Characterization of Myenteric Sensory Neurons in the Mouse Small Intestine

Yukang Mao,1,* Bingxian Wang,3,* and Wolfgang Kunze1,2
1Brain-Body Institute, 2Department of Psychiatry and Behavioral Neurosciences, and 3Department of Medicine, McMaster University, Hamilton, Ontario, Canada

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Mao, Yukang, Bingxian Wang, and Wolfgang Kunze. Characterization of myenteric sensory neurons in the mouse small intestine. J Neurophysiol 96: 998–1010, 2006; doi:10.1152/jn.00204.2006. We recorded from myenteric AH/Dogiel type II cells, demonstrated mechanosensitive responses, and characterized their basic properties. Recordings were obtained using the mouse longitudinal muscle myenteric plexus preparation with patch-clamp and sharp intracellular electrodes. The neurons had an action potential hump and a slow afterhyperpolarization (AHP) current. The slow AHP was carried by intermediate conductance Ca2+-dependent K+ channel currents sensitive to charybdotoxin and clotrimazole. All possessed a hyperpolarization-activated current that was blocked by extracellular cesium. They also expressed a TTX-resistant Na+ current with an onset near the resting potential. Pressing on the ganglion containing the patched neuron evoked depolarizing potentials in 17/18 cells. The potentials persisted after synaptic transmission was blocked. Volleys of presynaptic electrical stimuli evoked slow excitatory postsynaptic potentials (EPSPs) in 9/11 sensory neurons, but 0/29 cells received fast EPSPs. The slow EPSP was generated by removal of a voltage-sensitive K+ current. Patch-clamp recording with a KMeSO4-containing, but not a conventional KCl-rich, intracellular solution reproduced the single-spike slow AHPs and low input resistances seen with sharp intracellular recording. Cell-attached recording of intermediate conductance potassium channels supported the conclusion that the single-spike slow AHP is an intrinsic property of intestinal AH/sensory neurons. Unitary current recordings also suggested that the slow AHP current probably does not contribute significantly to the high resting background conductance seen in these cells. The characterization of mouse myenteric sensory neurons opens the way for the study of their roles in normal and pathological physiology.

INTRODUCTION

The ability of the intestine to function independently of the CNS has been attributed to the presence of the enteric nervous system (ENS) (Costa et al. 1998; Furness and Costa 1987). Consistent with this, the ENS of the guinea pig contains the components of an independent, integrative nervous system, including sensory, inter- and motor neurons (Kunze and Furness 1999). In the guinea pig, enteric sensory neurons (AH cells) have large oval somas with multiple long processes (Dogiel type II morphology) and they make up about 20–30% of enteric neurons. Because of their sensory role and large numbers in the gut, Dogiel type II neurons have become an object of considerable experimental interest (see Brookes 2001; Furness et al. 1998; Holzer 2001; Holzer et al. 2001; Kunze and Furness 1999). It is generally assumed that Dogiel type II neurons in species other than guinea pig are also sensory, although intrinsic sensory neurons have been directly identified only in the guinea pig where responses to chemical or mechanical stimulation were recorded under conditions of synaptic blockade (Bertrand et al. 1997; Kunze et al. 1995, 2000).

For historical reasons the great majority of electrophysiological recordings from enteric neurons have been made in the guinea pig small intestine. With the advent of knockout and transgenic technology, however, the mouse is becoming increasingly used in the study of physiology (Picciotto and Wickman 1998) including the activity of the intestine (see Bullard and Weaver 2002; Der et al. 2000; Gershon 1999; Spencer 2001). Despite this there have been few published reports (Bian et al. 2003; Furukawa et al. 1986; Nurgali et al. 2004; Ren et al. 2003) of nerve cell recording from the intact mouse enteric nervous system and these were done only in current-clamp mode. Nevertheless, neurons with clear AH cell electrophysiology have been recorded in mouse small intestine (Bian et al. 2003; Ren et al. 2003). The voltage-clamp device (Cole 1982) has been the conventional method for studying currents in enterocytes, intestinal myocytes, or interstitial cells of Cajal. Yet, reports of voltage-clamp recordings from myenteric neurons are scarce in species other than guinea pig. Three studies used cultured rat neurons (Franklin and Willard 1993; Haschke et al. 2002; Hirning et al. 1990) and one used cultured mouse neurons (Liu et al. 2002); all used the patch-clamp technique. Liu et al. (2002) reported that cultured mouse small intestinal myenteric neurons constitute an electrophysiologically homogeneous population that discharges phasically in response to prolonged depolarization.

The aim of the present experiments was to provide an initial description of mouse enteric AH cell electroresponsiveness and major somatic currents. Among guinea pig myenteric neurons, a slow afterhyperpolarization (AHP) current, a tetrodotoxin (TTX)-resistant persisting Na+ current and a hyperpolarization-activated cationic current are predominantly expressed in AH cells (Furness et al. 2004a; Kunze and Furness 1999). These currents profoundly influence AH cell electroresponsiveness and they were thus the ones we chose to initially investigate. We made recordings from intact ganglia because dissociation and isolation of enteric neurons erase currents that are expressed in situ (Rugiero et al. 2002, 2003) and disrupt natural synaptic connections. In preliminary experiments, we found that the technique of patch-clamp recording from myenteric neurons in the longitudinal muscle myenteric plexus preparation (LMMP) works as well for the mouse (Kunze et al. 2002) as it does for the guinea pig (Kunze et al. 2000). This

* Y. Mao and B. Wang contributed equally to this work.

Address for reprint requests and other correspondence: W. Kunze, St. Joseph’s Healthcare, Hamilton, North Tower, Room T3306, 50 Charlton Avenue East, Hamilton, Ontario, Canada L8N 4A6 (E-mail: kunzew@mcmaster.ca).
allowed us to make voltage- and current-clamp recordings from mouse myenteric neurons that could be directly compared with previous guinea pig data (Kunze et al. 2000; Rugiero et al. 2002) using the identical technique.

**METHODS**

**Preparation**

We used inbred C57BL/6 female mice (20–25 g) obtained from Charles River laboratories (http://www.criver.com). All procedures were in line with University of Tübingen and McMaster guidelines for the use and care of animals. A 2-cm segment of ileum was removed from deeply anesthetized [Na pentobarbitone, 70 mg kg⁻¹, administered intraperitoneally (IP)] mice, after which the animals were killed by exsanguination. The tissue was placed in a 2-ml recording dish lined with silastic and filled with oxygenated extracellular Krebs saline of the following composition (in mM): NaCl 118.1, KCl 4.8, NaHCO₃ 25, NaH₂PO₄ 1.0, MgSO₄ 1.2, glucose 11.1, and CaCl₂ 2.5. Nicardipine (2–3 μM) was routinely added to the saline to prevent spontaneous muscle contraction. The segment was opened along a line parallel to the mesenteric attachment and pinned flat, under moderate tension, mucosa uppermost. The myenteric plexus was exposed by dissecting away the mucosa, submucosa, and circular muscle. The recording dish was then mounted on an inverted microscope and the tissue continuously superfused (4 ml min⁻¹) with physiological saline, gassed with 95% O₂-5% CO₂, and warmed to 35–37°C. A single ganglion was prepared for patch clamping as described in Kunze et al. (2000); briefly, the ganglion was exposed for 10–15 min to 3 ml of warm, oxygenated, extracellular saline of the following composition (in mM): NaCl 118.1, KCl 4.8, NaHCO₃ 25, NaH₂PO₄ 1.0, MgSO₄ 1.2, glucose 11.1, and CaCl₂ 2.5. Nicardipine (2–3 μM) was routinely added to the saline to prevent spontaneous muscle contraction. The segment was opened along a line parallel to the mesenteric attachment and pinned flat, under moderate tension, mucosa uppermost. The myenteric plexus was exposed by dissecting away the mucosa, submucosa, and circular muscle. The recording dish was then mounted on an inverted microscope and the tissue continuously superfused (4 ml min⁻¹) with physiological saline, gassed with 95% O₂-5% CO₂, and warmed to 35–37°C. A single ganglion was prepared for patch clamping as described in Kunze et al. (2000); briefly, the ganglion was exposed for 10–15 min to 3 ml of 0.01–0.02% protease type XIV (Sigma, http://www.sigmapaldrich.com), then the upper surfaces of myenteric neurons were revealed by cleaning part of the ganglion with a fine hair until individual neuron soma became just visible. As described previously (Kunze et al. 2000; Rugiero et al. 2002) there was no evidence of cell swelling after this gentle treatment.

Other salines that were substituted for the standard extracellular Krebs saline were those in which CaCl₂ was replaced by 0.25 mM and MgCl₂ increased to 10 mM (saline for synaptic blockade), and another for which 90% of NaCl was replaced by N-methyl-d-glucamine-Cl (NMDG-Cl) (saline to remove Na⁺ currents). CdCl₂ (0.5 mM) or 2 mM CsCl was also added to some saline solutions, when, to prevent divalent cation precipitation, 10 mM HEPES buffer (pH 7.4) was added, bicarbonate and phosphate salts omitted, and NaCl adjusted to maintain osmolality. Aliquots of stock solutions of clotrimazole, charybdotoxin, and apamin (Sigma) were kept at −20°C.

**Electrophysiology**

Signals were measured in voltage- or current-clamp modes using an Axon Instruments Multiclamp 700A computer amplifier (Axon Instruments, http://www.axon.com) and a Digidata 1322A (Axon Instruments) digitizer was used for A/D conversion.

A bipolar stimulation electrode, constructed from two twisted 75-μm insulated stainless steel wires, was placed over one of the connecting internodal strands lying circumferentially to the ganglion being recorded from. Nerve fibers were electrically stimulated using 0.1-ms, 0.1- to 1-mA constant-current pulses delivered from an ISO-flex stimulus isolation unit (AMPI, http://www.ampi.co.il/).

Patch pipettes were pulled on a Flaming-Brown P97 (Sutter Instruments, http://www.sutter.com) electrode puller to produce micropipettes with resistances 4–6 MΩ. The error arising from uncompensated series resistance for a 130-mV voltage command was 2–4 MΩ for typical values of cell input and access resistances obtained in whole cell mode. Signals were low-pass, four-point Bessel filtered at 2 or 5 kHz, and then digitized at 5 or 20 kHz. Conventional sharp electrodes were made from thin-wall borosilicate glass and filled with 1 M KCl and 0.5% Neurobiotin.

Data were stored on computer and analyzed off-line. Voltage or current commands were delivered to the amplifier under computer control using Clampex 8 (Axon Instruments) software. To allow direct comparison with earlier work (Rugiero et al. 2002) using in situ patch-clamp recording from guinea pig myenteric neurons, patch pipettes were filled with a standard KCl-rich intracellular saline of the following composition (in mM): KCl 140–146, NaCl 10, CaCl₂ 1, MgCl₂ 2, HEPES 10, Na₂GTP 0.2, and EGTA 2, to which 0.2% (wt/vol) Neurobiotin had been added; pH was titrated to 7.3 using 0.1 M KOH. This solution had a predicted (Maxchelator; http://www. stanford.edu/~c-capton/maxcl.html) free [Ca²⁺] of 0.09 μM at 37°C (Bers et al. 1994). This value is close to the resting free intracellular [Ca²⁺] as estimated using Ca²⁺-sensitive dyes in guinea pig Dogiel type II neurons (Hillsley et al. 2000; Tatsunami et al. 1988).

A solution favoring preservation of the slow AHP similar to that recommended by Velumian and Carlen (1959) was used for synaptic blockade (in mM): KMeSO₄ 110–115, NaCl 9, CaCl₂ 0.09, MgCl₂ 1.0, HEPES 10, NaGTP 0.2, and BAPTA.K2 0.2 with 0.2% Neurobiotin and 14 mM KOH to bring the pH to 7.3. The same saline was used to perfuse the cytoplasmic face of inside-out patches, except that total Ca²⁺ was altered to produce free [Ca²⁺] of 0.1 or 0.5 μM. Total and free [Ca²⁺] were calculated using MaxChelator.

About +50 hPa pressure was applied to the pipette before its tip entered the extracellular saline; the pressure was maintained until the tip was in close apposition to a neuron membrane. Only recordings with seal resistances ≥4 GΩ were used and about half of these formed spontaneously (cf. Kunze et al. 2000) when pipette pressure was released; the rest were formed by applying mild (<10 hPa) suction. Whole cell recording mode was entered by further suction, then the amplifier was switched to current-clamp mode and brief current pulses designed to evoke a single action potential (AP) were injected by the patch pipette. Thus resting membrane potential, AP shape, and the existence of a slow AHP were all noted within seconds of rupturing the cell membrane. Access resistance and cell membrane resistance, capacitance, and time constants were periodically monitored by software programmed switching to the pClamp membrane test protocol, which injects square-wave pulses oscillating about the holding potential (V_hold). Quasi-steady-state current–voltage (I–V) plots were made using voltage-clamp mode and by slowly depolarizing the membrane (ramp speed = 25 mV s⁻¹) from an initial hyperpolarizing step.

Responses to local deformation in and around the ganglion-containing patched neurons were sought as previously described (Kunze et al. 2000). Briefly, after obtaining a stable whole cell recording, surfaces of the ganglion and surrounding muscle were gently and systematically prodded to depths of 25 and 50 μm from “touch” with a calibrated Von Frey hair (Kunze et al. 2000).

Descriptive statistics are given as means ± SD. When a statistical test was performed, the P value given is the probability of the test statistic being at least as extreme as the one observed if the null hypothesis of no difference is admitted.

**Histochemistry**

At the end of each recording, neurons were ionophoretically loaded with Neurobiotin by passing forty 500-ms duration, +0.1 nA current pulses by the patch pipette. The tissue was fixed in Zamboni’s fixative (2% vol/vol picric acid, 4% paraformaldehyde in 0.1 M Na₂HPO₄/ NaH₂PO₄ buffer, pH = 7.0) overnight at 4°C, and then cleared using three 10-min washes of DMSO followed by three 10-min washes with phosphate-buffered saline (PBS). The tissue was then exposed to streptavidin—Texas Red (Vector, http://www.vectorlabs.com), diluted 1:50, to reveal Neurobiotin. After a final rinsing, the tissue was mounted in PBS containing 80% glycerol and 0.1% NaN₃ and viewed under fluorescence epi-illumination on a Leitz DM RBE microscope.
Consequently, AH cells had a high resistance state near $R_{\text{avg}}$, as signaled by a conductance increase. Inward rectifier (Baidan et al. 1992; Hanani et al. 2000; Catterall 2000) current intensity, but negative to $V_{\text{rest}}$, was uniaxonal with Dogiel type I or filamentous soma shapes. All non-AH cells were uniaxonal. The correlation between morphology and electrophysiology was unambiguous. All 22 AH cells had Dogiel type I morphology with smooth oval somas and multiaxonal or pseudounipolar projections, having from two to four long processes that projected circumferentially. All non-AH cells were uniaxonal with Dogiel type I or filamentous soma shapes.

AH cells had a resting membrane potential ($V_{\text{rest}}$) of (mean ± SD) $-55 ± 7$ mV ($n = 32$). $V_{\text{rest}}$ did not change during recording periods of ±20 min. Input resistances ($R_{\text{in}}$) were calculated from instantaneous voltage deflections elicited by the injection of 500-ms-duration hyperpolarizing current pulses (Fig. 1). The slope (Fig. 1B) of the voltage–current ($V–I$) relation was extrapolated to $V_{\text{rest}}$ to give a resting input impedance ($R_{\text{in}}$) of $500 ± 52$ MΩ ($n = 30$). The membrane time constant ($\tau$) was $28 ± 8$ ms ($n = 32$).

All 32 AH cells showed a time-dependent sag in the voltage trace during hyperpolarizing current injection (Fig. 1A). Positive to $-90$ mV peak voltage response was linearly related to current intensity, but negative to $-90$ mV the onset of the inward rectifier (Baidan et al. 1992; Hanani et al. 2000; Rugiero et al. 2002) was signaled by a conductance increase. Consequently, AH cells had a high resistance state near $V_{\text{rest}}$, but when hyperpolarized input impedance decreased conspicuously. This trend was quantified by comparing slope conductances ($G_s$) at $V = 0$ and $-90$ mV taken from quasi-steady-state $I–V$ curves. These curves had an inflection close to $V_{\text{rest}}$ when $G_s$ approached 0 (Fig. 1C) or sometimes became negative (e.g., trace I, Fig. 7), matching the N-shaped $I–V$ relation described for guinea pig AH cells (Rugiero et al. 2002). For 29 AH cells, $G_s$ was $2.3 ± 0.7$ nS at $V_{\text{rest}}$ compared with $4.5 ± 1.9$ nS at $V = -90$ mV.

**RESULTS**

**Resting potentials and input resistances**

In agreement with previous LMMP patch-clamp studies that used the standard KCl-rich intracellular solution of Rugiero et al. (2002, 2003), we use the term AH cell (Hirst et al. 1974) to describe those neurons whose spikes had humps (Clerc et al. 1998; Kunze et al. 2000; Schutte et al. 1995) on the repolarization phase and expressed a slow AHP current as revealed by two successive voltage-ramp commands (see following text).

Results using the standard KCl-rich intracellular saline were taken from 32 myenteric AH cells in 31 ganglia from 30 animals. All neurons included for electrophysiological analysis had APs with a hump on the recovery phase. Cells that lacked AP humps were also recorded ($n = 31$) and none of these had a slow AHP current as tested for by the double voltage-ramp protocol; these S cells are not included in the present analysis. There were no cells recorded that had humps but lacked a slow AHP current or had the current but lacked the hump.

Twenty-two of the 32 AH cells and 17/31 non-AH cells recorded with the standard intracellular solution were injected with Neurobiotin and later recovered for morphological identification. The correlation between morphology and electrophysiology was unambiguous. All 22 AH cells had Dogiel type II morphology with smooth oval somas and multiaxonal or pseudounipolar projections, having from two to four long processes that projected circumferentially. All non-AH cells were uniaxonal with Dogiel type I or filamentous soma shapes.

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**The action potential and slow AH current**

Action potentials of the 32 AH cells recorded from using the standard KCl-rich patch pipette saline, had the characteristic shape of Dogiel type II neuron spikes; they were broad with a hump on the repolarization phase. The hump was always confirmed by the presence of an inflection in the time derivative of the voltage trace (Fig. 2A) (Clerc et al. 1998). Spikes had a peak amplitude and width at half-amplitude (half-width) of $102 ± 14$ mV and $2.7 ± 0.8$ ms. These parameters are the same as those reported for the guinea pig (Rugiero et al. 2002). An effective method for evoking slow AHP currents and measuring their voltage dependency has been to inject two successive, slow (25 mV s$^{-1}$) depolarizing (−110 to 0 mV) voltage ramps (Rugiero et al. 2002). The interval between the end of the first ramp and the beginning of the second ramp was 50 ms. The first ramp activates the current, the second describes the voltage relation during activation, and the difference between them gives the current ($I_{\text{KCa}}$) that was evoked (Rugiero et al. 2002). When this experiment was performed on mouse myenteric neurons with the standard KCl intracellular
solution, all (32/32) AH cells tested exhibited a distinct outward difference current. With voltage adjusted for a 9 mV junction potential (Gola and Niel 1993) the difference current reversed at the Nernst equilibrium potential for K\(^{+}\) (\(E_{K}\)) between −91 and −87 mV (88 ± 2 mV, \(n = 12\)). \(I_{KCa}\) was well fitted by the Goldman–Hodgkin–Katz (GHK) equation for K\(^{+}\) current

\[
P_{K}V\frac{F}{RT} \left( [K^+] - [K^+]e^{-VF/RT} \right)
\]

where \(R, T, F\) and \(V\) have their usual meanings. \([K^+]\) was set at 4.8 mM, but \([K^+]\), and K\(^{+}\) current permeability (\(P_{K}\)) were free to vary during the fitting process. For the current shown in Fig. 2B, \([K^+]\) = 140 mM and \(P_{K} = 0.32 \times 10^{-9}\) cm\(^3\) s\(^{-1}\). Although this current was present in all AH cells tested, \(P_{K}\) varied considerably between neurons (Rugiero et al. 2002), ranging from 0.0054 to 0.32 \(\times 10^{-9}\) cm\(^3\) s\(^{-1}\). For all 32 AH cells, \([K^+] = 144 ± 5\) mM and \(P_{K} = 0.13 ± 0.03 \times 10^{-9}\) cm\(^3\) s\(^{-1}\). This current was Ca\(^{2+}\) dependent because addition of the Ca\(^{2+}\) channel blocker CdCl\(_2\) (0.5 mM) to the extracellular saline (Fig. 2C) completely abolished it.

Only five of the 32 cells had a discernable slow AHP lasting \(\geq 2\) s (duration: 3.6 ± 1.1 s; amplitude: −2.3 ± 1.4 mV) after a single action potential. The proportion of single spike slow AHPs recorded with our standard solution patch pipettes (5/32) is less (\(P = 0.003\), Fisher’s exact test) than that (12/12) reported for sharp electrode recording by Ren et al. (2003), who also found a lower \(R_{m}\) value of 136 ± 121 MΩ than the 497 ± 52 MΩ given above (\(P = 0.001, t\)-test, two-tailed). We addressed this discrepancy by making recordings from a further 18 myenteric AH cells using various pipette configurations; all 18 neurons had Dogiel type II shape as revealed after injecting Neurobiotin. Sharp intracellular pipettes filled with 1 M KCl were used to record from nine neurons and whole cell patch-clamp recordings were made from another nine using pipettes filled with a slow AHP-favoring solution containing MeSO\(_4\) (methods). All 18 AH cells recorded with either method had single-spike slow AHPs (Fig. 3), which is a greater proportion (\(P = 0.001\), Fisher’s exact test, two-tailed) than that for standard pipette solution recording (5/32) (Table 1). For four of four AH cells, the slow AHP was unmitigated by exposure to 100 nM extracellular apamin for 20 min (Fig. 3B) (Kunze et al. 1994). After 20- to 30-min washout with normal Krebs solution, the same neurons were then exposed to 100 nM charybdotoxin for a further 20 min, which blocked the slow AHP. The block was irreversible for >30 min final washout (Kunze et al. 1994). Extracellular clotrimazole (20 \(\mu\)M) reversibly abolished the slow AHP for four of four AH cells (Fig. 3C) and after 5 to 10 min application, washout occurred within 3 min. Consistent with the current-clamp slow AHP data, AHP current availability as measured by K\(^{+}\) permeability (\(P_{K}\)) was doubled to 0.27 ± 0.02 \(\times 10^{-9}\) cm\(^3\) s\(^{-1}\) when KMeSO\(_4\) pipette solution was substituted for the KCl-rich one (Table 1). \(V_{rest}\) was comparable between recording modes, but sharp or KMeSO\(_4\) pipette recording was associated with a greater than threefold increase in background conductance (Table 1). In addition, \(R_{m}\) values for sharp and KMeSO\(_4\) pipette recordings were not discernably different. Sharp electrode recordings yielded smaller-amplitude APs than those of patch-clamp recordings, irrespective of the filling solution, yet AP half-widths were unaffected by recording modality (Table 1). Attenuation of AP amplitudes by sharp electrodes is a well-known phenomenon and can be ascribed to the poor high-frequency response of the electrode, and not damage to the neuron (Li et al. 2004).

Neurons recorded using the KMeSO\(_4\) solution or sharp electrodes were less excitable than those recorded with the standard patch-clamp solution. Action potential thresholds (rheobase) measured as the minimal intensity for 500-ms current pulses required to evoke single APs 50% of the time, were about four times lower for standard solution recordings (Table 1). Action potential firing accommodation was always tested with a 500-ms-duration positive current pulse at twofold rheobase intensity. For neurons recorded using the standard solution, 18/19 discharged with a tonic firing pattern (Fig. 3A), i.e., throughout the test pulse. Those recorded with sharp electrodes or KMeSO\(_4\) solution (17/18) discharged phasically (Fig. 3, B and C), i.e., accommodation occurred within the first 250 ms of the 500-ms stimulus pulse.

To minimize perturbation of the intracellular milieu while recording the slow AHP current, we also attempted to record...
the slow AHP ion channel (Fig. 4) from the nine neurons that were patched with the KMeSO₄ pipettes. After GΩ seals were formed, APs were evoked by passing 20 ms-duration inward current pulses by stepping the voltage clamp from 0 to −80 mV. Unitary currents caused by AHP channel opening (Kunze and Mueller 2002; Vogalis et al. 2002a) were detected after the AP in four of the nine cells (Fig. 4). Before the AP, the channel had a low open probability (P₀ = 0.05 ± 0.02, supposing three channels in the patch), but this increased to a maximum of 0.24 ± 0.12 postspike. All-points histograms made from the postspike openings (Fig. 4 C) were fitted with multiple Gaussians to reveal unitary currents of 1.6 ± 0.3 pA, which, assuming Vₑ = −60 ± 8 mV (Table 1), yielded a unitary conductance of 27 ± 6 pS. Ensemble averages of ≥10 individual traces gave postspike currents that activated rapidly but decayed to 0 pA over 4–10 s (Fig. 4 B)—a time course analogous to that for the whole cell slow AHP current (Vogalis et al. 2002a). After recording in cell-attached mode, inside-out patches were pulled from each of the active patches. Each cell was repatched with a new electrode for whole cell recording. The cytoplasmic face of the patch was exposed to a gravity-fed stream of the KMeSO₄ pipette solution whose [Ca²⁺] was either 0.1 or 0.5 mM (see METHODS). Channel openings were extremely rare with 0.1 mM cytoplasmic Ca²⁺ but when this occurred, the channel conductance was 62 ± 11 pS (0.05 < P < 0.001, Wilcoxon signed rank test for paired comparisons) compared with 9 ± 1 pS with 0.5 mM cytoplasmic Ca²⁺ (P > 0.05, Wilcoxon signed rank test for paired comparisons).

**TABLE 1. Effect of recording method on slow AHP current and action potential parameters**

<table>
<thead>
<tr>
<th></th>
<th>Slow AHP</th>
<th>Action Potential</th>
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<tr>
<td></td>
<td>No. of cells</td>
<td>Amplitude, mV</td>
</tr>
<tr>
<td>Whole cell KCl</td>
<td>5/32a</td>
<td>−2.3 ± 1.4</td>
</tr>
<tr>
<td>Sharp</td>
<td>9/9</td>
<td>−4.1 ± 1.2</td>
</tr>
<tr>
<td>Whole cell MeSO₄</td>
<td>9/9</td>
<td>−3.8 ± 3.7</td>
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Cell numbers in parentheses where they are less than the total recorded for that category. a Differs from that for sharp or KMeSO₄ pipettes (P < 0.001, Fisher’s exact test for proportions). b Differs from that for KCl or MeSO₄ pipettes (P < 0.05, Bonferroni–Holm test for pairwise comparisons). c Differs from that for sharp and KMeSO₄ pipettes (P < 0.05, Bonferroni–Holm test for pairwise comparisons). d Differs from that for KCl or MeSO₄ pipettes (P < 0.05, Bonferroni–Holm test for pairwise comparisons).
was increased to 0.5 μM the average number of open channels ($N_{Po}$; calculated from averaged unitary currents as in Kunze et al. (2000)) noticeably increased (Fig. 4D) and did not decrease even when the low Ca$^{2+}$ solution was again applied (cf. Vogalis et al. 2002b). For Fig. 4D the number of active channels in the patch was at least three, giving an upper limit for $P_o$ of 0.4. Current amplitudes changed with transpatch voltage (Fig. 4, Ea and F), although $N_{Po}$ was poorly voltage dependent. Single-channel conductances were calculated using the slope of the dashed line connecting single-channel open-state currents in all-points current histograms that were fitted with multiple Gaussians (Fig. 4Eb). The channel conductance was 26 ± 5 pS ($n = 4$), which is in the intermediate conductance class for $K_{Ca}$ channels recorded with 140 mM symmetrical K$^+$. The calcium sensitivity, conductance, and the poor voltage sensitivity together suggest that the slow AHP channel is related to the IK$_{Ca}$ (IK$_1$, KCa3.1 type) channel (IUPHAR 2002).

**Inward currents active near the resting potential**

**HYPERPOLARIZATION-ACTIVATED CURRENT.** Two inward currents that are active near $V_{rest}$ have been described in guinea pig AH cells: 1) a hyperpolarizing-activated cationic current ($I_h$), which produces the sag in membrane voltage during application of sustained negative current (Galligan et al. 1990; Rugiero et al. 2002); and 2) a TTX-resistant, persisting Na$^+$ current ($I_{Na,P}$), which results in a negative-going inflection in the steady-state $I-V$ graph (Rugiero et al. 2002). Therefore we tested whether similar currents are present in mouse myenteric AH neurons.

We examined the properties of the $I_h$ by injecting 1 to 2 s-duration hyperpolarizing voltage command pulses from a holding potential of −50 mV. All 32 AH cells exhibited a time-dependent inward current, which was further analyzed with voltage-step protocols for 24/32 cells. The $I_h$ amplitude was determined from the difference between the steady-state ($I_{ss}$) and instantaneous ($I_i$) currents (Fig. 5A), where $I_i$ was measured from a single exponential fit to the current trace extrapolated to the beginning of the step command (Fig. 5B).

Maximal $I_h$ ($I_{max}$) was determined by plotting $I_h$ amplitude against voltage, followed by fitting with a single-factor Boltzmann equation

$$I = \frac{I_{max}}{1 + e^{(V-V_{1/2})/k}}$$

to yield $I_{max}$ for each neuron (Fig. 5C). Fractional activation (open probability for the gating variable: $P_o = I/I_{max}$) was determined from the amplitude of instantaneous tail currents ($I_t$) using voltage steps as in Fig. 5A. To avoid contamination by capacitative transients and a transient outward rectifier, $I_t$ measurements were determined by fitting a single exponential to the tail current and extrapolating back to the time of offset of the step voltage command (e.g., Fig. 5F). Using this method,
A. steady-state $I_h$ for each step command was given by the difference between $I_{\text{on}}$ and $I_{\text{off}}$: 

$$I_h = I_{\text{on}} - I_{\text{off}}.$$ 

B. The curve fitted to tails and extrapolated to offset of prestep voltage commands to give instantaneous tail currents $I_h$. 

C. $I_h$ deactivation was measured from tail currents ($t_i$) after maximally activating $I_h$ with $-130$-mV presteps. Voltage protocol given in bottom panel. 

D. $I_h$ reversal potential ($E_h$) was estimated from intersection of linear fits to $I$ and $t_i$ currents. $E_h = -26$ mV for this neuron. 

$P_0$ was plotted against $V$ in Fig. 5D and the curve fitted with the Boltzmann equation, giving $V_{1/2} = -77$ mV and $k = 10$ mV for this neuron. Mean values ($n = 12$) of $P_0$ are plotted against command voltage in Fig. 6A, giving $V_{1/2} = -78 \pm 7$ mV and a slope factor $k = 11 \pm 4$ mV. 

The $I_h$ reversal potential ($E_h$) was measured from the intersection of two instantaneous $I$–$V$ relations (Lamas 1998). We used $I_h$ from the “on” current response for the protocol shown in Fig. 5A ($V_{\text{hold}} = -50$ mV) for one curve, and instantaneous tails for the protocol shown in Fig. 5E (hold = $-130$ mV) for the other curve. For the experiment shown in Fig. 5G, $E_h$ was $-26$ mV, and the average of 10 AH cells was $E_h = -28 \pm 3$ mV. The maximal conductance ($\bar{g}$) was calculated using the relation $I = \bar{g}P_0(V - E_h)$. From the Boltzmann fits described above, when $P_0 = 1$, $V = -140$ mV and $I_{\text{max}} = 205 \pm 96$ pA, and thus $\bar{g} = 2$ nS. Because $P_0 \approx 0.1$ at $V = -55$ mV (Fig. 6A), $I_h$ conductance near rest would be about $0.2$ nS. The variable step and prestep protocols shown in Fig. 5, A and E produced activation and deactivation traces, respectively, and these were well fitted with single-exponential functions yieldng time constants ($\tau$) at various potentials (Fig. 6B). The equation (Willms et al. 1999)

$$\tau = \frac{\tau_0 e^{V-V_0/k}}{1 + e^{V-V_0/k}},$$

was simultaneously fitted to the combined activation and deactivation $\tau$–$V$ plots of Fig. 6B, where $\tau_0$, $V_0$, $k$, and $\delta$ are free to vary during the fitting process. The fitting program gave $\tau_0 = 593 \pm 30$ ms, $V_0 = -73 \pm 7$ mV, $k = 9 \pm 1$ mV, and $\delta = 0.4 \pm 0.1$. The location of the peak of the $\tau$–$V$ plot did not differ from that for $V_{1/2}$ in the Boltzmann fit to the steady-state activation curve ($P = 0.1$; t-test, two-tailed). This suggests that the assumptions made in fitting the time constant data were reasonable. 

This current was reversibly blocked by addition of $2$ mM CsCl to the extracellular saline (middle trace compared with top trace in Fig. 6C). The inward rectifier ($I_{\text{Kir}}$) is manifest as a deviation from linearity in the instantaneous $I$–$V$ plot when $V < -90$ mV, and this was also blocked by extracellular CsCl (Fig. 6D). The current that remained over the voltage range...
from −140 to −40 mV was an essentially linear leak current of 3.9 nS, as indicated by the line joining the filled circles.

**PERSISTING, TTX-INSENSITIVE NA⁺ CURRENT.** All 32 cells had an inflection in the quasi-steady-state I–V curves generated by the first of the pair of voltage-ramp commands applied to each. In some cases, the inflection was sufficiently marked to produce a region of negative conductance just positive to $V_{\text{rest}}$ (e.g., trace 1 in Fig. 7). The inflection was comparable to that produced by the $I_{\text{Na},P}$ expressed in guinea pig AH cells (Rugiero et al. 2002). For 12 cells we extracted $I_{\text{Na},P}$ by blocking $I_h$ with extracellular Cs⁺. It was still present with 2 mM extracellular TTX present, but was eliminated when 130 mM of NaCl in the extracellular Krebs saline was replaced 1:1 with NMDG-Cl. The difference current (Fig. 7B) before and after the substitution showed that $I_{\text{Na},P}$ had an onset of $-56 \pm 2$ mV, $n = 12$ (i.e., close to $V_{\text{rest}}$). During 25 mV s⁻¹ depolarizing voltage ramp commands the current had a peak amplitude of $-150 \pm 60$ pA at $-25 \pm 3$ mV ($n = 12$).

**Direct (sensory) and synaptic activation of AH cells**

To determine whether the processes of AH cells are activated by mechanical stimulation, we pressed with a fine hair on the ganglion containing the neuron being monitored (Kunze et al. 2000). Pressing circumferentially from patched AH cells elicited depolarizations (Fig. 8A) that matched the generator-like potentials previously recorded from guinea pig myenteric neurons (Kunze et al. 2000). Equivalent responses were seen in 17/18 AH cells tested, 13 of which were filled with Neurobiotin and confirmed to be Dogiel type II neurons. The amplitude of the potentials ranged from 5 to 21 mV between cells but was consistent for repeat pressings on the same cell; for 17 AH cells they were $12 \pm 7$ mV. To ascertain whether responses were caused by activation of synapses or whether they resulted from direct transduction, we blocked all synaptic transmission by switching the extracellular saline to a low-Ca²⁺, high-Mg²⁺ one (Kunze et al. 1993). In each case, repeated pressing elicited consistent responses and synaptic blockade failed to lessen responses to mechanical distortion (e.g., Fig. 8, A and B). Receptive loci were confined to the myenteric plexus; pressing
pipette solution recordings are equivalent to those of guinea pig small intestine myenteric AH cells (Rugiero et al. 2002). This comparison is particularly cogent because both results were acquired with the same LMMP patch-clamp technique using identical pipette filling solutions. Conversely, MeSO₄ patch and intracellular sharp recordings produced $V_{rest}$, $R_{in}$, and AP firing adaptation comparable to those reported for guinea pig small intestine (Furness et al. 1998). Therefore differences in the small intestine resting behavior between these species (Bornstein et al. 2002) are unlikely to be attributable to the basic properties of their sensory neurons when these are in an unstimulated state.

The single-spike slow AHP in mouse AH cells
All 32 AH cells (standard KCl patch pipette solution) exhibited an outward current evoked by the depolarizing ramp.

DISCUSSION

Overall, the present work produced two main findings. First, as might have been expected by analogy with previous results from guinea pigs—but frankly was not known—mouse small intestine myenteric Dogiel type II AH cells are mechanosensitive neurons. Second, voltage- and current-clamp properties of mouse AH cells matched those of homologous cells in guinea pig to a noteworthy extent.

Basic whole cell properties
Values of $V_{rest}$, membrane $R_{in}$, and $\tau$, and AP parameters in mouse small intestine AH cells obtained by our standard patch

FIG. 8. Direct excitation of AH by mechanical stimulation during synaptic blockade. A: depolarization and APs in AH cell evoked by pressing on the ganglion with a fine hair about 60 μm from the soma (see C). Onset of pressing at downward and offset at upward arrowhead. B: responses from same neuron as in A were not attenuated after synaptic blockade with high (10 mM) Mg²⁺ and low (0.25 mM) Ca²⁺. C: digital image of Texas Red fluorescence from AH cell filled with Neurobiotin to reveal multiaxonal (Dogiel type II) shape; dashed line indicates edge of ganglion. Symbols indicate sites where pressing on the ganglion evoked (filled circle) or failed to evoke (open circles) excitatory responses. Responses to pressing at • shown in A and B.

directly onto adjacent longitudinal muscle, even to a degree that the patched soma moved relative to the pipette, never eliciting excitatory responses. Moreover, the receptive loci were directly in the path of circumferentially running processes of the neuron being tested (see Fig. 8C).

Synaptic activation was also tested by stimulating one of the internodal strands connected circumferentially to the ganglion being studied. No AH cells (0/29) received fast excitatory postsynaptic potentials (EPSPs) after single-pulse (0.1-ms duration) cathodal stimuli, although brief stimulus volleys at 20 Hz of 20–30 pulses evoked a prolonged slow EPSP in 9/11 AH cells tested. The slow EPSP was associated with a reduction in background conductance as is evident from the increase in the amplitude of voltage transients evoked by 10 pA hyperpolarizing-current pulses (Fig. 9A). In a simple attempt to detect the conductance that was involved, we recorded a quasi-steadystate $V$–$I$ curve just before (control) and during (test) a slow excitatory postsynaptic current (EPSC) evoked by internodal strand stimulation at 20 Hz (Fig. 9, B and C). When the junction potential was taken into account, the difference current (Fig. 9D) had a null current potential at $E_K$ (~90 mV). Gating for this current was voltage insensitive because it was well fitted by the GHK current equation for potassium, yielding $P_K = 0.019 \times 10^{-9} \text{ cm}^2 \text{s}^{-1}$, and $[K^+]_i = 151 \text{ mM}$.

FIG. 9. Synaptic input to AH cells. A: slow excitatory postsynaptic potential evoked in AH cell by 20 Hz volley of 0.1-ms cathodal pulses ($S$) applied to internodal strand. Downward deflections are electrotonic responses to 50 ms duration constant current pulses applied by the patch pipette. Increase in electrotonic response amplitudes after stimulation indicates decrease whole cell conductance. B: slow excitatory postsynaptic current (EPSC) evoked in another AH cell by 20-Hz stimulus volley ($S$) applied to internodal strand. Slow (25 mV s⁻¹) voltage command ramps were applied to the neuron before (1) and during (2) the slow EPSC. C: $V$–$I$ curves elicited by ramp command before (1) and during (2) slow EPSC shown in B. Trace (2 – 1) is the difference current. D: inverted difference current from C was fitted with the GHK equation for K⁺ currents. Resulting permeability value quantifies the reduction in a voltage-independent K⁺ current.

Basic whole cell properties
Values of $V_{rest}$, membrane $R_{in}$, and $\tau$, and AP parameters in mouse small intestine AH cells obtained by our standard patch

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This current was not gated by membrane voltage, reversed at $E_K$, and required the presence of extracellular calcium. These properties as well as its permeability at $0.13 \pm 0.03 \times 10^{-9}$ cm$^3$ s$^{-1}$ are commensurate with that of an identical current in guinea pig $0.17 \times 10^{-9}$ cm$^3$ s$^{-1}$ (Rugiero et al. 2002), which is responsible for the slow AHP. Probably the least invasive way to obtain information on whole cell currents is to record in the cell-attached mode. This can be done either by forming a macropatch containing enough channels to generate a substantial part of the whole cell current or by generating an ensemble average of unitary currents to reproduce the macroscopic current. Because we have not yet successfully made macropatches from the in situ myenteric plexus preparation, we used the latter method. Intermediate conductance calcium-dependent $K^+$ channels ($I_{KCa}$) have long been known to be present in dorsal root ganglion (DRG) sensory (Hay and Kunze 1994) and enteric neurons (Shen et al. 1992). In guinea pig AH cells, their opening generates the slow AHP current, which is charybdotoxin sensitive but apamin and voltage insensitive (Greffrath et al. 1998; Kunze and Mueller 2002; Kunze et al. 1994; Vogalis et al. 2002a). In mouse colon as in guinea pig, $I_{KCa}$ ($K_{C3.1}$) channel immunoreactivity has been localized to Dogiel type II neurons (Neylon et al. 2004), so it was expected that the $I_{KCa}$ channel would also be expressed in mouse small intestine AH cells. In fact, evidence for the slow AHP–generating $I_{KCa}$ channels was found in four of nine cells patched with KMeSO$_4$ solution pipettes. For each neuron, a single AP evoked a prolonged increase in $I_{KCa}$ channel opening ($P_{I_{K}}$) (Fig. 4). Before the AP, $P_{I_{K}}$ was extremely low, which means that the slow AHP–$I_{KCa}$ channel is unlikely to make a major contribution to the resting background conductance. Ensemble averages of post-AP channel activity formed simulacra of the single-spike slow AHP (see Fig. 4), demonstrating the role that $I_{KCa}$ has in slow AHP generation (Kunze and Mueller 2002; Vogalis et al. 2002a). Further evidence that the $I_{KCa}$ channels significantly contribute to the slow AHP came from the block of the slow AHP by extracellular charybdotoxin and clotrimazole. However, neither of these substances is specific for $I_{KCa}$ channels. Charybdotoxin also blocks large-conductance $K_{Ca}$ and some delayed rectifier channels, and clotrimazole blocks cytochrome P450 and calcium-release–activated Ca$^{2+}$ channels (Jensen et al. 1999). The combined single channel and whole cell data at least support the supposition that a large part of the slow AHP is generated by $I_{KCa}$ channel opening. Also, $I_{KCa}$ immunoreactivity has now been reported in rat (Furness et al. 2003) and human (Furness et al. 2004b) Dogiel type II cells; it therefore seems likely that the $I_{KCa}$ channel and slow AHP current are highly conserved across several species.

The presence of a slow AHP after a single AP of $\approx 2$-s duration has been the standard requirement for electrophysiological identification of AH/Dogiel type II cells (Bornstein et al. 1994; Hirst et al. 1974) in guinea pig. It is now apparent that, on its own, the slow AHP can be a somewhat fickle identifier of AH cells. Homologous neurons in pig small intestine (Cornelissen et al. 2000) and mouse large intestine (Nurgali et al. 2004) often do not exhibit a slow AHP, even when they are recorded with sharp intracellular electrodes and in the absence of sensory stimulation. It is known that recording conditions can influence the magnitude of the slow AHP; in particular, patch-clamp compared with sharp intracellular recording has been associated with decreased leak conductance, decreased postspike slow AHP, and increased electroresponsiveness (Gola and Niel 1993; Zhang et al. 1994; see also Furness et al. 2004a). Ren et al. (2003), recording with sharp electrodes from mouse small intestine myenteric neurons (presumed to be Dogiel II cells because each had a TTX-resistant AP), reported that the cells expressed pronounced single-spike slow AHPs. On the other hand, no slow AHPs were reported in patch-clamp recordings taken from any of 43 cultured mouse small intestine myenteric neurons (Liu et al. 2002). Such differences, and similar ones reported for guinea pig (Furness et al. 2004a), raise the question as to whether a slow-electrode–impalement Ca$^{2+}$ leak (Georgiou et al. 1987; Kudo and Ogura 1986) causes artificially high background conductance and Ca$^{2+}$ priming of the slow AHP. Alternatively, dialysis with patch pipette solutions, including Ca$^{2+}$ chelators such as EGTA might reduce Ca$^{2+}$-dependent K$^+$ currents (Staley 1992; Velumian and Carlen 1999). Therefore we expected that sharp electrodes might have artifactually facilitated the recording of single-spike AHPs because the slow AHP appears to depend on the priming of intracellular Ca$^{2+}$ stores (Hillsley et al. 2000) and because impalement produces shunt currents that can load the cell with calcium (Clements and Redman 1989; Spruston and Johnston 1992; Staley et al. 1992; Thurbon et al. 1998). Furthermore, measurements made using the Ca$^{2+}$ indicator fura-2 in hippocampal neurons have demonstrated an increase of $\approx 1$ $\mu$M in intracellular [Ca$^{2+}$$]_{i}$ that was caused by impalement with a fine microelectrode (Kudo and Ogura 1986). Yet our results argue that this is not the correct interpretation; sharp electrodes recorded a natural single-spike slow AHP, but this was inhibited by the standard EGTA-containing KCl-rich patch pipette saline. This was probably attributable to direct action of the anion on the AHP channel and to inappropriate buffering of intracellular free Ca$^{2+}$ (Velumian and Carlen 1999; Zhang et al. 1994). When sharp intracellular pipettes were used, all nine Dogiel type II cells tested had prominent single-spike AHPs. Furthermore, a slow AHP-favoring patch pipette solution of Velumian and Carlen (1999) produced recordings of slow AHPs equivalent to those recorded with sharp electrodes. In addition, the background conductance was also comparable with that from sharp recordings (see RESULTS and Table 1). The most parsimonious explanation for these outcomes would be that the standard patch solution reduced a physiological background conductance and sharp electrode recording did not cause substantial impalement leakage.

**Inward currents active near $V_{rest}$**

The $I_h$ and TTX-resistant Na$^+$ current ($I_{Na,p}$) are present mainly in AH cells in guinea pig and rat small intestine (Rugiero et al. 2002, 2003). Our results demonstrate that they are well represented in mouse AH cells, without excluding the possibility that they are expressed in S cells. For example, hyperpolarization-activated nucleotide-gated channel isoforms have been localized to some S and to AH cells in guinea pig, rat, and mouse myenteric plexuses (Xiao et al. 2004). Apart from the slow AHP currents, the $I_h$ has probably been studied in more detail than any other current in Dogiel type II neurons. Electrophysiological recording has shown that functional somatic $I_h$ channels are well expressed in guinea pig AH.
cells (Galligan et al. 1990; Rugiero et al. 2002; Xiao et al. 2004). We found that the $I_h$ was present in all 32 AH cells and we studied its kinetic properties in 24 of these cells. Remarkably, the Hodgkin–Huxley activation and deactivation parameters as well as their time constants closely match those taken from guinea pig (Galligan et al. 1990; Rugiero et al. 2002). However, the $I_h$ reversal potential ($E_{h}$) seemed to differ between species; it was $-28$ mV for mouse but $-40$ mV for the guinea pig myenteric neurons (Rugiero et al. 2002). Reversal potential measurements can be inaccurate because other unknown overlapping voltage-gated currents might contaminate them. We tried to minimize such problems by using the method of Lamas (1998) to estimate $E_{h}$ (see RESULTS), but this was not done for the previous guinea pig work. Nevertheless, if the apparent difference in reversal potentials is sustained by further experiments this would suggest that the $I_h\;Na^{+}:K^{+}$ permeability ratio is larger in mouse than in guinea pig. This possibly reflects the species differences between AH cells in $I_h$ channel isoform expression (Xiao et al. 2004).

The second major inward current, active near $V_{rest}$, was a TTX-resistant Na$^+$ current ($I_{Na,K}$). $I_{Na,P}$ is a relative newcomer among identified currents in AH cells but it has already been recorded in rat (Coste et al. 2004) and in guinea pig (Rugiero et al. 2002, 2003). For this current also, basic parameters such as voltage of first activation and maximal current were comparable between mouse and guinea pig. Based on gating characteristics and the presence of mRNA and positive immunostains it has been argued that $I_{Na,P}$ in AH cells is carried by the Na,1.9 Na channel isoform (Delmas and Coste 2003; Rugiero et al. 2003). Analogous currents are proposed to modulate the electroresponsiveness of small dorsal root ganglia (Herzog et al. 2001) and spinal motoneurons (Lee and Heckman 2001), especially by amplifying small depolarizations (Dib-Hajj et al. 2002) and they may similarly influence intrinsic enteric sensory neurons.

Sensory responses

One of the advantages of the LMMP patch-clamp recording technique is that it provides mechanical stability and thus distortion of the ganglion from which the recording is being made is possible without loss of seal or signal (Kunze et al. 2000). This is why direct identification of mechanosensory myenteric neurons by recording the sensory response was not achievable using sharp intracellular recording; close mechanical stimulation dislodged the electrode (Smith et al. 1992). For the mouse, as for the guinea pig (Kunze et al. 2000), we found that pressing on the ganglia containing the neuron being patched evoked excitatory responses (see RESULTS) that could be recorded at the soma for each of 14 AH cells tested. This was a direct (“sensory”) response because it persisted during synaptic blockade. The depolarization and discharge are not likely to be related to axonal injury because they could be repeatedly evoked from the same receptive locus (see RESULTS and Kunze et al. 2000) and because the distribution of receptive loci is punctate (see Fig. 6 in Kunze et al. 2000). In contrast, injury discharge would be expected to be elicited along the entire length of the path of the neurite; also, neurite damage ought to interfere with successive responses from the same locus.

Our recordings are consistent with the deduction made for guinea pig that AH/Dogiel type II cells are mechanosensory neurons that respond to tension (Kunze et al. 1998, 2000; Spencer and Smith 2004). They do not preclude the possibility that some mouse S cells might also have a sensory role, as is the case for guinea pig (Kunze et al. 1998; Spencer and Smith 2004). The present results are from the first recording of AH cell sensory responses in the mouse and thus set the stage for the study of the mechanotransducing mechanisms involved.

Synaptic input

When presynaptic fibers were electrically stimulated at 20 Hz, AH cells responded with slow EPSPs, although fast EPSPs were never discerned in any of 29 cells. Because we tested for synaptic input from only one intermodal strand per cell, it was not possible to absolutely rule out fast synaptic input to Dogiel type II neurons without more extensive and systematic stimulus–response mapping. Nonetheless, the present results agree with Bian et al. (2003), who also found that, in mouse small intestine, fast EPSP input is confined to S cells. Slow EPSPs seem to be a highly conserved AH cell property. They have been recorded in guinea pig small intestine AH/Dogiel type II cells (Hodgkiss and Lees 1984; Kunze et al. 1993; Takaki and Nakayama 1988; Wells and Maue 1993; Wood and Mayer 1979), in rat Dogiel type II cells (Brookes et al. 1988; Browning and Lees 1996), and in the one human AH cell recorded by Brookes et al. (1987). Furukawa et al. (1986) also reported slow EPSPs in AH cells of the mouse colon myenteric plexus.

In our experiments the slow EPSP was associated with a decrease in whole cell conductance, indicating that a background current had been reduced (Bertrand and Galligan 1995; Johnson et al. 1980). The difference current between quasi-steady-state $I–V$ curves made before and during the slow depolarization suggests that the slow depolarizing potential was caused by a reduction in a background $K^+$ current. Because it did not appear to be voltage dependent the slow EPSP current was probably analogous to the $K^+$ current(s) whose reduction underlies the slow EPSP in guinea pig small intestine AH cells (Bertrand and Galligan 1995; Johnson et al. 1980).

Functional implications and conclusion

We recorded from AH/Dogiel type II cells because the network of reciprocally connected intrinsic sensory neurons is thought to exert a critical influence over ENS processing (Bertrand and Thomas 2004; Kunze and Furness 1999) and may be the component where ENS functional plasticity and memory are expressed (Furness et al. 2000). A significant feature of our results was the extent to which mouse AH cell currents were quantitatively like those in guinea pig, attesting to a high degree of conservation of ion channels between these species. Direct mechanosensory responses were recorded from mouse AH cells that were similar to previous recordings from guinea pig, which had been, up to now, the only species where this was done. This result increases the likelihood that AH cells will be found to be intrinsic sensory neurons in other vertebrate species, including human.
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