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Loperamide-Induced Rat Prostate Relaxation Through Activation of Peripheral μ-Opioid Receptors

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Aims: The effect of μ-opioid receptor (MOR) agonist, loperamide on prostate relaxation and the role of potassium channel in this action were studied in isolated Wistar rat prostate. **Methods:** Tissue strips from rat prostate ventral lobe were hung in organ bath containing: group 1: standard Tyrode's solution (TS); group 2: TS with 1 μM naloxone; group 3: TS with 0.1 μM naloxonazine; and group 4: TS with 0.01–1 μM glybenclamide. The strips were pre-contracted with either 50 mM KCl or 1 μM phenylephrine. Dose–response study on the prostate strip was performed by cumulative administration of loperamide 0.1–10 μM into the organ bath. Western blot study was performed to detect the presence of MOR protein and adenosine triphosphate (ATP)-sensitive potassium channel (K_{ATP}) subunit Kir6.2 protein expressions in the prostate tissue. **Results:** Loperamide induced relaxation of the pre-contracted prostate strips in a dose-dependent fashion. Pre-treatment with 1 μM naloxone significantly inhibited the relaxation, thus suggesting activation of MOR in the loperamide effect. Pre-treatment with 0.1 μM naloxonazine inhibited relaxation only in the phenylephrine-contracted strips. The K_{ATP} channel blocker glybenclamide significantly inhibited the loperamide-induced relaxation, indicating involvement of K_{ATP} channels in mediating the prostate relaxation. Western blots showed the expression of MOR and Kir6.2 protein in the rat prostate. **Conclusions:** MOR and Kir6.2 are expressed in the rat prostate and loperamide induces rat prostate relaxation through activation of peripheral MOR. K_{ATP} channels are involved in mediating the effect of loperamide on the relaxation of prostate. *Neurourol. Urodynam. 30:468–471, 2011.* © 2011 Wiley-Liss, Inc.

Key words: loperamide; potassium channel blocker; prostate; μ-opioid receptor

INTRODUCTION

Opioid receptors are coupled to multiple effector systems that result in an array of biological effects, including analgesia, miosis, bradycardia, general sedation, hypothermia, insensitivity, and depression of flexor reflexes.[1] The existence of three distinct opioid receptors was originally deduced from the different pharmacologic effects of various opioid agonists and antagonists that selectively induce or inhibit different physiologic responses. Three separate receptors—μ (mu), δ (delta), and κ (kappa)—were identified on the basis of pharmacologic response, in vitro radioligand binding affinities, and in vivo localization of labeled drug in tissue homogenates or sections.[2,3] This allowed opioids to be grouped according to similarities in the activation of their receptor types. The μ-agonists are responsible for analgesic properties at both spinal and supraspinal sites, as well as for dependence, euphoria, respiratory depression, sedation, miosis, and tolerance. The δ-agonists, on the other hand, are associated with analgesia and euphoria, whereas the κ-agonists produce analgesia, miosis, sedation, and, in contrast to the other receptors, dysphoria.[4]

Loperamide is a phenylpiperidine derivative with a chemical structure similar to opiate receptor agonists such as diphenoxylate and haloperidol, exhibiting affinity and selectivity for the μ-opioid receptor (MOR).[5] Clinically it is used as an antidiarrheal medication. Loperamide works by a number of different mechanisms of action that decrease peristalsis and fluid secretion, resulting in longer gastrointestinal transit time and increased absorption of fluids and electrolytes from the gastrointestinal tract. Because of its low oral absorption and inability to cross the blood–brain barrier, loperamide has minimal central nervous system effects and no clinically significant analgesic activity.

Recent developments in opioid pharmacology and molecular biology have promised to make this class of compounds even more clinically useful. Since little attention had been paid on the role of peripheral opioid receptors in the lower urinary tract function, the purpose of this study was to elucidate the existence of MOR in the rat prostate, as well as the pharmacological effect and mechanism of loperamide on prostate contractility.

MATERIALS AND METHODS

Animals

Adult 3-month-old male Wistar rats (350–400 g) were obtained from the animal center of National Cheng Kung University Medical College in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health. The rats were kept in a temperature-controlled room (25 ± 1°C) with 12:12-hr light–

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dark cycle. Food and water were available ad libitum. The animal use protocol was approved by the Institutional Animal Care and Use Committee.

Tissue Strips Preparation

The rat was sacrificed by decapitation under anesthesia with 35 mg/kg intraperitoneal pentobarbital. The ventral prostate was removed via a midline abdominal incision and immediately placed in organ bath containing oxygenated (95% O_2 and 5% CO_2) Tyrode's solution (TS) (NaCl 125 mM, KCl 2.7 mM, $CaCl_2$ 1.8 mM, NaH_2PO_4 0.4 mM, $MgCl_2 \cdot 7H_2O$ 0.5 mM, $NaHCO_3$ 24 mM, and dextrose 5.6 mM; pH 7.4) at 37°C. Tissue strips about 8 mm × 5 mm were fashioned longitudinally from the prostate and hung with a basal tension of 1 g. Each preparation was connected to strain gauges (Grass FT03) and isometric tension was recorded on the computer equipped Chart Software (MLS023) of Powerlab (AD Instruments Pty, Ltd, Castle Hill, NSW, Australia). The pharmacological study started after equilibration for 1 hr.

Pharmacological Study

The strips were pre-contracted with either 50 mM KCl or 1 μM phenylephrine and divided into four groups according to the subsequent organ bath solutions: 1: standard TS; 2: TS with 1 μM naloxone (antagonist of opioid receptors); 3: TS with 0.1 μM naloxonazine (selective antagonist of opioid μ1-receptor); and 4: TS with 0.01–1 μM glybenclamide. The number of individual experiments (n) equaled to eight for each group. The drug additions in groups 2–4 did not significantly alter the strip basal tension. The drug concentrations used in the experiments had been determined in preliminary experiments as well as according to our previous study[6] to achieve maximum inhibitory effects on the loperamide responses. Dose–response study on the prostate strip was then performed by cumulative administration of 0.1–10 μM loperamide into the organ bath.

Western Blotting of MOR and Kir6.2 Protein Expressions

The prostate tissue was put in ice-cold homogenized buffer containing 10 mM Tris–HCl (pH 7.4), 20 mM EDTA, 10 mM EGTA, 20 mM β-glycerolphosphate, 50 mM NaF, 50 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride, and the protease inhibitors 25 μg/ml leupeptin and 25 μg/ml aprotinin. The mixture was centrifuged at 1,000*g* for 10 min at 4°C. The supernatant containing the membrane fraction was centrifuged at 48,000*g* for 30 min at 4°C. The supernatant was removed, and the pellet was re-suspended in Triton X-100 lysis buffer on ice for 30 min, homogenized, and then centrifuged at 14,010*g* for 20 min at 4°C. Finally, the supernatant was transferred to a new Eppendorf tube and stored at −80°C. The membrane extracts (20–80 μg) were separated by performing SDS–polyacrylamide gel electrophoresis, and the proteins were transferred onto a BioTrace™ polyvinylidene fluoride (PVDF) membrane (Pall Corporation, Pensacola, FL). Following blocking, the blots were developed using antibodies for MOR (Abcam, Cambridge, UK), and Kir6.2 (Santa Cruz Biotechnology, Santa Cruz, CA). The blots were subsequently hybridized using horseradish peroxidase-conjugated goat antigoat IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), and developed using the Western Lightning Chemiluminescence Reagent PLUS (PerkinElmer Life Sciences, Inc., Boston, MA). Rat brain and heart tissue were used as positive controls.

Statistical Analysis

The results were expressed as the mean ± standard error of the mean (SEM) for the number (n) of individual experiments performed. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. A probability level of $P < 0.05$ was required for statistical significance.

RESULTS

Under control condition (Figs. 1 and 2), loperamide induced relaxation of prostate tissue strips in a dose-dependent fashion from 0.1 to 10 μM. The tension reduction was up to 80% in KCl-contracted strips and 50% in phenylephrine-contracted strips. Pre-treatment with 1 μM naloxone in the organ bath significantly inhibited the relaxation responses, thus suggesting activation of MOR in the loperamide effect. On the other hand, pre-treatment with 0.1 μM naloxonazine in the organ bath inhibited the relaxation response only in the phenylephrine-contracted strips, but not the KCl-contracted strips (Fig. 1). The potassium channel blocker glybenclamide significantly inhibited the loperamide-induced relaxation in KCl-contracted strips at concentrations of 0.1–1 μM and in phenylephrine-contracted strips at 0.01–1 μM, indicating the involvement of potassium channels in mediating this

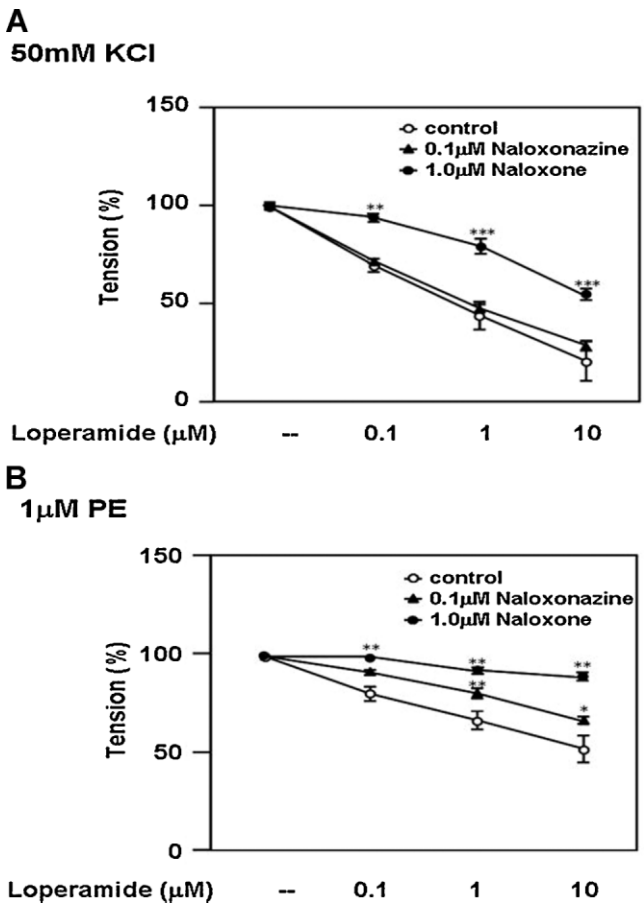

Fig. 1. Naloxone and naloxonazine inhibition of loperamide-induced relaxation in KCl pre-contracted prostate strips (upper); naloxone and naloxonazine inhibition of loperamide-induced relaxation in phenylephrine pre-contracted prostate strips (lower). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; the number of individual experiments, n = 8 in each group.

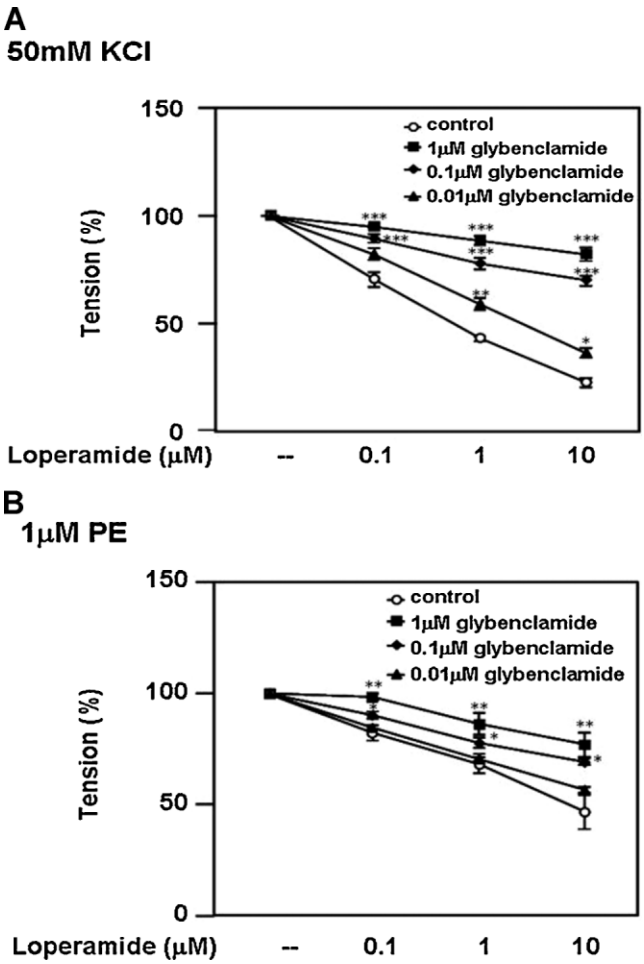

Fig. 2. Glybenclamide inhibition of loperamide-induced relaxation in KCl pre-contracted prostate strips (upper); glybenclamide inhibition of loperamide-induced relaxation in phenylephrine pre-contracted prostate strips (lower). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; the number of individual experiments, n = 8 in each group.

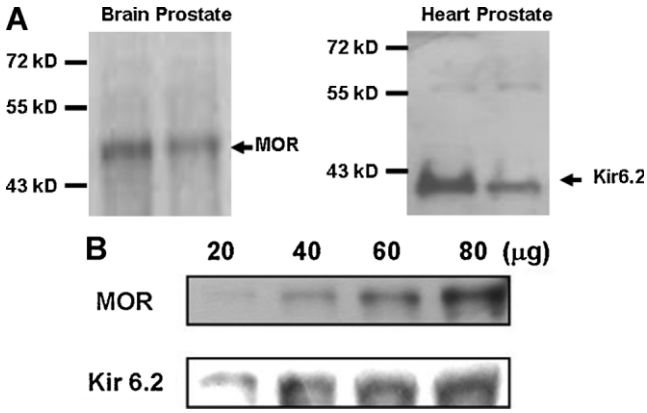

Fig. 3. Representative Western blots showing the presence of MOR and Kir6.2 protein expressions in the rat prostate. **A**: Immunoblots of 60 μg **tissue lysate from Wistar rat prostate against MOR antibody and Kir6.2 antibody. Whole brain and heart lysates were used as positive controls. B**: The expressions of MOR and Kir6.2 increased with the amount of loading protein. Lanes represent different loading protein amount: 20, 40, 60, and 80 μg, **respectively.**

relaxation (Fig. 2). The IC50 of naloxone and glybenclamide to reverse 10 μM loperamide-induced relaxation in phenylephrine-contracted strips was 1.0 ± 0.25 μM and 7.5 ± 1.7 nM, respectively.

Western blots demonstrated the expression of MOR as well as Kir6.2, which is an adenosine triphosphate (ATP)-sensitive potassium channel (K_{ATP}) subunit protein, in the rat prostate (Fig. 3A). The expression of each protein was raised with increments in the amount of loading protein (Fig. 3B).

DISCUSSION

Some previous studies have investigated the effects of opioids on the lower urinary tract function. Tramadol, a widely used analgesic and an opioid receptor agonist, significantly increased threshold pressure and micturition volume in conscious rat cystometry.[7] U-50488, a kappa opioid receptor agonist, could decrease detrusor-sphincter dyssynergia and improve voiding efficiency in conscious, spinal cord injured rats.[8] However, many of these opioid effects on the lower urinary tract have been attributed to actions on opioid receptors in the central nervous system.[9]

There is a paucity of studies on the possible role of peripheral opioid receptors in the prostate. Decreases of prostate and seminal vesicle weights were recently demonstrated in rats with cholestasis and attributed to increased endogenous opioids.[10] Treatment with naltrexone, an opioid receptor blocker, improved the weights of these two organs. Additionally, opioid peptides and opiergic neurons were found in the prostate gland and proposed to exert a role in tumor regulation. Opioid agonists inhibited cell growth in several systems, including the human prostate cancer cell line LNCaP.[11]

The current study has demonstrated MOR protein expression in the rat prostate. In addition, loperamide-induced rat prostate relaxation through activation of these peripheral MOR. Both naloxone and naloxonazine inhibited the loperamide-induced relaxation. Although μ-opioids share many common characteristics, there are also some pharmacological differences. Evidence over the years has suggested the existence of multiple MORs.[12] Two components of μ-agonist and μ-antagonist binding were identified. Both components exhibited high affinity for μ-agonists, but they could be readily distinguished by the μ-antagonists naloxonazine and naloxazone.[13] These two antagonists selectively blocked, in an irreversible fashion, the higher affinity binding both in vitro and in vivo. The utility of naloxonazine defined the pharmacological distinction between the naloxonazine-sensitive (μ-1) and the naloxonazine-insensitive (μ-2) sites. This study demonstrated that compared to 1.0 μM naloxone, 0.1 μM naloxonazine exhibited a significant but weaker inhibitory effect on the loperamide-induced prostate relaxation in the phenylephrine-contracted strips, but not in the KCl-contracted strips. These findings may suggest partial involvement of μ-1 receptor activation in the loperamide action. However, the actual subtyping for MORs is complicated. For example, studies of morphine-6β-glucuronide suggested that its actions involved yet another MOR subtype.[14] The cloning of MOR-1 gene,[15] provided one-way of further exploration at the molecular level. Recent studies have also identified a number of splice variants of this gene that appear to be important in the production of μ-opioid analgesia.[16]

The sulfonylurea compound glybenclamide is a commonly used drug in the treatment of type 2 diabetes mellitus. Besides promoting insulin secretion, the drug improves glucose control also by improving insulin sensitivity which may be

mediated through direct peripheral effects.[17] Its main pharmacological action mechanism consists of inhibition of the K_{ATP} channels, which leads to depolarization of pancreatic islet β-cells and insulin secretion. Linkage of drug molecules with surface receptor in the β-cell surface leads to reduction of conductance of the K_{ATP} channels.[18] The reduced K^+ efflux causes membrane depolarization and influx of Ca^{++} through Ca^{++} channels eventually causes insulin secretion. Similar action mechanism also leads to extrapancreatic actions of the drug at liver, skeletal muscle, heart muscle, and smooth muscle sites. In this study, the glybenclamide inhibited the loperamide-induced relaxation, suggesting involvement of K_{ATP} in mediating this relaxation. This notion was further supported by demonstration of Kir6.2 protein expression in the rat prostate. K_{ATP} channels are found in a wide variety of cell types, in which they couple intracellular metabolic changes to membrane electrical activity. K_{ATP} channels are hetero-octameric complexes consisting of four pore-forming subunits (inwardly rectifying potassium channel, subfamily J; Kir6.x) and four regulatory sulfonylurea receptor subunits (ATP-binding cassette protein, subfamily C; SURx).[19] Two Kir6.x (Kir6.1 and Kir6.2) and two SURx (SUR1 and SUR2) subunits have been identified, and their various combinations can give rise to different functional K_{ATP} channel subtypes.

Currently, two classes of drugs remain the mainstay medical treatments of clinical benign prostatic hyperplasia (BPH): (1) alpha-1 antagonists; (2) 5-alpha-reductase inhibitors. Systemic review has shown that alpha-1 antagonists could improve symptom scores by 30–40% and maximum flow rate by 15–30%.[20] On the other hand, 5-alpha-reductase inhibitors decreased prostate volume by 25%.[21] However, adverse reactions associated with these drugs are common. The main side effects of alpha-1 antagonists include: postural hypotension, dizziness, asthenia, somnolence, headache and ejaculatory difficulty.[22] For 5-alpha-reductase inhibitors, these include: erectile dysfunction, reduced libido, ejaculatory disorders, and breast tenderness.[23] The present study has shown that peripheral opioid receptors and agonists may play a role in the mediation of prostate relaxation. Thus, future development of new opioids that are devoid of central side-effects may be promising in the armamentarium for BPH treatments. Although up to date, MOR expression in the human prostate has not been reported, neuroendocrine opioid peptides including met-enkephalin, leu-enkephalin, and beta-endorphin have been demonstrated in the human prostate gland.[24] Thus, it appears that there should be some peripheral opioid mechanisms involving the human prostate. However, whether the current results on rats can be extrapolated to human will need further investigation.

CONCLUSIONS

MOR protein is expressed in the rat prostate. Loperamide induces rat prostate relaxation through activation of the prostatic MOR. Potassium channels are involved in mediating this loperamide effect.

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