Characteristics of TRK-130 (Naltalimide), a Novel Opioid Ligand, as a New Therapeutic Agent for Overactive Bladder

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ABSTRACT

We characterized TRK-130 (N-[[5R,6R,14S]-17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-yl]phthalimide; naltalimide), an opioid ligand, to clarify the therapeutic potential for overactive bladder (OAB). In radioligand-binding assays with cells expressing human μ-opioid receptors (MORs), δ-opioid receptors (DORs), or κ-opioid receptors (KORs), TRK-130 showed high selectivity for MORs (Ki for MORs, DORs, and KORs = 0.268, 121, and 8.97 nM, respectively). In a functional assay (cAMP accumulation) with cells expressing each human opioid receptor subtype, TRK-130 showed potent but partial agonistic activity for MORs [EC50 (E_{max}) for MORs, DORs, and KORs = 2.39 nM (66.1%), 26.1 nM (71.0%), and 9.51 nM (62.6%)], respectively. In isovolumetric rhythmic bladder contractions (RBCs) in anesthetized guinea pigs, TRK-130 dose-dependently prolonged the shutdown time (the duration of complete cessation of the bladder contractions) (ED30 = 0.0034 mg/kg i.v.) without affecting amplitude of RBCs. Furthermore, TRK-130 ameliorated formalin-induced frequent urination at doses of higher than 0.01 mg/kg p.o. in guinea pigs under the freely moving condition. Meanwhile, TRK-130 showed only a negligible effect on the gastrointestinal transit at doses of up to 10 mg/kg s.c. in mice. These results indicate that TRK-130 is a potent and selective human MOR partial agonist without undesirable opioid adverse effects such as constipation and enhances the storage function by suppressing the afferent limb of the micturition reflex pathway, suggesting that TRK-130 would be a new therapeutic agent for OAB.

Introduction

Overactive bladder (OAB) is defined as “urinary urgency, usually accompanied by frequency and nocturia, with or without urgency urinary incontinence, in the absence of urinary tract infection or other obvious pathology” (Haylen et al., 2010). OAB is a distressing condition and one of the most prevalent complaints among both male and female adults. The prevalence of OAB is estimated to be 10–20% of community residents in reports in the United States, Europe, and Japan (Milsom et al., 2001; Stewart et al., 2003; Homma et al., 2005). The prevalence of OAB increases as people age, and OAB is known to dramatically reduce people’s quality of life to the extent that it makes long-distance travel and adequate sleep difficult (Liberman et al., 2001). The therapeutic approaches for OAB include behavior therapy, electrostimulation, and pharmacologic therapy (Wein, 2003; Wein and Rackley, 2006; Cipullo et al., 2014). Although antimuscarinic drugs have been the current first-line pharmacotherapy for OAB, their persistence rates are low with less than 50 or 25% remaining on medication at 6 months or 1 year, respectively (Broström and Hallas, 2009; Sexton et al., 2011; Chancellor et al., 2013). The high rate of discontinuing antimuscarinic drugs is considered to be attributable to the concurrence of many types of adverse effects such as dry mouth, constipation, blurred vision, voiding dysfunction, and tachycardia (Yarker et al., 1995; Kelleher et al., 1997; Andersson, 2004) as well as the lack of efficacy of antimuscarinic drugs, in part due to the atropine-resistant contraction of the urinary bladder (Kelleher et al., 1997; Sahai et al., 2005; Sakakibara et al., 2011). Therefore, the development of new therapeutic agents with a mode of action different from that of antimuscarinic drugs has been eagerly anticipated. A number of clinical approaches to treat OAB with nonantimuscarinic mechanisms have proved to be beneficial, including β3-adrenergic receptor agonists (Ohlstein et al., 2012; Nitti et al., 2013). However, there is still no clinically proven pharmacotherapy for treating OAB that could act on the central nervous system (CNS), which controls lower urinary tract function. Moreover, recent advances in basic studies have revealed potential targets in the brain and spinal cord, including dopamine (Seki et al., 2001), GABA (Morikawa et al., 1992), serotonin (Kakizaki et al., 2001), and opioid receptors (Soulard et al., 1992; Pandita et al., 2003; Sexton et al., 2011; Chancellor et al., 2013).

Abbreviations: CHO, Chinese hamster ovary; CNS, central nervous system; DAMGO, [D-Ala², N-Me⁴, Gly⁵-ol]-enkephalin; DOR, δ-opioid receptor; DPDPE, cyclic [D-Pen², D-Pen⁵]-enkephalin; IBMX, 3-isobutyl-1-methylxanthine; KOR, κ-opioid receptor; MOR, μ-opioid receptor; OAB, overactive bladder; RBC, rhythmic bladder contraction; TRK-130/naltalimide, N-[[5R,6R,14S]-17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-yl]phthalimide; U-69593, (+)-(5α,7α,8β)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide.
Pehrsøn and Andersson, 2003; Pehrsøn et al., 2003; Holt et al., 2005), etc. Thus, drugs acting on the CNS have been proposed as an alternative pharmacotherapy to treat OAB.

Opioids have been well known to exert an inhibitory effect on the micturition reflex at various CNS sites, including the pontine micturition center (Noto et al., 1991), sacral parasympathetic nucleus (de Groat et al., 1983), and urethral sphincter motor nucleus in the spinal cord (Thor et al., 1989). Thus, opioid receptors are believed to be potential molecular targets for drugs acting on the CNS for OAB treatment. Although opioid receptor agonists such as morphine are known to inhibit the micturition reflex, as demonstrated by experimental studies, they cannot be used as therapeutic agents for OAB owing to their adverse effects. Recently, some opioid receptor agonists that were without such undesirable opioid features, but retained a voiding-suppressing action, were demonstrated (Soulard et al., 1992; Pandita et al., 2003; Pehrsøn and Andersson, 2003; Pehrsøn et al., 2003; Holt et al., 2005), suggesting the potential of opioid receptor agonists as a new therapeutic modality for OAB.

We have found the orally active morphinan derivative, TRK-130 (N-(5R,6R,14S)-17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphan-6-yl)phthalimide (Fig. 1), as a potent and selective human μ-opioid receptor (MOR) partial agonist without undesirable opioid adverse effects, such as constipation. In this study, we report the in vitro profiles of TRK-130 and its effects on lower urinary tract function in guinea pigs.

Materials and Methods

Drugs. TRK-130 was synthesized at Pharmaceutical Research Laboratories, Toray Industries (Kanagawa, Japan), according to a procedure, for example, described in previous literature (Simon et al., 1994). Morphine was obtained from Takeda Pharmaceutical (Osaka, Japan). [D-Ala², N-Me⁶, Gly³-ol]-enkephalin (DAMGO), cyclic [D-Pen², D-Pen⁵]-enkephalin (DPDPE), U-69593 ([(+)-(5α,7α,8β)-N-methyl-N-(7-1-pyrrolidinyl)-1-oxaspiro[4,5](dec-8-yl)]benzeneacetaldehyde), naloxone, forskolin, and oxybutynin were obtained from Sigma-Aldrich (St. Louis, MO). Buprenorphine (Lepetan injection) was obtained from Otsuka Pharmaceutical (Tokyo, Japan). [³H]Diprenorphine and [³H]naltrindole were obtained from PerkinElmer (Waltham, MA).

Radioligand Binding. The radioligand-binding studies were performed using a membrane preparation derived from human Otsuka Pharmaceutical (Tokyo, Japan). [³H]Diprenorphine solution in 5 mM Tris-HCl buffer (pH 7.4) (a final concentration of 2.2 μM) was used as a working solution of these drugs, and 20 μl [³H]naltrindole solution in 50 mM Tris-HCl buffer (pH 7.4) (a final concentration of 0.6 nM) was used as the tracer solution. A typical incubation mixture in a test tube for MOR and KOR consisted of 200 μl membrane suspension in 50 mM Tris-HCl buffer (pH 7.4), containing 5 mM MgCl₂, 2.2 μM work solution of these drugs, and 20 μl [³H]naltrindole solution in 50 mM Tris-HCl buffer (pH 7.4), containing 5 mM MgCl₂ (a final concentration of 0.9 nM). Incubation was performed at 25°C for 60 minutes for MOR and KOR and 120 minutes for DOR and terminated by filtration through a GF/B filter. The radioactivity trapped on the filter was determined using the LKB Betaplate Scintillation Counter to calculate the radioactivity bound to the receptors. Nonspecific binding was defined as the radiobound in the presence of 10 μM naloxone.

Cell Culture. CHO-K1 cells (host cells) were transfected with the cDNA for human MORs and KORS (from the amygdala or thalamus, cDNA Library; Clontech Laboratories, Mountain View, CA) in the pEF/myc/cytovector and for human KORS in the pCRII vector by the Lipofectamine transfection method. CHO-dhfr⁻ (cells) were transfected with the cDNA for human DORS (from the SK-N-SH cells) in the pCR3 vector by the Lipofectamine transfection method. Stably transfected clone was selected, and their expression was confirmed by radioligand-binding assay with their selective ligands, [³H]DAMGO, [³H]U-69593, and [³H]DPDPE (PerkinElmer), respectively. The CHO-K1 cells and CHO-dhfr⁻ cells, including those expressing human opioid receptors, were grown and maintained basically in a minimum essential medium with or without ribonucleosides and deoxyribonucleosides, respectively, in the presence of 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.6 mg/ml G418 disulfate in 5% CO₂ at 37°C. After CHO-dhfr⁻ and CHO-K1 cells were grown, the cells were rinsed with phosphate-buffered saline⁻ twice and then incubated in 0.53 mM EDTA/phosphate-buffered saline at room temperature until the cells were detached from culture flask. The cells were collected by centrifugation and suspended in Hanks’ balanced salt solution containing 0.5 mM IBMX (3-isobutyl-1-methylxanthine), 5 mM HEPES, and 0.1% bovine serum albumin (pH 7.4) (hereinafter referred to as stimulation buffer) to give a final concentration of 10,000 cells/μl. This suspension was used in the forskolin-stimulated cAMP accumulation assay described below.

cAMP Accumulation. The assay of forskolin-stimulated cAMP accumulation was performed using an AlphaScreen cAMP Assay Kit (PerkinElmer), according to the manufacturer’s instruction. In brief, a typical incubation mixture was prepared by the addition of 5 μl suspension containing CHO cells (7500–10,000 cells) and anti-cAMP receptor beads (1 U) in stimulation buffer to 5 μl drug solution (a prescribed final concentration) in stimulation buffer containing 100 μM forskolin. Incubation was performed in the dark at 25°C for 60 minutes. At the end of the incubation, 15 μl conditioned biotinylated-cAMP detection solution containing streptavidin donor beads was added to the incubation mixture (1 Uwell). The incubation mixture was further incubated in the dark at 25°C for 120 minutes. cAMP accumulation was quantitated as AlphaScreen signals using a Fusion-α Universal Microplate Reader (PerkinElmer). As a stock solution, TRK-130 was dissolved in 10% dimethyl sulfoxide solution containing methanesulfonic acid (1.1 molar equivalents to TRK-130); DAMGO, DPDPE, and morphine were dissolved in distilled water and U-69593 in ethanol. Each stock solution was serially diluted with stimulation buffer for use.

Distension-Induced Isovolumetric Rhythmic Bladder Contractions. This experiment was conducted as described previously, with some modification (Doi et al., 2000). Female Hartley guinea pigs (Japan SLC, Shizuoka, Japan; weighing 265–320 g) were anesthetized with an intraperitoneal injection of urethane (1.2 g/kg). The urinary bladder was exposed through a midline incision of the abdomen, and the urethra was ligated. A polyethylene tube (PE-100) was inserted into the bladder dome for recording the intravesical pressure and another for injection of saline into the bladder. Physiologic saline was injected with increments of each 0.2 ml into the bladder, and the

Fig. 1. Chemical structure of TRK-130.
injection was stopped when continuous isovolumetric rhythmic bladder contractions (RBCs) appeared to evaluate the effects of drug treatment under the threshold volume condition (Doi et al., 2000). Drugs were intravenously administered to the animals showing stable RBCs. Following drug administration, the intravesical pressure was recorded until the RBC reappeared with a cutoff time of 60 minutes. The parameters measured were the shutdown time (the duration of complete cessation of the bladder contractions) before and after drug administration and the maximum intravesical pressures at contractions immediately before and after drug administration. Drugs were dissolved in 5% xylitol solution (Otsuka Pharmaceutical) containing 0.02% citric acid and injected at a volume of 0.5 ml/kg.

Chemically-Induced Pollakiuria Model. Two days before the experiment, female Hartley guinea pigs (Japan SLC; weighing 280–353 g) received an intravesical instillation of formalin. Under ether anesthesia, a 4 Fr. Groshong catheter (Bard Access Systems, Salt Lake City, UT) was placed transurethrally. The tip of the catheter was positioned in the bladder. Urine was drained from the bladder, and 2.5% formalin saline solution (1 ml) was instilled into the bladder through the catheter for 1 minute, and then the solution was drained off. Animals with intravesical instillation of saline instead of formalin saline served as the saline-instilled control. A metabolic cage mounted on an electrical balance was used for measuring spontaneous voiding. Between the cage and the balance, a wire mesh was inserted to catch the feces. The balance was connected to a computer, and the digital output values of the balance were stored. A change in the output values of the balance was regarded as a voiding episode. After completion of the experiment, the sequential data obtained from the balance were analyzed to obtain the voiding parameters. On the day of the experiment, following a 30-minute acclimation period after placing the animals in the cages, water was given to the animals at a volume of 40 ml/kg. Following a 3-hour voiding measurement period after water loading (the predrug control session), the animals were subjected to oral administration with vehicle or TRK-130, followed by additional oral water loading at a volume

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Binding affinities of TRK-130 and morphine for human MOR, DOR, and KOR</th>
<th>Selectivity Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>$K_i$ Value</td>
<td>MOR</td>
</tr>
<tr>
<td>TRK-130</td>
<td>0.268 ± 0.012</td>
<td>121 ± 6</td>
</tr>
<tr>
<td>Morphine</td>
<td>11.5 ± 0.4</td>
<td>1517 ± 150</td>
</tr>
</tbody>
</table>

Fig. 2. Inhibitory effects of TRK-130, DAMGO, DPDPE, U-69593, morphine, and buprenorphine on forskolin-induced intracellular cAMP accumulation in cells stably expressing human MOR, DOR, or KOR. The values of intracellular cAMP accumulation represent the percentage of change from those recorded after stimulation with 50 μM forskolin only. Each plot represents the mean ± S.E.M. of six wells obtained from three independent experiments.
of 40 ml/kg. The voided volume was then measured for another 3 hours (the drug session). The percentage of the voiding parameters (the number of voiding episodes, mean urine volume per void, and total urine volume) obtained during the drug session to those obtained during the predrug control session was calculated and used to evaluate the drug effect. TRK-130 was dissolved in 5% xylitol solution (Otsuka Pharmaceutical) containing 0.02% citric acid and injected at a volume of 2 ml/kg.

**Gastrointestinal Transit.** Male ddY mice (Japan SLC; weighing 22.8–27.8 g) were made to fast on the day before use in the gastrointestinal transit experiment. This experiment was performed using a single-blinded study protocol. In the morning, on the day of the experiment, drugs were subcutaneously administered to the animals. Fifteen minutes after the administration, the animals were orally administered 5% gum arabic containing 10% charcoal (charcoal meal) at a dosing volume of 0.25 ml/body. Twenty minutes after the administration of the charcoal meal, the animals were euthanized by cervical dislocation. The small intestine from the pylorus to the cecum was removed from the body. The isolated intestine was straightened to measure its total length (centimeters) and the farthest distance (centimeters) that the charcoal meal traveled in the intestine. TRK-130 was dissolved in 5% xylitol containing 0.1% citric acid, and morphine and buprenorphine were dissolved in physiologic saline and injected at a volume of 10 ml/kg.

**Calculations and Statistics.** Results were expressed as mean ± S.E.M. or mean with 95% confidence intervals.

For the receptor-binding assay, the $K_i$ values were calculated from the equation,$K_i = \frac{IC_{50}}{1 + \frac{[\text{radioligand}]}{K_d}}$. The IC$_{50}$ value was determined using nonlinear logistic regression analysis. For the cAMP accumulation assay, the EC$_{50}$ values were determined using nonlinear optimization. For comparison of the $E_{\text{max}}$ values (the percentage to maximum inhibition of cAMP accumulation attained by the full agonist at the corresponding receptor subtypes), a multiple comparison was performed using the two-tailed Tukey test or two-tailed Steel-Dwass test. For RBCs, The ED$_{30}$ values were determined from the percentage of maximum possible effects $\% \text{maximal possible effect} = \left(\frac{\text{shutdown time in the drug-treated group} - \text{shutdown time in the vehicle-treated group}}{\text{cutoff time (60 minutes) - shutdown time in the vehicle-treated group}}\right) \times 100$ using a nonlinear logistic regression analysis. The cutoff time value of 60 minutes was used as the shutdown time when the disappearance of RBCs after the drug treatment lasted for longer than 60 minutes (cutoff time). The postdrug maximum intravesical pressure was converted into the percentage of the predrug value and used for analysis. The differences between the

<table>
<thead>
<tr>
<th>Compound</th>
<th>MOR EC$_{50}$</th>
<th>MOR $E_{\text{max}}$</th>
<th>DOR EC$_{50}$</th>
<th>DOR $E_{\text{max}}$</th>
<th>KOR EC$_{50}$</th>
<th>KOR $E_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nM</td>
<td>%</td>
<td>nM</td>
<td>%</td>
<td>nM</td>
<td>%</td>
</tr>
<tr>
<td>TRK-130</td>
<td>2.39 (1.85–3.09)</td>
<td>66.1 ± 3.9$^{abc}$</td>
<td>26.1 (22.4–30.5)</td>
<td>71.0 ± 1.9$^d$</td>
<td>9.51 (8.40–10.8)</td>
<td>62.6 ± 1.3$^{de}$</td>
</tr>
<tr>
<td>Morphine</td>
<td>19.9 (18.4–21.5)</td>
<td>100.0 ± 1.0</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>4.49 (4.11–4.91)</td>
<td>90.8 ± 1.4$^a$</td>
<td>8.00 (6.59–9.68)</td>
<td>62.2 ± 2.4$^d$</td>
<td>2.08 (1.53–2.68)</td>
<td>20.5 ± 0.7$^d$</td>
</tr>
<tr>
<td>DAMGO</td>
<td>2.07 (1.92–2.23)</td>
<td>100.0 ± 0.9</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>DPDPE</td>
<td>0.527 (0.456–0.608)</td>
<td>100.0 ± 0.7</td>
<td>1.64 (1.55–1.73)</td>
<td>100.0 ± 0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N.D., not determined.

$^aP < 0.05$ versus the corresponding full agonist by Steel-Dwass test.

$^bP < 0.05$ versus morphine by Steel-Dwass test.

$^cP < 0.05$ versus buprenorphine by Steel-Dwass test.

$^dP < 0.05$ versus the corresponding full agonist by Tukey test.

$^eP < 0.05$ versus buprenorphine by Tukey test.

TABLE 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>MOR $E_{\text{max}}$</th>
<th>DOR $E_{\text{max}}$</th>
<th>KOR $E_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRK-130</td>
<td>66.1 ± 3.9$^{abc}$</td>
<td>71.0 ± 1.9$^d$</td>
<td>62.6 ± 1.3$^{de}$</td>
</tr>
<tr>
<td>Morphine</td>
<td>100.0 ± 1.0</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>90.8 ± 1.4$^a$</td>
<td>62.2 ± 2.4$^d$</td>
<td>20.5 ± 0.7$^d$</td>
</tr>
<tr>
<td>DAMGO</td>
<td>100.0 ± 0.9</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>DPDPE</td>
<td>100.0 ± 0.7</td>
<td>1.64 (1.55–1.73)</td>
<td>100.0 ± 0.4</td>
</tr>
</tbody>
</table>

$^aP < 0.05$ versus the corresponding full agonist by Steel-Dwass test.

$^bP < 0.05$ versus morphine by Steel-Dwass test.

$^cP < 0.05$ versus buprenorphine by Steel-Dwass test.

$^dP < 0.05$ versus the corresponding full agonist by Tukey test.

$^eP < 0.05$ versus buprenorphine by Tukey test.
groups were analyzed using the Student’s *t* test, Aspin-Welch test, onetailed Williams’ test, or one-tailed Shirley-Williams’ test. For the 
pollakiuria model, the differences in the voiding parameters between 
the saline-instilled control group and the formalin-instilled vehicle 
treated group were analyzed using the Aspin-Welch test. The 
differences 
in the voiding parameters between the formalin-instilled vehicle-treated 
group and the formalin-instilled TRK-130–treated groups were 
analyzed using the one-tailed Shirley-Williams’ test or one-tailed 
Shirley-Williams’ test.

**Ethical Consideration.** All the animal experiments in this study 
were approved by the Animal Ethics Committee of the Research and 
Development Division, Toray, or Experimental Animal Care and Use 
Committee of Takeda.

### Results

**Binding Affinities of TRK-130 for Human MOR, DOR, and KOR.** In radioligand-binding assays, TRK-130 demonstrated selectivity for MOR over DOR and KOR (Table 1) with the *K*<sub>i</sub> (affinity) values of 0.268 nM for human MOR, 121 nM for human DOR, and 8.97 nM for human KOR. The *K*<sub>i</sub> values of morphine were 11.5 nM for human MOR, 1517 nM for human DOR, and 445 nM for human KOR.

**Agonist Activities of TRK-130 for Human MOR, DOR, and KOR.** In the experiment, DAMGO, DPDPE, and U-69593 were used as standard full agonists for human MOR, DOR, and KOR receptors, respectively.

In cells expressing human MORs, TRK-130 showed a concentration-dependent inhibition of intracellular cAMP accumulation induced by stimulation with forskolin (50 μM) (Fig. 2). The *E*<sub>max</sub> (efficacy) and *EC*<sub>50</sub> values (potency) were 66.1% 

### Table 3

**Effects of TRK-130, morphine, and oxybutynin on the intravesical pressure of distension-induced RBCs in anesthetized guinea pigs**

Values are expressed as means ± S.E.M. Three and eight animals treated with TRK-130 and morphine, respectively, had disappearance of bladder contractions for more than 60 minutes (cutoff time); therefore, these animals were excluded from the analysis.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>n</th>
<th>Predrug</th>
<th>Postdrug</th>
<th>% of Predrug Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mg/kg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.00125</td>
<td>6</td>
<td>18.4 ± 1.3</td>
<td>18.3 ± 1.1</td>
<td>99.7 ± 2.2</td>
</tr>
<tr>
<td>TRK-130</td>
<td>0.0005</td>
<td>5</td>
<td>18.6 ± 1.7</td>
<td>18.7 ± 1.8</td>
<td>99.9 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>5</td>
<td>25.0 ± 3.6</td>
<td>24.3 ± 3.1</td>
<td>98.3 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>3</td>
<td>22.0 ± 1.5</td>
<td>22.6 ± 2.5</td>
<td>102.2 ± 7.7</td>
</tr>
<tr>
<td>Morphine</td>
<td>0.25</td>
<td>10</td>
<td>24.7 ± 1.4</td>
<td>23.6 ± 1.4</td>
<td>95.7 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>9</td>
<td>27.2 ± 2.8</td>
<td>27.0 ± 2.9</td>
<td>99.0 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6</td>
<td>27.6 ± 2.8</td>
<td>27.0 ± 2.9</td>
<td>99.0 ± 1.6</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1</td>
<td>6</td>
<td>18.7 ± 2.0</td>
<td>19.4 ± 2.0</td>
<td>103.9 ± 1.6</td>
</tr>
<tr>
<td>Oxybutynin</td>
<td>1</td>
<td>6</td>
<td>22.7 ± 1.6</td>
<td>16.6 ± 1.6</td>
<td>72.7 ± 3.7</td>
</tr>
</tbody>
</table>

*P < 0.05 versus corresponding vehicle control by two-tailed Aspin-Welch test.
and 2.39 nM, respectively (Table 2). Morphine and buprenorphine also showed a concentration-dependent inhibition of intracellular cAMP accumulation, with the $E_{\text{max}}$ values of 100.0 and 90.8% and the $E_{50}$ values of 19.9 nM and 4.49 nM, respectively (Fig. 2; Table 2). In cells expressing human DORs and KORs, TRK-130 also concentration-dependently inhibited intracellular cAMP accumulation (Fig. 2). The $E_{\text{max}}$ and $E_{50}$ values were 71.0% and 71.1 nM for DOR and 62.6% and 9.51 nM for KOR, respectively (Table 2). The $E_{\text{max}}$ and $E_{50}$ values of buprenorphine were 62.2% and 2.08 nM for KOR, respectively (Fig. 2; Table 2). Because the inhibition effect of morphine did not reach its peak in the concentration range tested, its $E_{\text{max}}$ and $E_{50}$ values were not calculated in cells expressing DORs or KORs (Fig. 2; Table 2).

**Effects of TRK-130 on RBCs Induced by Bladder Distension.** Typical tracings of distension-induced RBCs in female guinea pigs and the effects of intravenous TRK-130 and oxybutynin at doses of 0.01 and 1 mg/kg, respectively, are shown in Fig. 3. The frequency of distension-induced RBCs is believed to be regulated by the micturition center in the CNS (Maggi et al., 1986). Intravenous administration with TRK-130 (0.00125–0.01 mg/kg) dose-dependently prolonged the shutdown time with an $ED_{30}$ value of 0.0034 mg/kg (Table 3) without affecting the maximum intravesical pressure (Table 4). Intravenous administration with morphine (0.25–1 mg/kg) also dose-dependently prolonged the shutdown time with an $ED_{30}$ value of 0.62 mg/kg (Table 3) without affecting the maximum intravesical pressure (Table 4). In contrast, intravenous administration with oxybutynin at a dose of 1 mg/kg significantly attenuated the maximum intravesical pressure (Table 4), although it did not show any effect on the generation of contractions (Table 3).

**Effects of TRK-130 on Chemically-Induced Pollakiuria Model.** The effects of oral administration with TRK-130 (0.003–0.03 mg/kg) on frequent urination (a status of increased number of voiding episodes and reduced urine volume per void) of conscious, freely moving female guinea pigs with formalin-induced pollakiuria are shown in Fig. 4. Administration with TRK-130 dose-dependently reduced the number of voiding episodes without affecting total urine production (data not shown), and the minimal effective dose was 0.01 mg/kg for either index (the number of voiding episodes and mean urine volume per void).

**Effects of TRK-130 on Gastrointestinal Transit.** Comparison with the vehicle control group in terms of the

![Fig. 4. Effects of oral administration of TRK-130 in freely moving guinea pigs with chemically induced pollakiuria. (A) Frequency of micturition and mean voided volume per micturition in 3-hour period immediately following TRK-130 administration. Open columns represent predrug control values of the respective parameters obtained during the predrug session, and filled columns represent postdrug values. (B) Percentage of changes in the frequency of micturition and mean voided volume per micturition obtained during drug session to those obtained during predrug control session. Each column represents mean ± S.E.M. of the results from 8 to 12 guinea pigs per group. *P < 0.05 versus intravesical injection of saline + vehicle control by Aspin-Welch test; †P < 0.025 versus vehicle control by one-tailed Williams test.](image)
gastrointestinal transit rate indicated no significant changes in the group subcutaneously treated with TRK-130 even at 10 mg/kg (Fig. 5). In contrast, comparison with the vehicle control group showed significant inhibition of the gastrointestinal transit in the group subcutaneously treated with morphine at any of the examined doses (0.3–10 mg/kg) (Fig. 5). Similarly, comparison with the vehicle control group showed significant inhibition of the gastrointestinal transit in the group subcutaneously treated with buprenorphine at any of the examined doses (0.03–1 mg/kg) (Fig. 5). Morphine showed approximately 90% inhibitions at maximum, whereas buprenorphine showed a plateau of approximately 50% inhibitions. From the relative gastrointestinal transit rates, the ID50 values of morphine and buprenorphine were estimated to be 1.01 and 0.326 mg/kg, respectively (Table 5). As for TRK-130, no ID50 value was calculated, because TRK-130 showed a less than 50% inhibition of the gastrointestinal transit rate at any of the doses examined.

Discussion

A series of the current studies was undertaken to characterize the pharmacological profile of TRK-130 in vitro and in vivo. To examine the affinities to MOR, DOR, and KOR, TRK-130 and morphine were tested at five to seven concentrations to estimate their binding inhibition constants (the $K_i$ values). Comparison of the estimated $K_i$ values indicated that TRK-130 had a higher affinity to any of the opioid receptor examined than morphine. In addition, both TRK-130 and morphine showed affinities to these different receptor subtypes in the order of $\mu > \kappa > \delta$. In the cAMP accumulation assay of MOR, the $E_{\text{max}}$ values of TRK-130 and buprenorphine, a typical MOR partial agonist (Selley et al., 1998; Huang et al., 2001), were 66.1 and 90.8%, respectively, which are significantly lower values compared with those of DAMGO, a standard MOR full agonist. This finding suggests that TRK-130 is a MOR partial agonist, similar to buprenorphine. The $E_{\text{max}}$ value of morphine was 100.0%, and the $E_{\text{max}}$ value of TRK-130 was also significantly lower compared with that of morphine and buprenorphine. The EC50 value for MOR was low in the order of DAMGO, TRK-130, buprenorphine, and morphine. It implies that potencies to human MOR were in the order of DAMGO > TRK-130 > buprenorphine >> morphine.

In cells expressing human DOR and KOR, the $E_{\text{max}}$ values of TRK-130 were significantly lower compared with those of DPDPE and U-69593, standard full agonists, respectively.

![Fig. 5. Effects of subcutaneous administration of TRK-130, morphine, and buprenorphine on the gastrointestinal transit in mice. Each column represents mean ± S.E.M. of the results from 10 mice per group. *P < 0.05 versus corresponding vehicle control by two-tailed Shirley-Williams’ test; †P < 0.05 versus corresponding vehicle control by two-tailed Williams’ test.](image-url)
This finding suggests that TRK-130 is also a DOR and KOR partial agonist. Each standard full agonist at the corresponding receptor subtypes induced no agonist activity in host cells. Taken together, these results demonstrate TRK-130 to be a potent and selective human MOR partial agonist.

Regarding RBCs induced by bladder distention, TRK-130 and morphine suppress the micturition reflex without affecting the contractile force of the detrusor, whereas oxybutynin attenuates the contractile force without affecting the generation of the voiding reflex in the same animal model. The current result of the effect of oxybutynin on RBCs is consistent with previous reports (Doi et al., 2000; Shimizu et al., 2001). In addition, we have performed an in vitro study and found that TRK-130 at 1 μM did not significantly inhibit electrical field stimulation-induced contractions in isolated rat bladder strips (data not shown). This concentration (1 μM) is considerably high compared with the plasma concentration (C_{min}) of 11.85 ng/ml (approximately 25 nM) after administration of TRK-130 at a dose of 0.1 mg/kg i.v., which is 10-fold greater than the highest dose (0.01 mg/kg) used in the present in vivo study. These results suggest that the site of action of TRK-130 lies in the afferent limb of micturition reflex pathway. Furthermore, TRK-130 exerts a more prominent suppressing effect on the micturition reflex than morphine because the effects of TRK-130 occurred at substantially lower doses than morphine. This may reflect the greater affinity and potency of TRK-130 for MOR. Taken together, these results strongly support the assumption that TRK-130 may enhance the storage function by modulating the afferent limb of the micturition reflex, in contrast to oxybutynin acting on the bladder efferent function and/or bladder contractility.

The intravesical instillation of formalin significantly increased voiding episodes compared with the instillation of vehicle and significantly decreased the mean urine volume per void. Compared with vehicle, oral administration with TRK-130 dose-dependently attenuated the effects of formalin on voiding parameters. TRK-130 at doses of 0.01 and 0.03 mg/kg significantly reduced voiding episodes and significantly increased urine volume per void, which was expressed as a percentage of the predrug values, in a conscious, freely moving animal model of pollakiuria induced by intravesical formalin administration. The effects of tramadol, which is well known to be a MOR agonist and an inhibitor of 5-hydroxytryptamine and noradrenaline reuptake, on micturition in normal and urinary frequent, conscious rats have been reported previously (Pandita et al., 2003; Pehrson and Andersson, 2003; Pehrson et al., 2003). Tramadol effectively inhibits micturition without decreasing the micturition pressure by mainly stimulating MORs. At the same time, tramadol is reported to have a diuretic effect by activating on KORs. Regarding diuresis, TRK-130 dose-dependently reduced frequent urination without affecting diuresis (data not shown). Furthermore, tramadol suppressed bladder pain-related behaviors in mice with chemically induced cystitis (Oyama et al., 2012). In the current study, we did not evaluate the effect of TRK-130 on chemically induced bladder pain. Thus, further investigations are needed to assess the potential of TRK-130 to relieve bladder pain. Taken together, these in vivo studies conducted in conscious and anesthetized guinea pigs indicate that TRK-130 has a favorable profile for the treatment of OAB.

It is well known that endogenous opioids are involved in the modulation of the micturition reflex mainly by the stimulation of MORs and DORs. The tonic enkephalinergic inhibition of the micturition reflex is believed to be mediated at several possible levels, including the peripheral bladder ganglia, sacral spinal cord, or brainstem (Noto et al., 1991). Studies using several types of MOR drugs have shown that the MOR modulation of bladder motility is exerted both at supraspinal and spinal sites in various species. Microinjection of the MOR agonist, DAMGO, into the ventrolateral periaqueductal gray in rats (Matsumoto et al., 2004) or fentanyl into the pontine micturition center in cats (Noto et al., 1991) inhibited reflex micturition. The intrathecal or intracerebroventricular administration of morphine also inhibited bladder motility, which was abolished by naloxone in rats (Dray and Metsch, 1984; Dray and Nunan, 1985). These previous findings suggest that MOR is a potential pharmacological target for OAB treatment by acting mainly on the afferent limb of the micturition reflex.

Although MOR agonists, such as morphine, are potential pharmacological drugs, they cannot be used as therapeutic agents for OAB due to adverse effects. In the current study, TRK-130 was tested for potential adverse effects on the gastrointestinal transit, which are typically shown with MOR agonists. TRK-130 had a negligible effect on the gastrointestinal transit at doses up to 10 mg/kg, when compared with the effects of morphine or buprenorphine. This result sets TRK-130 apart from the conventional MOR agonists.

In conclusion, we demonstrated that the orally active morphinan derivative, TRK-130, is a potent and selective human MOR partial agonist without undesirable opioid-related adverse effects, such as constipation, and that TRK-130 enhances the bladder storage function by modulating the afferent limb of the micturition reflex pathway in vivo. These findings suggest that TRK-130 would be a new therapeutic agent for OAB treatment and potentially have a better pharmacological profile than antimuscarinic drugs.

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References


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