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Participation of ATP-sensitive K^+ channels in the peripheral antinociceptive effect of fentanyl in rats

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Abstract

We examined the effect of several K^+ channel blockers such as glibenclamide, tolbutamide, charybdotoxin (ChTX), apamin, tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP), and cesium on the ability of fentanyl, a clinically used selective μ-opioid receptor agonist, to promote peripheral antinociception. Antinociception was measured by the paw pressure test in male Wistar rats weighing 180-250 g (N = 5 animals per group). Carrageenan (250 μg/paw) decreased the threshold of responsiveness to noxious pressure (Δ = 188.1 ± 5.3 g). This mechanical hyperalgesia was reduced by fentanyl (0.5, 1.5 and 3 μg/paw) in a peripherally mediated and dose-dependent fashion (17.3, 45.3 and 62.6%, respectively). The selective blockers of ATP-sensitive K^+ channels glibenclamide (40, 80 and 160 μg/paw) and tolbutamide (80, 160 and 240 μg/paw) dose dependently antagonized the antinociception induced by fentanyl (1.5 μg/paw). In contrast, the effect of fentanyl was unaffected by the large conductance Ca^{2+}-activated K^+ channel blocker ChTX (2 μg/paw), the small conductance Ca^{2+}-activated K^+ channel blocker apamin (10 μg/paw), or the non-specific K^+ channel blocker TEA (150 μg/paw), 4-AP (50 μg/paw), and cesium (250 μg/paw). These results extend previously reported data on the peripheral analgesic effect of morphine and fentanyl, suggesting for the first time that the peripheral μ-opioid receptor-mediated antinociceptive effect of fentanyl depends on activation of ATP-sensitive, but not other, K^+ channels.

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- K^+ channel
- μ-Opioid receptor agonist
- Glibenclamide

Introduction

Opioids can produce analgesia by inhibiting nociceptive input at supraspinal and spinal sites (1,2). In the central nervous system, the opening of K^+ channels seems to play a role in opioid-mediated antinociception, since the ATP-sensitive K^+ channel blockers (sulfonylureas) antagonize the antinociceptive effect of opioids (3-6). The antinociceptive effect of opioid agonists was also enhanced by ATP-sensitive K^+ channel openers such as pinacidil (7) and cromakalin (8). Evidence that some opioids induce opening of calcium-activated K^+ channels has also been obtained (9). In addition, the diver-

sity of K^+ channels (10) with different electrophysiological and pharmacological characteristics in neurons suggests that other types of K^+ channels may be involved in this effect.

Several studies have indicated that exogenous as well as endogenous opioids can act on peripheral nociceptors to produce an antinociceptive effect against the hyperalgesia induced by local inflammation (11,12). Although the presence of opioid receptors on the peripheral terminals of primary afferents has been demonstrated, the mechanism by which opioid agonists induce peripheral antinociception is unclear (13). It was previously shown that opioid receptors at peripheral sites are coupled with inhibitory G proteins since pertussis toxin inhibits the peripheral antinociception induced by morphine (14) and are also coupled to the L-arginine-NO-cGMP pathway (15,16). Morphine has been shown to exert its peripheral antinociceptive effect by also activating ATP-sensitive K^+ channels (17).

The purpose of the present study was to determine whether specific and non-specific K^+ channel blockers have any effect on the peripheral antinociception induced by fentanyl, since this drug is very potent and is extensively used in clinical practice (18). Thus, we tested the effects of glibenclamide and tolbutamide, sulfonylureas that specifically block ATP-sensitive K^+ channels (19), apamin, a selective blocker of small conductance Ca^{2+}-activated K^+ channels (20), charybdotoxin (ChTX), a blocker of large conductance Ca^{2+}-activated K^+ channels (21), and the non-selective K^+ channel blockers 4-aminopyridine (4-AP), tetraethylammonium chloride (TEA) and cesium (22).

Material and Methods

Animals

The experiments were performed on male Wistar rats weighing 180-250 g (from the Animal Facilities of the Federal University of Minas Gerais, CEBIO-UFMG). The animals were housed in a temperature-controlled room (23 ± 1ºC) on an automatic 12-h light/dark cycle (lights on at 6:00 am). All testing was conducted during the light phase (12:00 to 17:00 h). Food and water were freely available until the beginning of each experiment. Naive animals were used throughout.

Measurement of hyperalgesia

Hyperalgesia was induced in the hind paw by intraplantar administration of a carrageenan suspension (250 µg) and measured according to paw pressure test (23). An analgesia meter (Ugo-Basile, Varese, Italy) with a cone-shaped paw-presser with a rounded tip, which applies a linearly increasing force to the plantar surface of the paw, was used. The weight in grams required to elicit nociceptive responses such as paw flexion was taken to be the nociceptive threshold. A cut-off value of 300 g was used to prevent damage to the paw. The nociceptive threshold was always measured in the right hind paw (except when indicated) and reported as the average of three consecutive trials recorded before and 3 h after carrageenan injection. The result was calculated as the difference between these two averages (Δ of nociceptive threshold) and is reported in grams.

Experimental protocol

Fentanyl was administered once subcutaneously into the right hind paw 135 min after local injection of the carrageenan suspension. All the K^+ channel blockers were injected subcutaneously into the right hind paw. The sulfonylureas (glibenclamide and tolbutamide) were administered 5 min before fentanyl while all the other K^+ channel blockers were injected 30 min after fentanyl (5,24,25). In the protocol used to determine whether fentanyl was acting at central sites, carrageenan was injected into both hind paws

while fentanyl was administered 2 h later into the left or right paw and only the right paw was measured.

Drugs

The drug used as the hyperalgesic agent was lambda carrageenan (Sigma, St. Louis, MO, USA) and the μ-opioid receptor agonist was fentanyl (Janssen, Titusville, NJ, USA). The K^+ channel blockers and their suppliers were: glibenclamide, ChTX, apamin, TEA, 4-AP, and cesium (Sigma) and tolbutamide (ICN Biomedicals Inc., Aurora, OH, USA). Fentanyl and carrageenan were dissolved in isotonic saline and injected in a volume of 100 μl per paw. The K^+ channel blockers were dissolved in demineralized water immediately before use, with the exception of sulfonylureas which were dissolved in saline and 2% Tween, and injected in a volume of 50 μl per paw. For acidic or alkaline solutions of drops the pH was adjusted closer to 7.

Statistical analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA) followed by the Bonferroni test for multiple comparisons. Probabilities of less than 1% ($P < 0.01$) were considered to be statistically significant.

Results

Antinociceptive effect of fentanyl

The administration of fentanyl (0.5 to 3.0 μg) into the right hind paw produced an antinociceptive response against the hyperalgesia induced by prior local ipsilateral injection of carrageenan (Figure 1). Fentanyl at the dose of 1.5 μg, when administered into the left paw, did not produce an antinociceptive effect in the right paw, whereas fentanyl at the dose of 4.5 μg when injected into the left paw induced a potent antinociceptive effect in the contralateral paw (Figure 2).

Antagonism of fentanyl-induced antinociception by glibenclamide and tolbutamide

Glibenclamide (40, 80 and 160 μg/paw) significantly reduced the magnitude of fentanyl-induced antinociception (1.5 μg/paw) in a dose-dependent manner (Figure 3). As shown in Figure 4, the other sulfonylurea tested, tolbutamide (80, 160 and 240 μg/paw) also significantly inhibited the fentanyl-induced antinociceptive effect. Neither

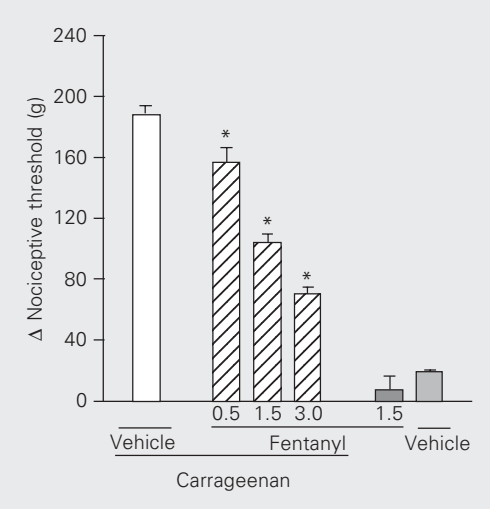

Figure 1. Fentanyl inhibition of the nociceptive threshold of carrageenan-induced hyperalgesia in rats. Fentanyl (μg) was administered intraplantarly 135 min after the local administration of 100 μl of a carrageenan suspension (250 μg). Each column indicates the mean ± SEM (N = 5). *$P < 0.01$ *vs* carrageenan + vehicle-injected control (ANOVA/Bonferroni test).

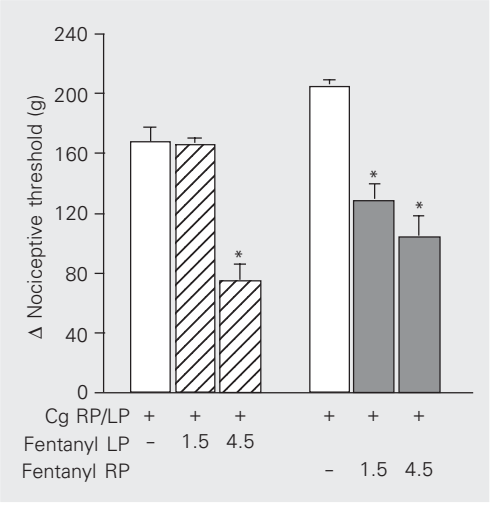

Figure 2. Exclusion of a central antinociceptive response to fentanyl. Fentanyl (μg) was administered into the right (RP) or left (LP) paw 135 min after carrageenan (Cg) administration into both hind paws. Each column indicates the mean ± SEM (N = 5). The symbols "–" and "+" indicate the absence and presence of treatment, respectively. *$P < 0.01$ *vs* carrageenan + vehicle-injected control (ANOVA/Bonferroni test).

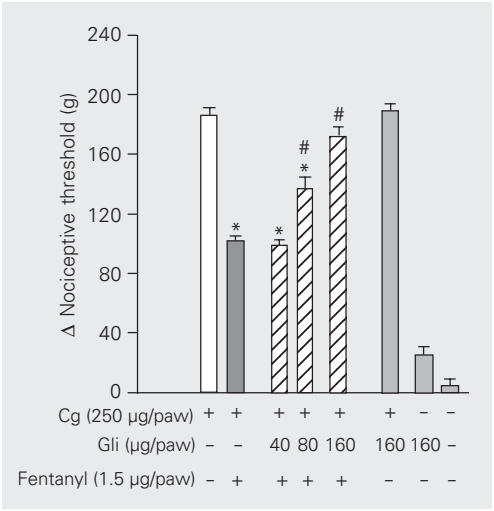

Figure 3. Antagonism induced by intraplantar administration of glibenclamide of the peripheral antinociception produced by fentanyl in hyperalgesic paws. Glibenclamide (Gli, µg/paw) was administered 5 min before fentanyl (1.5 µg). Each column indicates the mean ± SEM (N = 5). The symbols "–" and "+" indicate the absence and presence of treatment, respectively. *P < 0.01 compared to carrageenan (Cg) + vehicle-injected controls and #P < 0.01 compared to Cg + fentanyl + vehicle-injected controls (ANOVA/Bonferroni's test).

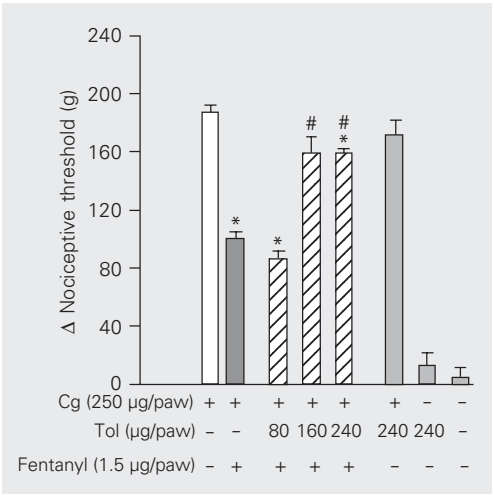

Figure 4. Antagonism induced by intraplantar administration of tolbutamide of the peripheral antinociception produced by fentanyl in hyperalgesic paws. Tolbutamide (Tol, µg/paw) was administered 5 min before fentanyl (1.5 µg). Each column indicates the mean ± SEM (N = 5). The symbols "–" and "+" indicate the absence and presence of treatment, respectively. *P < 0.01 compared to carrageenan (Cg) + vehicle-injected controls and #P < 0.01 compared to Cg + fentanyl + vehicle-injected controls (ANOVA/Bonferroni's test).

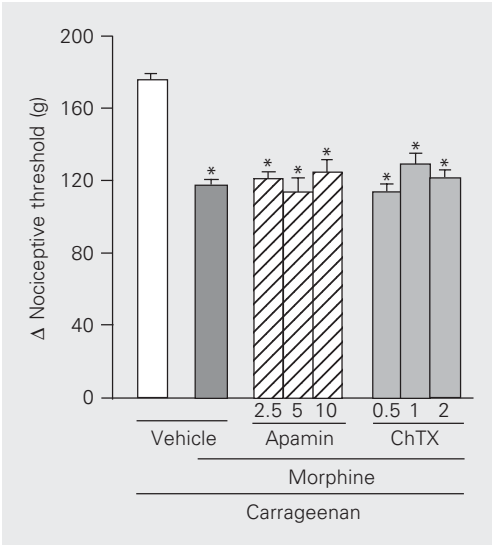

Figure 5. Inhibition by intraplantar administration of apamin (Apa, 10 µg), charybdotoxin (ChTX, 2 µg), 4-aminopyridine (4-AP, 50 µg), tetraethylammonium (TEA, 150 µg), and cesium (250 µg) of the peripheral antinociception induced by fentanyl in hyperalgesic paws. Antagonists were administered 30 min after fentanyl (1.5 µg). Each column indicates the mean ± SEM (N = 5). No statistically significant differences were detected between the groups treated with fentanyl + vehicle and fentanyl + Apa, ChTX, 4-AP, TEA, or cesium in any case. *P < 0.01 *vs* carrageenan + vehicle-injected control (ANOVA/Bonferroni's test).

sulfonylurea tested significantly modified the nociceptive threshold in control animals, or induced any overt behavioral effect at the doses used. Furthermore, the maximum dose of glibenclamide administered by the same route did not significantly alter the plasma glucose level (data not shown).

Effect of apamin, ChTX, 4-AP, TEA, or cesium on fentanyl-induced antinociception

Intraplantar injection of apamin (10 µg) had no significant effect on fentanyl-induced antinociception. ChTX (2.0 µg/paw) also failed to significantly counteract the antinociception induced by fentanyl (Figure 5). As also shown in the same Figure, 4-AP (50 µg/paw), TEA (150 µg/paw) and cesium (250 µg/paw) did not significantly modify the antinociception induced by fentanyl.

Discussion

It has been suggested that the molecular mechanism of peripheral (15) and central (16) analgesia induced by morphine involves activation of the L-arginine/nitric oxide/cGMP pathway. Morphine has been shown to exert its peripheral antinociceptive effect by activating ATP-sensitive K^+ channels (17). Recent studies carried out in our laboratory demonstrated that the peripheral antinociceptive action of the nitric oxide donor sodium nitroprusside (26) and dibutyryl cGMP (27) is associated with ATP-sensitive K^+ channels, thus establishing a link between the participation of the nitric oxide/cGMP pathway in the analgesia induced by certain drugs and the activation of ATP-sensitive K^+ channels.

The present findings demonstrate that the sulfonylureas glibenclamide and tolbutamide can reverse the peripheral antinociceptive effect induced by intraplantar administration of fentanyl in rats. Other K^+ channel blockers such as apamin, ChTX, 4-AP, TEA, and cesium did not exhibit any inhibitory

effect. The doses of the ineffective blockers are compatible with those used to examine the involvement of potassium channels in the inhibitory prejunctional effect of a μ-opioid agonist on peripheral sensory nerves *in vivo* (25), and in peripheral antinociception by morphine (17).

A growing number of both experimental and clinical studies have demonstrated that locally administered opioids produce pronounced analgesic effects by interacting with peripheral opioid receptors (11-13,28). According to Stein (29), μ-opioid agonists are more potent than δ or κ agonists in inducing peripheral antinociceptive effects. Thus, we used fentanyl because it has been described as an agonist of μ-opioid receptors (30) and has been extensively used as an analgesic (18).

Many strategies can be used to exclude the central effects of opioids (29). In the present study, we used the strategy of evaluating the efficacy of ipsi- versus contralateral paw administration because the route and site of administration would be the same. Carrageenan was administered into both hind paws, thus creating the same tissue conditions and providing an equal possibility that fentanyl would reach sites outside the injected paw. Since the nociceptive threshold was always measured in the right hind paw, fentanyl at a dose of 1.5 μg was ineffective when administered into the contralateral paw, suggesting that at this dose fentanyl has a peripheral site of action in inflamed tissue. This effect seems to be specific and receptor mediated, since 50 μg naloxone (when injected into the right paw, but not into the left), totally blocked the antinociceptive effect of fentanyl (data not shown).

Patch-clamp studies have shown that the sulfonylureas are selective inhibitors of ATP-sensitive K^+ channels in pancreatic ß-cells, cardiac myocytes and skeletal muscle cells (19). Indeed, the sensitivity to sulfonylureas, especially the potent glibenclamide, is commonly used to characterize the K_{ATP} channel (31). However, glibenclamide also blocks an ATP-independent K^+ current in a human neuroblastoma cell line (32) and a delayed rectifier K^+ current in neural and cardiac cells (33). Blockade of these currents might mimic the effects expected from K_{ATP} blockade, thus potentially confusing the interpretation of the results. Delayed rectifying K^+ channels are blocked by TEA, 4-AP and cesium (34) and if fentanyl were acting through the activation of these channels both sulfonylureas and these other blockers should reverse this effect.

It has been demonstrated that glibenclamide cannot bind directly to μ-, δ- or κ-opioid receptors because this drug cannot alter the binding of specific agonists of these receptors (35). The effect of sulfonylureas against fentanyl-induced antinociception should not be interpreted as a counteraction by a possible increased excitability induced by the blockers, since these drugs do not cause any hyperalgesic effect when administered alone. Our results are consistent with reports (36) describing glibenclamide as more potent in blocking ATP-sensitive K^+ channels than tolbutamide in pancreatic ß-cells and in smooth and cardiac muscle. In the present study, the maximum dose of glibenclamide (240 μg/paw) did not significantly alter the plasma glucose levels (data not shown). Furthermore, all sulfonylureas tested to date, when administered by the intracerebroventricular or intrathecal route, dose dependently antagonized the antinociception induced by systemic administration of fentanyl (4,37), suggesting that opening of ATP-sensitive K^+ channels in neurons of the central nervous system underlies the antinociceptive effect of fentanyl. Interestingly, peripheral antinociception of bremazocine, a κ-opioid, is not due to K^+ channel activation (38).

In the present study, apamin, a protein extracted from bee venom and a selective blocker of small conductance Ca^{2+}-activated K^+ channels (20), and ChTX, a toxin that

blocks large conductance calcium-activated K^+ channels (21), failed to antagonize the peripheral antinociceptive effect induced by the selective μ-opioid receptor agonist fentanyl. It was demonstrated that activation of ChTX-sensitive, but not apamin-sensitive K^+ channels may be the mechanism through which μ-opioid receptor agonists exert prejunctional modulation of sensory nerves in guinea pig airways (9). These investigators (9) also suggested that activation of the same type of K^+ channel could be involved in the modulation of pain sensation by opiates. Our results disagree with this hypothesis and exclude the involvement of both types of Ca^{2+}-activated K^+ channels in the peripheral antinociception induced by fentanyl. According to others (39), ChTX is not specific for the large conductance Ca^{2+}-activated K^+ channels, but blocks a number of other K^+ channels.

Our results show that 4-AP, TEA, and cesium administered intraplantarly had no significant effect on the peripheral antinociception induced by fentanyl. These drugs block different types of K^+ channels, including calcium-activated and voltage-dependent K^+ channels, although they are not specific for any of them in particular (22). Ocaña et al. (4) showed that 4-AP and TEA have no effect on the central antinociception induced by μ-opioid receptor agonists, including fentanyl. Finally, K^+ channels activated by μ-opioid agonists are not sensitive to 4-AP or TEA (40).

Thus, we have shown that two different sulfonylureas, glibenclamide and tolbutamide, antagonized the peripheral antinociceptive effect induced by the μ-opioid receptor agonist fentanyl in rats, suggesting that ATP-sensitive K^+ channels play an important role in this effect. It is important to consider that other potassium channels such as G protein-coupled channels might be involved. Since other K^+ channel blockers failed to reverse this effect it may be inferred that other types of K^+ channels such as large conductance Ca^{2+}-activated, small conductance Ca^{2+}-activated and voltage-dependent K^+ channels appear not to be involved in the peripheral antinociceptive effect of fentanyl.

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