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Differential effects of K^+ channel blockers on antinociception induced by α_2-adrenoceptor, $GABA_B$ and κ-opioid receptor agonists

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1 The effects of several K^+ channel blockers (sulphonylureas, 4-aminopyridine and tetraethylammonium) on the antinociception induced by clonidine, baclofen and U50,488H were evaluated by use of a tail flick test in mice.

2 Clonidine (0.125–2 mg kg^{-1}, s.c.) induced a dose-dependent antinociceptive effect. The ATP-dependent K^+ (K_{ATP}) channel blocker gliquidone (4–8 μg/mouse, i.c.v.) produced a dose-dependent displacement to the right of the clonidine dose-response line, but neither 4-aminopyridine (4-AP) (25–250 ng/mouse, i.c.v.) nor tetraethylammonium (TEA) (10–20 μg/mouse, i.c.v.) significantly modified clonidine-induced antinociception.

3 The order of potency of sulphonylureas in antagonizing clonidine-induced antinociception was gliquidone > glipizide > glibenclamide > tolbutamide, which is the same order of potency as these drugs block K_{ATP} channels in neurones of the CNS.

4 Baclofen (2–16 mg kg^{-1}, s.c.) also induced a dose-dependent antinociceptive effect. Both 4-AP (2.5–25 ng/mouse, i.c.v.) and TEA (10–20 μg/mouse, i.c.v.) dose-dependently antagonized baclofen antinociception, producing a displacement to the right of the baclofen dose-response line. However, gliquidone (8–16 μg/mouse, i.c.v.) did not significantly modify the baclofen effect.

5 None of the K^+ channel blockers tested (gliquidone, 8–16 μg/mouse; 4-AP, 25–250 ng/mouse and TEA, 10–20 μg/mouse, i.c.v.), significantly modified the antinociception induced by U50,488H (8 mg kg^{-1}, s.c.).

6 These results suggest that the opening of K^+ channels is involved in the antinociceptive effect of α_2 and $GABA_B$, but not κ-opioid, receptor agonists. The K^+ channels opened by α_2-adrenoceptor agonists seem to be ATP-dependent channels, whereas those opened by $GABA_B$ receptor agonists are not.

Keywords: Clonidine; baclofen; U50,488H; antinociception; K^+ channels; sulphonylureas; 4-aminopyridine; tetraethylammonium

Introduction

Agonists of μ- and δ-opioid receptors open K^+ channels in neurones (North, 1989) and produce antinociception in experimental animals (Porreca *et al.*, 1984). The opening of K^+ channels seems to play a role in opioid-mediated antinociception, since the specific ATP-dependent K^+ (K_{ATP}) channel blocker, glibenclamide (an antidiabetic sulphonylurea) dose-dependently antagonizes the antinociceptive effect of morphine (Ocaña *et al.*, 1990; 1993; Wild *et al.*, 1991; Narita *et al.*, 1992), whereas the K^+ channel activator pinacidil produces opposite effects (Vergoni *et al.*, 1992). Moreover, the order of potency of different sulphonylureas in blocking K_{ATP} channels in CNS neurones (Amoroso *et al.*, 1990) and in antagonizing morphine antinociception is the same (Ocaña *et al.*, 1993), strongly suggesting that the opening of K_{ATP} channels underlies the morphine antinociceptive effect. This type of K^+ channel is also involved in δ-receptor-mediated antinociception, since glibenclamide antagonizes the antinociceptive activity of [D-Pen^2, D-Pen^5]-enkephalin (Wild *et al.*, 1991).

Agonists of α_2-adrenoceptors and $GABA_B$ receptors also open K^+ channels in neurones (Morita & North, 1981; Christie *et al.*, 1987; Christie & North, 1988; Lacey *et al.*, 1988) and promote antinociception (Sawynok, 1987; Fornai *et al.*, 1990). However, whether K^+ channel opening plays a role in the antinociceptive effect of agonists of these receptors has not been tested. Electrophysiological studies have shown that the K^+ channels opened by α_2-adrenoceptor and μ-opioid receptor agonists in neurones appear to be identical, and are insensitive to some K^+ channel blockers such as tetraethylammonium (TEA) and 4-aminopyridine (4-AP) (North & Williams, 1985; Aghajanian & Wang, 1987). On the other hand, the K^+ conductances elicited by $GABA_B$ receptor agonists in neurones appear to be different, as they are antagonized by 4-AP and TEA (Inoue *et al.*, 1985; Stevens *et al.*, 1985).

In light of these facts it may be hypothesized that if K^+ channel opening underlies the antinociceptive effect of α_2-adrenoceptor and $GABA_B$ receptor agonists, K^+ channel blockers would be expected to antagonize this antinociception. Moreover, taking into account the results of the electrophysiological studies cited above, a differential sensitivity of α_2-adrenoceptor- and $GABA_B$ receptor-mediated antinociception to K^+ channel blockers would be expected. In the present study we evaluated the effect of the i.c.v. administration of sulphonylureas, TEA and 4-AP on the antinociception induced by clonidine, an α_2-adrenoceptor agonist, and baclofen, a $GABA_B$ receptor agonist.

Finally, to test the specificity of the effects of the K^+ channel blockers used, we evaluated whether these drugs antagonize the antinociception elicited by U50,488H, a κ-opioid receptor agonist (Von Voightlander *et al.*, 1983; Clark & Pasternak, 1988). Activation of κ receptors does not open K^+ channels but does close Ca^{2+} channels (Werz & MacDonald, 1984; Cherubini & North, 1985). Consequently, if the effects of sulphonylureas, TEA and 4-AP are due to K^+ channel blockade, they should not modify antinociception induced by U50,488H.

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Methods

Female CD-1 mice (Charles River, Spain) weighing 25–30 g were used for all experiments. The animals were housed in a temperature-controlled room (21 ± 1°C), with air exchange every 20 min and a standard 12 h light/dark cycle (lights on at 08 h 00 min and off at 20 h 00 min). The experiments were performed from 09 h 00 min to 15 h 00 min. Food and water were available *ad libitum* up to the beginning of the experiments. Naïve animals were used throughout. At all times the mice were handled in accordance with current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983).

The tail flick test was run as previously described (Ocaña & Baeyens, 1991). Briefly, the animals were restrained in a plexiglass tube and placed on the tail flick apparatus (LI 7100, Letica, S.A., Spain). A noxious beam of light was focussed to the tail about 4 cm from the tip, and the latency to removal was recorded automatically to the nearest 0.1 s. The intensity of the radiant head source was adjusted to yield baseline latencies between 3 and 5 s; this intensity was never changed and any animal in which baseline latency was outside the pre-established limits was excluded from the experiments. The cut-off time was 10 s.

Two baseline tail flick latencies were recorded within 20 min before all injections. Then the solvent or drug was administered and tail flick latencies were measured 10, 20, 30, 45, 60, 90 and 120 min after treatment. The area under the curve of antinociception against time (AUC) was calculated for each animal according to the method of Tallarida & Murray (1987). The degree of antinociception in each animal was calculated according to the formula: % antinociception = $[(AUCt - AUCc)\ (AUCmax - AUCc)^{-1}] \times 100$, where AUCt and AUCc are the areas under the curve for treated and control animals respectively, and AUCmax is the area under the curve of maximum possible antinociception (10 s in each determination). Furthermore, to illustrate the time-course of the antinociceptive effect of the treatments, the degree of antinociception at each time was calculated according to the formula: % antinociception = $[(LTT\text{-}LTB)\ (CT\text{-}LTB)^{-1}] \times 100$, where LTT is the latency time in treated mice, LTB is the baseline latency time and CT is the cut-off time (10 s).

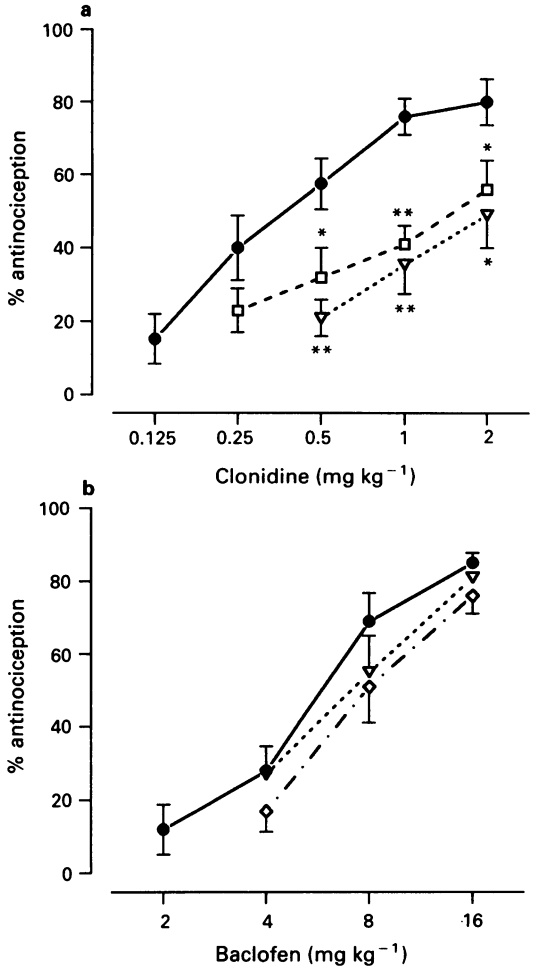

Figure 1 Effects of i.c.v. treatment with different doses of gliquidone on the antinociceptive effect of clonidine and baclofen (administered s.c.) in a tail flick test in mice. (a) Effects of clonidine + solvent (●), clonidine + gliquidone 4 μg/mouse (□) and clonidine + gliquidone 8 μg/mouse (▽). Each point represents the mean ± s.e.mean of the values obtained in 8–12 animals. Statistically significant differences in comparison with clonidine + solvent: $^{*}P < 0.05$, $^{**}P < 0.01$ (Newman Keuls test). (b) Effects of baclofen + solvent (●), baclofen + gliquidone 8 μg/mouse (▽) and baclofen + gliquidone 16 μg/mouse (◇). Each point represents the mean ± s.e.mean of the values obtained in 8–12 animals. No statistically significant differences in comparison with baclofen + solvent were found (Newman Keuls test).

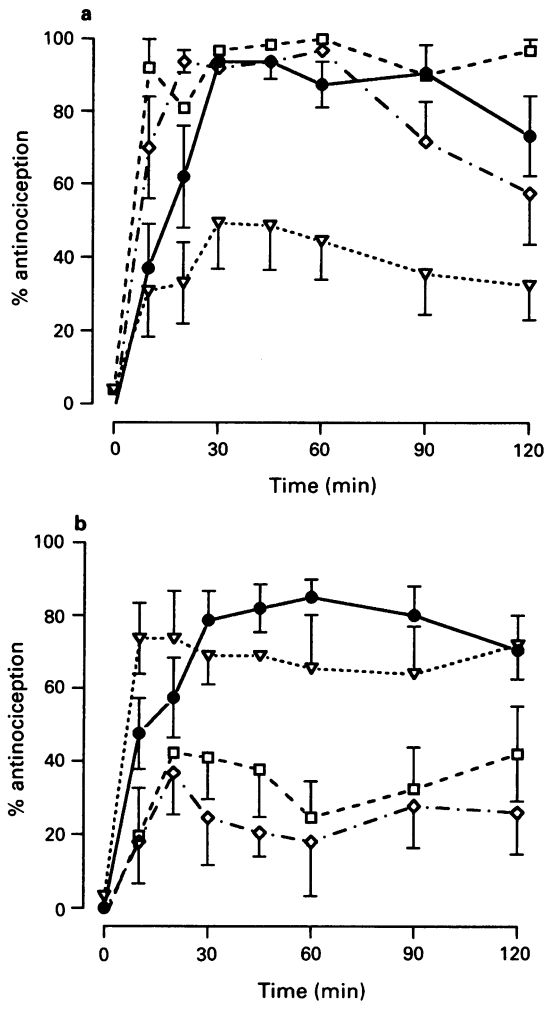

Figure 2 Time-course of the antinociceptive effect of clonidine (1 mg kg⁻¹, s.c.) and baclofen (8 mg kg⁻¹, s.c.) associated to different K^+ channel blockers in a tail flick test in mice. (a) Effects of clonidine (●), clonidine + gliquidone (8 μg/mouse, i.c.v.) (▽), clonidine + tetraethylammonium (TEA) (20 μg/mouse, i.c.v.) (□) and clonidine + 4-aminopyridine (4-AP) (25 ng/mouse, i.c.v.) (◇). Each point represents the mean ± s.e.mean of the values obtained in 8–12 animals. (b) Effects of baclofen (●), baclofen + gliquidone (8 μg/mouse, i.c.v.) (▽), baclofen + TEA (20 μg/mouse, i.c.v.) (□) and baclofen + 4-AP (25 ng/mouse, i.c.v.) (◇). Each point represents the mean ± s.e.mean of the values obtained in 8–12 animals.

Table 1 Effects of different sulphonylureas on the antinociception induced by clonidine (1 mg kg^{-1}, s.c.) in a tail flick test in mice

Treatment[a]	*% antinociception*[b]
Clonidine + solvent	76.24 ± 5.09
Clonidine + gliquidone 2	45.09 ± 5.97**
Clonidine + gliquidone 4	41.25 ± 5.12**
Clonidine + glipizide 20	56.61 ± 6.25*
Clonidine + glipizide 40	52.06 ± 7.11*
Clonidine + glibenclamide 40	61.09 ± 7.11
Clonidine + glibenclamide 80	48.36 ± 7.39*
Clonidine + tolbutamide 80	62.09 ± 5.40
Clonidine + tolbutamide 160	48.93 ± 9.52*

[a]The numbers represent the dose (μg/mouse) of each sulphonylurea administered i.c.v.
[b]The values represent the mean ± s.e.mean of the results obtained in 8–12 animals.
Statistically significant differences in comparison to clonidine + solvent: *$P < 0.05$; **$P < 0.01$ (Newman Keuls test).

Once baseline latencies were obtained, the animals received a s.c. injection of clonidine, baclofen, U50,488H or their solvent, and an i.c.v. injection of one of the K^+ channel blockers or their solvents at time 0; the degree of antinociception was then measured during 2 h as described above. The s.c. injections were made in the interscapular region. The i.c.v. injections were administered to gently restrained unanaesthetized mice in Hamilton microlitre syringes, according to the method previously described (Robles *et al.*, 1992). After the antinociceptive test was finished, the trajectory of the i.c.v. injection was evaluated in each animal, and the results from those in which it was incorrect were discarded.

The drugs used and their suppliers were as follows: clonidine HCl (Sigma), baclofen (Sigma), *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pirrolidynyl]cyclohexyl) benzeneacetamide methanesulfonate salt (U50,488H) (Sigma), tetraethylammonium bromide (Sigma), 4-aminopyridine (Sigma) and the sulphonylureas glibenclamide, tolbutamide (both Sigma), gliquidone (Europharma, S.A.) and glipizide (Far-

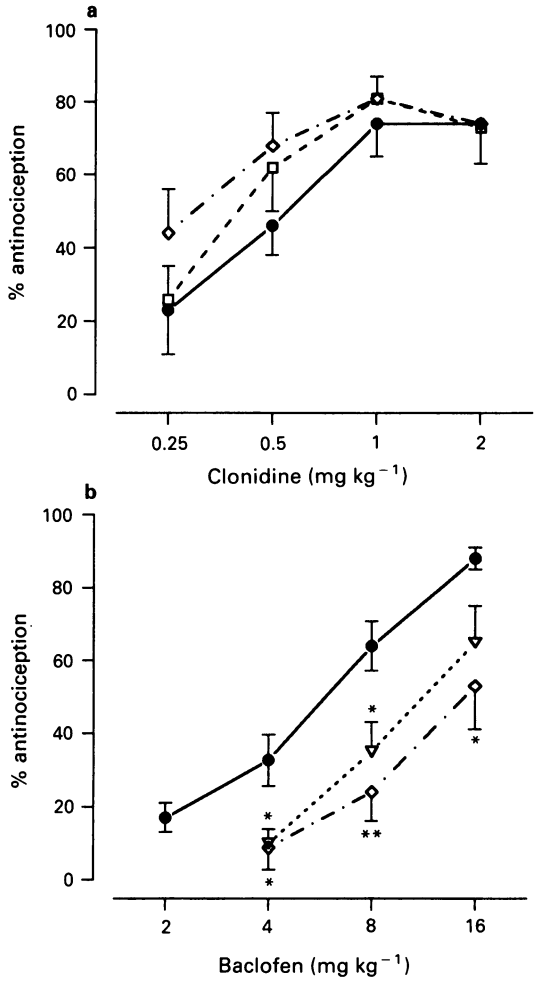

Figure 3 Effects of i.c.v. treatment with different doses of 4-aminopyridine (4-AP) on the antinociceptive effect of clonidine and baclofen (administered s.c.) in a tail flick test in mice. (a) Effects of clonidine + solvent (●), clonidine + 4-AP 25 ng/mouse (◇) and clonidine + 4-AP 250 ng/mouse (□). Each point represents the mean ± s.e.mean of the values obtained in 8–12 animals. No statistically significant differences in comparison with clonidine + solvent were found (Newman Keuls test). (b) Effects of baclofen + solvent (●), baclofen + 4-AP 2.5 ng/mouse (▽) and baclofen + 4-AP 25 ng/mouse (◇). Each point represents the mean ± s.e.mean of the values obtained in 8–12 animals. Statistically significant differences in comparison with baclofen + solvent: *$P < 0.05$; **$P < 0.01$ (Newman Keuls test).

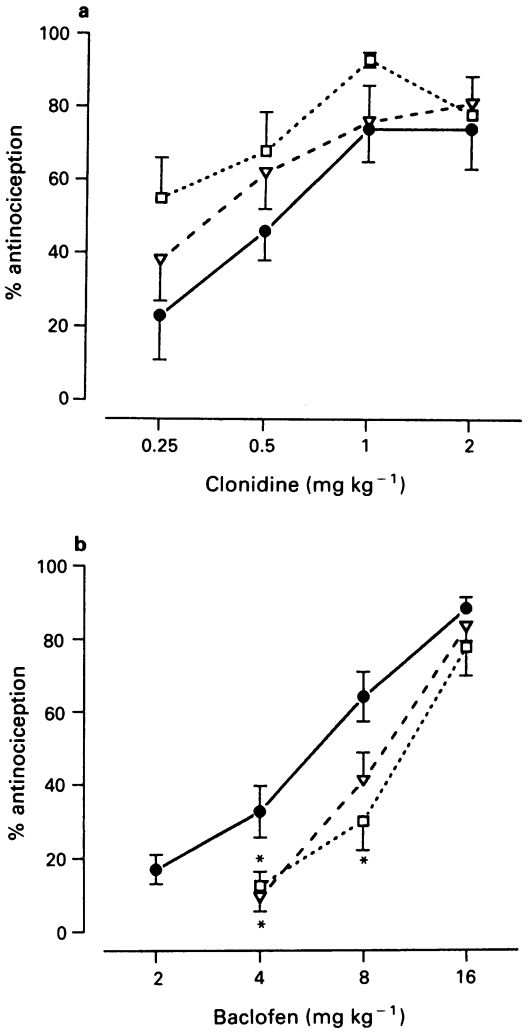

Figure 4 Effects of i.c.v. treatment with different doses of tetraethylammonium (TEA) on the antinociceptive effect of clonidine and baclofen (administered s.c.) in a tail flick test in mice. (a) Effects of clonidine + solvent (●), clonidine + TEA 10 μg/mouse (▽) and clonidine + TEA 20 μg/mouse (□). Each point represents the mean ± s.e.mean of the values obtained in 8–12 animals. No statistically significant differences in comparison with clonidine + solvent were found (Newman Keuls test). (b) Effects of baclofen + solvent (●), baclofen + TEA 10 μg/mouse (▽) and baclofen + TEA 20 μg/mouse (□). Each point represents the mean ± s.e.mean of the values obtained in 8–12 animals. Statistically significant differences in comparison with baclofen + solvent: *$P < 0.05$ (Newman Keuls test).

mitalia Carlo Erba). Clonidine, baclofen and U50,488H were dissolved in demineralized water and injected subcutaneously in a volume of 5 ml kg^{-1}. All sulphonylureas were dissolved in 1% Tween 80 in demineralized water, whereas TEA and 4-AP were dissolved in demineralized water. All K^+ channel blockers were injected intracerebroventricularly in a volume of 5 µl per mouse. Control animals received the same volume of solvents.

Differences between the values in control and K^+ channel blocker-treated groups were analysed with analysis of variance (ANOVA) followed by a Newman Keuls test, and were considered significant when P was below 0.05.

Results

Effects of sulphonylureas on clonidine- and baclofen-induced antinociception

Both clonidine (0.125–2 mg kg^{-1}) and baclofen (2–16 mg kg^{-1}) induced a dose-dependent antinociception after subcutaneous administration to mice (Figures 1a and b). Gliquidone (4 and 8 µg/mouse, i.c.v.) significantly reduced clonidine antinociception, dose-dependently displacing to the right the clonidine dose-response line (Figure 1a). In contrast, gliquidone (8 and 16 µg/mouse, i.c.v.), i.e. at doses even greater than those used with clonidine, did not significantly modify baclofen-induced antinociception (Figure 1b). A representative example of the time-course of the antinociceptive effects of clonidine and baclofen plus gliquidone (8 µg/mouse, i.c.v.) is illustrated in Figure 2.

All sulphonylureas tested (gliquidone, glipizide, glibenclamide and tolbutamide) signficantly antagonized clonidine antinociceptive activity (Table 1). Considering the minimum dose of each sulphonylurea necessary to antagonize the clonidine effect, the order of potency was: gliquidone > glipizide > glibenclamide > tolbutamide (Table 1). None of the sulphonylureas significantly modified tail flick latency in control animals (data not shown), or induced any overt behavioural effect at the doses used.

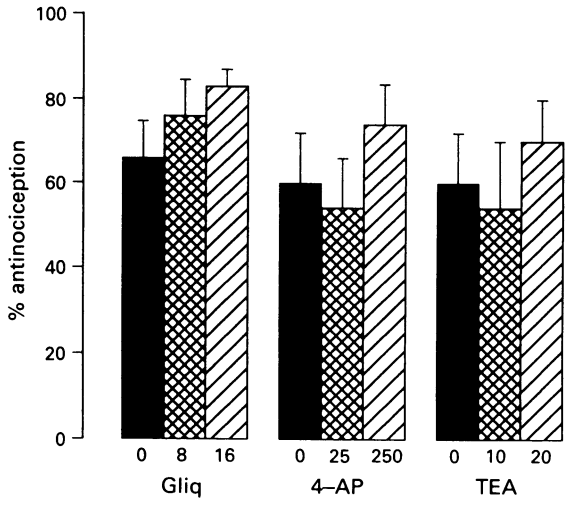

Figure 5 Effects of different K^+ channel blockers (Gliq = gliquidone; 4-AP = 4-aminopyridine; TEA = tetraethylammonium) on U50,488H-induced antinociception in a tail flick test in mice. The solid columns represent the effect of U50,488H (8 mg kg^{-1}, s.c.) + solvents. The doses of Gliq and TEA (µg/mouse), and those of 4-AP (ng/mouse) are shown below the columns. All the K^+ channel blockers were injected i.c.v. No statistically significant differences in comparison with U50,488H + solvent were found in any case (Newman Keuls test). Each column represents the mean ± s.e.mean of the values obtained in 8–12 animals.

Effects of 4-aminopyridine and tetraethylammonium on clonidine- and baclofen-induced antinociception

The i.c.v. administration of both 4-AP (2.5 and 25 ng/mouse) and TEA (10 and 20 µg/mouse) significantly antagonized the antinociceptive activity of baclofen, producing a displacement to the right of the baclofen dose-response line (Figures 3b and 4b). In contrast, neither 4-AP (25 and 250 ng/mouse, i.c.v.) nor TEA (10 and 20/µg mouse, i.c.v.) significantly modified the antinociceptive activity of clonidine (Figures 3a and 4a). An example of the time-course of the antinociception induced by clonidine and baclofen given with TEA (20 µg/mouse, i.c.v.) and 4-AP (25 ng/mouse, i.c.v.) is shown in Figure 2.

Neither TEA nor 4-AP significantly modified tail flick latency in control animals (data not shown), but the highest doses of both drugs produced signs of excitation in some animals (increased locomotor activity and number of explorations, groomings and rearings).

Effects of K^+ channel blockers on U50,488H-induced antinociception

As shown in Figure 5, i.c.v. administration of the K^+ channel blockers gliquidone (8 and 16 µg/mouse), 4-AP (25 and 250 ng/mouse) and TEA (10 and 20 µg/mouse) failed to modify significantly the antinociception induced by U50,488H (8 mg kg^{-1}, s.c.).

Discussion

Several kinds of K^+ channels with different electrophysiological characteristics and pharmacological sensitivities have been described in neurones (Halliwell, 1990; Aronson, 1992). Clonidine and other agonists of α_2-adrenoceptors open K^+ channels in neurones (Morita & North, 1981; Christie *et al.*, 1987; Tatsumi *et al.*, 1990) and produce antinociception (Fornai *et al.*, 1990). The possible relationship between these two effects was previously unknown, although it was suggested that noradrenaline, acting through α_2-adrenoceptors, may inhibit nociceptive input to the spinal cord by increasing potassium conductance in substantia gelatinosa neurones (North & Yoshimura, 1984). Our results show that clonidine-induced antinociception is antagonized by different sulphonylureas. All sulphonylureas tested to date specifically block ATP-dependent K^+ channels (Amoroso *et al.*, 1990; Cook & Quast, 1990; Schmid-Antomarchi *et al.*, 1990). Therefore, our results suggest that the opening of these K^+ channels is involved in clonidine antinociception. In support of this idea, it is interesting to note that the order of potency of different sulphonylureas in blocking ATP-dependent K^+ channels in CNS neurones – gliquidone > glipizide > glibenclamide > tolbutamide (Amoroso *et al.*, 1990; Schmid-Antomarchi *et al.*, 1990) – is the same order of potency as we found for blocking clonidine antinociception.

Neither TEA nor 4-AP antagonized clonidine-induced antinociception. That the lack of effect is not due to the use of low doses of the drugs, or to any methodological pitfall, is shown by the finding that the same or even lower doses of these K^+ channel blockers antagonize baclofen-induced antinociception, when administered by the same methods. Consequently, our results suggest that K^+ channels sensitive to TEA and 4-AP are not involved in the clonidine effect. These results were expected, as none of these K^+ channel blockers antagonizes the K^+ conductances induced by α_2-adrenoceptor agonists in neurones (North & Williams, 1985).

Various electrophysiological studies have suggested that the K^+ channels opened by α_2-adrenoceptor, μ- and δ-opioid receptor agonists are the same (Andrade & Aghajanian, 1985; North & Williams, 1985; Aghajanian & Wang, 1987; Tatsumi *et al.*, 1990). The present data confirm this idea, as sulphonylureas, but neither TEA nor 4-AP, antagonized cloni-

dine-induced antinociception, which is exactly the same pattern of activity as is shown by these K^+ channel blockers against morphine antinociception (Ocaña *et al.*, 1990; 1993; Wild *et al.*, 1991; Narita *et al.*, 1992) and the antinociception induced by [D-Pen^2, D-Pen^5]-enkephalin (Wild *et al.*, 1991), a proposed ∂_1-opioid receptor agonist (Mattia *et al.*, 1992). Taken together, these results suggest that α_2-adrenoceptors, μ- and ∂_1-opioid receptors are coupled to the same class of K^+ channels (probably K_{ATP} channels), and that the opening of these channels is involved in the antinociceptive effect of agonists of these receptors.

The antinociceptive effect of baclofen was antagonized by TEA and low doses of 4-AP. The degree of antagonism by TEA was lower than that caused by 4-AP, suggesting that 4-AP-sensitive K^+ channels play a more important role than TEA-sensitive channels in baclofen antinociception. These results are in agreement with the findings of electrophysiological studies showing that 4-AP markedly antagonized or even abolished the K^+ conductances induced by baclofen in neurones (Inoue *et al.*, 1985; Stevens *et al.*, 1985; Ogata *et al.*, 1987), whereas TEA did not antagonize (Inoue *et al.*, 1985) or only partially antagonized them (Stevens *et al.*, 1985; Lacey *et al.*, 1988). In addition, it has been shown that baclofen activates a 4-AP-sensitive K^+ channel in hippocampal neurones that mediates a voltage-dependent transient outward K^+ current with the characteristics of an A-current (Saint *et al.*, 1990). Bearing in mind these data it is tempting to assume that 4-AP antagonizes the baclofen antinociceptive effect by blocking this current. However, neither 4-AP nor TEA are specific blockers of a particular type of K^+ channel (Cook & Quast, 1990; Halliwell, 1990); consequently, considering only the sensitivity of baclofen-induced antinociception to these K^+ channel blockers, it is difficult to deduce what type of K^+ channel may underly this baclofen effect.

The antinociceptive effect of baclofen was not antagonized by gliquidone. The lack of effect of gliquidone cannot be an artifact, as under identical experimental conditions, even lower doses of gliquidone antagonized clonidine- (present study) and morphine-induced antinociception (Ocaña *et al.*, 1993). Consequently our results suggest that K_{ATP} channels are probably not involved in the antinociceptive effect of baclofen. Other drugs show a pattern of sensitivity to K^+ channel blockers similar to that of baclofen, the antinociception induced by [D-Ala^2]deltorphin II, a proposed ∂_2-opioid receptor agonist (Mattia *et al.*, 1992), being insensitive to glibenclamide but antagonized by TEA (Wild *et al.*, 1991).

It was previously shown that sulphonylureas did not antagonize U50,488H-induced antinociception (Narita *et al.*, 1992; Ocaña *et al.*, 1993); moreover, neither glibenclamide nor TEA antagonized the antinoception produced by U69,593 (Wild *et al.*, 1991), another κ-opioid receptor agonist. Our present results confirm, and extend these findings, since none of the K^+ channel blockers we tested antagonized U50,488H-induced antinociception. This lack of effect was expected, as U50,488H does not open K^+ channels in neurones, but does close Ca^{2+} channels (Cherubini & North, 1985; Christie & North, 1988; Xiang *et al.*, 1990). The lack of antagonism of the U50,488H effect by the different K^+ channel blockers is interesting, because it suggests that these drugs do not antagonize, in an unspecific and indiscriminate way, the antinociception induced by any drug. Instead, K^+ channel blockers seem to antagonize specifically only the antinociception due to drugs that activate receptors linked to K^+ channels.

In conclusion, our study shows that the antinociceptive effect of clonidine and baclofen are differentially antagonized by K^+ channel blockers. This suggests (1) that the opening of K^+ channels plays a role in antinociception, and (2) that different K^+ channels underlie the antinociceptive effect of α_2-adrenoceptor and $GABA_B$-receptor agonists.

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