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Potassium Currents of Olfactory Bulb Juxtaglomerular Cells: Characterization, Simulation, and Implications for Plateau Potential Firing

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Abstract

Odor identity is encoded by the activity of olfactory bulb glomeruli, which receive primary sensory input and transfer it to projection neurons. Juxtaglomerular cells (JGCs) may influence glomerular processing via firing of long lasting plateau potentials. Though inward currents have been investigated, little is known regarding potassium current contribution to JGC plateau potentials. We pursued study of these currents, with the overarching goal of creating components for a computational model of JGC plateau potential firing.

In conditions minimizing calcium-activated potassium current ($I_{K(Ca)}$), we used whole cell voltage clamp and *in vitro* slice preparations to characterize three potassium currents in rat JGCs. The prominent component I_{Kt1} displayed rapid kinetics ($\tau_{10\%-90\% \text{ rise}}$ 0.6–2ms, $\tau_{\text{inactivation}}$ 5–10ms) and was blocked by high concentration 4-AP (5mM) and TEA (40mM). It had half maximal activation at –10mV ($V_{1/2\text{max}}$) and little inactivation at rest. I_{Kt2} , with slower kinetics ($\tau_{10\%-90\% \text{ rise}}$ 11–15ms, $\tau_{\text{inactivation}}$ 100–300ms), was blocked by low concentration 4-AP (0.5mM) and TEA (5mM). The $V_{1/2\text{max}}$ was 0mV and inactivation was also minimal at rest. Sustained current I_{Kt3} showed sensitivity to low concentration 4-AP and TEA, and had $V_{1/2\text{max}}$ of +10mV. Further experiments, in conditions of physiologic calcium buffering, suggested that $I_{K(Ca)}$ contributed to I_{Kt3} with minimal effect on plateau potential evolution. We transformed these characterizations into Hodgkin-Huxley models that robustly mimicked experimental data. Further simulation demonstrated that I_{Kt1} would be most efficiently activated by plateau potential waveforms, predicting a critical role in shaping JGC firing.

These studies demonstrated that JGCs possess a unique potassium current profile, with delayed rectifier (I_{Kt3}), atypical A-current (I_{Kt1}), and D-current (I_{Kt2}) in accordance with known expression patterns in OB glomeruli. Our simulations also provide an initial framework for more integrative models of JGC plateau potential firing.

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Keywords

olfactory bulb; glomerulus; potassium current; calcium current; plateau potential; simulation

1. INTRODUCTION

The glomerulus is a critical locus for odorant processing in the olfactory bulb (OB). Each glomerulus receives input via olfactory sensory neurons (OSNs) expressing the same odorant receptor (Ressler et al., 1994; Vassar et al., 1996; Mombaerts et al., 1996). Within each glomerulus, this sensory input is transferred axodendritically to excitatory mitral and tufted (M/T) cells, the OB projection neurons (Shepherd et al., 2004). The pattern of activated glomeruli encodes odor identity and characteristics (Stewart et al., 1979; Rubin and Katz, 1999; Mori et al., 1999; Johnson and Leon, 2000; Xu et al., 2003).

Juxtglomerular cells (JGCs), whose somata lie within the glomerular border, consist of periglomerular cells (PGCs), external tufted cells (ETCs), and short axon cells (SACs) (Pinching and Powell, 1971c; Shipley and Ennis, 1996). JGCs influence glomerular processing via primarily dendrodendritic and axodendritic interactions with OSNs, M/T cells, and other JGCs (Pinching and Powell, 1971a; Pinching and Powell, 1971b).

Some JGCs fire bursts or plateau potentials in response to brief input (Wellis and Scott, 1990; McQuiston and Katz, 2001; Hayar et al., 2004a; Zhou et al., 2006). Bursts, generated by ETCs (McQuiston and Katz, 2001; Hayar et al., 2004a), may coordinate glomerular excitation. Anatomically, ETCs are superficial projection neurons with extensively branched primary dendrites within the glomerulus, implying numerous dendrodendritic interactions (Pinching and Powell, 1971b; Pinching and Powell, 1971c; McQuiston and Katz, 2001; Hayar et al., 2004a; Zhou et al., 2006; Antal et al., 2006). ETCs bursts are generated spontaneously and rhythmically, and such ETCs mutually couple via gap junctions (Hayar et al., 2004a; Hayar et al., 2005; Kosaka and Kosaka, 2005). Evidence supports that such superficial tufted cells excite glomerular interneurons (Hayar et al., 2004b; Murphy et al., 2005) and mitral cell populations (De Saint Jan et al., 2009), and that PGC population activity prevents glomerular activation via inhibition of ETCs (Gire and Schoppa, 2009).

The function of JGC plateau potentials, primarily a feature of PGCs and some ETCs (McQuiston and Katz, 2001; Zhou et al., 2006), is unclear. They can also be generated rhythmically at frequencies of 2–3Hz at room temperature, possibly converting high frequency input to such low frequency output (Zhou et al., 2006). PGC diversity (Kosaka et al., 1998) suggests that plateau potentials could have differential effects based on neurochemical identity.

Less is known of the ionic mechanism of JGC plateau potentials than that of ETC bursts. JGC plateau potentials are low voltage-activated (LVA) events, not mediated by intracellular calcium, and not blocked by fast sodium channel blockade (Zhou et al., 2006). They are eliminated by nickel, not cadmium, implying their generation by T-type calcium currents (McQuiston and Katz, 2001; Zhou et al., 2006) that are likely dendritically located (Zhou et al., 2006). This LVA calcium current differs from that of GABAergic PGCs, which are blocked by dihydropyridines and cadmium, and not by nickel (Murphy et al., 2005). This contrasts with rat ETC bursts, which are driven by persistent sodium current ($I_{Na,P}$) and independent of low threshold calcium spikes (Hayar et al., 2004a), and mouse ETC bursts, which are mediated by $I_{Na,P}$ and LVA calcium current (Liu and Shipley, 2008). Lastly, ETCs and PGCs possess hyperpolarization activated current (I_h) (Cadetti and Belluzzi,

2001). I_h promotes ETC burst rhythmicity (Liu and Shipley, 2008), and may also play a role in plateau potentials (Zhou et al., 2006).

There is less information regarding potassium current in JGCs. A calcium-dependent potassium current (BK), characterized in cultured OB neurons (Egan et al., 1993), partially mediates ETC burst termination when activated by high voltage activated (HVA) calcium currents (Liu and Shipley, 2008). In regards to PGCs, prior work in rat defined one population with A-type and delayed rectifier potassium current and another with predominantly A-type current (Puopolo and Belluzzi, 1998). A similar mixture was demonstrated in frog PGCs (Bardoni et al., 1996). Additionally, tonically spiking dopaminergic mouse PGCs possess a delayed rectifier (Pignatelli et al., 2005). Beyond this, the nature of potassium currents specifically in regards to plateau potential-firing JGCs is not known.

In this study we aimed to characterize voltage-gated potassium currents in plateau potential-firing rat JGCs to create a computer simulation of total potassium current and enable prediction of each current's contributions to plateau potential firing.

2. EXPERIMENTAL PROCEDURES

2.1 Animal use and care

Study approval in regards to animal experimentation was performed by the Yale University Animal Care and Use Committee. Sprague Dawley (*Rattus norvegicus*) rats, 12 to 21 days old, were anesthetized with 1.2g/kg urethane (intraperitoneal) until unresponsive to tail pinch, and decapitated according to aforementioned committee guidelines. The number of animals used were minimized.

2.2 Slice preparation and solutions

Olfactory bulbs were extracted in chilled (4°C) and oxygenated (95% O_2 , 5% CO_2) artificial cerebrospinal fluid (ACSF). ACSF contained 124mM NaCl, 2mM $CaCl_2$, 3mM KCl, 1.25mM NaH_2PO_4 , 26mM $NaHCO_3$, 1.3mM $MgSO_4$, and 10mM dextrose at pH 7.4. Horizontal slices of 400 μ m thickness were made using a rotorslicer (Dosaka) and immediately immersed in oxygenated ACSF bath at 34°C for 15 to 20 minutes. Slices were then kept in oxygenated ACSF at room temperature for up to 8 hours.

D-APV, CNQX, TTX were dissolved in ddH₂O as stock solutions. TEA and 4-AP were dissolved in ACSF at concentrations used, with pH adjusted accordingly. All were stored in refrigeration except TTX, which was placed in -20°C.

2.3 Current clamp recording

JGCs were recorded in whole cell mode. Current clamp recordings were made different intracellular solutions. The Kgluconate-EGTA internal solution consisted of 130mM Kgluconate, 10mM HEPES, 0.2mM EGTA, 4mM MgATP, 0.3mM Na_3GTP , and 10mM $Na_2phosphocreatine$ and adjusted to pH 7.3–7.4 with KOH. Another solution was used for determining plateau potential voltage measurements without junction potential error and consisted of: 130mM KCl, 10mM HEPES, 0.2mM EGTA, 4mM MgATP, 0.3mM Na_3GTP , and 10mM $Na_2phosphocreatine$ and adjusted to pH 7.3–7.4 with KOH (KCl-EGTA internal solution). Another was used to test chelation of internal Ca^{2+} on the plateau potential and was similar to KCl internal solution except with exchange of KCl with 70mM K-Gluconate and 20mM K_4BAPTA (Kgluconate-BAPTA internal solution).

Cells were visually identified via an infrared camera (Hamamatsu C2400-07ER) on an upright microscope (Olympus BX50WI) employing differential interference contrast microscopy and 40X water immersion objective. Plateau potential-firing JGCs were predominantly identified by their large cell body at the deep border of the glomerulus. Our impression is that this primarily biased our recordings to external tufted cells and larger periglomerular cells that, identified morphologically in prior studies, fired plateau potentials (McQuiston and Katz, 2001; Zhou et al., 2006). Indeed, in a separate unpublished study we have demonstrated that certainly a subgroup of these neurons are excitatory, like external tufted cells (results not shown). Current clamp whole cell recordings were made using Axoclamp 2A and 2B amplifiers in bridge balance current clamp mode. Pclamp 9.0 software was used for data acquisition at 10–20kHz sampling rate. Borosilicate glass tube with filament (1.20mm outer/0.69mm inner diameter; Sutter) was pulled on P-97 Flaming/Brown micropipette puller (Sutter). Pipette resistances were 8–12M Ω . Recording chamber with ACSF was heated to 33–37°C.

2.4 Voltage clamp recording

For voltage clamp characterization of potassium current, we used the Kgluconate-BAPTA intracellular solution. In some cases, where internal Ca²⁺ chelation was not needed, we used the Kgluconate-EGTA internal solution. The theoretical reversal potential for K⁺ was -97mV .

The ACSF used for slice preparation was the same used for recordings. Isolation of outward current of the plateau potential-firing JGCs was achieved by pharmacologic blockade of voltage-gated Na⁺ current with 1 μM extracellular TTX and I_h with 1mM extracellular CsCl. For calcium current blockade, after attempting several combinations of Cd²⁺ and Ni⁺, we ultimately chose high concentration Ni⁺ (1mM) alone to better preserve cell viability and because at this concentration, Ni⁺ will block both HVA calcium currents and LVA calcium current of JGCs (Zhou et al., 2006). To suppress synaptic input we added blockers of NMDA receptors (50 μM D-APV) and AMPA receptors (20 μM CNQX).

To study individual potassium currents we used extracellular TEA and 4-AP. Extracellular TEA-Cl replaced NaCl in equimolar concentrations. 4-AP was prepared separately as a stock solution, with pH was adjusted with HCl before adding it to ACSF.

Pipettes were pulled, as described above, to 3–5M Ω (initial series resistance below 20M Ω). Tips were coated with dental wax to reduce pipette capacitance. An axoclamp 200B amplifier was used for whole cell voltage clamp recordings in continuous single electrode voltage clamp (cSEVC) mode.

JGCs were visualized and identified as above then confirmed as plateau potential-firing in gigaseal mode by testing responses to olfactory nerve (ON) input or current injection. The ON layer was preserved in our acute slices, and its stimulation was elicited by placing a large tip glass pipette (1M Ω) filled with ACSF or a concentric bipolar electrode (25 μm diameter tip) that was connected to an electrical isolator, which controlled current amplitude of stimulation. Nerve was stimulated with one shock, of 0.05–0.2ms width and less than 0.1mA amplitude.

After JGC identification, pipette capacitance was compensated and membrane was broken. The cell was dialyzed at resting potential for several minutes prior to recording. Series resistance (R_s), with membrane capacitance (C_m), was compensated to at least 80% correction and prediction with 10 μs lag for kinetics and activation/inactivation experiments. If uncompensated R_s went above 20M Ω the recording was discontinued.

Currents elicited from voltage protocols were P/5 leak subtracted online. Data were sampled and filtered at 2 or 5kHz based on presumed Nyquist rate dependent on low passfiltering by the series resistance at $1/2\pi R_s C_m$ (with uncompensated R_s usually less than 20M Ω , this cutoff frequency was typically much higher than ~250Hz). When possible, repeats of a protocol were performed on the same cell and averaged for analysis. All voltages reported were corrected for a liquid junction potential of 13mV between ACSF and Kgluconate internal solutions, measured according to a KCl salt bridge-based method (Neher, 1992). All experiments were performed at 33–37°C.

2.5 Computer simulation

Currents were assumed to be generated by homogeneous conductances undergoing state transitions according to a Hodgkin-Huxley scheme for activation (m) and inactivation (h) (Hodgkin and Huxley, 1952):

$$m(V_m, t) = m_\infty (1 - e^{-t/\tau_m}), \quad [1]$$

where

$$m_\infty(V_m) = \tau_m(V_m) / (\tau_m(V_m) + \tau_m(V_m)) \quad [2]$$

$$\tau_m = 1 / (\tau_m(V_m) + \tau_m(V_m)) \quad [3]$$

for activation (m) and analogous equations for inactivation (h). Current amplitude was determined by the following:

$$I = G * m^X(V_m, t) * h^Y(V_m, t) * (V_m - E_{rev}) \quad [4]$$

We determined experimental values to simulate these equations based on modifications of prior methods (Connor and Stevens, 1971c; Huguenard and McCormick, 1992). Briefly, during voltage clamp experiments, $m_\infty^X(V_m)$ was assumed to be equivalent to $G/G_{max}(V_m)$ obtained by the steady state activation protocol. This involved measuring peak current (I_{peak}) generated at different test potentials from a given holding potential. This holding potential was chosen where current was maximally recovered from inactivation, and required the assumption that activation occurred much faster than inactivation to establish peak current. This held true in the case of the transient currents. Conductance value was determined by the following and normalized for each cell by dividing by the maximal conductance:

$$G = I_{peak} / (V_{test} - E_{rev}) \quad [5]$$

The value of X was determined for each transient current by fitting Boltzmann functions of the appropriate power to $G/G_{max}(V_m)$ and then examining how well macroscopic current generated in NEURON simulations fit experimentally recorded traces (see below and Results). Similarly, $h_\infty(V_m)$ was assumed to be equivalent to I/I_{max} , derived from the steady state inactivation protocol which generated I/I_{max} for a given test potential as a function of holding potential. Test potential had been chosen to maximize activation and isolation of the current of interest. We assumed $Y = 1$, as in most conductance models.

The time constants, $\tau_m(V_m)$ and $\tau_h(V_m)$ could be constructed by measuring the rate of activation/deactivation and inactivation/recovery from inactivation, respectively. Time constant of activation was determined from the 10%–90% rise time of currents elicited in the activation protocol as follows. Because different schemes were used ($X=1,2,3\dots$), converting the rise time (t_{rise}) to a time constant required different conversion factors for each value of X , derived by solving for $t_{rise} = t_{90}-t_{10}$ in the following system of equations:

$$0.9 I_{max} = I_{max} (1 - e^{-t_{90}/\tau_m})^X \quad [6]$$

$$0.1 I_{max} = I_{max} (1 - e^{-t_{10}/\tau_m})^X \quad [7]$$

This lead to a scaling factor as follows:

$$\tau_m = t_{rise} / \ln((1 - (0.1)^{1/X}) / (1 - (0.9)^{1/X})) \quad [8]$$

We were unable to accurately measure time constant of deactivation via a fixed depolarization and stepped hyperpolarization protocol. Instead we used fitting of the time constant of activation function to known characterizations of each current to replace the missing values, assuming that time constant of deactivation was on the same order of magnitude as time constant of activation. Time constant of inactivation was determined from monoexponential fits to decay of currents elicited in the activation protocol. Time constant of recovery from inactivation was determined by monoexponential fits to recovery of current after different durations of a hyperpolarized holding potential. The function $\tau_h(V_m)$ was constructed by combining these two functions.

The NEURON simulation environment version 5.9 (Hines and Carnevale, 1997) was used to simulate currents and a single compartment neuron containing these currents. The morphological parameters of the cell were chosen to match capacitive transients produced in these cells in current clamp, as well as capacitance in ranges seen in voltage clamp ($L = 43\mu m$ and diameter = $43\mu m$). Passive parameters were $R_a = 35.4 \text{ ohm} \cdot \text{cm}$, $C_m = 1\mu F/\text{cm}^2$, $G_{leak} = 0.000155 S/\text{cm}^2$, $E_{leak} = -63.5 \text{ mV}$. Currents were modeled using equations fit to $m_{\infty}^X(V_m)$, $h_{\infty}^Y(V_m)$, $\tau_m(V_m)$, and $\tau_h(V_m)$ wherever possible, and incorporated in NMODL. For simulations generated completely *de novo* (I_{kt1} , I_{kt2}), the following differential equation was used in NEURON to actively compute $m(V_m, t)$, and analogously for $h(V_m, t)$:

$$m' = (m_{\infty}(V_m) - m(V_m, t)) / \tau_m \quad [9]$$

For I_{kt3} , we based simulations on code used in a previously published model (Mainen et al., 1995). Simulation runs were computed with time step of 0.025ms.

2.6 Statistical Analysis

Numerical averages are presented with standard error values, unless otherwise noted. P-values were obtained by Welch's unpaired T-test. Correlations were evaluated using Pearson coefficient, with r values presented.

3. RESULTS

3.1 Characterization of the JGC plateau potential

Plateau potential-firing JGCs were identified as in described in the Methods. These neurons showed multiple spike discharges in response to ON stimulation while in gigaseal mode (Figure 1Ai). Immediately upon break-in, these neurons also displayed similar multiple spike discharges over a large slow membrane depolarization, or plateau potential (Figure 1Aii). The example shown in Figure 1A is a recording with Kgluconate-EGTA internal solution and similar results were found with the Kgluconate-BAPTA internal solution. For accurate voltage measures characteristics without junction potential error, we also made recordings with a KCl-based intracellular solution (Figure 1B, $n = 7$). The important markers to characterize this firing pattern (resting membrane potential, spike threshold, and plateau level) are listed in Table 1. These values were similar in a small subset when bath-applied antagonists of NMDA, AMPA, and GABAA receptors were added ($n = 4$, data not shown). These measurements are also indicated in subsequent plots of activation/inactivation parameters of the currents under examination later in this study.

3.2 Isolation of three kinetically different components (I_{kt1} , I_{kt2} , I_{kt3}) of JGC potassium current

Outward current was isolated as described in the Methods. JGCs likely express both pure voltage gated and Ca^{2+} -activated potassium currents. Under normal physiologic conditions, intracellular calcium dynamics are complex and therefore difficult to control. To minimize the contribution of Ca^{2+} -activated potassium currents, we used a Kgluconate-BAPTA internal solution as opposed to Kgluconate-EGTA internal solution (see Methods). At this level of buffering (20mM BAPTA), intracellular calcium concentrations would be maintained very low, resulting in a right-shifting of the activation curve of large conductance (BK) and elimination of small conductance (SK) Ca^{2+} -activated potassium currents (Barrett et al., 1982; Marty and Neher, 1985; Grissmer et al., 1992). Prior work in our laboratory (Zhou et al., 2006) has demonstrated that plateau potentials persist in this intracellular solution. We readdress Ca^{2+} -activated currents later in this study.

Representative outward current traces, induced by voltage step test potentials (V_{test}), are shown in Figure 2A ($n = 19$). The membrane potential (V_m) was held at $-93mV$ (V_{hold}) in order to allow full deinactivation of potential inactivating currents. As indicated by the arrows, the outward current decay had three time constants to its decay that we hypothesized to correspond to three distinct currents: a rapid component (I_{kt1} ; $5ms < \tau_1 < 10ms$), a slower component (I_{kt2} ; $100ms < \tau_2 < 300ms$) and a sustained component that did not show clear inactivation (I_{kt3} ; $\tau_3 \gg 500ms$). Such a pattern was most evident at V_{test} above $-3mV$. We used exponential fitting to the current decay to extract the magnitude of each component, as in other studies (Huguenard et al., 1991; Foehring and Surmeier, 1993; Dong and White, 2003). These three component recordings were stable and did not exhibit rundown for at least 8 minutes (Figure 2B, $n = 3$).

Were these three distinct currents or one current with three different decay rates? A current that has multiple intrinsic decay rates would possess the constituent components in consistent ratios. Figure 2C is a three dimensional plot of current amplitudes of each current for all cells recorded ($n = 21$). Pearson correlation between pairs of currents demonstrated no consistent relationship from cell to cell (Pearson $r_{ikt1-ikt2} = 0.5512$, $r_{ikt1-ikt3} = 0.1662$, $r_{ikt2-ikt3} = 0.7546$). Note also that there was no clustering, which would have been suggestive of JGC subtypes. Figure 2D shows the average percent contribution of each current to the total outward current ($n = 21$). On average, I_{kt1} was found in higher quantities than the other two currents. Conductance values were calculated as described in the Methods

and were normalized by cell surface area, derived from whole cell C_m compensation indicated on the amplifier (see Methods).

We also tested if these components showed distinct voltage dependence to their activation. We calculated the activation curve for each component by dividing I-V curves by the driving force and then normalizing conductance values. Comparison of these activation curves (Figure 2E) suggests that each I_k shows distinct V_m dependencies from the others ($n = 19$). From the above approaches, it is evident that the bulk current is comprised of at least three distinct kinetic components.

We then attempted to separate these components, first by altering V_{hold} . I_{kt1} shows rapid kinetics similar to the classically described A-current (Connor and Stevens, 1971a; Neher, 1971), the amplitude of which is sensitive to V_{hold} and requires a V_m less than $-80mV$ for complete deinactivation. We tested if the amplitude of I_{kt1} , as well as the currents, would reduce at depolarized V_{hold} by comparing the current traces between two V_{hold} conditions: $-63mV$ and $-93mV$ (Figure 2F). The amplitudes of all components showed a small decrease likely related to the decrease in driving force, but no clear difference in overall current shapes. These data suggest that I_{kt1} was not a typical A-current and likely had a right shifted activation curve, and that varying V_{hold} would not be useful to separate the components. To further assess the differences between these currents, we turned to pharmacology and further voltage clamp characterization.

3.3 Pharmacological analysis of I_{kt1} , I_{kt2} , and I_{kt3}

We first used the potassium channel blocker 4-AP extracellularly both low (0.5mM) and high (5mM) concentrations. Classically, extracellular 4-AP at millimolar levels are required to eliminate the A-current, whereas the slower inactivating D-current (or I_{Aslow}) is sensitive to submillimolar levels of the same (Storm, 1988). Figure 3A shows three currents: bulk potassium current elicited by V_{test} to $+37mV$ from a V_{hold} of $-93mV$, bulk potassium current elicited by the same test pulse in the presence of 0.5mM 4-AP, and the difference between those currents ($n = 4$). Clearly, addition of 0.5mM 4-AP led to reduction of I_{kt2} , leaving only the fast inactivating I_{kt1} and sustained I_{kt3} . Figure 3B shows I_{kt2} as the complete set of such difference currents evoked by a range of test potentials ($n = 4$). Note that also appearing in the difference current was the sustained I_{kt3} . Application of 5mM 4-AP further blocked the rapidly inactivating I_{kt1} , as clearly shown in Figure 3C ($n = 4$). Overall sensitivities of each component to 4-AP are graphed in Figure 3D, showing that I_{kt1} was pharmacologically distinct from I_{kt2} and I_{kt3} ($n = 5$).

We also used the potassium channel blocker TEA at low (5mM) and high (40mM) extracellular concentrations to separate the currents. TEA has been used to separate variants of delayed rectifier current (Huguenard and Prince, 1991) and to distinguish the A-current from delayed rectifier current (Connor and Stevens, 1971b). Figure 4A shows outward current evoked in response to depolarization to $+37mV$ from $-93mV$ before and after application of 5mM TEA ($n = 4$). Again, the difference current contained components resembling I_{kt2} and I_{kt3} . Figure 4B shows this difference current in response to a range of test potentials ($n = 4$), which also appeared to be I_{kt2} . Both difference currents were grouped together for simulation of I_{kt2} .

When higher concentrations of TEA (40mM) were added extracellularly, both fast I_{kt1} and sustained I_{kt3} were reduced (Figure 4C, $n = 4$). Overall sensitivities of each component to TEA are graphed in Figure 4D ($n = 4$). Sensitivity of I_{kt1} to TEA at low concentrations was significantly different than that of I_{kt3} but not I_{kt2} .

3.4 Voltage dependent inactivation properties of I_{kt1} and I_{kt2}

The voltage dependence of inactivation for I_{kt1} and I_{kt2} was examined by the voltage protocol in Figure 5A ($n = 8$). To extract the magnitude of current for each temporal component, we again fit triexponential decay to the currents and extracted each component (I_{kt1} , I_{kt2} , I_{kt3}). Overlap of normalized traces from the inactivation protocol showed significant similarity, likely owing to good voltage clamp and homogeneity of the two current groups in this voltage range (Figure 5Bi). In Figure 5Bii, this result is quantified across all neurons tested in this manner ($n = 8$), showing that time constant of inactivation ($\tau_{inactivation}$) was consistent for both I_{kt1} and I_{kt2} over a wide range of holding potentials. Inactivation was determined by normalized current magnitude by maximum current amplitude elicited within the protocol (I/I_{max}). The inactivation curve generated for each current is shown in Figure 5C (I_{kt1}) and 5D (I_{kt2}). We overlaid activation curves for each current as well. For I_{kt1} , the activation curve was generated from the data in Figure 2. For the I_{kt2} activation curve, we combined data from the 0.5mM 4-AP-sensitive and 5mM TEA-sensitive difference currents from Figure 3 and Figure 4, respectively ($n = 6$). These curves demonstrate that both activation and inactivation varied smoothly with voltage in the manner of voltage-gated conductances. Note also that I_{kt1} and I_{kt2} show near complete deinactivation at resting membrane potential. However, note that at action potential threshold and at plateau potential amplitude, I_{kt1} is activated but I_{kt2} is not. It was difficult to maintain stable recordings for measuring recovery from inactivation (Figure 5E), but time constants were approximately 150ms for I_{kt2} and <10ms for I_{kt1} ($n = 2$). Nonetheless we felt that even minimal data points would greatly assist function fitting for our simulations.

3.5 Influence of intracellular Ca^{2+} on JGC potassium currents

We next aimed to study the properties of the bulk outward current in more natural calcium buffering conditions. This was done mainly because we wanted to determine if the currents studied above were altered in conditions of lower intracellular calcium buffering. To do this, we again performed voltage clamp characterization of outward current in JGCs with the Kgluconate-EGTA (0.2mM) internal solution instead of the Kgluconate-BAPTA internal solution ($n = 8$). In the majority of cells, the three currents were easily identified (I_{kt1} , I_{kt2} , I_{kt3}), as shown in Figure 6A. Shown in figure 6B is a comparison of average total peak I_{kt1} , I_{kt2} , and I_{kt3} elicited in the 0.2mM EGTA-buffered condition versus 20mM BAPTA-buffered condition, normalized by capacitance of the recorded neuron. Note that in the 0.2mM EGTA-buffered condition, there was a near significant ($p=0.057$) increase in sustained I_{kt3} . We repeated the activation and inactivation protocols for I_{kt1} , as shown in Figure 2A and Figure 5A, and the resulting curves are shown in Figure 6C. For comparison, overlapped are the activation and inactivation curve derived with the Kgluconate-BAPTA internal solution. There was no difference between the two sets of activation curves, but a statistically significant difference in inactivation at two voltages: $-43mV$ and $-53mV$. We considered this difference to be minor regarding plateau potential mechanism, as this change would not likely alter steady state availability of I_{kt1} at rest nor affect inactivation properties at the plateau potential voltage. In figure 6D and 6E we compare the 10%–90% rise time and $\tau_{inactivation}$, respectively, between each condition ($n = 5$). Again, there is no statistically significant difference in kinetics of I_{kt1} between the two conditions. For I_{kt2} , we were able to compare $\tau_{inactivation}$ between each state and also found no statistically significant difference (Figure 6F, $n = 5$).

Based on these data, it was evident that though I_{kt1} and I_{kt2} were overall not significantly altered, there was an increase in sustained current. This could have been related to the rise in BK and SK current when Ca^{2+} was buffered by EGTA and not BAPTA. These currents can affect plateau potential shape. BK current has been shown to influence action potential repolarization (Storm, 1990) and therefore could affect interspike interval and spike width.

SK current mediates slow afterhyperpolarizations (Storm, 1990) and could therefore affect plateau duration. To examine whether increased prominence of I_{kt3} was meaningful, we tested if spike features and plateau potential shape were altered when transitioning from natural Ca^{2+} -buffering states to those governed by 20mM BAPTA. We dialyzed these JGCs with Kgluconate-BAPTA internal solution for up to 20 minutes (Figure 7A, $n = 6$) and analyzed the effect on spike characteristics (delay to initial spike, interspike intervals, spike amplitude, spike width; Figure 7B, $n = 6$) and plateau shape (plateau duration, plateau amplitude; Figure 7C, $n = 6$). Our analyses indicated that none of these features were significantly altered. Therefore, it seems that although we were lacking analysis of BK and SK currents, they likely do not play a significant role in shaping the plateau potential itself. However, we cannot rule out that these currents play a role in shaping afterhyperpolarizations or rhythmicity of plateau potential firing. Thorough characterization of such currents, however, is beyond the scope of this study.

3.6 Hodgkin-Huxley computer simulation of I_{kt1} , I_{kt2} , I_{kt3}

We next turned to simulating these three currents using our data with two aims. First, we sought further evidence that these components could be generated by voltage-gated conductances as dictated by Hodgkin-Huxley mechanistic schemes. Second, we wanted to use simulation to overcome our inability to clearly separate these three potassium currents via steady state voltage manipulations and pharmacology.

To model I_{kt1} we converted steady state voltage dependence and kinetics into forms readily fit by functions for the m and h particles according to the Hodgkin Huxley model originally described for the fast Na^+ current (See Methods). Typically, simulations of such fast currents, like the A-current, have used one or four m particles to describe the rate of activation and one h particle for the rate of inactivation (Connor and Stevens, 1971c; Huguenard and McCormick, 1992; Bhalla and Bower, 1993). Shown in Figure 8A is the activation curve again, with $m(V_m)$ shown for mh , m^2h , m^3h , and m^4h schemes (without exponentiation to show differences). In Figure 8B we show the inactivation curve with a smooth fit for $h(V_m)$. To describe activation kinetics for this current, we converted 10%–90% rise time to τ_m for each scheme and shifted known τ_m curves for other currents (Huguenard and McCormick, 1992) to fit our data (See Methods). In our studies, deactivation was difficult to study because of the imperfect separation of the multiple current components as well as the rapid activation of the currents involved. Instead, we assumed that $\tau_{deactivation}$ was on the same order of magnitude as activation and used interpolation of curve fitting to replace missing values in those voltage regions. The converted data and curves are shown in Figure 8C. We used an identical approach for τ_h , by combining curves for $\tau_{inactivation}$ and $\tau_{recovery}$ from inactivation, for which we only had one point. All three schemes employed the same $\tau_h(V_m)$, and the combined data and fit are shown in Figure 8D.

To identify which model was most suitable, we simulated voltage clamp of each model for I_{kt1} in separate passive single compartment JGCs. Figure 8E shows fits of each model to the average normalized experimental I_{kt1} gleaned from isolation with 5mM 4-AP ($n = 6$). Demonstrated is current elicited at a high test potential ($V_{test} = +37mV$). From these comparisons, it was evident that the m^4h model was the best. Figure 8F shows the same comparison, but at a lower test potential ($V_{test} = -13mV$). Again, the m^4h model achieved closest approximation. The equations for this simulation are in Table 2 and Table 3.

We used the same approach to generate simulations of I_{kt2} . Prior models of slower potassium currents, such as the D-current, have employed mh , m^2h , and m^3h schemes (Miyasho et al., 2001; D'Angelo et al., 2001; Golomb et al., 2007). Figure 9A through 9D show function fits for activation, inactivation, τ_m , and τ_h , respectively. As mentioned above,

for simulation purposes, we assumed that I_{kt2} component sensitive to 0.5mM 4-AP contributed equally to that blocked by 5mM TEA, and combined these experimental data to make and test the model. Figure 9E shows average normalized current elicited at V_{test} of +37mV ($n = 4$) versus those generated by each model. From this, it is evident that the m^2h method is most appropriate. Equations for this model are as shown in Table 2 and Table 3.

For I_{kt3} , we assumed it was a homogeneous population of delayed rectifier currents. Simulation of this constant current was much simpler, using no inactivation and assuming a scheme with one m particle based on previous work simulating delayed rectifiers (Mainen et al., 1995). The fit of the model activation curve to the experimental average ($n = 19$) is shown in Figure 9F. The function for $\tau_m(V_m)$ is shown in Figure 9G. Equations are in Table 2.

Employing these models, we were able to recreate the total bulk current typically found in these JGCs. Suprathreshold and subthreshold traces of simulated bulk outward current are shown in Figure 10A. The currents generated from this activation curve look remarkably similar to those generated by physiologic experiments presented in Figure 2A.

In the absence of a complete model of JGC plateau potentials to assess the contribution of each conductance, we instead examined the responses of each single compartment model with the membrane clamped to a typical JGC plateau potential waveform, as shown in Figure 10B. To remove effects of the magnitude of each current found in these neurons, which would bias importance to I_{kt1} , the same density was used for each current. Notably, I_{kt1} begins to activate at an earlier point during the evolution of the plateau waveform than the other two currents and also generates a much larger current throughout the plateau potential stimulation, implying that it is more efficiently stimulated by the plateau potential waveform. The inset shows an expansion of the region during the first two spikes. Both I_{kt1} and I_{kt3} peak just after the first spike, whereas I_{kt2} reaches its peak after the second spike. After the plateau potential level is reached, all currents are reduced but I_{kt2} and I_{kt3} remain much smaller than I_{kt1} . That I_{kt1} is more influential to plateau potential shape than the other two potassium currents is further corroborated in our companion study, in which each conductance is selectively removed from a single compartment model of these JGCs (Masurkar and Chen, 2011).

4. DISCUSSION

In this study, we identified three populations of potassium current in plateau potential -firing JGCs that are primarily distinguished by their kinetics and activation voltage range. The most distinct and prominent component I_{kt1} was notable for rapid kinetics ($\tau_{10\%-90\% \text{ rise}}$ 0.6–2ms, $\tau_{inactivation}$ 5–10ms), half maximal activation at –10mV ($V_{1/2max}$), and distinct pharmacology (sensitivity to high concentration 4-AP and TEA). I_{kt2} , the least prominent, showed slower kinetics ($\tau_{10\%-90\% \text{ rise}}$ 11–15ms, $\tau_{inactivation}$ 100–300ms), more depolarized activation ($V_{1/2max}$ was 0mV), and pharmacology different to I_{kt1} (sensitive to low concentration 4-AP and TEA). The sustained current I_{kt3} , in intermediate quantities, had the most depolarized activation ($V_{1/2max}$ of +10mV) and was pharmacologically similar to I_{kt2} . In conditions of physiologic calcium buffering, I_{kt3} showed a near-significant increase, suggesting that $I_{K(Ca)}$ partially contributed to bulk potassium current. Current clamp experiments evaluating plateau potential features with increasing calcium buffering demonstrated that $I_{K(Ca)}$ likely plays roles other than shaping key plateau potential voltage features. Characterizations were successfully transformed to create three current simulations that, when combined at appropriate ratios, mimicked bulk JGC potassium current. Clamping membrane potential to plateau potential waveforms revealed that I_{kt1} was the most effectively activated current.

The currents described herein showed some similarity to classical descriptions and currents or channels previously described in the olfactory glomerulus. The kinetics and pharmacology of I_{kt1} is similar to A-type potassium current (Connor and Stevens, 1971a; Neher, 1971) but with an activation curve right-shifted by 20mV. Indeed, granule cells of the olfactory bulb also possess an A-type current with depolarized activation curve (Schoppa and Westbrook, 1999). I_{kt1} also reflects the mixture of A-type currents found in the olfactory bulb glomerular layer, including Kv4.2, Kv4.3, Kv3.3, and Kv1.4 (Serodio and Rudy, 1998; Weiser et al., 1994; Veh et al., 1995). Of these, Kv3.3 and Kv4.2 similarly activate at more depolarized potentials (Pak et al., 1991; Vega-Saenz de Miera et al., 1992), and both Kv4.2 and Kv4.3 have similar inactivation rates (Blair et al., 1991; Franqueza et al., 1999). Kv3.3 and Kv4.2 have similar 4-AP sensitivity (Blair et al., 1991; Rudy et al., 1999), and Kv1.4 shows similar TEA sensitivity (Philipson et al., 1991). Note that bulk potassium current in bursting mouse ETCs also contained a rapidly decaying component, which also could be the A-current (Liu and Shipley, 2008).

Slower inactivating current I_{kt2} shows similar kinetics and 4-AP sensitivity to the classical D-current, though with activation curve shifted in the depolarizing direction (Storm, 1988; McCormick, 1991). A similar high threshold D-current has also been described in CA1 pyramidal and preBotzinger complex neurons (Chen and Johnston, 2004; Metz et al., 2007; Hayes et al., 2008). Kv1 family channels may comprise some D-currents (Scott et al., 1994), and like I_{kt2} , they activate at more depolarized voltages than classical D-current (Snyders et al., 1993; Grissmer et al., 1994). Kv1.2, 1.3, and 1.4 are expressed in OB tufted cells (Veh et al., 1995).

Noninactivating I_{kt3} likely reflects a mixture of currents comprising delayed rectifier current (Huguenard and Prince, 1991; Martina et al., 1998; Baranauskas et al., 1999; Rudy and McBain, 2001). Of the Kv2 and Kv3 channels that are thought to make up this current (Martina et al., 1998; Baranauskas et al., 1999), Kv2.1, Kv2.2, and Kv3.1 are expressed in the OB glomerular layer (Hwang et al., 1993; Ozaita et al., 2002). Per our data, I_{kt3} may also be composed of some $I_{K(Ca)}$ current. There is physiological and immunohistochemical evidence of such currents in the olfactory bulb (Egan et al., 1993; Sausbier et al., 2006; Sailer et al., 2004), of which BK plays a role in ETC bursting (Liu and Shipley, 2008). Because I_{kt2} and I_{kt3} possess similar pharmacologic profiles, I_{kt3} could also partially represent a sustained component of I_{kt2} . Though voltage-gated potassium currents have characterized with two time constants of decay (Huguenard and McCormick, 1992), our impression is that these are predominantly separate currents because I_{kt2} and I_{kt3} were found in variable ratios and possessed unique activation curves.

The minimal role for $I_{K(Ca)}$ in this study does not minimize the overall potential role for such currents in JGC physiology. The lack of effect could be due to the plateau potential features specifically chosen for analysis, or could be due to coupling of $I_{K(Ca)}$ to calcium currents not involved in plateau potential generation. For example, in mouse ETCs, BK currents do not lengthen low threshold spikes but terminate spontaneous bursts and control spike width (Liu and Shipley, 2008). Additionally, JGC plateau potentials are followed by an afterhyperpolarization, which could be functionally important and mediated by $I_{K(Ca)}$.

The finding that A-type I_{kt1} is much more activated during a plateau waveform voltage clamp is suggestive of its prominent role in shaping the plateau waveform itself during its natural evolution. Beyond being found in higher quantities than I_{kt2} and I_{kt3} , the importance of I_{kt1} suggested is in line with its properties within the voltage range of the plateau potential itself. I_{kt1} is deinactivated at rest and could be activated by the LVA calcium current that likely mediates the plateau potential. Based on our prior study of the voltage dependence of the plateau potential (Zhou et al., 2006), this LVA calcium current likely has an activation

curve depolarized to match I_{kt1} , such that the A-current modulates the rate at which the calcium conductance is activated to control plateau duration. I_{kt1} also displays significant overlap between its activation and inactivation curves, underscoring the possibility that it could function as a window current in this region (Wang and Schreurs, 2006). Contrastingly, I_{kt2} is less than 5% activated at the plateau potential level and activates slowly, unlikely to influence initial spikes and plateau potential onset. This current is probably recruited with suprathreshold activity and thus during plateau potentials with only one or two small suprathreshold spikes, little I_{kt2} will be activated to influence plateau potential shape. Additionally, the delayed rectifier I_{kt3} , activating at even more depolarized potentials, likely requires larger amplitude or a higher number of spikes to be recruited.

We acknowledge that the limitations to our study stem from three areas: space clamp, possible transmitter-mediated current modulation, and definitive cell-type characterization. First, as with any single-electrode voltage clamp study of whole, branched neurons, space clamp will inevitably distort voltage dependence and kinetics of the currents studied herein. However, we are encouraged by the similarity of our characterizations to known currents found in the olfactory bulb, specifically in the glomerulus itself. Additionally, though individual kinetics may be distorted, the distinctiveness of each potassium current is likely still valid. Regarding neurotransmitter effects, GABAergic (Karpuk and Hayar 2008; Hayar and Ennis 2007) and cholinergic input (Pignatelli and Belluzzi 2008) can increase certain potassium conductances and alter JGC firing properties, neither of which were specifically blocked in our experiments. Though we cannot exclude the possibility that current ratios or properties could be altered in the absence of these inputs, we have data to support persistence of JGC plateau potentials with ionotropic and metabotropic GABA and glutamergic inputs blocked (unpublished, not shown). Lastly, we did not anatomically distinguish plateau potential-firing PGCs and ETCs. Morphological differences between the two groups may influence plateau potential mechanism, and should be pursued in future studies. Nevertheless, in our data set we did notice relative homogeneity in both plateau potential characteristics and current composition.

The literature is replete with plateau potential mechanisms mainly involving potassium currents other than A-type, not limited to delayed rectifier in subthalamic L-type plateau potentials (Otsuka et al., 2001; Otsuka et al., 2004), $I_{K(Ca)}$ in L-type plateau potentials in motoneurons (Booth et al., 1997) and dorsal horn neurons (Russo and Hounsgaard, 1996) and P-type plateau potentials of Purkinje cells (De Schutter and Bower, 1994), as well as D-current in R-type plateau potentials of Purkinje cells (Miyasho et al., 2001). The proposed functional prominence of the A-current in JGC plateau potentials resembles mechanisms suggested in another study of P-type calcium plateau potentials in Purkinje cells, in which amplitude is guided by A-current and delayed rectifier (Genet and Delord, 2002). However the mechanism in this study differs in its relative independence from the latter.

5. CONCLUSION

In summary, we describe three populations of potassium current (I_{kt1} , I_{kt2} , I_{kt3}) that make up the majority of bulk outward current in plateau potential-firing JGCs. Each of these currents is defined by particular kinetics and voltage dependence of gating, as well as distinct pharmacology, that correlates with channels known to be expressed in the OB glomerular layer. I_{kt1} has qualities most similar to that of the A-current, but with right-shifted voltage dependence. I_{kt2} is similar in kinetics and pharmacology to the D-current, but also with right-shifted gating. I_{kt3} likely is a composed of several delayed rectifier type currents, and may also include SK and BK current in conditions of low intracellular Ca^{2+} buffering. Using our voltage clamp characterization, we have been able to achieve robust simulations based on Hodgkin-Huxley schemes for all three currents (m^4h scheme for I_{kt1} , m^2h for I_{kt2} , m for

I_{kt3}) that would be useful for future models of JGCs. The features delineated in these models, as well as the current amplitudes measured experimentally, predict that inward conductance in JGC plateau potentials is dominated by atypical A-current I_{kt1} .

RESEARCH HIGHLIGHTS

- Olfactory juxtaglomerular cells fire plateau potentials of unclear mechanism.
- We recorded their potassium current, showing A-type, D-type, and delayed rectifier.
- We created simulations of each to analyze their behavior during plateau potentials.
- Simulations supported that A-current is critical to plateau potential generation.

Abbreviations

4-AP	4-aminopyridine
TEA	tetramethylammonium
TTX	tetrodotoxin
BAPTA	1,2-bis(o-aminophenoxy)ethane- N,N,N',N'-tetraacetic acid
EGTA	glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid
D-APV	D-2-amino-5-phosphonovaleric acid
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
AMPA	2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid
NMDA	N-Methyl-D-aspartic acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
ATP	Adenosine-5'-triphosphate
GTP	Guanosine-5'-triphosphate

GLOSSARY

Olfactory bulb	The first area of the brain to receive and process primary olfactory sensory information from olfactory sensory neurons
Glomerulus	A spherical 50–100µm of neuropil and the locus of information transfer between olfactory sensory neurons and output neurons of the olfactory bulb, as well as the site of modulation of this transfer by interneurons. Several hundred glomeruli form the first layer of the olfactory bulb
Mitral cell	An excitatory output neuron of the olfactory bulb, whose axons project to olfactory cortex, and whose dendrites are within the glomerulus and cell body is deep to the glomerular layer
Tufted cell	Another excitatory output neuron of the olfactory bulb, whose soma is more superficial than that of a mitral cell

Juxtglomerular cell	A group of neurons whose cell bodies lie around the border of a glomerulus. They include external tufted, periglomerular, and short axons cells
External tufted cell	The most superficially located tufted cell
Periglomerular cell	A chemical heterogeneous group of interneurons within the glomerular layer show dendrites and axons participate in synaptic interactions with other glomerular processes. They can be GABAergic, dopaminergic, or release other neurotransmitters
Short axon cell	An excitatory interneuron thought mediate interglomerular inhibition

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Figure 1. Features of juxtaglomerular cell (JGC) plateau potentials in current clamp

(A) (i) Response of a JGC to olfactory nerve (ON) stimulation in cell-attached mode shows multi-spike discharges with Kgluconate-EGTA internal solution. (ii) The same neuron after seal rupture displays the same firing pattern in whole cell configuration (B) Current injection induced a plateau potential with KCl internal solution, allowing measurement of features (arrows) with minimal junction potential error. These values are in Table 1.

Figure 2. Potassium current of plateau potential-firing JGCs contained three kinetic components: I_{kt1} , I_{kt2} , I_{kt3}

(A) Bulk potassium currents (above) elicited by a range of test potentials (below) displayed rapid (τ_1), slower (τ_2), and very slow (τ_3) rates of decay ($n = 19$) (B) By exponential fitting, each time constant was associated to a current (I_{kt1} , I_{kt2} , I_{kt3}). A plot of the ratios of each current's amplitude in time ($I(t)$) to its amplitude at the beginning of recording ($I(0)$) demonstrates stability of recordings for several minutes ($n = 3$) (C) Three dimensional plot of current amplitudes for I_{kt1} , I_{kt2} , and I_{kt3} for each cell shows lack of clear clustering and lack of consistent ratios (Pearson $r_{ikt1-ikt2} = 0.5512$, $r_{ikt1-ikt3} = 0.1662$, $r_{ikt2-ikt3} = 0.7546$) (D) The average percent contribution of each current to total current, over all recordings, showed

that I_{kt1} was found in higher quantities than I_{kt2} and I_{kt3} ($n = 20$, $*p < 0.0001$, $**p < 0.002$, $***p < 0.05$, $p < 0.0001$ by ANOVA) (E) Converting currents elicited in (A), with each component extracted by exponential peeling, to activation as a function of test potential revealed that each component had a distinct voltage dependence of activation ($n = 19$, $*p < 0.001$ by ANOVA). (F) Bulk outward current elicited at two different holding potentials are similar in shape

Figure 3. Sensitivity of I_{kt1} , I_{kt2} , I_{kt3} to extracellular 4-AP

(A) Potassium current before (black) and after (red) application of 0.5mM 4-AP, and the difference current (blue), revealed that a slow component similar to I_{kt2} was extracted ($n = 4$) (B) Full set of 0.5mM 4-AP difference currents elicited by a range of test potentials ($n = 4$) (C) Potassium current after application of 0.5mM 4-AP (black), after application of 5mM 4-AP (red), and difference current (blue) ($n = 4$) (D) Graph of 4-AP sensitivity of each component of outward current in JGCs shows that I_{kt1} has more distinct sensitivity ($n = 5$, * $p < 0.02$, ** $p < 0.05$)

Figure 4. Sensitivity of I_{kt1} , I_{kt2} , I_{kt3} to extracellular TEA

(A) Potassium current before (black) and after (red) application of 5mM TEA, and the difference current (blue). Slow component similar to I_{kt2} was extracted ($n = 4$) (B) Full set of 5mM TEA difference currents elicited by a range of test potentials (C) Potassium current after application of 5mM TEA (black), after application of 40mM TEA (red), and difference current (blue) ($n = 4$). Both I_{kt1} and I_{kt3} are partially blocked by 40mM TEA. (D) Graph of TEA sensitivity of each component of outward current in JGCs shows that I_{kt1} has more distinct sensitivity ($n = 4$, $*p < 0.05$)

Figure 5. Inactivation of I_{kt1} and I_{kt2}

(A) Bulk potassium current (above) elicited from varied holding potentials to a given test potential (below), i.e. inactivation protocol ($n = 8$) (B) (i) Overlay of normalized bulk currents elicited by an inactivation protocol (ii) Quantification of result in Figure 5Bi showing consistency of time constant of inactivation for I_{kt1} and I_{kt2} over several holding potentials ($n = 8$). (C) Superposition of activation and inactivation curves for I_{kt1} . Activation curve currents extracted in Figure 2A ($n = 19$). Inactivation curve is based currents extracted in Figure 5A ($n = 8$). (D) Superposition of activation and inactivation curves for I_{kt2} . Activation curve is from Figure 4D ($n = 8$). Inactivation curve is based currents extracted in Figure 5A ($n = 8$). (E) Protocol for recovery from inactivation (below) and currents elicited (above) demonstrate recovery time constants of $<10\text{ms}$ for I_{kt1} and $\sim 150\text{ms}$ for I_{kt2} ($n = 2$)

Figure 6. Effects of differential calcium buffering on outward currents, spike features, and plateau potential shape

(A) Bulk potassium current (above) elicited by different test potentials (below) in conditions of intracellular dialysis with 0.2 mM EGTA ($n = 5$) (B) Average amplitude of each current, normalized by cell capacitance, when intracellular Ca^{2+} buffering achieved by 20 mM BAPTA (black) or 0.2 mM EGTA (red, $n = 5$, note I_{kt3} : $p=0.057$) (C) Activation and inactivation of I_{kt1} , as functions of test potential and holding potential, respectively, in conditions of intracellular dialysis with 0.2 mM EGTA (red, $n = 5$). Superimposed are analogous curves for I_{kt1} measured when Ca^{2+} buffering achieved by 20 mM BAPTA (black, see Figure 5C), showing similarity at most points. $*p<0.01$ (D) Time constant of inactivation for I_{kt1} as a function of test potential, when Ca^{2+} buffering achieved by 0.2 mM EGTA (red, $n = 5$). Superimposed is analogous curve for 20 mM BAPTA condition (black, $n = 19$, data from Figure 2A), showing similarity (E) 10%–90% rise time for I_{kt1} as a function of test potential, when Ca^{2+} buffering achieved by 0.2 mM EGTA (red, $n = 5$). Superimposed is analogous curve for 20 mM BAPTA condition (black, $n = 19$, data from Figure 2A), showing similarity. (F) Time constant of inactivation for I_{kt2} as a function of test potential, when Ca^{2+} buffering achieved by 0.2 mM EGTA (red, $n = 5$). Superimposed is analogous curve for 20 mM BAPTA condition (black, $n = 8$, combined data from Figure 4Eii), showing similarity.

Figure 7. Effects of differential calcium buffering on JGC plateau potential shape: spikes and plateau potential

(A) Plateau potential immediately after break-in (black) and after dialysis for 20minutes with 20mM BAPTA (gray) ($n = 6$) (B) Values of spike shape features as a function of time dialyzed with 20mM BAPTA. Only first interspike interval seems affected. (C) Values of plateau shape features, as a function of time dialyzed with 20mM BAPTA, are relatively constant.

Figure 8. Simulation of I_{kt1}

(A) Four Boltzmann schemes for $m_{\infty}(V_m)$ that when raised to the appropriate power ($X=1,2,3,4$) overlap well with measured I_{kt1} activation curve, $G/G_{max}(V_m)$. For clarity, shown is each function prior to this exponentiation. (B) Boltzmann fit for $h_{\infty}(V_m)$ matches measured $I/I_{max}(V_m)$ (C) Four schemes for $\tau_m(V_m)$ based on measured 10%–90% rise time, scaled for each scheme as in Methods (D) Function fit for $\tau_h(V_m)$ that overlaps well with measures $\tau_{inactivation}(V_m)$ and $\tau_{recovery}$ from inactivation (E) Simulated currents based on all four schemes compared to average current elicited by a test potential of +37mV ($n = 6$). The m^4h scheme seemed most similar. (F) Simulated currents based on all four schemes compared to average current elicited by a test potential of -13mV ($n = 6$). The m^4h scheme seemed most similar. Shown on all graphs are measured values in Table 1. Function equations are in Table 2 and 3.

Figure 9. Simulation of I_{kt2} and I_{kt3}

(A) Four Boltzmann schemes for $m_{\infty}(V_m)$ that when raised to the appropriate power ($X=1,2,3,4$) overlap well with measured I_{kt2} activation curve, $G/G_{max}(V_m)$. For clarity, shown is each function prior to this exponentiation. (B) Boltzmann fit for $h_{\infty}(V_m)$ to match measured I_{kt2} $I/I_{max}(V_m)$ (C) Four schemes for $\tau_m(V_m)$ based on measured I_{kt2} 10%–90% rise time, scaled for each scheme as in Methods (D) Function fit for $\tau_h(V_m)$ that overlaps well with measure I_{kt2} $\tau_{inactivation}(V_m)$ and $\tau_{recovery}$ from inactivation (E) Simulated currents based on all four schemes compared to average current elicited by a test potential of +37mV ($n=4$). The m^2h scheme seems most similar. (F) Boltzmann scheme for $m_{inf}(V_m)$ that overlaps well with measured I_{kt3} activation curve, $G/G_{max}(V_m)$, based on exponential fitting

to bulk current (Figure 2A, $n = 19$) (**G**) $\tau_m(V_m)$ based on prior model for delayed rectifier (Mainen et al., 1995) . Shown on all graphs are measured values in Table 1. Function equations are in Table 2 and 3.

Figure 10. Simulation of total bulk current and responses of I_{kt1} , I_{kt2} , I_{kt3} to plateau potential waveform

(A) Simulated bulk outward current (above) elicited by a range of test potentials (below), as in Figure 2A. Shown on all graphs are measured values in Table 1. (B) Overlay of sample JGC plateau potential trace and simulated response of each current to voltage clamped to the JGC plateau potential (black – I_{kt1} , red – I_{kt2} , blue – I_{kt3}). Inset is an expansion of the area including the first two JGC spikes. Note prominence of I_{kt1} .

Table 1

Voltage parameters related to JGC plateau potential shape

Resting potential (V_{rest})	$-57.5 \pm 5.8 \text{ mV}$
Action potential threshold (V_{Apthresh})	$-25.3 \pm 6.7 \text{ mV}$
Plateau potential level (V_{plateau})	$-26.5 \pm 2.7 \text{ mV}$

Table 2

Activation equations for models of JGC potassium currents

Scheme		$m_{\text{inf}}(V_m)$	$t_m(V_m)$
I_{kt1}	m^4h	$\frac{1}{1 + e^{\frac{-(V_m + 39.1)}{18.56}}}$	$\frac{e^{(0.17 * 0.35 * (V_m + 31.5))}}{0.54(3 + e^{(0.17(V_m + 31.5))})} + 0.23$
	m^2h	$\frac{1}{1 + e^{\frac{-(V_m + 9.83)}{13.18}}}$	$\frac{e^{(0.562 * 0.978 * (V_m + 17.91))}}{0.2(23 + e^{(0.562(V_m + 17.91))})} + 2.45$
I_{kt3}	m	$\frac{\alpha(V_m)}{\alpha(V_m) + \beta(V_m)}$	$\frac{1}{\alpha(V_m) + \beta(V_m)}$
		$\alpha(V_m) = \frac{0.6(V_m - 40)}{1 - e^{\frac{-(V_m - 40)}{16.7}}}$ $\beta(V_m) = \frac{-0.012(V_m - 40)}{1 - e^{\frac{(V_m - 40)}{16.7}}}$	

Table 3

Inactivation equations for models of JGC potassium currents

scheme		$h_{inf}(V_m)$	$\tau_h(V_m)$
I_{kt1}	m^4h	$\frac{1}{1+e^{\frac{(V_m+39.57)}{3.68}}}$	$\frac{e^{(0.6*0.8*(V_m+16))}}{0.2(0.000001+e^{(0.6(V_m+16))})}+5$
I_{kt2}	m^2h	$\frac{1}{1+e^{\frac{(V_m+40.3)}{12.3}}}$	$\frac{e^{(0.695*0.966*(V_m+16))}}{0.00818(5.945 \times 10^{-8}+e^{(0.695(V_m-21.04))})}115.9$