AMTB, a TRPM8 channel blocker: evidence in rats for activity in overactive bladder and painful bladder syndrome

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Lashinger ES, Steinga MS, Hieble JP, Leon LA, Gardner SD, Nagilla R, Davenport EA, Hoffman BE, Laping NJ, Su X. AMTB, a TRPM8 channel blocker: evidence in rats for activity in overactive bladder and painful bladder syndrome. Am J Physiol Renal Physiol 295: F803–F810, 2008. First published June 18, 2008; doi:10.1152/ajprenal.90269.2008.—The activation of the TRPM8 channel, a member of the large class of TRP ion channels, has been reported to be involved in both normal bladder and painful bladder syndrome, although an endogenous activator has not been identified. In this study, N-(3-aminopropyl)-2-[(3-methylphenyl)methyl]oxy]-N-(2-thienylmethyl)benzamide hydrochloride salt (AMTB) was evaluated as a TRPM8 channel blocker and used as a tool to evaluate the effects of this class of ion channel blocker on volume-induced bladder contraction and nociceptive reflex responses to noxious bladder distension in the rat. AMTB inhibits icilin-induced TRPM8 channel activation as measured in a Ca2+ influx assay, with a pIC50 of 6.23. In the anesthetized rat, intravenous administration of AMTB (3 mg/kg) decreased the frequency of volume-induced bladder contractions, without reducing the amplitude of contraction. The nociceptive response was measured by analyzing both visceromotor reflex (VMR) and cardiovascular (pressor) responses to urinary bladder distension (UBD) under 1% isoflurane. AMTB (10 mg/kg) significantly attenuated reflex responses to noxious UBD to 5.42 and 56.51% of the maximal VMR response and pressor response, respectively. The ID50 value on VMR response was 2.42 ± 0.46 mg/kg. These results demonstrate that TRPM8 channel blocker can act on the bladder afferent pathway to attenuate the bladder micturition reflex and nociceptive reflex responses in the rat. Targeting TRPM8 channel may provide a new therapeutic opportunity for overactive bladder and painful bladder syndrome.

BLADDER SENSATION, which can be perceived as either urgency or pain, is initiated by primary afferent nerve endings of the dorsal root ganglia (DRG) in the bladder wall. The signal from these neurons is relayed to the spinal cord and higher centers of the central nervous system. The bladder afferent pathway functions both in normal micturition and in pathological states such as overactive bladder (OAB) and bladder pain, where afferent sensitivity is increased. One of the common clinical tests for bladder sensitivity involves the intravesical infusion of cold water, which is more likely to induce a micturition reflex in OAB vis-à-vis normal subjects (2, 7, 9, 22). Bladder pain can exist without bladder hyperactivity; in these subjects, cold water induces pain without a micturition reflex (23). The TRPM8 ion channel is calcium permeable and was initially known as the cold menthol receptor-1 (CMR-1). It is known to open in response to lowered temperature (21, 25) and cooling agents, such as menthol and icilin (1). The expression of TRPM8 is consistent with a role in the initiation of pain or the micturition reflex, with message being concentrated in the urothelium lining the bladder as well as in the DRG (29). Studies in a variety of animal models have shown that either cold or intravesical menthol will induce micturition or increase bladder contraction frequency in a spontaneously contracting bladder (8, 19). This is confirmed by several independent observations of decreased cold sensitivity in knockout mice lacking the TRPM8 channel (4). A recent immunohistochemical study in humans showed the density of TRPM8 channel protein to be elevated in bladder afferent nerve fibers of OAB patients vis-à-vis normal subjects, with an even higher density in patients with bladder pain. In OAB patients, there was a positive correlation between TRPM8 density and voiding frequency (23).

These observations suggest that a blocker of the TRPM8 channel could be effective for treatment of OAB and bladder pain. Some, but not all, channel blockers of TRPV1 also have affinity for TRPM8 (1, 34), but selective TRPM8 channel blockers were not identified. Recent patents report several structural classes containing examples of compounds having potent TRPM8 channel blockade activity (13–16). However, the pharmacology and selectivity of these TRPM8 channel blockers were not reported. N-(3-aminopropyl)-2-[(3-methylphenyl)methyl]oxy]-N-(2-thienylmethyl)benzamide hydrochloride salt (AMTB) was synthesized as an example of the compounds described in these patents.

Thus, the objectives of the present study were to characterize AMTB as a TRPM8 channel blocker and to use it as a tool compound to evaluate the role of this channel in bladder micturition contraction. Furthermore, to characterize the involvement of TRPM8 channel in bladder function, the effect of AMTB on visceromotor reflex (VMR) and cardiovascular (pressor) responses to noxious bladder distension (UBD) was evaluated. This model has been demonstrated to reliably measure drug effects on bladder afferent signal transmission (31).

MATERIALS AND METHODS

Female adult Sprague-Dawley rats (weighing 150–250 g) were used in this study. The experimental protocol was approved by the Institutional Animal Care and Use Committee of GlaxoSmithKline Pharmaceuticals, King of Prussia, PA.

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TRPM8 antagonist. AMTB (MW: 465.79) was synthesized by the Department of Medicinal Chemistry, GlaxoSmithKline. The chemical structure of AMTB is shown in Fig. 1.

Functional evaluation of TRPM8 using FLIPR. 1 × Hank’s balanced salt solution (Sigma, St. Louis, MO) was supplemented with 20 mM HEPES (Sigma). Test compounds were diluted with 65% balanced salt solution (Sigma, St. Louis, MO) was supplemented with 20 mM HEPES and 0.01% CHAPS (Sigma) for final concentration of 50 μM. The TRPM8 channel activator icilin was purchased from Sigma. The TRPM8 cDNA matches NCBI sequence NM_024080 and was inserted in a pcDNA3.1D mammalian expression vector. A stable cell line was created by transfection of HEK293 cells with the vector carrying the full-length TRPM8 cDNA. Parental HEK293 cells were transfected with lipofectamine 2000 as per manufacturer’s recommendations with 2 μg plasmid. After 24-h incubations, cells were trypsinized and plated at low density in DMEM containing 10% FCS and 1% G418 Sulfate (MediaTech, Manassas, VA). After 12 days, clonal cell lines were isolated and characterized by FLIPR assays.

htTRPM8 HEK293 stable cells were grown at 37°C, 5% CO2 using DMEM/F-12 (HAM’S) 1:1 with t-glutamine with 15 mM HEPES, pH 7.3, supplemented with 10% heat-inactivated fetal bovine serum and 1% of 50 mg/ml G418. After 12 days, clonal cell lines were isolated and characterized by FLIPR assays.

htTRPM8 HEK293 stable cells were seeded into CellCoat 384 well black, μClear bottom, poly-D-lysine-coated microplates (Greiner Bio-One, Frickenhausen, Germany) at a density of 15,000 cells per well and incubated for 48 h at 37°C. Following the incubation and baseline readouts, cells were then stimulated immediately and kept at −20°C before analysis. Analysis of plasma samples was performed using liquid chromatography/tandem mass spectrometry (LC/MS/MS) detection. Rat plasma samples were thawed, plasma proteins were precipitated with 200 μl of 95/5 acetonitrile/methanol, and the resulting mixture was vortex-mixed for 2 min followed by centrifugation for 30 min at 2,000 g. Using a sensitive and selective LC/MS/MS method on an HPLC PAL autosampler (CTC Analytics, Zwingen, Switzerland) coupled to a Sciex API5000 triple-quadrupole mass spectrometer (Applied Biosystems, Foster City, CA), samples were analyzed for quantitative concentrations of AMTB. Analytical standards for AMTB (1–2,500 ng/ml) were prepared in rat plasma to ensure accurate calibration of the mass spectrometer for each biological matrix.

Gene expression by TaqMan study. Rats (n = 9) were anesthetized initially with 3% isoflurane and euthanized by exsanguination. The DRGs at level L6/S1 and T13/L1 and bladders were removed immediately and kept at −80°C. Tissues were homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA) and after phase separation with chloroform; total RNA was extracted using the RNaseasy Mini Kit (Qiagen) following the manufacturer’s instructions. Any genomic DNA contamination was removed using DNase I (Ambion, Austin, TX). RNA samples were judged to be free of genomic DNA by no amplification in a standard TaqMan assay using 10 ng of RNA and TaqMan master probe oligonucleotides. The RNA was quantified using Ribogreen RNA quantitation reagent (Invitrogen) and converted to cDNA by reverse transcription utilizing the High Capacity cDNA Archive Kit (Applied Biosystems). The equivalent of 10 ng mRNA per well was arrayed into 384-well plates using a Biomek FX robot (Beckman Coulter) and quantitative RT-PCR was carried out using a 7900HT Sequence Detector System (Applied Biosystems) in a 5-μl reaction volume. TaqMan Universal PCR Master Mix 2× (Applied Biosystems) and universal PCR conditions recommended by the manufacturer were followed. All primers and probes (FAM, TAMRA) are listed in Table 1. To normalize the data, samples were scaled relative to each other by the geometric mean of the set of valid housekeeper data points for that sample. Each data point was then expressed as the ratio of the housekeeper abundance in the sample to the average of that housekeeper in all samples and marked invalid if

Table 1. Primers used in TaqMan study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Probe</th>
</tr>
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<tbody>
<tr>
<td>TRPM8</td>
<td>GAGCTTAGAGGAGACACAAAAATGATG</td>
<td>GGCTGGGATTGAGCAAAACA</td>
<td>ACTCACAGCCCTTGCTCACACTTCCGT</td>
</tr>
<tr>
<td>ACTB</td>
<td>GAGCTATGAGGATGCTGAC</td>
<td>AGTTTCTAGGATGCGACAGGAG</td>
<td>CATCAGCATAGGACATCGGCTCC</td>
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<tr>
<td>GAPDH</td>
<td>CAAGAATCATCGGCAAACTTGG</td>
<td>GGGCCATCCAGGTCTCTCAG</td>
<td>ACCAGAGTGCTGCAACTCGGCA</td>
</tr>
<tr>
<td>PPIA</td>
<td>TTAATGGCATGCGCAAGACCTGA</td>
<td>CGCAAATGCTGATGCGTCTCTTTC</td>
<td>CCAAGAAGCGCAGTGCTGGCAATCGA</td>
</tr>
</tbody>
</table>

Fig. 1. Chemical structure of N-(3-aminopropyl)-2-[(3-methylphenyl)(methyl)oxy]-N-(2-thienylmethyl)benzamide hydrochloride salt (AMTB).
it had statistically inconsistent behavior with the other housekeepers in those samples with similar tissue types. The housekeeping genes used were β-actin (ACTB), GAPDH, and Cyclophilin (PPIA), and the data were floored at a relative abundance of 30. The mean and SE of each group were calculated using Prism 4 (GraphPad Software, San Diego, CA). An ANOVA was performed to compare the expression in bladder to DRG also using Prism 4.

In vivo bladder rhythmic contraction. All surgical procedures were performed under 3% isoflurane anesthesia. Core body temperature of the rats was maintained at 36°C through use of a circulating hot water pad placed under the rat with a feedback-controlled system. Rats (n = 22) were euthanized upon completion of experimental procedures by an intravenous overdose of pentobarbital sodium (120 mg/kg, Vortech Pharmaceuticals, Dearborn, MI). In each animal, one jugular vein was cannulated with polyethylene tubing (PE50) for intravenous administration of compound or vehicle, and a tracheostomy tube was inserted to facilitate respiration. A cannula (PE50) was placed into the bladder via the urethra and the urethra was ligated to ensure an isovolumetric bladder. Occasionally, blood was visually observed in urine. In this case, the rat was not used for the experiment and was euthanized. Upon completion of surgical procedures, slow intravenous infusion of urethane (1.2 g/kg, Ethyl Carbonate, Sigma) was given over 15 min. Saline bladder infusion procedures were begun 30 min after final dose of urethane.

The urethral cannula was connected with a T connector and linked with a low volume transducer (ADInstruments MLT0380D, Colorado Springs, CO). The signal was amplified through a DC amplifier (ADInstrument, ML119). The other end of the T connector was linked to a 20-ml syringe with a perfusion pump. For the pharmacological study on bladder rhythmic contraction, the saline infusion into bladder was at a rate of 50 μl/min to induce micrurition reflex (here defined as bladder contraction with amplitude $>$10 mmHg). The infusion rate was then lowered to 10 μl/min until 3-5 rhythmic bladder contractions were established and infusion was terminated. The vehicle (1% DMSO with PEG200) or AMTB was administered intravenously after a 15-min control period. Following administration, the bladder rhythmic contraction was recorded for 20 min. Three parameters of bladder rhythmic contraction were evaluated: frequency/interval, basal pressure, and amplitude of the bladder rhythmic contraction. The mean controls were calculated by the averages of readouts during the last 5-min interval of the control period. The inhibition of contraction frequency by AMTB was calculated by the mean response in every 5 min after injection.

In vivo VMR and pressor responses to UBD. Rats (n = 13) were anesthetized initially with 3% isoflurane. To measure blood pressure, the right carotid artery was catheterized with PE50 tubing. The arterial catheter was linked with a low volume transducer (ADInstruments, MLT0380D) and signal was amplified through a DC amplifier (ADInstrument, ML119). One jugular vein was cannulated with polyethylene tubing for intravenous administration of compound or vehicle. PE90 tubing was inserted into the urinary bladder via the urethra and secured by a tight ligature around the distal urethral orifice. The bladder catheter was also linked to a bladder distension control device. The bladder was distended with saline by regulating air inflow into a Mariott bottle from a valve interface distension control device (University of Iowa, Bioengineering, B482C-1) (30). Two needle electrodes were sutured into the oblique abdominal musculature just above the inguinal ligament. Abdominal contractions were quantified by action potentials of electromyographic activity. Action potentials were initially amplified through a low-noise AC differential amplifier (ADInstrument, EC4–400), processed using the AD data-acquisition program (PowerLab 16/30, ML880). Raw action potentials of myoelectric activities, bladder pressure, and blood pressure were displayed on-line continuously. All data were analyzed off-line using the AD power lab program (Chart 8, Colorado Springs, CO).

Following completion of the surgical preparation, isoflurane anesthesia was reduced until flexion reflex response could be evoked by pinch of the foot without spontaneous escape behaviors (~1% isoflurane).

For UBD, all rats received a series of at least 6 phasic bladder distensions at 60 mmHg for 30 s at 3-min intervals to evaluate response stability to repeated bladder distension. Drug or vehicle (1% DMSO with PEG200) was administered intravenously after recovery. The inhibition of contraction frequency by AMTB was calculated by the mean response in every 5 min after injection.

Table 2. Pharmacokinetic parameters for AMTB following intravenous infusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose, mg/kg</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>Cmax, ng/ml</td>
<td>240 ± 60</td>
</tr>
<tr>
<td>Time Cmax is achieved, h</td>
<td>0.41 ± 0.13</td>
</tr>
<tr>
<td>Half-life, h</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Mean residence time, h</td>
<td>0.75 ± 0.14</td>
</tr>
<tr>
<td>Steady-state volume of distribution, l/kg</td>
<td>100 ± 22</td>
</tr>
<tr>
<td>Steady-state volume of distribution, l/kg</td>
<td>4.5 ± 1.3</td>
</tr>
<tr>
<td>AUC0–∞, μg·h−1·ml−1</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>AUC0–∞, μg·h−1·ml−1</td>
<td>0.17 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. Cmax, maximum concentration; AUC0–∞, area under the plasma concentration-time curve to the last time point with quantifiable drug; AUC0–∞, extrapolated area under the plasma concentration-time curve.
DMSO with PEG 200) was administered only after four consistent responses were elicited.

The electromyographic activity was integrated and calculated as the area under the curve (AUC). The VMR response to the stimulus was defined as the increase in electromyographic activity during UBD from the baseline activity before each response. Pressor response was quantified as the peak change in mean arterial pressure during UBD compared with the average level during a baseline period immediately before UBD. Following drug administration, response was modified to the percentile of mean control response: the average of four UBD responses before drug treatment.

Data analysis. All data were expressed as means ± SE. Results were analyzed with Student’s t-test or ANOVA with repeated measures by Prism 4 (GraphPad Software). A value of $P < 0.05$ was considered statistically significant.

RESULTS

Determination of antagonist activity of AMTB at TRPM8 channels. A FLIPR assay was used to measure Ca$^{2+}$ influx, induced by TRPM8 channel opening by icilin in cells expressing the recombinant channel (Fig. 2). The pEC$_{50}$ for this response was $6.91 ± 0.02$ ($6.95 - 6.88$), which is consistent with published data from mouse TRPM8 expressed in HEK293 cells (1). In a HEK293 stable cell line expressing TRPA1, icilin is inactive up to 15 $\mu$M. AMTB produced a concentration-related inhibition in icilin-activated Ca$^{2+}$ influx in HEK293 cells expressing TRPM8 (Fig. 2). The pIC$_{50}$ of AMTB was $6.23 ± 0.02$ ($6.27 - 6.19$).

To demonstrate specificity, similar experiments were used to show that AMTB had no antagonist activity against capsaicin in cells expressing hTRPV1 ($n = 6$); the mean pIC$_{50}$ was <4.6. Similarly, AMTB had no antagonist activity against GSK4 in cells expressing hTRPV4 ($n = 6$). The pIC$_{50}$ was 5.4 in one experiment and <4.6 in the other 5.

Pharmacokinetic studies. The time course for the plasma levels of AMTB during and following an intravenous infusion of 1 mg/kg is shown in Fig. 3. The Cmax value for AMTB was obtained $25$ min postdosing ($240 ± 60$ ng/ml). Following administration of AMTB, the corresponding systemic exposure...
Gene expression by quantitative PCR. The three housekeeping genes (β-actin, GAPDH, and cyclophilin) were expressed in a stable manner to each other across all of the samples allowing for proper normalization. The localization of mRNA of TRPM8 in rat bladder and DRG normalized to the mean of housekeeping genes is shown in Fig. 4. The TRPM8 mRNA expression level was consistently high in DRG isolated either at the junction of lumbar and thoracic (T13-L1) or lumbar and sacral (L6-S1) spinal cord. Data were pooled in Fig. 4. In contrast, the mean expression level in bladder smooth muscle was 4.6-fold lower than that in DRG.

In vivo bladder rhythmic contraction. Isovolumetric bladder rhythmic contraction was established to evaluate AMTB. Figure 5 is an example of raw trace recording in a rat before and after intravenous bolus dosing with AMTB (3 mg/kg). Data from these experiments are summarized below. Figure 5A demonstrates the intervals between contractions enlarged postinjection (P < 0.05, paired Student’s t-test). In some cases, weak inhibition of the amplitude of the first bladder contraction after drug administration was observed. However, evaluation of the mean amplitude of the first contraction after the inhibition by AMTB showed no statistical difference (Fig. 5B). Basal bladder pressure tended to increase throughout the experiment; however, as shown in Fig. 5C, the magnitude of this increase did not differ between vehicle- and AMTB-treated groups.

Figure 6A summarizes the mean frequency of the rhythmic contraction expressed as a percent of control values before and after intravenous doses. The administration of AMTB dose dependently inhibited the frequency of the rhythmic contraction (P < 0.05, 2-way ANOVA). The mean contraction amplitude was not attenuated, even at a dose of 10 mg/kg, which produced a 70% reduction in contraction frequency (P < 0.05, unpaired Student’s t-test). Figure 6, B and C, shows the mean frequency and amplitude during the 10-min interval following intravenous dosing. In vivo VMR and pressor responses to UBD. Reflex responses to repeated UBD to noxious levels were reproducible, as has been shown previously (30, 31). AMTB at a dose of 3 mg/kg or lower did not attenuate the mean blood pressure. However, at 10 mg/kg, AMTB reduced the mean blood pressure from 110.41 ± 6.00 to 87.62 ± 7.01 mmHg (P < 0.01, paired Student’s t-test). AMTB produced a dose-related inhibition of both the VMR and blood pressure responses to bladder distention. Figure 7 summarizes the dose-response functions to noxious UBD following AMTB administration. Summary data show that the VMR response was more sensitive to inhibition than the pressor response. An unpaired t-test showed a stronger effect of AMTB at 3 mg/kg on the VMR response than that on the pressor response (P < 0.001). The ID$_{50}$ values of AMTB (mg/kg) on pressor and VMR responses to noxious UBD (60 mmHg, 20 s in 3 min) were 10.89 ± 1.04 (8.74–13.04, 95% confidence intervals) and 2.42 ± 0.46 (AUC$_{0-\infty}$) was 0.17 ± 0.04 μg·h$^{-1}$·ml$^{-1}$. Other pharmacokinetic parameters are shown in Table 2.
First effective dose to show statistical significance, where the baseline response before administration of drugs is defined as 100%.

Student’s t-test. Each value represents the mean of experiments ± SE. The significance of differences between the test and control values was determined by ANOVA, mmHg. The significance of differences between the test and control values was determined by ANOVA, mmHg. The significance of differences between the test and control values was determined by ANOVA, mmHg.

DISCUSSION

There is a need for improved therapy for OAB, since the only approved drugs are the muscarinic antagonists, with limited efficacy and bothersome side effects. Clinical trials are underway with compounds acting by several novel mechanisms; however, positive results have been limited to studies with very small patient populations (10). Modulation of the activity of bladder afferent nerves would be ideal, since the ability of bladder smooth muscle to contract would not be impaired. Based on localization, function, and regulation in disease, a TRPM8 channel blocker may be a way to achieve this goal. An additional target for a TRPM8 antagonist would be painful bladder syndrome (PBS).

PBS is defined by the International Continence Society as “the complaint of suprapubic pain related to bladder filling, accompanied by other symptoms such as increased day-time and night-time frequency and night-time frequency, in the absence of proven urinary infection or other obvious pathology” (24). This condition is now commonly referred to as PBS/IC, where interstitial cystitis (IC) is reserved for patients with histologic or cystoscopic diagnoses (11). PBS/IC is more common than previously thought (18). Current oral therapy is limited to relatively nonspecific agents such as pentosan polysulfate sodium or antihistamines and is not very effective. Intravesical therapy or surgery is often required (24).

Our TaqMan studies confirm the localization of the TRPM8 channel in rat DRG as reported by Stein et al. (29). The rat should be a suitable model for the characterization of the role of a TRPM8 channel blocker on bladder afferent activity since the human DRG also has this channel. Although the TRPM8 channel is present in rat bladder urothelium (29), it was difficult to detect in a homogenate of rat bladder. Thus, the localization of the channel responsible for the cold response in human hyperactive bladder has not been established. Nevertheless, based on the abundance of clinical and experimental data, it is likely that the excitatory action of either cold or a TRPM8 opener such as menthol is mediated by a similar mechanism in rat and humans. There is also evidence for regulation of the TRPM8 channel by inflammatory mediators and nerve injury (6, 20). The localization of the TRPM8 channel in human afferent nerves, its upregulation in OAB and PBS/IC, and the correlation between density and bladder symptoms make a strong case for this channel as a viable therapeutic target.

We characterized AMTB as a TRPM8 channel blocker and used it as a tool compound to test the feasibility of using a TRPM8 channel blocker for the treatment of bladder hypersensitivity disorders. While compounds of this class have been recently reported in the patent literature (14–16), no data on their efficacy in bladder models were reported. In our FLIPR assay, AMTB has only moderate potency as a human TRPM8 channel blocker (pIC50 = 6.23). Although its selectivity profile has not been determined, it does achieve selectivity for TRPM8 vs. TRPV1 and TRPV4. It is the first TRPM8 channel blocker to be characterized and should be useful as a tool compound for in vitro and in vivo studies.

AMTB inhibits the frequency of volume-induced rhythmic contraction in the anesthetized rat, without reducing the amplitude of this response. This is the opposite profile to that which we observed with tolterodine, a muscarinic acetylcholine antagonist, which reduced the amplitude, with a slight increase in contraction frequency (data not shown). This profile for tolterodine in the rhythmic contraction model has been reported by other investigators (12, 32). There are little data in this model with agents acting via inhibition of afferent nerve activity. A delta-opiate receptor agonist has been shown to be active, presumably via inhibition of the micturition reflex at a spinal or higher level (12). In a similar model using the guinea pig, NK-1 antagonists will produce a profile like AMTB, decreasing frequency without a reduction in amplitude (26). Several agents presumed to act on bladder smooth muscle, such as β3-adrenoceptor agonists (17, 32, 35) and K+ channel openers (5, 27, 33), decrease contraction frequency in the rat.

All compounds active in this model will produce an abolition of spontaneous bladder contractions following intravenous administration; indeed, one way to evaluate activity in this model is to measure the length of time contractions are shutdown following dosing. The shutdown is due to reduction of bladder tone to a level below the...
threshold for induction of spontaneous contraction. This phenomenon does not imply that a compound will abolish the micrituration reflex in a model where voiding is not prevented. In the rhythmic contraction study, obstruction or irritation/inflammation of urethra by the transurethral catheterization could eliminate or sensitize a urethral afferent-mediated excitatory bladder reflex. Thus, this model is a readout of the micrituration reflex induced by bladder over-distension, but not the urodynamics under conditions of physiology or pathology of bladder overactivity.

The effects of AMTB on VMR and pressor responses are suggestive of an effect on sensory nerve activity. AMTB was a more potent inhibitor of the VMR vis-à-vis the pressor response to UBD (Fig. 7). A similar profile was produced by morphine and mexiletine, a sodium channel blocker (30), as well as a CaV2.2 channel blocker (31), all of which were more potent against the VMR. An explanation for this potency difference is not clear. AMTB at 10 mg/kg produced a modest reduction of blood pressure, which does not contribute to its antinociceptive effect, since we showed that reduction of blood pressure per se does not inhibit the response to UBD (30). The threshold bladder pressure for inducing the VMR response is higher than that of the pressor response (31), and VMR is considered to be a more reliable readout for the nociceptive response of the bladder.

AMTB effects on VMR

It should be noted that voiding-associated abdominal wall response (3) is triggered by urethral afferent nerves during normal voiding (28). This response differs from the passive VMR produced by noxious bladder distension (60 mmHg), which is attributed to pain sensation. To what degree the voiding-associated VMR response contributes to the nociceptive VMR response to 60 mmHg bladder distension remains to be determined. In addition, the specific site(s) of action of AMTB in the periphery or central nervous system requires further investigation via local delivery of AMTB. Moreover, based on the work of Lindstrom and colleagues, the cold receptors are located on bladder C-fiber afferents (19). Additional experiments using capsaicin or RTX pretreatment or single nerve recording would be useful to clarify whether AMTB suppresses different afferent nerves (C fibers or Aδ fibers). In any case, the potent activity of AMTB against the VMR response suggests utility in PBS/IC.

In conclusion, AMTB represents the first tool compound available for the evaluation of the role of the TRPM8 channel in animal models. Potency needs to be improved, and selectivity for other receptors and ion channels needs to be determined, but AMTB, at a dose which produces minimal cardiovascular effects, can inhibit volume-induced rhythmic contraction and also inhibit the response to noxious bladder stimulation. The rhythmic contraction model may be predictive of OAB efficacy and the bladder nociceptive model predictive of efficacy in PBS/IC. Based on the known ability of cold to stimulate a hyperactive bladder or to induce pain in susceptible patients, a TRPM8 channel blocker could represent a novel therapeutic agent offering clear advantages over currently available drugs.

REFERENCES


