Identification of a spontaneously active, Na+-permeable channel in guinea pig gallbladder smooth muscle

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Submitted 28 March 2005; accepted in final form 24 May 2005

GALLBLADDER SMOOTH MUSCLE (GBSM) is a key regulator of biliary function, extending in response to gallbladder filling and contracting postprandially to deliver bile to the intestines. Ca2+ activity by controlling the level of Ca2+ and contracting postprandially to deliver bile to the intestines. The treatment of gallbladder disorders. Ca2+ phasic contractions of individual GBSM bundles (28). GBSM action potential generation is critically dependent on the resting membrane potential, which is approximately −50 mV (12, 16, 28, 29, 30), and is −35 mV more positive to the equilibrium potential for K+ (approximately −85 mV). Hyperpolarization of the membrane potential, mediated by the activation of ATP-sensitive K+ channels, reduces the frequency of GBSM action potentials and associated spontaneous GBSM contractions, indicating the requirement of a membrane potential substantially positive to equilibrium potential for K+ for action potential generation in GBSM (9, 12, 29, 30).

Despite the functional importance of the resting membrane potential in GBSM, an understanding of the ionic currents involved in generating and controlling this membrane potential is lacking. We hypothesized that a spontaneously active, depolarizing conductance exists in GBSM, functioning to drive the resting membrane potential to a depolarized level sufficient to facilitate action potential generation. Here we have identified and characterized the properties of a novel spontaneously active, predominately Na+-mediated cation current (Icat) in guinea pig GBSM, which is reduced by external Ca2+, Mg2+, and Gd3+. Icat contributes tonically to the membrane potential of GBSM cells, facilitating electrical and contractile activity of this tissue.

METHODS

Tissue preparation. Male guinea pigs (250–350 g) were killed either by isoflurane or halothane overdose, followed by exsanguination. This procedure was reviewed and approved by the Office of Animal Care Management at the University of Vermont. The entire gallbladder was removed and placed in ice-cold physiological saline solution (PSS, for composition see Solutions and drugs). The gallbladder was pinned to the bottom of a petri dish containing nominally Ca2+-free dissection/dissociation solution (DS; see below).

GBSM single cell isolation. The GBSM tissue was cut into small strips (1 mm wide and 5–7 mm long). Several muscle strips were placed in a vial containing 2 ml DS supplemented with 1 mg/ml BSA, 1 mg/ml papain (Worthington Biochemical, Freehold, NJ), and 1 mg/ml dithioerythritol and incubated for 20 min at 37°C. After that, the tissue was placed in 2 ml DS containing 1 mg/ml BSA, 1 mg/ml collagenase (type II from Sigma), and 100 µM CaCl2 for 5–6 min at 37°C. After the incubation, the digested tissue was washed several times in DS medium and then dispersed with gentle trituration through the tip of a fire-polished Pasteur pipette. Several drops of the solution containing the dissociated cells were then placed in a recording chamber. Most cells were elongated and had a bright, shiny appearance when examined using phase-contrast microscopy.

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Whole cell current recordings. The conventional whole cell patch-clamp method was employed without the use of leak subtraction. GBSM cells were held at 0 mV, stepped briefly to −100 mV for 100 ms, and then ramped from −100 to +100 mV for 1 s. The contribution of K⁺ and VDCC currents to the ramp currents was eliminated by omitting K⁺ to remove the K⁺ currents and by adding 1 μM nifedipine in the bathing solution, respectively. A holding potential of 0 mV was employed to inactivate VDCC and voltage-dependent K⁺ channels. \( I_{\text{cat}} \) was measured as the difference between the current in the presence and absence of extracellular Na⁺ [i.e., ± N-methyl-D-glucamine (NDMGI)]. The current traces of 5–10 pulses were recorded, and the data were averaged for the controls and drug effects. In a separate series of experiments, the amphotericin-perforated whole cell configuration of the patch-clamp technique was employed (10) to evaluate the steady-state \( I_{\text{cat}} \) at the GBSM resting membrane potential (−50 mV) under physiological ionic conditions. Ramp protocols (−100 to +100 mV) were also applied using a holding potential of −50 mV. Whole cell currents were recorded using pCLAMP version 9.2 software through a Digidata 1322A and an Axopatch 200 amplifier (Axon Instruments) filtered at 500 Hz. All experiments were conducted at 22°C.

Membrane potential recording with intracellular microelectrodes. Gallbladders were removed from the mucosal layer, pinned out with serosal side up to the bottom of a Sylgard-lined recording chamber, and placed on the stage of an inverted microscope (Nikon Diaphot). Oxygenated (95% O₂–5% CO₂) PSS (for composition, see Solutions and drugs) was superfused over the tissue (10 ml/min), and the temperature was maintained between 35 and 37°C at the recording site. To facilitate intracellular recordings, tissue movement was reduced by adding wortmannin (200–400 nM) to the PSS solution. Smooth muscle bundles were visualized at ×200 with Hoffman Modulation Contrast optics (Modulation Optics, Greensville, NY). Glass microelectrodes were pulled on a Flaming/Brown glass puller (Sutter Instrument, Novato, CA) and had a resistance of 50–120 MΩ when filled with 2 M KCl. Transmembrane potential was measured with a negative-capacity compensation amplifier Axoclamp 2A (Axon Instruments, Union City, CA). The electrical signals were acquired using PowerLab hardware and software (version 5.02; AD Instruments, Castle Hills, Australia). To inhibit the Na⁺ conductance through the GBSM \( I_{\text{cat}} \) channel, NaCl in the bathing PSS was replaced with an equimolar amount of NMDG while other ions and pH were kept constant.

Solutions and drugs. PSS was made daily and contained (in mM) 121 NaCl, 5.9 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 25 NaHCO₃, 1.2 Na₂HPO₄, and 8 glucose and aerated with 95% O₂–5% CO₂ to obtain pH 7.4. DS contained (in mM) 80 monosodium glutamate, 55 NaCl, 6 KCl, 10 glucose, 10 HEPES, and 2 MgCl₂, pH adjusted to 7.3 with NaOH. The extracellular (bath) solution used in the conventional whole cell experiments contained (in mM) 124.2 NaCl, 3 NaHCO₃, 1.8 CaCl₂, 0.5 MgCl₂, 10 glucose, and 10 HEPES, pH adjusted to 7.4 with NaOH; the intracellular (pipette) solution contained (in mM) 140 aspartic acid, 0.5 MgCl₂, 0.77 GTP, 5 Na₂ATP, 10 HEPES, and 10 EGTA, pH adjusted to 7.2 with NaOH. K⁺ was omitted from the above solutions to eliminate contaminating K⁺ currents. The calculated equilibrium potential for Na⁺ in these solutions was −3 mV. In ion substitution experiments, NMDG was substituted for Na⁺ in the bath solution.

To differentiate effectively between the presence of cation and Cl⁻ currents in the GBSM cells, K⁺-free solutions with Na⁺ as the only monovalent cation and Ca²⁺ as the only divalent cation and with unique reversal potentials for Na⁺ (+45 mV) and Cl⁻ (−82 mV) were used. These solutions, each 300 mosmol/kg H₂O, contained the following (in mM): bath, 120 NaCl, 50 mannitol, 10 glucose, 2 CaCl₂, and 10 HEPES, pH 7.2 (NaOH); and pipette, 120 NMDG, 120 aspartic acid, 50 mannitol, 5 NaCl, 1 Na₂ATP, 5 EGTA, and 5 HEPES, pH 7.2 (after titration with NaOH, the total Na⁺ concentration was 21 mM). In ion substitution experiments, NMDG was substituted for Na⁺ in the bath solution.

Physiological K⁺-containing extracellular (bath) solution used in the perforated whole cell patch-clamp experiments contained (in mM) 134 NaCl, 6 KCl, 1 MgCl₂, 2 CaCl₂, 10 glucose, and 10 HEPES, pH adjusted to 7.4 with NaOH; and the intracellular (pipette) solution contained (in mM) 110 potassium aspartate, 30 KCl, 10 NaCl, 1 MgCl₂, 10 HEPES, and 0.05 EGTA, pH adjusted to 7.2 with NaOH and supplemented with 200 μg/ml amphotericin B. All chemicals and drugs used were obtained from Sigma-Aldrich (St. Louis, MO).

Statistics. Summary data are presented as means ± SE for \( n \), the number of cells isolated from different animals. Statistical analysis of drug effects and the difference between treatment groups were determined using paired Student’s t-test. A P value of <0.05 was considered significant.

RESULTS

\( I_{\text{cat}} \) is present in the GBSM cells. To establish whether \( I_{\text{cat}} \) was present in GBSM cells, the conventional whole cell patch-camp method was used to record membrane currents by applying a ramp protocol (see METHODS). The external bath solution contained physiological concentrations of Na⁺, Mg²⁺, Ca²⁺, and Cl⁻ at pH 7.4. The contribution of K⁺ channels to the ramp currents was eliminated by omitting K⁺ from both external and internal solutions, whereas the VDCC currents were eliminated by (I) blocking the channels with 1 μM nifedipine and 2) inactivating VDCC by using a holding potential of 0 mV. Furthermore, omitting K⁺ from both external and internal solutions eliminates the contribution of the Na⁺–K⁺ pump, equivalent to blocking the pump with ouabain. Under these conditions, an inward current was observed at −50 mV (−0.21 ± 0.03 pA/pF; \( n = 20 \); Fig. 1). This current reversed at −1.8 ± 1.7 mV (\( n = 20 \)), close to the calculated equilibrium potential for Na⁺ (~3 mV). These findings suggest that a Na⁺ influx contributed to the inward ramp current.

To investigate further the ion selectivity of this conductance, Na⁺ in the bath solution was substituted with NMDG, a large relatively impermeant cation. This ion substitution caused a negative shift in the reversal potential of the ramp current (from −1.7 ± 2 mV for Na⁺ to −25.3 ± 2.5 mV for NMDG, \( P < 0.0005, \ n = 5 \)). Na⁺ substitution with NMDG also reduced the amplitude of the inward current at −50 mV by 65.4% (from −0.26 ± 0.05 to −0.09 ± 0.02 pA/pF; \( P < 0.0005, \ n = 5 \); Fig. 1), indicating that a tonic depolarizing Na⁺ current is functional at the GBSM resting membrane potential. The values of the ramp current were rapidly restored to control levels by a return to the Na⁺-containing bath solution.

Our results indicate that a Na⁺ permeable channel is active at the GBSM resting membrane potential; thus, this \( I_{\text{cat}} \) conductance could play an important role in the regulation of GBSM excitability and contractility.

\( I_{\text{cat}} \) is blocked by extracellular gadolinium. Gd³⁺ is known to partially inhibit nonselective \( I_{\text{cat}} \) channels. In GBSM, the inward ramp current was significantly reduced in the presence of 50 μM extracellular Gd³⁺ (control, 0.23 ± 0.04 pA/pF; 50 μM Gd³⁺, 0.16 ± 0.03 pA/pF at −50 mV, \( n = 4, \ P < 0.05 \); Fig. 2). These results support the idea that a Na⁺-permeable channel exists in GBSM.

\( I_{\text{cat}} \) is augmented by exclusion of extracellular divalent ions. Omitting Ca²⁺ and Mg²⁺ from the external bath solution (plus the addition of 100 μM EDTA) led to a significant increase in...
the amplitude of the ramp-induced inward current, an increase from \(-0.26 \pm 0.03\) to \(-0.88 \pm 0.16\) pA/pF at \(-50\) mV \((n = 6, P < 0.01, \text{Fig. 3})\). The values of the ramp current were rapidly restored to control levels by a return to the Ca\(^{2+}\) and Mg\(^{2+}\)-containing bath solution. Thus \(i_{\text{cat}}\) conductance is increased after omitting external divalent cations, revealing a large inward current mediated predominately by Na\(^{+}\).

**Separation of cation and chloride currents in GBSM: role of extracellular Ca\(^{2+}\).** To determine further the role of extracellular Ca\(^{2+}\) and if the ramp current recorded in K\(^{+}\)-free solutions was the result of the activity of cation and/or Cl\(^{-}\) channels, solutions with Na\(^{+}\) as the only monovalent cation and Ca\(^{2+}\) with the only divalent cation were utilized (see METHODS). These special K\(^{+}\)-free solutions have distinctive equilibrium potentials for Na\(^{+}\) and Cl\(^{-}\) (Na\(^{+}\) = +45 mV, Cl\(^{-}\) = \(-82\) mV). Under these conditions, the majority of the outward ramp current between \(-100\) and \(+100\) mV should be the result of influx of Cl\(^{-}\) and the majority of the inward current due to influx of Na\(^{+}\). With the use of these solutions, the inward ramp current was increased by reducing extracellular Ca\(^{2+}\) from 2 mM to 100 \(\mu\)M (control \(-0.21 \pm 0.05\) pA/pF; 100 \(\mu\)M Ca\(^{2+}\), \(-0.36 \pm 0.06\) pA/pF at \(-50\) mV; \(n = 8, P < 0.05, \text{Fig. 4}\)). Whereas the outward current at \(+50\) mV and the reversal potential (control \(+8.9 \pm 2.4\) mV; 100 \(\mu\)M Ca\(^{2+}\), \(+12.4 \pm 3.5\) mV; \(n = 8, P > 0.05, \text{Fig. 4}\)) were largely unaffected. Furthermore, as expected, replacement of bath Na\(^{+}\) with NMDG inhibited the increased inward current from \(-0.36 \pm 0.06\) to \(+0.05 \pm 0.04\) pA/pF at \(-50\) mV (\(n = 8, P < 0.0001, \text{Fig. 4}\)) and shifted the reversal potential from \(+12.4 \pm 3.5\) to \(-5.74 \pm 7.11\) mV (\(n = 8, P < 0.0001, \text{Fig. 4}\)). These results further confirm the presence of an inward \(i_{\text{cat}}\) in GBSM, mediated predominantly by Na\(^{+}\) influx and inhibited by extracellular Ca\(^{2+}\).

**Role of \(i_{\text{cat}}\) in maintaining the GBSM membrane potential.**

To evaluate the physiological role of \(i_{\text{cat}}\) conductance in the regulation of GBSM membrane potential, we employed the perforated configuration of the whole cell patch-clamp technique, with physiological ionic gradients that included the presence of K\(^{+}\) (see METHODS). Voltage-clamp experiments were performed to measure the steady-state \(i_{\text{cat}}\) at the resting GBSM membrane potential (\(-50\) mV). Replacement of Na\(^{+}\) in
the bathing solution with NMDG caused a shift of the steady-state current at −50 mV from a net inward to a net outward current (control −0.29 ± 0.1 pA/pF, NMDG +0.16 ± 0.05 pA/pF, n = 8; P < 0.005; Fig. 5). This was completely reversed on returning to control Na⁺-containing bath solution (−0.26 ± 0.08 pA/pF, n = 8; Fig. 5).

Utilizing the perforated patch-clamp technique, we applied the ramp protocol (from −100 to +100 mV for 1 s; holding potential = −50 mV) in the presence of the same physiological solutions. The contribution of VDCC currents was eliminated by blocking the channels with 1 μM nifedipine. Again, under perforated patch-clamp conditions, an inward current was observed at −50 mV (Fig. 6). Na⁺ substitution with NMDG reduced the amplitude of this inward current, indicating that, under physiological conditions, a tonic depolarizing Na⁺ current is functional at the level of the GBSM resting membrane potential. These results suggest that Icat influences the resting membrane potential of GBSM cells.

Control of GBSM cell membrane potential by Icat conductance. Current-clamp experiments were performed to evaluate the physiological role of Icat in the regulation of GBSM cell membrane potential utilizing the perforated-patch configuration of the whole cell patch-clamp technique, with physiological ionic gradients. Under current-clamp conditions, the average membrane potential of GBSM cells was −28 ± 3.1 mV (n = 14). Replacement of external Na⁺ with NMDG caused −33 mV hyperpolarization, resulting in an average membrane potential of −61 ± 5.5 mV (n = 14; P < 0.0001; Fig. 7). Analogous to the effects on NMDG on the holding current under voltage clamp, the membrane potential measured under current clamp was rapidly restored to control levels by returning to a Na⁺-containing bath solution (−30 ± 3.7 mV; n = 14; P < 0.0001; Fig. 7). Together, these data suggest a prominent role of Na⁺-permeable Icat in the maintenance of the GBSM membrane potential.
Regulation of resting membrane potential and spontaneous action potentials by $I_{\text{cat}}$ conductance in intact GBSM tissue. To elucidate further the functional role of $I_{\text{cat}}$ in GBSM, we performed membrane potential recordings from intact GBSM preparations (at 37°C) using conventional intracellular microelectrodes. Inhibition of the $I_{\text{cat}}$ conductance should hyperpolarize the membrane potential, inhibit action potentials, and thereby decrease GBSM contractility.

The average resting membrane potential recorded from intact GBSM preparations was $-47.0 \pm 4.1$ mV ($n = 5$), which is similar to previous reports (12, 16, 28, 29, 30). All muscle preparations showed spontaneous electrical activity, exhibited in the form of action potentials (Fig. 8A). Reducing Na$^+$ from 147 to 26 mM (NMDG replacement) in the PSS caused a hyperpolarization and inhibition of spontaneous action potentials ($n = 5$; Fig. 8). NMDG induced $\sim$10 mV hyperpolarization (from control $-47.0 \pm 4.1$ mV for Na$^+$ to $-57 \pm 3.9$ mV for NMDG; $n = 5$; $P < 0.0005$; Fig. 8B). Return to physiological Na$^+$-containing PSS solution resulted in a fast recovery of the resting membrane potential and spontaneous action potentials to the control level ($n = 4$).

The membrane potential measurements indicate that the inhibition of $I_{\text{cat}}$ conductance by NMDG is associated with membrane hyperpolarization and inhibition of the action potentials. Collectively, these data suggest a central role of Na$^+$-mediated $I_{\text{cat}}$ in the maintenance of the GBSM resting membrane potential and spontaneous action potential generation.

Muscarinic stimulation by carbachol does not activate $I_{\text{cat}}$ in GBSM. In gastrointestinal smooth muscle tissue, muscarinic receptors (M2 subtype) are linked to $I_{\text{cat}}$ channels (5, 23, 13, 20). Stimulation of these muscarinic receptors by agonists leads to activation of $I_{\text{cat}}$ channels, which results in sustained influx of Na$^+$ as well as presumably Ca$^{2+}$. This causes a membrane depolarization to a degree sufficient to activate VDCC and initiate action potentials (for comprehensive reviews, see Refs. 13 and 20).

To test whether muscarinic stimulation leads to activation of $I_{\text{cat}}$ in GBSM, we studied the effect of carbachol under perforated whole cell patch-clamp conditions and using solutions with physiological ion concentrations. Carbachol (10 µM) did not change the steady-state $I_{\text{cat}}$ measured at the resting GBSM membrane potential level ($-50$ mV; control $-0.055 \pm 0.019$ pA/pF, carbachol $-0.038 \pm 0.031$ pA/pF, $n = 4$; $P > 0.05$). Carbachol (10 µM) did not change the amplitude of the ramp-induced inward current ($n = 4$; Fig. 9); therefore, our data do not support the concept that ACh acts at least in part by stimulating $I_{\text{cat}}$ in GBSM cells. Perhaps other regulatory mechanisms are involved in the control of $I_{\text{cat}}$ in this tissue, and their role and physiological significance remain to be established.

**DISCUSSION**

In this study, we provide the first evidence for the presence of a spontaneously active, Na$^+$-mediated $I_{\text{cat}}$ in guinea pig GBSM. This $I_{\text{cat}}$ conductance is active under physiological conditions, regulating the resting membrane potential and therefore GBSM contractile activity. The $I_{\text{cat}}$ increases after omission of external Ca$^{2+}$ and Mg$^{2+}$, revealing an inward current mediated predominately by Na$^+$ that is reduced by externally applied Gd$^{3+}$.

**Physiological role of spontaneously active $I_{\text{cat}}$ channel in smooth muscle.** A number of $I_{\text{cat}}$ channels have been identified in smooth muscle (for a review, see Ref. 4), including 1) receptor-operated channels, activated by excitatory agonists (14), 2) store-operated channels, activated by depletion of intracellular Ca$^{2+}$ stores (1), and 3) stretch- or swelling-

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Fig. 5. Contribution of $I_{\text{cat}}$ to the membrane potential of GBSM cells. A: representative trace of the steady-state current recorded at a holding potential ($V_h = -50$ mV) from a GBSM cell under physiological ionic gradients using the perforated whole cell technique. Inhibition of GBSM $I_{\text{cat}}$ by replacement of external Na$^+$ with NMDG reduces the net inward current in a reversible manner. Outward current deflections are spontaneous transient outward currents flowing through the large-conductance Ca$^{2+}$-activated K$^+$ channels. B: summarized data for normalized current recorded at $-50$ mV from 8 different GBSM cells isolated from 6 different animals ($**P < 0.005$).

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Fig. 6. Perforated whole cell patch-clamp recordings of $I_{\text{cat}}$ mediated by Na$^+$ and blocked by NMDG. A: average traces of GBSM whole cell currents recorded under physiological ionic gradients in response to a ramp protocol in the presence of external Na$^+$ and after Na$^+$ replacement by NMDG. External Na$^+$ replacement by NMDG demonstrates a reduction of the inward ramp current. VDCC were blocked with 1 µM nifedipine. B: NMDG-sensitive current, obtained as difference currents from the original traces in A.
activated channels that are activated by changes in pressure (19, 25). Although these cation channels contribute to the effects of various stimuli in smooth muscle, cation channels not requiring stimulation for activity have only recently been identified (4).

A small number of reports identify the presence of spontaneously active cation currents in freshly isolated smooth muscle cells. These have been limited to vascular smooth muscles (2, 3, 26, 27) and urinary bladder smooth muscle (21), which, similarly to GBSM, exhibits spontaneous action potentials and phasic contractions (17). The tonic cation currents, described previously, consistent with our data, demonstrate an inhibition by extracellular Ca\(^{2+}\) and Mg\(^{2+}\). In intact visceral smooth muscle preparations (gastrointestinal and urinary bladder), removal of external Ca\(^{2+}\) causes membrane depolarization (6, 15). In urinary bladder smooth muscle, the inhibitory effect of Ca\(^{2+}\) and Mg\(^{2+}\) on the I\(_{\text{cat}}\) channel decreases steeply over the range of the action potential (−50 to 0 mV) and becomes voltage independent at membrane potentials negative to −60 mV (21). Furthermore, these authors have demonstrated that the trivalent cation Gd\(^{3+}\), used as an I\(_{\text{cat}}\) channel inhibitor, shares a similar blocking mechanism with Ca\(^{2+}\) and Mg\(^{2+}\) (21). Although the I\(_{\text{cat}}\) in GBSM shows lesser sensitivity to Gd\(^{3+}\) in comparison with previous reports (14, 21, 25), I\(_{\text{cat}}\) channels similar to the one described here are commonly expressed in phasic smooth muscles, providing a tonic depolarizing influence that regulates the resting membrane potential and action potentials (for review, see Ref. 4).

Suppression of GBSM I\(_{\text{cat}}\) current by polyvalent cations demonstrates substantial similarity to the transient potential receptor (TRP) channel superfamily (4, 7, 22). It is likely that members of the TRP channel superfamily encode the genes responsible for the molecular basis of GBSM I\(_{\text{cat}}\) channel. Consistently, we have identified mRNA transcripts for TRP superfamily members expressed in guinea pig GBSM (B. Horowitz and G. M. Mawe, unpublished observations).

**Physiological role of spontaneously active I\(_{\text{cat}}\) channel in GBSM**. The resting membrane potential in intact guinea pig GBSM preparations is around −50 mV (Fig. 8; also see Refs. 12, 16, 28, 29, and 30); therefore, the spontaneously active cation current described in this study may drive the membrane potential to values more depolarized than the K\(^{+}\) equilibrium potential (approximately −85 mV), thus contributing to the resting membrane potential. The maintenance of the resting membrane potential in GBSM cells is critical to the generation of action potentials, which underlie spontaneous contractile activity in this tissue. This has been demonstrated by the activation of ATP-sensitive K\(^{+}\) channels evoking hyperpolarization and subsequent inhibition of spontaneous action potentials of the GBSM (9, 12, 29, 30). VDCC mediate the upstroke of the GBSM action potential; however, pharmacological inhibition of these channels has little effect on the resting membrane potential (28). Our results, reported here, support the concept that the depolarizing activity of I\(_{\text{cat}}\) contributes to

Fig. 7. Contribution of I\(_{\text{cat}}\) to the resting membrane potential in isolated GBSM cells. A: original membrane potential recordings from a single GBSM cell recorded under current-clamp conditions and physiological ionic gradients in the presence of external Na\(^{+}\) and after Na\(^{+}\) replacement by NMDG. External Na\(^{+}\) replacement by NMDG demonstrates a reduction of the resting membrane potential, which rapidly returns to its initial values after restoring the physiological Na\(^{+}\). B: summarized data from 14 different GBSM cells isolated from 6 different animals (****P < 0.0001).

Fig. 8. Contribution of I\(_{\text{cat}}\) to the resting membrane potential and action potential generation in intact GBSM preparations. A: original intracellular microelectrode recordings of spontaneous action potentials from an intact GBSM preparation in the presence of physiological Na\(^{+}\) concentration and after Na\(^{+}\) replacement with NMDG. Decreasing Na\(^{+}\) concentration in the PSS solution hyperpolarizes the cell membrane and disrupts production of spontaneous action potentials. B: summarized data from 5 different GBSM preparations isolated from 5 different animals (****P < 0.0005).

Fig. 9. Muscarinic stimulation by carbachol does not activate I\(_{\text{cat}}\) in GBSM. Average traces of perforated whole cell patch-clamp currents were recorded in response to a ramp protocol under control conditions and in the presence of 10 μM carbachol. All recording conditions were as in Fig. 6.
the maintenance of the resting membrane potential and functions to increase GBSM contractility, thus maintaining gallbladder tone. Because the $I_{\text{cat}}$ channel observed here is both Na$^+$ and Ca$^{2+}$ permeable, indirect effects via Na$^+$/Ca$^{2+}$ exchanger and Na$^+$/K$^+$-ATPase cannot be ruled out completely.

In many types of smooth muscle, activation of muscarinic receptors causes a depolarization via an opening of $I_{\text{cat}}$ channels, which in turn activates VDCC. In the current study, we tested whether a similar mechanism is involved in GBSM neurons, which in turn activates VDCC. In the current study, we tested whether a similar mechanism is involved in GBSM responsiveness to cholinergic stimulation and found that the $I_{\text{cat}}$ reported here was not affected by the muscarinic agonist carbobalol (10 $\mu$M). This may be due to the fact that muscarinic activation of $I_{\text{cat}}$ channels typically involves the activation of M$_2$ muscarinic receptors (5, 13, 20, 23), whereas the M$_3$ muscarinic receptor is the principal subtype involved in GBSM contraction (8, 24).

In conclusion, the data presented here indicate that, under physiological conditions, a spontaneously active, predominately Na$^+$-mediated current plays a fundamental role in the control of the GBSM excitability and contractility. Thus we present the first identification and characterization of an $I_{\text{cat}}$ conductance in GBSM cells and provide an electrophysiological fingerprint of this current for future dissection of its physiological role.

ACKNOWLEDGMENTS

We thank Dr. Kevin Thorneloe (Merck Research Laboratories) for the discussions on cation currents and Drs. Thomas Heppner and Scott Earley for critical evaluations of the manuscript.

GRANTS

This study was supported by National Institutes of Health Grants NS-26995 (to G. M. Mawe), and DK-53832 and DK-65947 (to M. T. Nelson), and by a GlaxoSmithKline Young Investigator Grant of The National Kidney Foundation (to G. V. Petkov).

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