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Review

Potassium channels and pain: present realities and future opportunities

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Abstract

Four families of potassium channels with different structures, functional characteristics and pharmacological sensitivity, are distinguished in neurons: voltage-gated (K_v), calcium-activated (K_{Ca}), inward rectifier (K_{ir}) and two-pore (K_{2P}) K^+ channels. During the last 15 years, numerous studies have demonstrated that the opening of some of these K^+ channels plays an important role in the antinociception induced by agonists of many G-protein-coupled receptors (α_2-adrenoceptors, opioid, $GABA_B$, muscarinic M_2, adenosine A_1, serotonin 5-HT_{1A} and cannabinoid receptors), as well as by other antinociceptive drugs (nonsteroidal antiinflammatory drugs [NSAIDs], tricyclic antidepressants, etc.) and natural products. Several specific types of K^+ channels are involved in antinociception. The most widely studied are the ATP-sensitive K^+ channels (K_{ATP}), members of the K_{ir} family, which participate in the antinociception induced by many drugs that activate them in both the central and the peripheral nervous system. The opening of G-protein-regulated inwardly rectifying K^+ channels (GIRK or $K_{ir}3$), $K_v1.1$ and two types of K_{Ca} channels, the small- and large-conductance calcium-activated K^+ channels (SK and BK channels, respectively), also play a role in the antinociceptive effect of different drugs and natural products. Recently, drugs that open K^+ channels by direct activation (such as openers of neuronal K_v7 and K_{ATP} channels) have been shown to produce antinociception in models of acute and chronic pain, which suggests that other neuronal K^+ channels (e.g. $K_v1.4$ channels) may represent an interesting target for the development of new K^+ channel openers with antinociceptive effects.

Keywords: K^+ channels; Antinociception; G-protein-coupled receptor; Opioid; Nonsteroidal antiinflammatory drugs; Tricyclic antidepressant

Contents

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1. Introduction

During the 1970s and 1980s, numerous studies demonstrated that agonists of several G-protein-coupled receptors (such as μ- and δ-opioid receptors, α_2-adrenoceptors, $GABA_B$ receptors, etc.) open specific K^+ channels in neurons (reviewed in North, 1989) and produce antinociception (see references below). These antecedents led to the hypothesis that K^+ channel opening was involved in the antinociceptive effect of agonists of G-protein-coupled receptors; consequently, it was expected that blockers of specific K^+ channels would antagonize the antinociception induced by these agonists. The earliest evidence in support of this hypothesis was published in 1990 (Ocaña et al., 1990). Subsequently, many studies have used the same experimental strategy to show that certain K^+ channel blockers antagonize the antinociceptive effect of many drugs. A drawback of this experimental approach is the lack of specific blockers for several K^+ channels.

At the end of the 1990s, new experimental strategies were used to identify the K^+ channels involved in antinociception. The antinociceptive effects of drugs were tested either in animals pretreated with antisense oligodeoxyribonucleotides (ODN) to knockdown the expression of a particular K^+ channel protein (Galeotti et al., 1997a), or in animals genetically modified to knockout the expression of a specific K^+ channel (Clark and Tempel, 1998). In both cases, if the antinociception induced by the drug was reduced, it was concluded that the absent or downregulated K^+ channel was activated by the drug under study to induce its antinociceptive effect.

In recent years, it has become evident that K^+ channel opening is involved in the antinociception induced by numerous drugs. Consequently, K^+ channels themselves have begun to be considered as targets for the development of new antinociceptive drugs, and drugs that open K^+ channels by directly interacting with them (K^+ channel openers) have been tested in models of acute and chronic pain.

This review is devoted mainly to the role of K^+ channel opening in the antinociception induced by several antinociceptive drugs (agonists of receptors coupled to G proteins, nonsteroidal anti-inflammatory drugs, tricyclic antidepressants, etc.) and by K^+ channel openers. However, since there are many types of K^+ channels that differ in structure, functional characteristics and pharmacological sensitivity, it is useful to begin with a brief review of the different types of K^+ channels and their sensitivity to drugs.

2. Types of K^+ channels

Neuronal K^+ channels selectively enable extremely rapid diffusive flow of K^+, down its electrochemical gradient, across the plasma membrane. They play a key role in controlling neuronal activity and signal propagation throughout the nervous system (Shieh et al., 2000; MacKinnon, 2003). The opening of K^+ channels is regulated by a wide array of stimuli such as changes in membrane voltage or intracellular levels of certain ions, small organic molecules and proteins (e.g. Ca^{2+}, ATP, cAMP or G-protein subunits) (MacKinnon, 2003; Roosild, 2004). Recently, the International Union of Pharmacology (IUPHAR) proposed a standardized K^+ channel nomenclature based in the structural and phylogenetic relationships of the proteins that constitute the channels (Gutman et al., 2003). This classification is intended to unify and update the different classifications published previously, and distinguishes four different types of K^+ channels known as voltage-gated (K_v), calcium-activated (K_{Ca}), inward rectifier (K_{ir}) and two-pore (K_{2P}) K^+ channels.

2.1. Voltage-gated K^+ channels

Voltage-gated K^+ channels are very numerous. They comprises 12 different families (named K_v1 to K_v12), many consisting of more than 1 channel. For instance, the K_v1 family comprises eight different channels (named $K_v1.1$ to

K_v1.8) (Gutman et al., 2003). All K_v channels share the same structural characteristics and are tetramers of α subunits (Fig. 1A and B). The α subunits are proteins with six transmembrane domains and N and C termini regions located intracellularly (Fig. 1A). The fifth and sixth transmembrane domains and the region between them (P region) constitute the K^+ channel pore-forming motif (Fig. 1B) (Gutman et al., 2003; Roosild et al., 2004), whereas the fourth transmembrane domain is positively charged and is part of the voltage sensor that permits the opening of the channel when the cell is depolarized (MacKinnon, 2003; Roosild et al., 2004) (Fig. 1A). Near the N terminus of the protein there is a cytoplasmic domain (named T1) that is involved in mediating the assembly of the subunits, in modulating voltage sensitivity of the channel, and in interacting with auxiliary β subunits (K_vβ) (Fig. 1A) (Roosild et al., 2004).

Many synthetic drugs are able to block K_v channels. Two of them, 4-aminopyridine and tetraethylammonium, have frequently been used in nociception studies; however, they are not selective for a particular type of K_v channel (and can even block some K_{Ca} channels at high concentrations) (Mathie et al., 1998; Kaczorowski and Garcia, 1999). Many toxins from scorpions and other sources are K_v blockers, and they offer the advantage of greater specificity. For instance, some scorpion venoms (e.g. margatoxin, kaliotoxin or agitoxin) block some K_v1 channels, but not K_v2, K_v3 or K_v4 channels (Kaczorowski and Garcia, 1999). On the other hand, some spider venoms (such as hanatoxins) preferentially block K_v2 channels (Shiau et al., 2003). None of these toxins have been used to modulate the effect of antinociceptive drugs, but such a use may be of interest. Neuronal K_v7 channels (K_v7.2–K_v7.5 also known as KCNQ2–5) can be modulated by many drugs, which include both openers (retigabine, flupirtine, BMS-204352 [(±)-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2*H*-indol-2-one)]) and blockers (linopirdine, XE-991 [10,10-bis(4-pyridinylmethyl)-9(10*H*)-anthracenone], etc.) (Gribkoff, 2003). The openers of neuronal K_v7 channels produce antinociception (see Section 6.1 below).

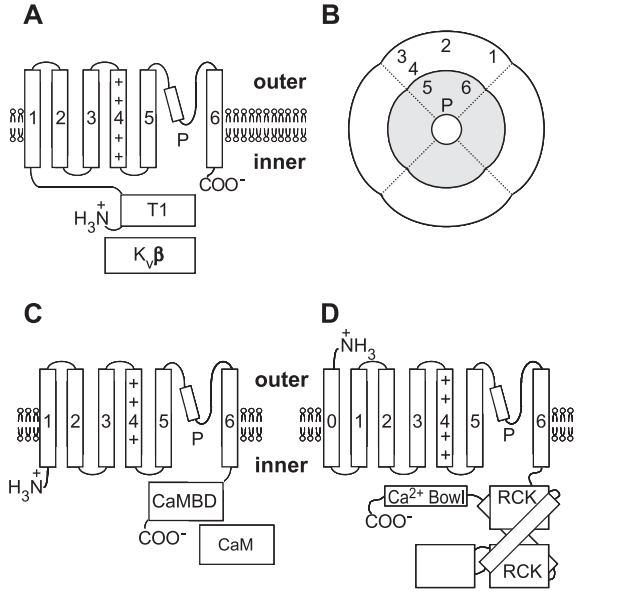

Fig. 1. Schematic representation of the structure of voltage-gated (K_v), small- and large-conductance calcium-activated K^+ channels (SK and BK, respectively). (A) The structure of the K_v channel α subunit contains six transmembrane domains (1–6), a pore-forming loop (P) and an N termini intracellular domain (T1), which permits interaction with the β subunits (K_vβ). (B) Schematic quaternary structure of the K_v channels: The channel is a tetramer of α subunits, each α subunit has a pore-forming motif (grey) constituted by the P-loop and transmembrane domains 5 and 6. (C) The structure of the α subunit of SK channels is similar to that of the K_v channels; however, the fourth transmembrane domain is less charged and the C-terminal intracellular domain contains a calmodulin-binding domain (CaMBD), which permits interaction with calmodulin (CaM) and its regulation by calcium. The quaternary structure of SK channels is similar to that of the K_v channels illustrated in B. (D) The BK α subunits contain seven transmembrane domains (0–6), a P-loop and a complex C termini intracellular domain that contains two RCK (regulated conduction of K^+) domains and a 'Ca^{2+} bowl' that permits regulation of the BK channel by Ca^{2+}. The quaternary structure of the BK channels is similar to that shown in B.

2.2. Calcium-activated K^+ channels

Calcium-activated K^+ channels are not so numerous as the K_v channels and have been classified in three different families on the basis of channel conductance. They are usually named large- (big, BK), intermediate- (IK) and small- (SK) conductance calcium-activated K^+ channels (Vergara et al., 1998). Several subtypes of BK and SK channels have been described (Vergara et al., 1998; Gutman et al., 2003). The common characteristic in all K_{Ca} channels is that they are activated when the cytoplasmic concentration of Ca^{2+} is increased, but BK channels are also voltage sensitive (Vergara et al., 1998; Vogalis et al., 2003).

The overall architecture of the SK channel α subunit is very similar to that of the K_v channels: six transmembrane domains (1–6) and a P region, with intracellular N and C termini (Fig. 1C). These elements undergo tetramerization to form a functional channel (Fig. 1B) (Vergara et al., 1998; Vogalis et al., 2003). However, the SK channels have a less charged fourth transmembrane domain and are voltage-insensitive. Moreover, the SK channel possess a C-terminal domain, called the calmodulin-binding domain (CaMBD), which allows the channel to interact with calmodulin and to be regulated by Ca^{2+} (Fig. 1C) (Roosild et al., 2004). The SK channels are blocked by apamin, dequalinium and scyllatoxin (Vogalis et al., 2003; Liegeois et al., 2003). Openers of SK channel have recently been described (Liegeois et al., 2003). The structure of the IK channels is not so well known as that of the other K_{Ca} channels, but seems to show high homology with SK channel structure (Vergara et al., 1998; Vogalis et al., 2003). The IK channels are insensitive to apamin but blocked by charybdotoxin and clotrimazole (Vergara et al., 1998).

The BK channel α subunits also share with the K_v channels the six transmembrane domains and the P region structure, and also constitute tetramers. However, unlike K_v channels, BK channels have extracellular N-termini owing to an additional transmembrane domain (termed 0) that precedes the six domains homologous to those in K_v channels (Fig. 1D). The C-termini is cytosolic and contains a region termed the "calcium bowl", which binds calcium and participates in the activation of the channel, as well as two RCK (regulates conduction of K^+) domain structures (Fig. 1D). The BK channels are stimulated by NS1619 (1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl) phenyl]-5-(trifluoromethyl)-2*H*-benzimidazol-2-one) and blocked by charybdotoxin (which also blocks IK channels and some K_v channels) and iberiotoxin (which blocks only BK channels) (Nardi et al., 2003). Charybdotoxin has usually been used to identify the involvement of BK channels in the antinociception induced by many drugs, but since iberiotoxin is a more selective blocker of these channels, it seems that this drug is preferable for this purpose.

2.3. Inward rectifier K^+ channels

Inward rectifier K^+ channels are also numerous. They comprises seven different families ($K_{ir}1$–$K_{ir}7$) and several of these families comprise different subtypes of K^+ channels (e.g. $K_{ir}3.1$–$K_{ir}3.4$) (Nichols and Lopatin, 1997; Gutman et al., 2003). Their structure is simpler than that of K_v and K_{Ca} channels, since they contain only the pore-forming motif of the K^+ channels, i.e., two transmembrane domains and a P region (Fig. 2A). The N and C termini of these channels are located in the cytoplasm (Fig. 2A), and the functional channel is a tetramer of these pore-forming subunits (Fig. 2B) (Nichols and Lopatin, 1997; Gutman et al., 2003).

Two families of these K_{ir} channels are particularly important for nociception: the G-protein-regulated inward rectifier K^+ channels (GIRK or $K_{ir}3$) and the ATP-sensitive K^+ (K_{ATP}) channels (formed with $K_{ir}6$). The GIRK channels contain cytoplasmic structures located at both the N and C termini that permit interaction with the $G_{\beta\gamma}$ subunits of G-proteins (Fig. 2A) (Mark and Herlitze, 2000; Roosild et al., 2004). A blocker of GIRK channels, tertiapin, has been used in some nociception experiments (Marker et al., 2004). The functional K_{ATP} channels are constituted by two subunits, the $K_{ir}6$ channel and the so-called SUR (*SU*lfonylurea *R*eceptor) subunit, a 17-transmembrane-domain protein that contains two nucleotide binding folds (NBF) (Fig. 2E) (Aguilar-Bryan et al., 1998). Several subtypes of $K_{ir}6$ ($K_{ir}6.1$ and 6.2) and SUR (SUR1, 2A and 2B) subunits have been identified. This permits several combinations to constitute the functional K_{ATP} channels, which are tetramers of $K_{ir}6$-SUR complexes. The SUR protein permits blockade of the K_{ATP} channels by sulfonylureas, their opening by several K_{ATP} openers (cromakalim, pinacidil, minoxidil, nicorandil) (Aguilar-

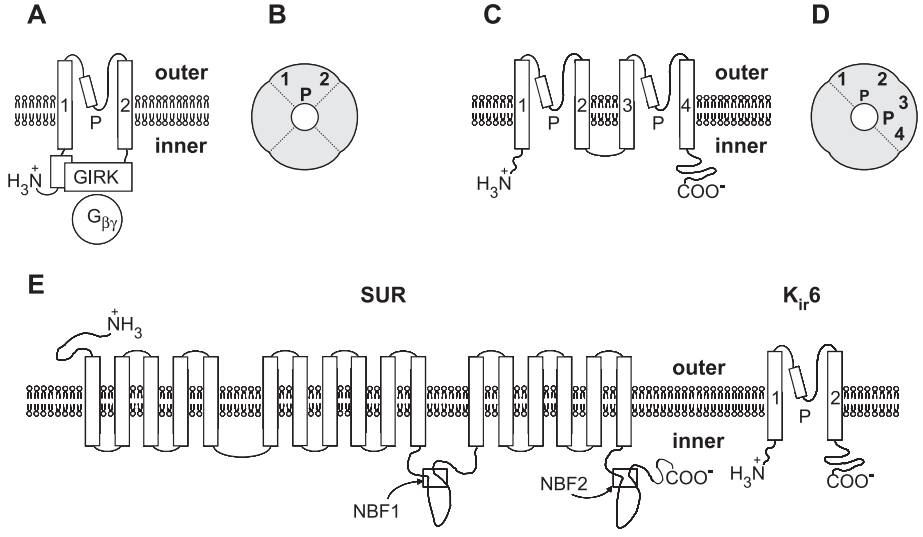

Fig. 2. Schematic representation of the structure of inward rectifier (K_{ir}) and two-pore (K_{2P}) K^+ channels. (A) The structure of the G-protein-regulated inwardly rectifying K^+ channel (GIRK or $K_{ir}3$ channel) comprises only two transmembrane domains and a P-loop. The N and C termini are intracellular and contain domains that interact with the $G_{\beta\gamma}$ subunits of G-proteins, which regulate channel opening. (B) Schematic quaternary structure of the GIRK channels: The channel is a tetramer of α subunits (equivalent to that shown in grey in Fig. 1B). (C) The K_{2P} channel α subunits are constituted by four transmembrane domains and two P-loops, with intracellular N and C termini. (D) The quaternary structure of K_{2P} channels consists of a dimer of α subunits. (E) Structure of the K_{ATP} channels: The functional channel is constituted by a $K_{ir}6$ channel and a SUR (*SU*lfonylurea *R*eceptor) subunit. The $K_{ir}6$ channel has two transmembrane domains, a P-loop and intracellular N and C termini. The N termini of the $K_{ir}6$ channel interact with the C termini of the SUR subunit. The SUR subunit has 17 transmembrane domains and two nucleotide binding folds (NBF). The SUR subunit permits the regulation of the channel by G-proteins and several K_{ATP} channel openers and blockers. The quaternary structure of the K_{ATP} channel consists of a tetramer of $K_{ir}6$-SUR complexes. The four $K_{ir}6$ subunits form a pore similar to that shown in B, which is surrounded by four SUR subunits.

Bryan et al., 1998) and regulation of K_{ATP} channel activity by G-proteins (Wada et al., 2000).

2.4. Two-pore K^+ channels

The family of two-pore K^+ channels consists, at present, of 14 members (Gutman et al., 2003; Kim, 2003). The α subunits that constitute the K_{2P} channels contain two pore-forming motifs linked in tandem; therefore, the α subunit is constituted by four transmembrane domains and two P regions (Fig. 2C). Functional K_{2P} channels are thought to be composed of dimers of α subunits (Fig. 2D) (Gutman et al., 2003; Kim, 2003). The pharmacological characteristics of these channels are not well known for the moment (Kim, 2003). Moreover, no studies have evaluated the possible role of these channels in nociception, but at least one of these channels (K_{2P}5.1 or KCNK5) is highly expressed in the superficial layers (laminae I and II) of the spinal cord dorsal horn (Gabriel et al., 2002), a key point in the nociception pathway.

3. Role of K^+ channels in antinociception induced by opioid receptor agonists

During the 1980s many electrophysiological studies demonstrated that agonists of μ- and δ-opioid receptors open inward rectifier K^+ channels in neurons through the activation of $G_{i/o}$ proteins (North, 1989). The $G_{i/o}$ proteins are able to open two different types of K_{ir} channels: the K_{ATP} (Sanchez et al., 1998; Wada et al., 2000) and the GIRK (Mark and Herlitze, 2000) channels. It is therefore logical that many studies have evaluated the involvement of these two types of K_{ir} channels in opioid-induced antinociception. Some studies have also evaluated the role of different K_v and K_{Ca} channels in this type of antinociception.

3.1. μ-Opioid receptors

The first study to suggest a role for K^+ channel opening in μ-opioid-induced antinociception showed that the K_{ATP} channel blocker glibenclamide (i.c.v.) antagonized the antinociception induced by subcutaneous morphine in a hot plate test (Ocaña et al., 1990). Similar results were obtained later in a tail flick test in mice and rats (Ocaña et al., 1993, 1995; Roane and Boyd, 1993). The antagonism by glibenclamide of morphine antinociception can not be explained by a direct effect of the sulfonylurea in the μ-opioid receptor, since glibenclamide did not displace the μ-opioid receptor agonist [^{3}H]DAMGO ([D-Ala^2, N-Me-Phe^4, Gly^5-ol]enkephalin) from its binding sites (Raffa and Codd, 1994). Moreover, morphine was unable to displace [^{3}H]glibenclamide from its binding sites in mouse brain (Narita et al., 1992), which suggests that the interaction between glibenclamide and morphine was not due to the binding of morphine to the sulfonylurea receptor in the K_{ATP} channel.

Not only glibenclamide but all sulfonylureas tested (i.c.v. injected) dose-dependently antagonized the antinociception induced by s.c. morphine (Table 1). The only difference between the sulfonylureas in inducing this effect was in their potency: gliquidone>glipizide>glibenclamide>tolbutamide (Ocaña et al., 1993). This order of potency is the same as that found for the blocking of K_{ATP} channels in neurons by these drugs (Amoroso et al., 1990; Schmid-Antomarchi et al., 1990), which suggests that the antagonism of morphine-induced antinociception by sulfonylureas is due to the blockade of K_{ATP} channels. Further support for the role of K_{ATP} channels in morphine-induced antinociception was

Table 1
Changes in the antinociception induced by agonists of several G-protein-coupled receptors when they were associated with different K^+ channel-acting drugs (K_{ATP} channel blockers [sulfonylureas], K_{ATP} channel openers [cromakalim, diazoxide, minoxidil or nicorandil], tetraethylammonium [TEA], 4-aminopyridine [4-AP], apamin or charybdotoxin), or when they were administered to animals pretreated with antisense ODN to K_v1.1 channels or to GIRK2 knockout animals

Receptor activated to induce antinociception	K_{ATP} blocker	K_{ATP} opener	TEA	4-AP	Apamin	Charybdotoxin	K_v1.1 ODN	GIRK2 knockout
μ-Opioid (morphine)[a]	↓	↑	=	=	=	=	↓	↓
α_2-Adrenoceptor	↓	↑	=	=	=	↓	↓	↓
Adenosine A_1	↓	↑	=	=	?	?	?	?
Serotonin 5-HT_{1A}	↓	↑	=	=	?	?	?	?
Muscarinic M_2	↓	↑	?	?	?	↓	?	↓
δ_1-Opioid	↓[b]	↑	=	?	↓	=	?	?
$GABA_B$	=	=	↓	↓	?	?	↓	↓
δ_2-Opioid	=	?	↓	?	?	?	?	?
κ-Opioid	=	=	=	=	↓	=	?	↓
Cannabinoid	=	?	=	=	↓[b]	=	?	↓
μ-Opioid (fentanyl)[a]	=	=	=	=	?	?	?	?

The antinociception induced by the agonists of the receptors is classified as reduced (↓), enhanced (↑) or unchanged (=). A question mark (?) indicates that there are no data available.

[a] Different results were obtained when μ-opioid receptors were activated with morphine and fentanyl.

[b] Different results were obtained with the i.c.v and i.t. administration of K^+ channel blockers (see text for details).

obtained with the use of K_{ATP} channel openers: the i.c.v. administration of cromakalim and pinacidil potentiated the antinociception induced by s.c. morphine in the tail flick test (Vergoni et al., 1992; Ocaña et al., 1995, 1996) (Table 1). The potentiation induced by cromakalim was abolished by gliquidone (Ocaña et al., 1996), which suggests that this interaction was due to an effect on the K_{ATP} channels.

Both supraspinal and spinal mechanisms are involved in the antinociception induced by subcutaneous morphine; therefore, additional experiments were performed to evaluate the effects of K_{ATP} channel blockers and openers on these mechanisms. The antinociception induced by i.c.v. morphine in hot plate and tail flick tests was antagonized by i.c.v. glibenclamide (Wild et al., 1991; Narita et al., 1992; Kamei et al., 1994; Raffa and Martinez, 1995) and was potentiated by the i.c.v. administration of two K_{ATP} channel openers (cromakalim and diazoxide) (Narita et al., 1993; Lohmann and Welch, 1999b) (Table 1). Coadministration of glibenclamide abolished potentiation by the K_{ATP} channel openers of the antinociceptive effect of morphine (Lohmann and Welch, 1999b). These results support a role for K_{ATP} channel opening in morphine-induced antinociception at the supraspinal level.

The spinally mediated antinociceptive effect of morphine (i.t.) was also antagonized by i.t. glibenclamide in different models of pain in rodents (Welch and Dunlow, 1993; Kang et al., 1997, 1998a,b; Yang et al., 1998). On the other hand, the antinociception induced by epidural morphine in a tail flick test in rats was potentiated by the epidural administration of the K_{ATP} channel openers nicorandil and levcromakalim, and glibenclamide was able to abolish this potentiation (Asano et al., 2000). Together, these results suggest that opening of the K_{ATP} channels also plays an important role in the spinal antinociceptive effect of morphine. Moreover, since the supraspinally induced antinociceptive effect of morphine is mainly naloxonazine-sensitive (i.e. μ_1-opioid receptor-mediated), whereas the spinally induced is mainly naloxonazine-insensitive (i.e. μ_2-opioid receptor-mediated) (Pasternak, 2001), it seems apparent that both μ_1- and μ_2-opioid receptor activation promotes antinociception through the opening of K_{ATP} channels.

Opioid receptors are located not only in the central nervous system but also in the peripheral terminals of primary afferents, and activation of the peripheral μ-opioid receptors produces local antinociception (Janson and Stein, 2003). Rodrigues and Duarte (2000) showed that intraplantar administration in the rat paw of low doses of morphine was able to antagonize the mechanical hyperalgesia induced by carrageenan in the paw through the activation of peripheral opioid receptors. The local (intraplantar) administration of the K_{ATP} channel blockers glibenclamide and tolbutamide antagonized the antihyperalgesic effect of morphine in this model (Rodrigues and Duarte, 2000) (Table 1). Glibenclamide was more potent than tolbutamide in inducing antagonism, which is consistent with their order of potency for blocking K_{ATP} channels in neurons (Amoroso et al., 1990; Schmid-Antomarchi et al., 1990). Intraplantar glibenclamide was also able to antagonize the local antinociception induced by the intraplantar administration of morphine in the formalin test (Ortiz et al., 2002; Granados-Soto et al., 2002), which also suggests that K_{ATP} channels are involved in the peripheral antinociception induced by morphine.

In summary, many studies have suggested that the opening of K_{ATP} channels plays an important role in the antinociception induced by morphine at supraspinal, spinal and peripheral (primary afferent nerve ending) levels. The opening of K_{ATP} channels by morphine could be due to its ability to activate $G_{i/o}$ proteins since both G_{α} and $G_{\beta\gamma}$ subunits of these proteins are able to activate K_{ATP} channels (Sanchez et al., 1998; Wada et al., 2000).

In addition to K_{ATP} channels, another type of K_{ir} channel has also been implicated in the antinociceptive effect of morphine, mainly through studies in mutant mouse strains in which subtypes of $K_{ir}3$ channels (also known as GIRK or G-protein-regulated inward rectifier K^+ channels) are altered or lacking. The first study was performed in *weaver* mutant mice, which have a mutation in the pore-forming region of the GIRK2 subunits. In these animals the antinociceptive effect of the i.p. administration of morphine was lower in both the hot plate and the tail flick test in comparison to control animals (Ikeda et al., 2000). More recently, mutant mice lacking each of the GIRK subunits have been produced. This has permitted a detailed study of the role of each type of GIRK in morphine-induced antinociception. In the GIRK1 and GIRK2 knockout animals, the antinociceptive effect of morphine after i.t. administration was reduced, which correlates with a dramatic reduction in the expression of both subunits in the superficial layers of the spinal cord dorsal horn. These results suggest that these channels are important for the expression of the spinal antinociceptive effect of morphine (Marker et al., 2004). The subcutaneous administration of morphine also produced less antinociception both in the hot plate and the tail flick test in GIRK2 knockout animals in comparison to wild-type littermates (Marker et al., 2002; Mitrovic et al., 2003) (Table 1). However, there were no differences in the antinociceptive effect of s.c. morphine between the GIRK2 knockout animals and their wild-type littermates in the formalin test (Mitrovic et al., 2003). These results suggest that the role of GIRK2-containing channels differs in different types of pain.

The s.c. administration of morphine also produced less antinociception in the hot plate test in GIRK3 knockout animals, but the reduction was not as evident as in GIRK2 knockout animals (Marker et al., 2002). Moreover, since the reduction in the antinociception induced by s.c. morphine in GIRK2 knockout animals and in GIRK2/3 double knockout mice was similar (Marker et al., 2002), it seems that GIRK3 subunits are not particularly important for s.c. morphine-

induced antinociception. Furthermore, morphine i.t. had similar effects in GIRK3 knockout and wild-type animals (Marker et al., 2004), which suggest that the role of GIRK3-containing channels in the spinal antinociceptive effect of morphine is limited. In summary, activation of K^+ channels that contain GIRK1 or GIRK2, but not GIRK3, subunits appears to play a significant role in the antinociception induced by morphine.

Only one study has used a pharmacological approach to evaluate the role of GIRK channels in morphine-induced antinociception. The results are congruent with those obtained in knockout animals: the i.t. administration of tertiapin (a blocker of GIRK channels) antagonized the antinociception induced by i.t. morphine in a tail flick test (Marker et al., 2004). Whether a similar interaction occurs at supraspinal level is unknown.

Some studies have also evaluated the possible role of K_{Ca} channels in morphine-induced antinociception. The SK channel blocker apamin (administered i.t.) was unable to modify the antinociceptive effect of i.t. morphine in the tail flick test (Welch and Dunlow, 1993); similarly, intraplantar apamin did not modify the peripherally mediated antinociception induced by the intraplantar administration of morphine in different pain tests (Rodrigues and Duarte, 2000; Ortiz et al., 2002; Granados-Soto et al., 2002) (Table 1). The BK channel blocker charybdotoxin was also unable to modify the spinal and peripheral antinociceptive effects of morphine (Welch and Dunlow, 1993; Rodrigues and Duarte, 2000; Ortiz et al., 2002; Granados-Soto et al., 2002) (Table 1). Therefore, although the number of studies published thus far is still small, all of them suggest that the opening of K_{Ca} channels does not play an important role in morphine-induced antinociception.

Few studies have evaluated the role of K_v channels in the antinociceptive effect of morphine. In this case, the pharmacological tools used (4-aminopyridine and tetraethylammonium) are not specific blockers of any particular type of K_v channel and, consequently, no clear conclusions can be drawn. The i.c.v. and i.t. administration of 4-aminopyridine and tetraethylammonium did not modify the antinociception induced by s.c. morphine in the tail flick test (Ocaña et al., 1990, 1995; Welch and Dunlow, 1993), or the spinal antinociception induced by i.t. morphine in this test (Welch and Dunlow, 1993). Moreover, both K^+ channel blockers (administered in the footpad) were unable to antagonize the peripheral antinociception induced by morphine in the carrageenan test (Rodrigues and Duarte, 2000). Therefore, it seems that 4-aminopyridine- and tetraethylammonium-sensitive K^+ channels are not involved in morphine-induced antinociception (Table 1).

A totally different experimental approach, the use of antisense ODN and knockout animals, has been used to evaluate the role of $K_v1.1$ channels in the antinociceptive effect of morphine. The i.c.v. administration of an antisense ODN targeting the mouse $K_v1.1$ mRNA produced dose-dependent antagonism of morphine-induced antinociception in a hot plate test, an effect that was simultaneous with the inhibition of $K_v1.1$ gene expression (Galleotti et al., 1997b) (Table 1). The effect of the antisense ODN appears to be specific, since a degenerated ODN which did not affect $K_v1.1$ gene expression did not antagonize morphine antinociception (Galleotti et al., 1997b). In agreement with these results, it was shown that the antinociceptive effect of low doses of morphine in two thermal tests (hot plate and paw-withdrawal) was lower in $K_v1.1$ knockout animals than in their wild-type littermates (Clark and Tempel, 1998).

In conclusion, many studies suggest that the antinociception induced by morphine is mediated by the opening of K_{ATP} and GIRK channels. Some studies also suggest a role for $K_v1.1$ channels, whereas BK and SK channels do not appear to play a significant role (Table 1).

Two studies have compared the effects of K^+ channel-acting drugs on the antinociception induced by several agonists of μ-opioid receptors. The antinociceptive effect of morphine, methadone and buprenorphine was antagonized by the i.c.v. administration of K_{ATP} channel blockers (glibenclamide and gliquidone) and was potentiated by the K_{ATP} channel opener cromakalim (Ocaña et al., 1995; Raffa and Martinez, 1995), but was not modified by 4-aminopyridine and tetraethylammonium (Ocaña et al., 1995). On the other hand, the antinociception induced by fentanyl and its analogues (sufentanil, alfentanil and carfentanil) was not modified by K_{ATP} channel openers or blockers, or by 4-aminopyridine and tetraethylammonium (Ocaña et al., 1995; Raffa and Martinez, 1995). These results are interesting because they suggest that not all agonists of μ-opioid receptors open K_{ATP} channels to induce antinociception, and point toward the existence of subgroups of these drugs that may be definable on the basis of their sensitivity to K_{ATP} channel-acting drugs.

3.2. δ-Opioid receptors

Electrophysiological studies suggested that the K^+ channels opened by agonists of μ- and δ-opioid receptors are similar (North, 1989). Therefore, it was not unexpected that K^+ channel-acting drugs were able to modify δ-opioid receptor-mediated antinociception.

The peripherally mediated antinociceptive effect of the intraplantar administration of [D-Pen^2,D-Pen^5]enkephalin (DPDPE) in a model of mechanical hyperalgesia induced by prostaglandin E_2 was abolished by the K_{ATP} channel blocker glibenclamide administered in the footpad (Picolo et al., 2003). This antagonism can not be explained by an antagonistic effect of glibenclamide on δ-opioid receptors, since glibenclamide did not displace [^{3}H]DPDPE from its binding sites (Raffa and Codd, 1994). Similarly, the antinociception induced by the i.c.v. administration of DPDPE in a tail flick test was antagonized by i.c.v. glibenclamide (Wild et al., 1991; Kamei et al., 1994) and was potentiated by cromakalim and diazoxide (i.c.v.

injected) (Lohmann and Welch, 1999b) (Table 1). These results suggest that the opening of K_{ATP} channels is involved in the peripheral and supraspinal antinociceptive effect of DPDPE. In contrast to these results, the antinociception induced by i.t. DPDPE was not significantly antagonized by glibenclamide i.t. (Welch and Dunlow, 1993) (Table 1), which suggests that the role of K_{ATP} channels in the spinal and supraspinal antinociceptive effect of DPDPE are different.

The possible involvement of GIRK channels in the antinociception mediated by δ-opioid receptors has not been evaluated, and very few studies have tested the effect of blockers of K_v and/or K_{Ca} channels on this antinociception. However, it has been reported that the i.c.v. administration of tetraethylammonium did not modify the supraspinal antinociceptive effect of DPDPE (Wild et al., 1991), and that the antinociception induced by i.t. DPDPE was antagonized by i.t. apamin but not by i.t. charybdotoxin (Welch and Dunlow, 1993) (Table 1). None of these results have been replicated or refuted by any other study.

Pharmacological experiments suggest that two types of δ-opioid receptors (δ_1 and δ_2) exist. DPDPE is considered an agonist of δ_1-opioid receptors, whereas [D-Ala^2]deltorphin II is considered an agonist of δ_2-opioid receptors (Quock et al., 1999). Interestingly, the antinociceptive effect of these two agonists showed differential sensitivity to K^+ channel blockers. The antinociception induced by i.c.v. DPDPE was antagonized by i.c.v. glibenclamide but not by tetraethylammonium, whereas [D-Ala^2]deltorphin II-induced antinociception was antagonized by i.c.v. tetraethylammonium but not by glibenclamide (Wild et al., 1991) (Table 1). These results suggest the hypothesis that each type of δ-opioid receptors opens different types of K^+ channels to induce antinociception. To date, however, no further studies have tested this hypothesis.

Another interesting observation is that although DPDPE produced similar antinociceptive effects in the tail flick test in nondiabetic and streptozotocin-induced diabetic mice (Kamei et al., 1994), the role of K_{ATP} channel opening in DPDPE-induced effects in these two types of mice appears to be different. The i.c.v. administration of glibenclamide antagonized the antinociceptive effect of i.c.v. DPDPE in nondiabetic but not in diabetic mice (Kamei et al., 1994). The effect of i.c.v. cromakalim in diabetic mice was also altered, which suggests that brain K_{ATP} channels may have been impaired in the diabetic mice (Kamei et al., 1994).

In summary, several studies suggest that opening of the K_{ATP} channels is involved in the antinociception induced by agonists of δ_1-opioid receptors, and a role for SK channels opening in this antinociception has also been suggested (Table 1). However, additional studies are needed to define the involvement of other K^+ channels in δ-opioid receptor-mediated antinociception and the possible differences between the K^+ channels involved in the antinociceptive effect of δ_1- and δ_2-opioid receptor agonists.

3.3. κ-Opioid receptors

The first study that tested the effect of K^+ channel blockers on the antinociceptive effect of agonists of κ-opioid receptors showed that the antinociception induced by the i.c.v. administration of U69,593 ((5α,7α,8β)-(+)-*N*-methyl-*N*-(7-[1-pyrrolidinyl]-1-oxaspiro[4.5]dec-8-yl)-benzeneacetamide) was not antagonized by glibenclamide or tetraethylammonium injected i.c.v. (Wild et al., 1991). Subsequently, numerous studies further suggested that the opening of K_{ATP} channels is not relevant for the centrally mediated antinociceptive effect of κ-opioid receptor agonists, since glibenclamide i.c.v. and i.t. was also unable to modify the antinociception induced by i.c.v. and i.t. U50,488H (*trans*-(±)-3,4-dichloro-*N*-methyl-*N*-(2[1-pirrolidynyl]cyclohexyl)benzeneacetamide methanesulfonate salt) in hot plate and tail flick tests (Narita et al., 1992; Welch and Dunlow, 1993). Moreover, the antinociception induced by s.c. U50,488H in the tail flick test was not modified by any of several sulfonylureas (glibenclamide, gliquidone, glipizide or tolbutamide) administered i.c.v. (Ocaña and Baeyens, 1993; Ocaña et al., 1993) (Table 1). The only discrepant results have been obtained with i.c.v. cromakalim, which did not modify the antinociceptive effect of U50,488H in two studies (Narita et al., 1993; Ocaña et al., 1996) but enhanced such effect in another (Lohmann and Welch, 1999b). However, in this latter study the potentiation by cromakalim of U-50488H-induced antinociception was not antagonized by i.c.v. glibenclamide, which suggest that K_{ATP} channels were not involved in the interaction. K_{ATP} channels also do not appear to play a role in the peripheral antinociception induced by agonists of κ-opioid receptor, since the antinociception induced by the intraplantar administration of U50,488H in a model of mechanical hyperalgesia induced by prostaglandin E_2 was not modified by the intraplantar administration of glibenclamide (Picolo et al., 2003).

Very few studies have tested the possible role of other K^+ channels (GIRK, K_v and K_{Ca} channels) in κ-opioid-mediated antinociception. The i.c.v. and i.t. administration of 4-aminopyridine and tetraethylammonium did not modify the antinociception induced by s.c. and i.t. U50,488H (Welch and Dunlow, 1993; Ocaña and Baeyens, 1993) (Table 1). Weak antagonism of U50,488H-induced antinociception (i.t.) was observed with i.t. apamin, but i.t. charybdotoxin was ineffective (Welch and Dunlow, 1993) (Table 1). More recently, a reduction in the antinociceptive effect of i.p. U50,488H was observed in mice with a mutation in the pore-forming region of the GIRK2 subunit (Ikeda et al., 2000). Whether a similar reduction occurs in animals lacking each of the GIRK subunits has not been reported.

In conclusion, it seems clear that opening of the K_{ATP} channels does not play a role in the antinociceptive effect of agonists of κ-opioid receptors (Table 1). On the other hand, the opening of GIRK2-containing channels and perhaps

spinal SK channels may be involved in κ-opioid-mediated antinociception, but more studies are needed to confirm the role of these channels in this antinociception.

4. Role of K^+ channels in the antinociception induced by agonists of non-opioid G-protein-coupled receptors

Agonists of many G-protein-coupled receptors share with opioid receptor agonists the ability to open K^+ channels (North, 1989) and to produce antinociception. Since K^+ channel-acting drugs are able to modulate opioid-induced antinociception, they are also expected to modulate the antinociception induced by agonists of non-opioid G-protein-coupled receptors.

4.1. α_2-Adrenoceptors

Several sulfonylureas (administered i.c.v.) antagonized the antinociception induced by s.c. clonidine in tail flick and hot plate tests (Ocaña and Baeyens, 1993; Galeotti et al., 1999b). The order of potency of the sulfonylureas in antagonizing clonidine-induced antinociception was: gliquidone>glipizide>glibenclamide>tolbutamide (Ocaña and Baeyens, 1993), which agrees with their order of potency in blocking K_{ATP} channels (Amoroso et al., 1990; Schmid-Antomarchi et al., 1990). The antinociception induced by other α_2-adrenoceptor agonists (guanabenz, tizanidine and dexmedetomidine) was also antagonized by sulfonylureas (Raffa and Martinez, 1995; Galeotti et al., 1999b; Asano et al., 2000), which suggests that this interaction is common to many agonists of α_2-adrenoceptors (Table 1). It has also been shown that glibenclamide (administered i.c.v. and i.t.) antagonized the antinociception induced by i.c.v. and i.t. clonidine (Raffa and Martinez, 1995; Yamazumi et al., 2001), as well as that induced by i.t. norepinephrine in different nociceptive test (Kang et al., 1998b; Yang et al., 1998). These results suggest that both supraspinal and spinal antinociception induced by α_2-adrenoceptor agonists are antagonized by K_{ATP} channel blockers. In contrast, the antinociception induced by s.c. clonidine and guanabenz was enhanced by several K_{ATP} channel openers (cromakalim, minoxidil, pinacidil) administered i.c.v. (Ocaña et al., 1996; Galeotti et al., 1999b), and the same results were obtained when i.t. nicorandil was associated to i.t. clonidine (Yamazumi et al., 2001) (Table 1). Moreover, the potentiation by cromakalim of clonidine-induced antinociception was dose-dependently antagonized by gliquidone (Ocaña et al., 1996), which further suggests that the interaction takes place at the K_{ATP} channels. In conclusion, many studies have shown that K_{ATP} channel-acting drugs modulate the antinociception induced by α_2-adrenoceptor agonists, and the findings suggest that opening of the K_{ATP} channel is involved in the antinociceptive effect of these drugs.

The GIRK channels also appears to play a key role in the antinociception induced by α_2-adrenoceptor agonists, since the effect of s.c. and i.p. clonidine was markedly reduced in GIRK2 knockout animals both in hot plate and tail flick tests (Mitrovic et al., 2003; Blednov et al., 2003) (Table 1). Interestingly, the loss of clonidine-induced antinociception in these animals was greater than the reduction in antinociception produced by morphine (Mitrovic et al., 2003), which suggests that GIRK2-containing channels may play a more significant role in α_2-adrenoceptor-mediated antinociception than in μ-opioid-mediated.

Only a few studies have evaluated the role of other K^+ channels in the antinociceptive effect of α_2-adrenoceptor agonists. A knockdown of $K_v1.1$ channels induced by antisense ODN treatment reduced the antinociception induced by s.c. clonidine and guanabenz (Galeotti et al., 1999b) (Table 1). The effect of the antisense sequence seems to be specific since a degenerated ODN was ineffective (Galeotti et al., 1999b). It has also been shown that blockers of several K_v channels such as 4-aminopyridine and tetraethylammonium, and the SK channel blocker apamin (all administered i.c.v.) did not modify clonidine-induced antinociception (Ocaña et al., 1993; Galeotti et al., 1999b) (Table 1). On the other hand, the spinal antinociception induced by clonidine (administered i.t.) was antagonized by i.t. charybdotoxin (Yamazumi et al., 2001) (Table 1). Unfortunately, these results are based in a single study and have not been confirmed or refuted by other laboratories; therefore, solid conclusions must await further research.

In summary, it seems that similar K^+ channels (K_{ATP}, GIRK2 and $K_v1.1$) appears to be involved in the antinociception induced by agonists of α_2-adrenoceptors and μ-opioid receptors (Table 1).

4.2. Adenosine A_1 and serotonin 5-HT_{1A} receptors

The i.c.v. administration of several sulfonylureas antagonized the antinociception induced by the agonist of adenosine A_1 receptors R-PIA ((−)-N^6-(2-phenylisopropyl)-adenosine) (Ocaña and Baeyens, 1994) and the agonist of serotonin 5-HT_{1A} receptors 8-OH-DPAT ((±)-8-hydroxy-2-(di-*n*-propylamino)tetralin) (Robles et al., 1996). In both cases, the order of potency of the sulfonylureas in antagonizing antinociception was: gliquidone>glipizide>glibenclamide (Ocaña and Baeyens, 1994; Robles et al., 1996), which is the same order of potency of these drugs in blocking K_{ATP} channels (Amoroso et al., 1990; Schmid-Antomarchi et al., 1990). Gliquidone (administered i.c.v.) also antagonized the antinociception induced in a hot plate test by other agonists of 5-HT_{1A} receptors (buspirone, tandospirone and lesopitron) (Robles et al., 1996). In contrast, the opener of K_{ATP} channels cromakalim (administered i.c.v.) potentiated the antinociception induced by R-PIA and all the agonists of serotonin 5-HT_{1A} receptors (8-OH-DPAT, buspirone, tandospirone and lesopitron) (Ocaña and Baeyens, 1994; Robles et al., 1996) (Table 1). Both the opposite effects of sulfonylureas and

cromakalim and the order of potency of the sulfonylureas in modulating the antinociceptive effect of the agonists of adenosine A_1 and serotonin 5-HT_{1A} receptors suggest that K_{ATP} channels are involved in the antinociception mediated by these receptors. On the other hand, the i.c.v. administration of 4-aminopyridine and tetraethylammonium did not significantly modify the antinociception induced by R-PIA and 8-OH-DPAT (Ocaña and Baeyens, 1994; Robles et al., 1996) (Table 1). Therefore, the modulation of the antinociceptive effect of agonists of adenosine A_1 and serotonin 5-HT_{1A} receptors by the K^+ channel-acting drugs studied is the same as that observed with morphine and clonidine (Table 1). However, no studies have evaluated the role of K_{Ca}, $K_v1.1$ or GIRK channels in the antinociception mediated by agonists of these receptors. Such studies are likely to yield interesting results.

4.3. Muscarinic and dopamine receptors

The first study to suggest a role for K^+ channels in the antinociception mediated by muscarinic receptors showed that the antinociceptive effect of i.c.v. pilocarpine was antagonized by i.c.v. glibenclamide (Raffa and Martinez, 1995). It was later shown that the antinociception induced in a tail flick test by i.t. bethanecol and carbachol was antagonized by glibenclamide and potentiated by nicorandil (both administered i.t.) (Kang et al., 1997; Yamazumi et al., 2001). These results suggest that the antinociception induced by muscarinic receptor agonists both at the supraspinal and the spinal levels is dependent on K_{ATP} channel opening.

It has also been shown that the antinociception induced in a hot plate test by s.c. oxotremorine (probably through the activation of muscarinic M_2 receptors) was dramatically reduced in GIRK2 knockout animals (Blednov et al., 2003), and that the i.t. administration of charybdotoxin antagonized the antinociception induced in a tail flick test by i.t. bethanecol (Yamazumi et al., 2001). Therefore, several kinds of K^+ channels (K_{ATP}, GIRK2 and BK) seem to be involved in the antinociceptive effect of muscarinic receptor agonists (Table 1).

Only a couple of studies have evaluated the role of K^+ channels in the antinociception induced by agonists of dopamine receptors. Both studies found that the antinociception induced by i.t. apomorphine (a mixed dopamine D_1/D_2 receptors agonist) was antagonized by i.t. glibenclamide (Kang et al., 1998a; Hu et al., 1999). Obviously, further studies are needed to better characterize the role of K^+ channels in dopamine receptor-mediated antinociception.

4.4. Cannabinoid receptors

Only a few studies have evaluated the role of K^+ channels in the antinociception induced by agonists of cannabinoid receptors. They show that the types of K^+ channels involved in cannabinoid receptor-mediated antinociception resemble only those known to be involved in κ-opioid receptor-mediated antinociception (Table 1).

The antinociception induced in the tail flick test by the cannabinoid receptor agonists Δ^8- and Δ^9-tetrahydrocannabinol, CP 55,940 ((−)-*cis*-3-[2-hydroxy-4(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol), anandamide and fluoroanandamide (administered i.c.v. and i.t.) was not antagonized by the K^+ channel blockers glibenclamide, 4-aminopyridine, tetraethylammonium or charybdotoxin (administered i.t. and i.c.v.) (Welch et al., 1995a,b) (Table 1). These results suggest that K_{ATP}, BK and the K_v channels sensitive to 4-aminopyridine and tetraethylammonium do not play a role in the supraspinal and spinal antinociception mediated by cannabinoid receptors. The i.c.v. administration of apamin also was ineffective in modifying the antinociception induced by i.c.v. Δ^8-tetrahydrocannabinol, Δ^9-tetrahydrocannabinol and CP 55,940 (Welch et al., 1995b). However, the i.t. administration of apamin antagonized the antinociception induced by the i.t. administration of these drugs (Welch et al., 1995a,b) (Table 1). It therefore seems that SK channels at the spinal (but not supraspinal) level are involved in the antinociceptive effect of cannabinoid receptor agonists. Additionally, it has been shown that the antinociception induced by i.p. WIN 55,212-2 [(*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate] (another cannabinoid receptor agonist) in the hot plate test was reduced in GIRK2 knockout animals (Blednov et al., 2003), which suggests that these channels also play a role in cannabinoid receptor-mediated antinociception.

In conclusion, only GIRK2-containing channels and spinal SK channels appear to be involved in the antinociception mediated by cannabinoid receptors (Table 1).

4.5. $GABA_B$ receptors

As found for other agonists of G-protein-coupled receptors, the antinociception induced by i.p. baclofen (an agonist of $GABA_B$ receptors) was markedly reduced in GIRK2 subunit knockout animals (Blednov et al., 2003) and in animals pretreated during several days with an antisense ODN that lowered $K_v1.1$ gene expression (Galeotti et al., 1997b) (Table 1). However, in contrast to most agonists of G-protein-coupled receptors, the antinociception induced by baclofen was not modified by the i.c.v. administration of the K_{ATP} channel-acting drugs gliquidone and cromakalim, whereas it was antagonized by i.c.v. 4-aminopyridine and tetraethylammonium (Ocaña and Baeyens, 1993; Ocaña et al., 1996) (Table 1). This opposite pattern of response to K^+ channel-acting drugs cannot be explained by methodological pitfalls, since under the same experimental conditions gliquidone and cromakalim modulated clonidine-induced antinociception, whereas 4-aminopyridine and tetraethylammonium did not (Ocaña and Baeyens, 1993; Ocaña et al.,

1996). The effect of K_{Ca} channel blockers in the antinociception induced by $GABA_B$ receptor agonists has not been tested, but the results of such experiments would be interesting in the light of the particular pattern of sensitivity to K^+ channel blockers seen in the antinociceptive effect of baclofen.

In conclusion, the K^+ channels involved in $GABA_B$-mediated antinociception appear to include $K_v1.1$ and GIRK2-containing channels, and also the K_v channels blocked by 4-aminopyridine and tetraethylammonium but not K_{ATP} channels (Table 1).

5. Role of K^+ channels in the antinociception induced by other drugs and natural products

5.1. Nonsteroidal antiinflammatory drugs (NSAIDs)

The peripherally mediated antinociception induced in the formalin test by the intraplantar administration of low doses of ketorolac and diclofenac was antagonized by the local (intraplantar) administration of sulfonylureas (Lázaro-Ibáñez et al., 2001; Ortiz et al., 2002, 2003b). Similar results have been obtained with dipyrone and diclofenac in a model of mechanical hyperalgesia induced by prostaglandin E_2 (Alves and Duarte, 2002; Alves et al., 2004b). In all these studies, the order of potency of the sulfonylureas in antagonizing antinociception was glibenclamide>tolbutamide, which corresponds to their potency in blocking K_{ATP} channels (Amoroso et al., 1990; Schmid-Antomarchi et al., 1990). Moreover, the intraplantar administration of the K_{ATP} channel openers pinacidil and diazoxide also produced dose-dependent antinociception that was blocked by sulfonylureas (Ortiz et al., 2002; Alves et al., 2004a). Taken together, these findings suggest that opening of the K_{ATP} channels located in the primary afferent nerve endings produces antinociception and represents an important step in the antinociceptive effect of some NSAIDs. However, opening of the K_{ATP} channels is not involved in the antinociception induced by all NSAIDs in all pain tests, as shown by the finding that sulfonylureas were not able to antagonize the peripheral antinociception induced by dipyrone in the formalin test (Beirith et al., 1998; Ortiz et al., 2003a) and the antinociception produced by indomethacin in several pain models (Ortiz et al., 2003b, Alves et al., 2004b).

The possible role of non-K_{ATP} channels in the peripheral antinociception induced by NSAIDs has also been evaluated. Blockers of K_v (4-aminopyridine and tetraethylammonium), BK (charybdotoxin) and SK (apamin and dequalinium) channels were unable to modify the peripheral antinociception induced by dipyrone and indomethacin (Alves and Duarte, 2002; Ortiz et al., 2003b). However, contradictory results have been reported with blockers of K_{Ca} channels associated to diclofenac. Charybdotoxin and apamin antagonized the effect of diclofenac in the formalin test (Ortiz et al., 2003b), whereas charybdotoxin and dequalinium did not modify the effect of diclofenac against mechanical hyperalgesia induced by prostaglandin E_2 (Alves et al., 2004b). No explanation has been suggested thus far for these contradictory results, except the different pain models used in both studies.

In summary, opening of the K_{ATP} channels appears to play a significant role in the peripheral antinociception induced by several (but not all) NSAIDs, whereas opening of the K_v and K_{Ca} channels does not seem to be involved in this antinociception in most cases.

5.2. Activators of the nitric oxide–cyclic GMP pathway

It is well known that nitric oxide (NO), an activator of soluble guanylate cyclase, can open K^+ channels in several tissues by increasing the intracellular concentration of cyclic GMP (cGMP) (see references in Soares and Duarte, 2001). Therefore, it was hypothesized that activation of the NO–cGMP pathway could induce antinociception through the opening of K^+ channels. This hypothesis was confirmed when the antinociception induced by sodium nitroprusside (an NO donor) and dibutyryl-cGMP (a membrane permeable analogue of cGMP) was found to be antagonized by sulfonylureas (glibenclamide and tolbutamide) and potentiated by diazoxide (Soares et al., 2000; Soares and Duarte, 2001; Alves et al., 2004a). On the other hand, blockers of K_v (4-aminopyridine, tetraethylammonium) and K_{Ca} (charybdotoxin, apamin) channels did not modify the antinociception induced by sodium nitroprusside or dibutyryl-cGMP (Soares et al., 2000; Soares and Duarte, 2001). These results suggest that K_{ATP} channels are involved in the antinociception induced by activators of the NO-cGMP pathway and are of interest because they offer a mechanistic explanation for the differential involvement of K_{ATP} channels in the antinociception induced by some NSAIDs.

Dipyrone-, ketorolac- diclofenac- and rofecoxib-induced antinociception is mediated by activation of the NO–cGMP pathway (Lázaro-Ibáñez et al., 2001; Alves and Duarte, 2002; Ortiz et al., 2003b; Déciga-Campos and López-Muñoz, 2004) and is antagonized by K_{ATP} channel blockers (Lázaro-Ibáñez et al., 2001; Alves and Duarte, 2002; Ortiz et al., 2002, 2003b; Alves et al., 2004b; Déciga-Campos and López-Muñoz, 2004), whereas indomethacin-induced antinociception is not due to activation of the NO–cGMP pathway (Ortiz et al., 2003b) and is also insensitive to K_{ATP} channel blockers (Ortiz et al., 2003b; Alves et al., 2004b). Interestingly, the antinociception induced by morphine and by snake venom from *Crotaluss durissus terrificus* is also due to activation of the NO–cGMP pathway and is mediated by K_{ATP} channel opening (Rodrigues and Duarte, 2000; Picolo et al., 2003), which further supports the notion that a NO–cGMP–K_{ATP} channel pathway may play a significant role in the antinociceptive effect of different drugs.

5.3. Tricyclic antidepressants, H_1-antihistamines and other drugs

Several tricyclic antidepressants and H_1-antihistamines share the ability to induce antinociception that is antagonized by pretreatment with pertussis toxin (an irreversible blocker of G_i and G_o proteins) (Galeotti et al., 1999a, 2001). This indicates that the activation of G-protein-coupled receptors is involved in their antinociceptive effect. Since many activators of G-protein-coupled receptors produced antinociception through the opening of K^+ channels, it seemed justified to test whether this is also the case for the antinociception induced by these drugs. It was found that the antinociception induced in the hot plate test by the s.c. administration of amitriptyline and clomipramine was antagonized by gliquidone, tetraethylammonium and apamin, and potentiated by minoxidil and pinacidil (all administered i.c.v.) (Galeotti et al., 2001). Moreover, the i.c.v. administration of a $K_v1.1$ antisense ODN was able to reduce brain levels of $K_v1.1$ mRNA and to antagonize the antinociception induced by the tricyclic antidepressants, whereas a degenerated ODN was unable to induce either of these effects (Galeotti et al., 1997a). These results suggest that the opening of K_{ATP}, $K_v1.1$ and SK channels was involved in the antinociception induced by amitriptyline and clomipramine.

The antinociception induced in the hot plate test by several H_1-antihistamines (pyrilamine, diphenhydramine and promethazine, administered s.c.) showed the same pattern of sensitivity to K^+ channel blockers and openers as was observed with tricyclic antidepressants (Galeotti et al., 1999a); however, the antinociception induced by the H_1-antihistamines was not antagonized by a $K_v1.1$ antisense ODN (Galeotti et al., 1999a). These results suggest that only K_{ATP} and SK channel opening appears to play a role in the antinociception induced by pyrilamine, diphenhydramine and promethazine.

Homotaurine also induces antinociception, and its effect appears to be due, at least in part, to mechanisms dependent on the activation of $GABA_B$ and μ-opioid receptors (see Serrano et al., 2001); therefore, homotaurine-induced antinociception is expected to be modulated by K^+ channel-acting drugs. The i.c.v. administration of gliquidone, tetraethylammonium and 4-aminopyridine antagonized the antinociceptive effect of homotaurine in the acetic acid-induced writhing test and especially in the tail flick test (Serrano et al., 2001). These results are interesting because morphine-induced antinociception (μ-opioid receptor-mediated) is antagonized by gliquidone but not by tetraethylammonium and 4-aminopyridine, whereas the opposite pattern of antagonism was observed with baclofen ($GABA_B$ receptor-mediated) (see references in Sections 3.1 and 4.5). Therefore, the pattern of sensitivity to K^+ channel blockers shown by homotaurine was that expected for a drug that activates both mechanisms.

Only one study have evaluated the role of K^+ channels in the antinociception induced by antiepileptic drugs. The antiallodynic effect of i.t. gabapentin (in a model of neuropathic pain induced by ligation of L5 and L6 spinal nerves) was dose-dependently antagonized by i.t. administered glibenclamide, apamin and charybdotoxin, but was not modified by margatoxin (Mixcoalt-Zecualt et al., 2004). These results suggest that opening of K_{ATP}, SK and BK channel appears to be involved in gabapentin effect.

5.4. Natural products

Several natural products (gallic acid ethyl ester, MV8612 and polygodial) induce antinociception in different models of pain, that is antagonized by pertussis toxin (Mendes et al., 2000; Santos et al., 1999, 2003). This property suggests that mechanisms that activate $G_{i/o}$-protein-coupled receptors play a role in their antinociceptive effect, and point to the hypothesis that K^+ channel opening may also be involved in this effect. When this hypothesis was tested, the antinociception induced in the formalin test by gallic acid ethyl ester (a small molecule isolated form the aerial part of the plant *Phyllantus urinaria*) was antagonized by i.t. administered glibenclamide, charybdotoxin and apamin, but not by tetraethylammonium (Santos et al., 1999). On the other hand, the antinociception induced by MV8612 (a pregnane isolated from the rhizome of the plant *Mandevilla velutina*) was antagonized by the i.t. administration of charybdotoxin and apamin, but not by glibenclamide or tetraethylammonium (Santos et al., 2003). Polygodial (a sesquiterpene isolated from the bark of *Drymis winteri*) induced antinociception in the formalin test, and this effect was not antagonized by any of the K^+ channel blockers tested (glibenclamide, charybdotoxin, apamin and tetraethylammonium) (Mendes et al., 2000). Therefore, although all these natural products activate $G_{i/o}$ protein-dependent mechanisms, their pattern of sensitivity to K^+ channel blockers is very different. This is not unexpected, since, as summarized in Table 1, the agonists of receptors coupled to G-proteins showed different patterns of sensitivity to K^+ channel blockers.

The involvement of K^+ channels in the antinociception induced by two other natural products (resveratrol and *Crotalus durissus terrificus* venom) has been also evaluated. Resveratrol (a phytoalexin present in grapes and wines) produces antinociception, which may be related to its ability to block cyclooxygenases 1 and 2 (Granados-Soto et al., 2002). The peripheral antinociception induced in the formalin test by the intraplantar administration of resveratrol was not modified by intraplantar sulfonylureas (glibenclamide, glipizide and tolbutamide), but was antagonized by charybdotoxin, apamin, 4-aminopyridine and tetraethylammonium (Granados-Soto et al., 2002). Exactly the opposite pattern of sensitivity to these K^+ channel blockers was observed when their effects were tested on the antinociception induced by *C. durissus terrificus* venom (a mixed δ- and κ-opioid receptor agonist) in models of mechanical

hyperalgesia induced by prostaglandin E_2 and carrageenan (Picolo et al., 2003).

6. Antinociceptive effects of K^+ channel openers in different pain models

6.1. Openers of K_v channels

The opener of neuronal K_v7 channels ($K_v7.2$–7.5, also known as KCNQ2–5) retigabine has been evaluated in models of acute and chronic pain. Discrepant results have been reported in the tail flick test: in one study, retigabine was devoid of effect (Blackburn-Munro and Jensen, 2003), whereas in another it produced antinociception (Dost et al., 2004). However, this effect was not reversed by linopirdine (a neuronal K_v7 channel blocker), which suggest that the antinociceptive effect in the tail flick test was not due to the opening of K_v7 channels (Dost et al., 2004). In contrast, retigabine antagonized the second phase of pain induced by formalin (Blackburn-Munro and Jensen, 2003), and the hyperalgesia induced by carrageenan (Passmore et al., 2003). In both studies, the antinociception was reversed by the neuronal K_v7 channel blocker XE-991 (Blackburn-Munro and Jensen, 2003; Passmore et al., 2003). Retigabine is also active in several models of chronic pain, and at least in some of them the effect is reversed by K_v7 channel blockers. Retigabine inhibited the mechanical allodynia induced by repeated i.m. injections of an acid solution (Nielsen et al., 2004), and also reduced the hyperalgesia and allodynia (to mechanical or thermal stimuli) in different models of neuropathic pain, such as the chronic constriction and spared nerve injury models (Blackburn-Munro and Jensen, 2003), and the L5-spinal nerve ligation model (Dost et al., 2004). In this latter model the antiallodynic effect was reversed by the K_v7 channel blocker linopirdine (Dost et al., 2004), which suggests that the effect of retigabine was due to opening of neuronal K_v7 channels.

Part of the effects of retigabine are produced spinally, as shown by the finding that when directly applied to the spinal cord it inhibited the Aδ and C fibre-mediated response of dorsal horn neurones to noxious (electrical, mechanical and thermal) stimuli, as well as the 'windup' discharge of these neurons induced by repetitive stimulation of their peripheral receptive fields (Passmore et al., 2003). Interestingly, the inhibition of windup was greater in neuropathic (L5-spinal nerve ligation) than in normal animals (Passmore et al., 2003).

In summary, numerous studies have shown that retigabine produces antinociception in different models of pain through the activation of neuronal K_v7 channels, and is particularly active in models of chronic and neuropathic pain.

Since homomeric $K_v1.4$ channels predominate in Aδ and C fibres arising from nociceptive small diameter neurones of the dorsal root ganglia (Rasband et al., 2001), it is expected that $K_v1.4$ channel openers may have antinociceptive activity. This hypothesis has not been tested to date.

6.2. Openers of K_{Ca} channels

An opener of BK channels designated NS1619 (Olesen et al., 1994) has also been tested in models of nociception. The intraplantar administration of NS1619 does not inhibit mechanical hyperalgesia induced by prostaglandin E_2 (Alves et al., 2004a). This lack of effect can not be ascribed to methodological problems, because in the same study other drugs produced antinociception. Moreover, the i.c.v. administration of NS1619 produced weak antinociception (less that 50% of the maximum possible effect) in the phenylquinone-induced writhing test, whereas several K_{ATP} channel openers produced 100% antinociception (El-Mabrouki and Baeyens, unpublished data). Therefore, it seems that NS1619 has no marked antinociceptive effects. However, more studies using other pain tests are needed to more accurately characterize the possible antinociceptive effects of this BK channel opener. Recently, openers of SK channels have been described (Liegeois et al., 2003), but none of them have been tested in pain models.

6.3. Openers of inward rectifier K^+ channels

As described above, the opening of K_{ATP} and GIRK channels plays an important role in the antinociceptive effect of many agonists of G-protein-coupled receptors. Therefore, opener of these channels are expected to show antinociceptive effects. No openers of GIRK channels are available, but this channel appears to be a good target for the development of new analgesic drugs. On the other hand, the antinociceptive effects of K_{ATP} openers have been characterized in numerous studies.

Discrepant results were obtained when the effect of K_{ATP} channel openers was tested in hot plate and tail flick tests. The i.c.v. administration of cromakalim reportedly produced weak antinociception in the hot plate test (<25% of the maximum possible effect) in one study (Narita et al., 1993). However, no effect in this test was observed in several studies with the i.c.v. administration of cromakalim (Robles et al., 1996), pinacidil or minoxidil (Galeotti et al., 1999a,b, 2001). Similar discrepancies have been observed in the tail flick test: several studies found no antinociceptive effect after the i.c.v., i.t. and epidural administration of different K_{ATP} channel openers (Vergoni et al., 1992; Ocaña and Baeyens, 1994; Ocaña et al., 1995, 1996; Asano et al., 2000; Yamazumi et al., 2001), whereas other studies found dose-dependent antinociception after the i.c.v. and i.t. administration of the same K_{ATP} channel openers (Welch and Dunlow, 1993; Kamei et al., 1994; Lohmann and Welch, 1999a,b; Campbell and Welch, 2001; Zushida et al., 2004). Methodological differences may explain these discrepancies. In all studies in which K_{ATP}

channel openers produced antinociception, the effect was evaluated 10 min after their administration, whereas in the studies that reported no effect, this interval was longer. It is therefore possible that the antinociceptive effect of K_{ATP} channel openers in this test is of a very short duration. In studies that observed antinociceptive effects, the antinociception was obtained after both the i.c.v. and i.t. administration of cromakalim, minoxidil, pinacidil or diazoxide, and in all cases the effect was reversed by the K_{ATP} channel blocker glibenclamide (Welch and Dunlow, 1993; Kamei et al., 1994; Lohmann and Welch, 1999a,b; Campbell and Welch, 2001; Zushida et al., 2004). These data indicate that the antinociceptive effect of K_{ATP} openers was produced at both the spinal and supraspinal levels, and that it was probably due to their effect on K_{ATP} channels. Interestingly, the antinociception induced by all K_{ATP} channel openers was reversed by naloxone, μ-opioid receptor antagonists (CTOP [D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2] and β-funaltrexamine) and δ-opioid receptor antagonists (ICI 174,864 [*N*,*N*-diallyl-Tyr-Aib-Aib-Phe-Leu]; naltrindole and naltriben) (Welch and Dunlow, 1993; Lohmann and Welch, 1999b; Campbell and Welch, 2001; Zushida et al., 2004), as well as by pretreatment with antisense ODNs to the μ- and the δ-opioid receptors (Lohmann and Welch, 1999a). These results suggest that K_{ATP} channel opener-induced antinociception stimulates mechanisms that produce antinociception through μ- and δ-opioid receptor activation. The exact mechanism of this activation is unknown, but at least in the case of minoxidil is not due to the release of endogenous β-endorphin or [Leu^5]enkephalin (Campbell and Welch, 2001). On the other hand, nor-binaltorphimine (a κ-opioid receptor antagonist) inhibited the antinociception induced by diazoxide and pinacidil but not that produced by minoxidil and cromakalim (Welch and Dunlow, 1993; Lohmann and Welch, 1999a,b; Campbell and Welch, 2001; Zushida et al., 2004). An antisense ODN to the κ-opioid receptors also antagonized the antinociceptive effect of diazoxide (Lohmann and Welch, 1999a). Therefore, it seems that κ-opioid-mediated mechanisms are involved only in pinacidil- and diazoxide-induced antinociception.

K_{ATP} channel openers also appear to activate channels located at the primary afferent nerve endings to produce peripheral antinociception. The intraplantar administration of diazoxide and pinacidil produced a prolonged antinociceptive effect in the paw pressure test (Picolo et al., 2003), inhibited second phase pain induced by formalin (Ortiz et al., 2002), and reduced mechanical hyperalgesia induced by prostaglandin E_2 and carrageenan (Picolo et al., 2003; Alves et al., 2004a). These effects were antagonized by glibenclamide (Ortiz et al., 2002; Picolo et al., 2003; Alves et al., 2004a), which suggests that are due to the activation of K_{ATP} channels.

In summary, K_{ATP} channel openers have been shown to produce supraspinal and spinal antinociceptive effects of short duration, and peripheral antinociceptive effects of long duration.

7. Concluding remarks

The main conclusions to be drawn from this review are two. First, the opening of K^+ channels in the peripheral and central nervous system is an important mechanism in the antinociception induced by many types of drugs and natural products. Second, drugs that open certain K^+ channels by directly interacting with them (e.g. K_{ATP} and neuronal K_v7 channel openers) induce antinociception; consequently, other neuronal K^+ channels (e.g. GIRK, $K_v1.1$ and especially $K_v1.4$ channels) may represent an interesting target for the development of new K^+ channel openers with antinociceptive activity.

This review also highlights some areas of research that merit further study. The possible role of K^+ channel opening in the antiallodynic and antihyperalgesic effects of drugs (e.g. antiepileptic drugs) in models of neuropathic pain deserves more attention. Similarly, the involvement of K_{Ca} channel opening in the antinociception induced by drugs at the supraspinal level remains for the most part unexplored to date. Furthermore, the availability of relatively specific blockers of GIRK channels (e.g. tertiapin), K_v1 channels (e.g. margatoxin, kaliotoxin, agiotoxin), K_v2 channels (e.g. hanatoxins) or neuronal K_v7 channels (e.g. linopirdine) offer potentially fruitful possibilities for exploring the role of these channels in the antinociception induced by known antinociceptive drugs.

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