5-HT_{1A} receptors (Zifa and Fillion, 1992; Haj-Dahmane et al., 1994). Lesopitron and tandospirone were slightly less potent than buspirone in the hot plate test, and they also showed less affinity for 5-HT_{1A} receptors in our experiments and in previous studies (Zifa and Fillion, 1992; Costall et al., 1992; Haj-Dahmane et al., 1994). These findings indirectly suggest that the antinociception induced by 5-HT_{1A} receptor agonists may be mediated by 5-HT_{1A} receptors. In fact, the antinociceptive effect of 8-OH-DPAT was antagonized by 5-HT_{1A} receptor blockers in some experiments (Cervo et al., 1994; Xu et al., 1994; but see Millan, 1994 for an opposing view).

As would be predicted from electrophysiological studies which demonstrate that activation of 5-HT_{1A} receptors is followed by neuronal hyperpolarization and K^+ outflow (Andrade et al., 1986; Penington et al., 1993), our results show that the K^+ channel opener cromakalim (Longman and Hamilton, 1992) facilitates, whereas the K^+ channel blocker, gliquidone (Edwards and Weston, 1993) antagonizes, the antinociception induced by all 5-HT_{1A} receptor agonists tested in the present study. The modulation was apparently very selective, as only openers and blockers of K_{ATP} channels modify 5-HT_{1A}-induced antinociception, whereas 4-aminopyridine and tetraethylammonium, blockers of a wide range of non-ATP-dependent K^+ conductances (Cook and Quast, 1990), failed to modify 8-OH-DPAT antinociception. Furthermore, K_{ATP} channel openers and blockers produce opposite effects, and the order of potency for the blockade of antinociception by the sulfonylureas (glipizide < gliquidone) coincides with their potency as K_{ATP} channel blockers (Amoroso et al., 1990; Schmid-Antomarchi et al., 1990). Interestingly, sulfonylureas failed to abolish the antinociceptive effect of 5-HT_{1A} receptor agonists, suggesting that in addition to K_{ATP} channel opening, some other mechanisms are involved in this effect. Finally, none of the K_{ATP} channel-acting drugs modified the binding of [^{3}H]8-OH-DPAT to brain membranes, indicating that modulation of antinociception induced by 5-HT_{1A} receptor agonists does not take place at the receptor level.

The modulation by K_{ATP} channel-acting drugs of 5-HT_{1A} receptor agonist-induced antinociception does not correlate with 5-HT_{1A}-dependent K^+ conductances described in dorsal raphe neurons, which are not sensitive to sulfonylureas (Penington et al., 1993), but are blocked by 4-aminopyridine (Haj-Dahmane et al., 1991). Furthermore, the electrophysiological characteristics of 8-OH-DPAT-activated K^+ conductances in the hippocampus are similar to those induced by baclofen through $GABA_B$ receptors (Andrade et al., 1986; Innis et al., 1988), and the K^+ conductances induced by baclofen are sensitive to 4-aminopyridine and tetraethylammonium (Inoue et al., 1985; Stevens et al., 1985). Several facts may explain the apparent contradiction between the electrophysiological and the behavioral data. Firstly, our method (e.g. the use of inappropriate doses or times of treatment for these K^+ channel blockers) may not have allowed us to detect any effect of 4-aminopyridine or tetraethylammonium against 5-HT_{1A} receptor agonist-induced antinociception. However, this does not appear to be the case, as we previously showed that both 4-aminopyridine and tetraethylammonium, at the same doses and times of treatment as in the present experiments, antagonized baclofen-induced antinociception (Ocaña and Baeyens, 1993). An alternative explanation for the apparent discrepancy is that 5-HT_{1A} receptor agonists may induce antinociception through mechanisms different

from 5-HT_{1A} receptor activation. Evidence in support of this hypothesis comes from reports that noradrenaline depletion antagonized 8-OH-DPAT antinociception (Archer et al., 1987), and that α_2-adrenoceptor antagonists inhibited 8-OH-DPAT-induced antinociception, whereas 5-HT_{1A} antagonists failed to do so under the same conditions (Millan and Colpaert, 1991; Millan, 1994). If α_2-adrenoceptors do indeed mediate the antinociceptive activity of 5-HT_{1A} receptor agonists, modulation of this activity by K_{ATP} channel-acting drugs could be attributed to their effect on α_2-adrenoceptor-induced K^+ conductances, because the antinociception elicited by α_2-adrenoceptor agonists is antagonized by sulfonylureas but not by 4-aminopyridine or tetraethylammonium (Ocaña and Baeyens, 1993; Raffa and Martinez, 1995). However, it was recently shown that 5-HT_{1A} receptor antagonists, but not α_2-adrenoceptor antagonists, inhibit 8-OH-DPAT-induced antinociception (Cervo et al., 1994), contradicting previously reported results (Millan and Colpaert, 1991). In addition, at least in the case of buspirone, both the drug and its metabolites behave as antagonists, not as agonists, of α_2-adrenoceptors, and reduce clonidine antinociception (Cao and Li, 1994). This raises further doubts about the involvement of α_2-adrenoceptor activation as the mechanism underlying the antinociceptive effect of 5-HT_{1A} receptor agonists.

Finally, a third explanation for the discrepancies between the electrophysiological and behavioral data is that 5-HT_{1A} receptors regulate more than one type of K^+ channels in neurons. In this connection it has been shown that agonists of 5-HT_{1A} receptor and adenosine A_1 receptors open the same kind of K^+ channels in the hippocampus (Zgombick et al., 1989), and adenosine A_1 receptor agonists are known to open K_{ATP} channels (Kirsch et al., 1990; Li and Henry, 1992). Interestingly, the antinociception induced by adenosine A_1 receptor agonists is modulated by K_{ATP} channel-acting drugs (Ocaña and Baeyens, 1994) in a way similar to the antinociception induced by 5-HT_{1A} receptor agonists (present results). Furthermore, the firing of central serotonergic neurons is also modulated by K_{ATP} channels (Haj-Dahmane et al., 1993). This suggests that, despite their similarities in some electrophysiological studies, the K^+ channels involved in 5-HT_{1A}- and $GABA_B$-mediated antinociception may be different, and that 5-HT_{1A} receptor agonists behave like μ- and δ_1-opioid, adenosine A_1 and α_2-adrenoceptor agonists, all of them producing K_{ATP}-sensitive antinociception (see Introduction for references).

In summary, this study shows that the antinociception induced by 5-HT_{1A} receptor agonists in the hot plate test in mice is selectively modulated by K_{ATP} channel-acting drugs. This further supports a role for K^+ channels in antinociception, and suggests that a common cellular transduction mechanism is shared by several antinociceptive drugs.

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