ELSEVIER

European Journal of Pharmacology 384 (1999) 147–152

ejp

www.elsevier.nl/locate/ejphar

Antisenses to opioid receptors attenuate ATP-gated K^+ channel opener-induced antinociception

Alka B. Lohmann, Sandra P. Welch *

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Box 980613, Richmond, VA 23298-0613, USA

Received 18 March 1999; received in revised form 28 September 1999; accepted 1 October 1999

Abstract

The ATP-gated K^+ channel openers diazoxide, levcromakalim and morphine induce cell hyperpolarization by opening the K^+ channels and enhancing K^+ efflux. This hyperpolarization decreases intracellular Ca^{2+} levels, lessening neurotransmitter release thus leading to antinociception. Previous findings implicate the release of endogenous opioids as the mediator of the antinociceptive effects of ATP-gated K^+ channel openers. Antisense oligodeoxynucleotides to the opioid receptor clones, which decrease the number of available receptors, were used to demonstrate the involvement of endogenous opioids in diazoxide- and levcromakalim-induced antinociception. Antisense to all three opioid receptors attenuated the effect of diazoxide, suggesting that diazoxide is inducing the release of endogenous opioids activating the μ(MOR-1)-, δ(DOR-1)-, and κ(KOR-1)-opioid receptors. Antisense to the μ-opioid receptor clone and δ-opioid receptor clone attenuated levcromakalim-induced antinociception, indicating that endogenous opioids acting at the μ- and δ-opioid receptors are potential candidates for the mediation of the antinociceptive effects of levcromakalim.

Keywords: K^+ channel, ATP-gated; Opioid; Antinociception; Antisense ligonucleotide; Intracerebroventricular; (Mouse)

1. Introduction

The openers of ATP-gated K^+ channels are a class of drugs that share with opioids the ability to open the K^+ channels and enhance K^+ efflux from the cell (Duty and Weston, 1990). Openers of ATP-gated K^+ channels include diazoxide, cromakalim and the active L-isomer of cromakalim, levcromakalim. These drugs are used clinically for hypertension and angina, respectively. Central administration of ATP-gated K^+ channels openers produces dose-dependent antinociception (Narita et al., 1993; Welch and Dunlow, 1993; Ocana et al., 1995; Lohmann and Welch, 1999). Glyburide, a potent sulfonylurea, acts as an antagonist by allosterically closing ATP-gated K^+ channels (Amoroso et al., 1990). Previous findings in our laboratory have demonstrated that the opiate antagonists also attenuate the antinociceptive effects of the K^+ channel openers when administered both intrathecally (i.t.) and intracerebroventricularly (i.c.v.) (Welch and Dunlow, 1993; Lohmann and Welch, 1999). This suggests that the ATP-gated K^+ channel openers either interact directly with the opioid receptors or release an endogenous opioid, which can bind to its specific opioid receptor. Lack of cross-tolerance between the ATP-gated K^+ channel openers and morphine indicates that there is not a direct interaction between the openers and the opioid receptors (Welch and Dunlow, 1993). Therefore, the ATP-gated K^+ channel openers are likely inducing the release of endogenous opioids.

Evidence of receptor-specific binding has been provided by the use of antisense oligonucleotide experiments. Antisense sequences down-regulate the protein by blocking translation of the messenger RNA (mRNA) into its proper protein, in this case, the receptor. The antisense oligonucleotide is composed of a sequence that is complimentary to the portion of the mRNA that contains the initiation codon or other critical regions of the mRNA. The binding of the antisense oligonucleotide to the mRNA disables the binding of the ribosomes necessary for translation (Lodish et al., 1995) and can also increase mRNA degradation.

* Corresponding author. Tel.: +1-804-828-8424; fax: +1-804-828-2117.

E-mail address: swelch@hsc.vcu.edu (S.P. Welch)

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00698-6

Upon administration of MOR-1 receptor antisense, morphine-induced analgesia was attenuated in mice (Rossi et al., 1994) and in rats (Rossi et al., 1997). Mismatch oligonucleotide to the μ-opioid receptor clone, which differs from the antisense by only two base pairs, was used as the control for antisense specificity and showed no alteration in morphine-induced antinociception (Rossi et al., 1994, 1997). Similar methods were used to demonstrate trans-(±)-3,4-dichloro-*N*-methyl1-*N*-[2-(1-pyrrolodinyl)-cyclohexyl]benzeneacetamide methane sulfonate (U50,488H) specificity for the κ-opioid receptor and [D-Pen^{2,5}]enkephalin (DPDPE) specificity for the δ-opioid receptor (Chien et al., 1994; Wang et al., 1996). In the above experiments, a decrease in specific opioid receptor number was shown to accompany the administration of the opioid-specific antisense probe. Our protocol in mice was based upon the results of the above studies in both mice and rats, utilizing similar antisense probes and doses equal to or higher than those previously described.

ATP-gated K^+ channel antagonists and opioid receptor-selective antagonists have also been utilized to demonstrate the mechanisms of ATP-gated K^+ channel- and opioid-induced antinociception. Glyburide, a potent sulfonylurea, antagonizes the antinociceptive effects of morphine (Ocana et al., 1990; Wild et al., 1991). However, glyburide does not possess significant affinity for the μ-opioid receptor nor does it alter the receptor's affinity for its ligand, morphine (Raffa and Codd, 1994). This suggests that morphine's antinociceptive effects involve the opening of ATP-gated K^+ channels (North et al., 1987; Chavkin, 1988). The entire class of sulfonylureas has the same effect on morphine-induced antinociception (Amoroso et al., 1990; Schmid-Antomarchi et al., 1990). Glyburide has similar effects on DPDPE-induced antinociception. On the other hand, no alteration of the antinociceptive effects of κ-opioid receptor ligands is seen with glyburide administration (Ocana et al., 1993; Raffa and Codd, 1994). Previous experiments in our laboratory performed with opioid receptor antagonists suggest that diazoxide interacts with all three classes of opioid receptors while levcromakalim-induced antinociception involves only the μ- and δ-opioid receptors (Welch and Dunlow, 1993; Lohmann and Welch, 1999).

In our effort to implicate the release of endogenous opioids as the mediators of the ATP-gated K^+ channel opener-induced antinociception, antisense oligonucleotides to the opioid receptors were employed. Since ATP-gated K^+ channel opener-induced antinociception is opioid receptor antagonist-sensitive, we hypothesize that antisense directed toward discrete opioid receptors would also attenuate the antinociceptive effects of the ATP-gated K^+ channel openers. Since our work with the ATP-gated K^+ channel openers was performed i.c.v., we elected to administer the antisense probes i.c.v. in order to best maximize the effect of the probes in the brain. The doses chosen for the antisense probes were those doses that showed a selective block of the antinociceptive effect of the selective opioid receptor agonist for the opioid receptor of interest. For instance, the dose of DOR-1 receptor antisense utilized was based upon experiments showing that the dose blocked DPDPE-induced antinociception, but not μ- or κ-opioid-induced antinociception. All doses used were equal to or higher than those previously shown to down-regulate selective opioid receptors (Chien et al., 1994; Wang et al., 1996).

2. Materials and methods

2.1. Intracerebroventricular (i.c.v.) injections

I.c.v. injections were performed according to method of Pedigo et al. (1975). Ether-anesthetized male ICR mice, 21–24 g, were injected 2 mm caudal and 2 mm lateral at a 45° angle from the bregma. This area was exposed by a small incision made between the ears of the animal. Injections of 5 μl were made at a depth of 2 mm into the third lateral ventricle. Hamilton 50-μl syringes and 26 gauge 3/8 in. needles were used. A tubing cover was applied to the needle to ensure injections were made at the proper depth.

K^+ channel openers, diazoxide and levcromakalim were prepared in 100% dimethyl sulfoxide (DMSO). Administration of DMSO i.c.v. produced less than 15% maximum possible effect (MPE) and induced no overt signs of toxicity in the mice in these short-term acute experiments. Opioid receptor agonists, morphine, U50,488H and DPDPE were prepared in distilled water.

Time-course studies and previous findings from our laboratory were used to ascertain peak antinociception as tested by the tail—flick test. Peak time for the K^+ channel openers was 10 min after injection.

Antisense experiments were performed following the protocol of Chien et al. (1994). Antisense or mismatch to the cloned μ-, δ- or κ-opioid receptor or dH_2O vehicle was administered on days 1, 3 and 5. Antisense doses of 50 μg/mouse for the μ-opioid receptor clone, 25 μg/mouse for the δ-opioid receptor clone and 20 μg/mouse for the κ-opioid receptor clone were administered i.c.v. The same doses were used for the mismatch administrations. Antisense and mismatch sequences to the opioid receptors are listed in Table 1. For the multiple

Table 1
Antisense and mismatch oligodeoxynucleotide sequences to the opioid receptors

Receptor	Sequence	Oligodeoxynucleotide (5′ to 3′)
μ (MOR-1)	Antisense	CGC CCC AGC CTC TTC CTC T
μ (MOR-1)	Mismatch	CGC CCC GAC CTC TTC CCT T
δ (DOR-1)	Antisense	AGA GGG CAC CAG CTC CAT
δ (DOR-1)	Mismatch	CGA GCG CAA CAG CTG CAT
κ (KOR-1)	Antisense	GGT GCC TCC AAG GAC TAT CGC
κ (KOR-1)	Mismatch	GGA GCC TGC AAG GTC TAT GGC

antisense, mismatch and vehicle injections the animals were anesthetized in methoxyflurane (Metofane®). The area where the incision was made was shaved to avoid fur accumulating in the exposed area. Wound clips were used to keep the incision closed between injections. On day 6, the animals were anesthetized in ether and doses of diazoxide, levcromakalim or appropriate opioid receptor agonist that produced approximately 60% MPE were administered i.c.v.

2.2. The tail flick test

The D'Amour and Smith (1941) tail–flick test was used to assess antinociception. Reaction times of 2 to 4 s were employed for the control, while a time of 10 s was used as the cutoff. Quantification of effect was done using the % MPE formula:

$$\%\text{MPE} = 100 \times [(\text{test} - \text{control}) / (10 - \text{control})]$$

(Harris and Pierson, 1964). Using 3–8 mice/dose, a % MPE was calculated for each animal. The mean effect and standard error of the mean (S.E.M) were calculated for every dose using the % MPE for each mouse.

2.3. Statistical analysis

Two-tailed unpaired *t*-tests were used to determine significant differences between control and treatment animal groups for each opioid receptor antisense treatment. P values of less than 0.05 were deemed significant.

2.4. Drugs

U50,488H was purchased from Research Biomedicals International (Natick, MA). DPDPE was purchased from Bachem Bioscience (King of Prussia, PA). Diazoxide and DMSO were purchased from Sigma (St. Louis, MO). Levcromakalim was kindly provided by Dr. P.G. Treagust at SmithKline Beecham Pharmaceuticals (Worthing, West Sussex, UK). Morphine was obtained from the National Institute of Drug Abuse (NIDA). The unmodified antisense and mismatch oligonucleotides were constructed by and purchased from Operon Technologies (Alameda, CA). Ether and Metofane® were purchased from J.T. Baker and Mallinckrodt Veterinary, respectively.

3. Results

The antisense oligodeoxynucleotide to the μ-opioid receptor significantly attenuated morphine-induced antinociception (1 μg/mouse). The % MPE was decreased to 19 (±3)% in the antisense-treated animals from a value of 68 (±5)% in the vehicle-treated animals. The mismatch sequence to the μ-opioid receptor did not significantly attenuate the antinociceptive capabilities of morphine (Fig. 1,Panel A). DPDPE-induced (2 μg/mouse) and U50,488H-induced (10 μg/mouse) antinociception were unaffected by both the MOR-1 receptor antisense and mismatch oligonucleotides (Fig. 1, Panel A). The MOR-1 receptor antisense significantly antagonized diazoxide-induced antinociception (100 μg/mouse). The vehicle-treated animals demonstrated 74 (±12)% MPE while the antisense-treated group had a value of only 38 (±6)% MPE. No attenuation of diazoxide-induced antinociception was demonstrated in the μ-opioid receptor mismatch-treated animals (Fig. 1, Panel B). The other ATP-gated K^+ channel agonist tested, levcromakalim, was also significantly attenuated by MOR-1 receptor antisense. The antinociceptive effect of 100 μg/mouse levcromakalim was decreased significantly from 68 (±8)% MPE with vehicle administration to 18 (±4)% with antisense treatment. Mismatch oligodeoxynucleotide to the μ-opioid receptor had no effect on levcromakalim-induced antinociception (Fig. 1, Panel B).

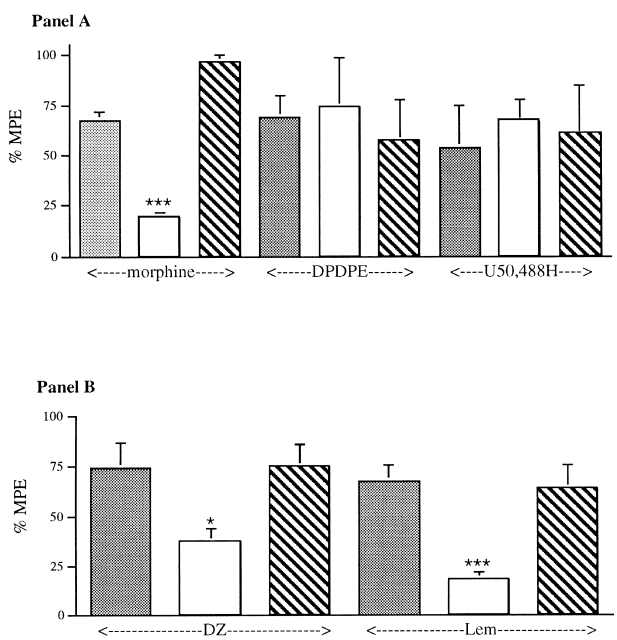

Fig. 1. Effects of antisense to the μ-opioid receptor on opioid- and ATP-gated K^+ channel opener (K_{ATP})-induced antinociception. Panel A: Antisense to the μ-opioid receptor (white bars), when administered i.c.v. on days 1, 3 and 5, significantly attenuated the antinociceptive effects of morphine (i.c.v.), a μ-opioid receptor-specific agonist, but not DPDPE (DOR-1) or U50,488H (KOR-1) (vehicle-gray bars). Mismatch oligodeoxynucleotides to the μ-opioid receptor (striped bars) had no effect on morphine-, DPPDE- or U50,488H-induced antinociception. Panel B: Antisense to the μ-opioid receptor (white bar), when administered i.c.v. on days 1, 3 and 5, significantly attenuated the antinociceptive effects of both diazoxide (DZ) and levcromakalim (Lem) (vehicle-gray bars). Mismatch oligodeoxynucleotides to the μ-opioid receptor (striped bars) had no effect on either DZ- or Lem-induced antinociception. The % MPE and significant antagonism were determined as described in Section 2. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Antisense and mismatch oligodeoxynucleotides to the δ-opioid receptor were also tested. Significant and opioid-selective attenuation of the antinociceptive effects of the δ-opioid receptor-specific agonist, DPDPE, was seen in the antisense-treated animals. A drop in % MPE from 69 (±11)% with vehicle administration to 27 (±7)% with antisense treatment was demonstrated. Mismatch to the δ-opioid receptor clone had no effect on the DPDPE-induced antinociception (Fig. 2, Panel A). In addition, the DOR-1 receptor antisense and mismatch had no effect on morphine- or U50,488H-induced antinociception (Fig. 2, Panel A).

Diazoxide-induced (100 μg/mouse) antinociception was significantly antagonized by DOR-1 receptor antisense treatment. Vehicle-treated animals were observed to have an antinociceptive effect of 86 (±5)% MPE, while antisense-treated animals had an antinociceptive effect of 46 (±13)% MPE. The δ-opioid receptor clone mismatch administration had no effect on diazoxide-induced antinociception (Fig. 2, Panel B). Additionally, levcromakalim-induced antinociception was significantly attenuated by DOR-1 receptor antisense but not the δ-opioid receptor mismatch sequence. Vehicle-treated animals had an antinociceptive effect of 68 (±8)% MPE while the antisense-treated group had an antinociceptive effect of 25 (±8)% MPE (Fig. 2, Panel B).

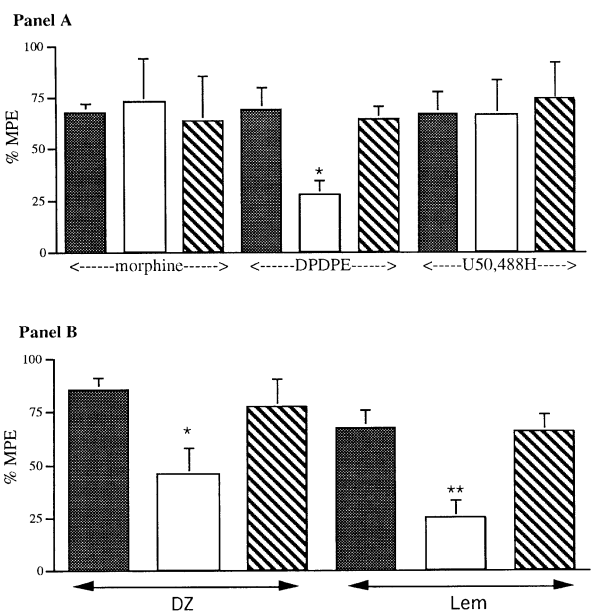

Fig. 2. Effects of antisense to the δ-opioid receptor on opioid- and K_{ATP}-induced antinociception. Panel A: Antisense to the δ-opioid receptor (white bars), when administered i.c.v. on days 1, 3 and 5, significantly attenuated the antinociceptive effects of DPDPE, a δ-opioid receptor-specific agonist, but not morphine (MOR-1) or U50,488H (KOR-1) (vehicle-gray bars). Mismatch oligodeoxynucleotides to the δ-opioid receptor (striped bars) had no effect on DPDPE-, morphine- or U50,488H-induced antinociception. Panel B: Antisense to the δ-opioid receptor (white bars), when administered i.c.v. on days 1, 3 and 5, significantly attenuated the antinociceptive effects of both DZ and Lem (vehicle-gray bars). Mismatch oligodeoxynucleotides to the δ-opioid receptor (striped bars) had no effect on either DZ- or Lem-induced antinociception. The % MPE and significant antagonism were determined as described in Section 2. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

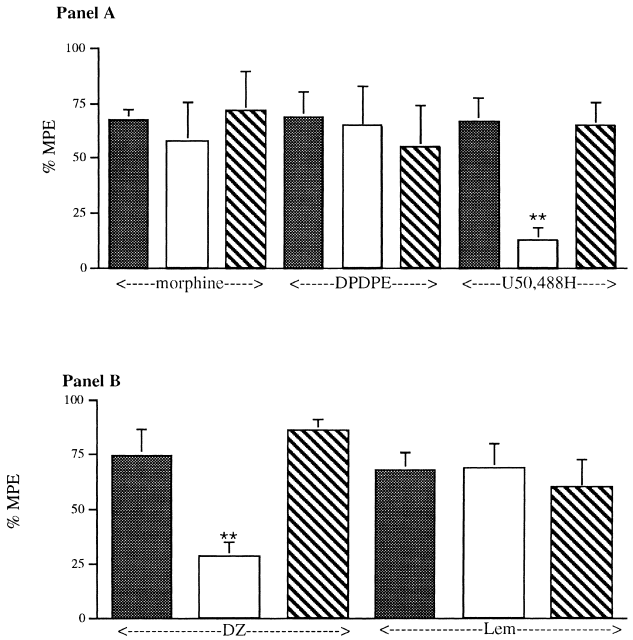

Fig. 3. Effects of antisense to the κ-opioid receptor on opioid- and K_{ATP}-induced antinociception. Panel A: Antisense to the κ-opioid receptor (white bars), when administered i.c.v. on days 1, 3 and 5, significantly attenuated the antinociceptive effects of U50,488H, a κ-opioid receptor-specific agonist, but not morphine (MOR-1) or DPDPE (DOR-1) (vehicle-gray bars). Mismatch oligodeoxynucleotides to the κ-opioid receptor clone (striped bars) had no effect on U50,488H-, morphine- or DPDPE-induced antinociception. Panel B: Antisense to the κ-opioid receptor clone (white bars), when administered i.c.v. on days 1, 3 and 5, significantly attenuated the antinociceptive effects of DZ but not Lem (vehicle-gray bars). Mismatch oligodeoxynucleotides to the κ-opioid receptor (striped bars) had no effect on either DZ- or Lem-induced antinociception. The % MPE and significant antagonism were determined as described in Section 2.

The antisense sequence to the κ-opioid receptor significantly and opioid-selectively antagonized the antinociception produced by U50,488H. Vehicle-treated animals demonstrated 67 (±11)% MPE, while the animals that received antisense exhibited 13 (±6)% MPE. Mismatch oligodeoxynucleotide to κ-opioid receptor had no effect on U50,488H-induced antinociception (Fig. 3, Panel A). KOR-1 receptor antisense and mismatch-treated animals demonstrated no alteration in either morphine- or DPDPE-induced antinociception (Fig. 3, Panel A).

Diazoxide-induced antinociception was significantly attenuated by KOR-1 receptor antisense treatment. The % MPE was reduced from 74 (±12)% in the vehicle-treated animals to 28 (±7)% for the antisense-administered animals. κ-Opioid receptor mismatch had no effect on the antinociceptive capabilities of diazoxide (Fig. 3, Panel B). In contrast, KOR-1 receptor antisense had no effect on levcromakalim-induced antinociception. Mismatch administered animals also demonstrated no significant attenua-

tion of the antinociception produced by levcromakalim (Fig. 3, Panel B).

4. Discussion

Central administration of the tested ATP-gated K^+ channel openers, diazoxide and levcromakalim, produces dose-dependent antinociception (Narita et al., 1993; Welch and Dunlow, 1993; Ocana et al., 1995; Lohmann and Welch, 1999). Attenuation of this antinociception by opioid receptor antagonists implicates the release of endogenous opioids as a mediator of the observed effects (Welch and Dunlow, 1993; Lohmann and Welch, 1999). Antisense to the opioid receptors demonstrated that the ATP-gated K^+ channel openers may release endogenous opioids and it is the release of these endogenous opioids that mediates the antinociceptive effects of the openers. In addition, a differential effect of diazoxide and levcromakalim on the release κ opioids, most likely the dynorphins, appears probable.

The administration of MOR-1 receptor clone antisense oligodeoxynucleotides significantly attenuated the effects of both of the ATP-gated K^+ channel openers. This finding indicates that both diazoxide and levcromakalim quite possibly produce antinociception via the release of endogenous opioids acting at the μ-opioid receptor. It should be noted that δ-opioid receptor agonists bind the μ-opioid receptor but with a much lesser affinity. Therefore, we cannot decisively conclude that only endogenous opioids acting at the μ-opioid receptor are released (Hayes, 1986). Since antisense to the δ-opioid receptor clone significantly attenuated both diazoxide- and levcromakalim-induced antinociception, both ATP-gated K^+ channel openers appear to produce antinociception through the release of either [Met^5]enkephalin or [Leu^5]enkephalin or another endogenous opioid with δ-opioid receptor affinity. Antisense to the third opioid receptor, the *kappa*, demonstrated differences between diazoxide- and levcromakalim-induced antinociception. Since only the effects of diazoxide were attenuated by antisense to the κ-opioid receptor, there is evidence that diazoxide produces its antinociception via dynorphin release, while this particular endogenous opioid plays no role in levcromakalim-induced antinociception. It is known that the dynorphins are the endogenous ligands for the κ-opioid receptor (Reisine and Pasternak, 1996). Previous work done in this laboratory using opioid receptor antagonists produced similar results. Nor-binaltorphimine, a κ-opioid receptor-specific antagonist, attenuated diazoxide- but not levcromakalim-induced antinociception (Welch and Dunlow, 1993; Lohmann and Welch, 1999).

In summary, the data presented here demonstrate that the ATP-gated K^+ channel openers, diazoxide and levcromakalim produce antinociceptive effects via the release of endogenous opioids which interact with opioid receptors. Use of antisense probes, which were shown in critical experiments to be receptor selective, differentially blocked the effects of diazoxide versus that of levcromakalim. In our effort to determine which endogenous opioids are specifically involved, we hypothesize that diazoxide induces the release of β-endorphin, enkephalins and/or dynorphins, while levcromakalim induces the release of only β-endorphin and/or enkephalins.

It is known that K^+ channel openers, like opioids, produce an increase in K^+ efflux from the cell and thus hyperpolarize the cell's membrane potential. This causes a decrease in Ca^{+2} entry, which lessens the amount of neurotransmitters released by the cell. We envision K^+-ATP-induced release of endogenous opioids as involving a disinhibition of inhibitory processes in either a descending or ascending pathway, yet to be determined. It is possible that this disinhibition allows the release of endogenous opioids and thus explains ATP-gated K^+ channel opener-induced antinociception.

Opioids are used clinically to alleviate numerous types of pain, including neuropathic pain and pain due to cancer. Unfortunately, there are numerous negative side effects associated with both acute and chronic opioid use. It has been shown that one opioid can enhance the analgesia produced by another opioid (Vaught and Takemori, 1979; Sutters et al., 1990; Vanderah et al., 1996). Therefore, it is possible that the combination of ATP-gated K^+ channel openers with opioids (at low doses of both drugs) may produce similar efficacy to that of the higher doses of opioids when administered alone. It should be noted that such an enhancement of antinociception has been demonstrated (Narita et al., 1993; Ocana et al., 1996; Lohmann and Welch, 1999).

Interestingly, the ATP-gated K^+ channel openers are still effective in opioid tolerant animals (Welch and Dunlow, 1993), which have a down-regulation of opioid receptors (Bernstein and Welch, 1998). Hence, the K^+ channel openers may be useful in morphine-tolerant patients. However, antisense to the opioid receptors, which presumably down-regulates the number of available opioid receptors, significantly attenuates ATP-gated K^+ channel opener-induced antinociception. It is intriguing that one method of down-regulation of opioid receptors causes a decrease in antinociception and another does not. It is possible that such a discrepancy can be explained by the extent of down-regulation following antisense treatment versus that following tolerance to the opioids. Alternatively, the possibility that the ATP-gated K^+ channel openers have additional mechanisms that are independent of opioids can not be ruled out.

Acknowledgements

This work was funded by NIDA grants: K02DA00186 and DA01647.

References

Amoroso, S., Schmid-Antomarchi, H., Fosset, M., Lazdunski, M., 1990. Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive K^+ channels. Science 247, 852.

Bernstein, M.A., Welch, S.P., 1998. Mu opioid receptor down-regulation and cAMP-dependent protein kinase phosphorylation in a mouse model of chronic morphine tolerance. Mol. Brain Res. 55 (2), 237–242.

Chavkin, C., 1988. Electrophysiology of opiates and opioid peptides. In: Pasternak, G.W. (Ed.), The Opiate Receptors. Humana Press, NJ, p. 273.

Chien, X., Brown, G., Pan, Y.X., Pasternak, G.W., 1994. Blockade of U50,488H analgesia by antisense oligodeoxynucleotides to a κ-opioid receptor. Eur. J. Pharmacol. 253, R7–R8.

D'Amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.

Duty, S., Weston, A.H., 1990. K^+ channel openers: pharmacological effects and future uses. Drugs 40, 785–791.

Harris, L.S., Pierson, A.K., 1964. Some narcotic antagonists in the benzomorphan series. J. Pharmacol. Exp. Ther. 143, 141–148.

Hayes, A.G., 1986. Mode of action of opioid drugs. In: Levy, J., Budd, K. (Eds.), Opioids. Royal Society of Medicine Services, London, pp. 1–7.

Lodish, H., Baltimore, D., Berk, A., Zipusky, S.L., Matsudaira, P., Darnell, J., 1995. Molecular Cell Biology. 3rd edition Scientific American Books, New York.

Lohmann, A.B., Welch, S.P., 1999. ATP-gated K^+ channel openers enhance opioid antinociception: indirect evidence for the release of endogenous opioid peptides. Eur. J. Pharmacol., in press.

Narita, M., Takemori, K., Kawashima, N., Funada, M., Kamei, J., Suzuki, T., Misawa, M., Magasse, H., 1993. Activation of central ATP-sensitive K^+ channels produces the antinociception and spinal noradrenaline turnover-enhancing effect in mice. Psychopharmacologia 113, 11–14.

North, R.A., Williams, J.T., Surprenant, A., Christie, M.J., 1987. μ and δ receptors belong to a family of receptors that are coupled to K^+ channels. Proc. Natl. Acad. Sci. 84, 5487–5491.

Ocana, M., Del Pozo, E., Barrios, M., Robles, M., Baeyens, J.M., 1990. An ATP-dependent potassium channel blocker antagonizes morphine analgesia. Eur. J. Pharmacol. 186, 377–378.

Ocana, M., Del Pozo, E., Baeyens, J.M., 1993. ATP-dependent K^+ channel blockers antagonize morphine- but not U50,488H-induced antinociception. Eur. J. Pharmacol. 230, 203–207.

Ocana, M., Del Pozo, E., Barrios, M., Baeyens, J.M., 1995. Subgroups among mu- opioid receptor agonists distinguished by ATP-sensitive K^+ channel-acting drugs. Br. J. Pharmacol. 114, 1296–1302.

Ocana, M., Barrios, M., Baeyens, J.M., 1996. Cromakalim differentially enhances antinociception induced by agonist of $Alpha_2$ adrenoceptors, g-aminobutyric $acid_b$, *mu* and *kappa* opioid receptors. J. Pharmacol. Exp. Ther. 276, 1136–1142.

Pedigo, N.W., Dewey, W.L., Harris, L.S., 1975. Determination and characterization of the antinociceptive activity of intraventricularly administered acetylcholine in mice. J. Pharmacol. Exp. Ther. 193, 845–852.

Raffa, R.B., Codd, E.E., 1994. Lack of glibenclamide or TEA affinity for opioid receptors: further evidence for in vivo modulation of antinociception at K^+ channels. Brain Res. 650, 146–148.

Reisine, T., Pasternak, G., 1996. Opioid analgesics and antagonists. In: Hardman, J.G., Limbird, L.E. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics. 9th Edition McGraw-Hill, NY, pp. 521–556.

Rossi, G.C., Pan, Y.-X., Cheng, J., Pasternak, G.W., 1994. Blockade of morphine analgesia by an antisense oligonucleotide against the mu receptor. Life Sci. 52, PL375–PL379.

Rossi, G.C., Leventhal, L., Pan, Y.-X., Cole, J., Su, W., Bodnar, R.J., Pasternak, G.W., 1997. Antisense mapping of MOR-1 in rats: distinguishing between morphine and morphine-6β-glucuronide antinociception. J. Pharmacol. Exp. Ther. 281, 109–114.

Schmid-Antomarchi, H., Amoroso, S., Fosset, M., Lazdunski, M., 1990. K^+ channel openers activate brain sulfonylurea-sensitive K^+ channel and block neurosecretion. Proc. Natl. Acad. Sci. 87, 3489.

Sutters, K.A., Miaskowski, C., Taiwo, Y.O., Levine, J.D., 1990. Analgesic synergy and improved motor function produced by combinations of μ–δ and μ–κ opioids. Brain Res. 530, 290–294.

Vanderah, T.W., Bernstein, R.N., Yamamura, H.I., Hruby, V.J., Porreca, F., 1996. Enhancement of morphine by a CCKB antagonist in mice is mediated via opioid *delta* receptors. J. Pharmacol. Exp. Ther. 278, 212–219.

Vaught, J.L., Takemori, A.E., 1979. Differential effects of leucine and methionine enkephalin on morphine-induced analgesia, acute tolerance and dependence. J. Pharmacol. Exp. Ther. 208, 86–90.

Wang, H.Q., Kampine, J.P., Tseng, L.F., 1996. Antisense oligonucleotides to a δ-opioid receptor messenger RNA selectively blocks the antinociception induced by intracerebroventricularly administered δ-, but not μ-, ε- or κ-opioid receptor agonists in the mouse. Neuroscience 75, 445–452.

Welch, S.P., Dunlow, L.D., 1993. Antinociceptive activity of intrathecally administered potassium channel openers and opioid agonists: a common mechanism of action? J. Pharmacol. Exp. Ther. 267, 390–399.

Wild, K.D., Vanderah, T., Mosberg, H.I., Porreca, F., 1991. Opioid δ receptor subtypes are associated with different potassium channels. Eur. J. Pharmacol. 193, 135–141.