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CALCIUM CURRENTS OF OLFACTORY BULB JUXTAGLOMERULAR CELLS: PROFILE AND MULTIPLE CONDUCTANCE PLATEAU POTENTIAL SIMULATION

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Abstract—The olfactory glomerulus is the locus of information transfer between olfactory sensory neurons and output neurons of the olfactory bulb. Juxtaglomerular cells (JGCs) may influence intraglomerular processing by firing plateau potentials that support multiple spikes. It is unclear what inward currents mediate this firing pattern. In previous work, we characterized potassium currents of JGCs. We focus here on the inward currents using whole cell current clamp and voltage recording in a rat *in vitro* slice preparation, as well as computer simulation. We first showed that sodium current was not required to mediate plateau potentials. Voltage clamp characterization of calcium current (I_{Ca}) determined that I_{Ca} consisted of a slow activating, rapidly inactivating ($\tau_{10\%\text{-}90\%\ rise}$ 6–8 ms, $\tau_{inactivation}$ 38–77 ms) component I_{cat1}, similar to T-type currents, and a sustained ($\tau_{inactivation}$>>500 ms) component I_{cat2}, likely composed of L-type and P/Q-type currents. We used computer simulation to test their roles in plateau potential firing. We robustly modeled I_{cat1} and I_{cat2} to Hodgkin-Huxley schemes (m^3h and m^2, respectively) and simulated a JGC plateau potential with six conductances: calcium currents as above, potassium currents from our prior study (A-type I_{kt1}, D-type I_{kt2}, delayed rectifier I_{kt3}), and a fast sodium current (I_{Na}). We demonstrated that I_{cat1} was required for mediating the plateau potential, unlike I_{Na} and I_{cat2}, and its $\tau_{inactivation}$ determined plateau duration. We also found that I_{kt1} dictated plateau potential shape more than I_{kt2} and I_{kt3}. The influence of these two transient and opposing conductances suggests a unique mechanism of plateau potential physiology.

Key words: olfactory bulb, glomerulus, calcium current, potassium current, plateau potential, simulation.

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Abbreviations: CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; D-APV, D-2-amino-5-phosphonovaleric acid; EGTA, glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid; ETCs, external tufted cells; GTP, guanosine-5'-triphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; JGCs, juxtaglomerular cells; LVA, low voltage activated; MES, methanesulfonate; NMDA, N-methyl-D-aspartic acid; OB, olfactory bulb; TEA, tetramethylammonium; TTX, tetrodotoxin; VGCCs, voltage dependent calcium currents.

Odor identity and concentration are represented by the combinatorial activation of distinct sets of glomeruli at the surface of the olfactory bulb (OB) (Stewart et al., 1979; Rubin and Katz, 1999; Mori et al., 1999; Johnson and Leon, 2000; Xu et al., 2003). Each glomerulus is a processing unit, receiving unique olfactory nerve input from olfactory sensory neuron (OSN) axons expressing the same olfactory receptor, which is then modulated by interactions with dendrites of interneurons and the projection neurons, mitral and tufted (M/T) cells (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996).

Juxtaglomerular cells (JGCs) are a heterogeneous group of neurons whose somata are near the border of an individual glomerulus, receiving input from OSNs and participating in complex synaptic interactions with other JGCs and M/T cells (Pinching and Powell, 1971a,b). JGCs are made up of interneurons such as periglomerular cells (PGCs) and short axon cells (SACs), as well as a subgroup of superficial tufted cell called external tufted cells (ETCs) (Pinching and Powell, 1971c; Shipley and Ennis, 1996).

In response to brief input, certain JGCs fire long lasting responses (Wellis and Scott, 1990; McQuiston and Katz, 2001; Hayar et al., 2004a; Zhou et al., 2006). One type of long lasting response is the spike burst, generated specifically by ETCs in a spontaneous and rhythmic fashion (McQuiston and Katz, 2001; Hayar et al., 2004a). In rat, ETC bursts are driven by persistent sodium current $I_{Na,P}$ (Hayar et al., 2004a), and in mouse by $I_{Na,P}$ and a low voltage activated (LVA) calcium current showing features of both T-type and L-type calcium current (Liu and Shipley, 2008). They also possess hyperpolarization-activated current (I_h) that may promote burst rhythmicity (Cadetti and Belluzzi, 2001; Liu and Shipley, 2008).

These ETC bursts may coordinate glomerular excitation through extensively branched primary dendrites giving numerous dendrodendritic synapses to other glomerular neurons (Pinching and Powell, 1971b,c; McQuiston and Katz, 2001; Hayar et al., 2004a; Zhou et al., 2006; Antal et al., 2006), as well as on physiological evidence that they can excite glomerular interneurons and mitral cells (Hayar et al., 2004b; Murphy et al., 2005; De Saint Jan et al., 2009) and engage in mutually electrical coupling (Hayar et al., 2005; Kosaka and Kosaka, 2005). In addition, inhibitory PGC activity prevents glomerular excitation by suppressing this critical ETC pathway (Gire and Schoppa, 2009).

Alternatively, some PGCs and ETCs fire plateau potentials spontaneously at low frequencies (McQuiston and Katz, 2001; Zhou et al., 2006). Despite extensive work on ETC burst mechanism and function, less is known regard-

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ing JGC plateau potential physiology. Input-output relationships in these neurons suggest that they can convert high frequency synaptic input into low frequency output (Zhou et al., 2006), but the network effect of this is unknown.

The mechanism of plateau potential firing is also less clear than the burst mechanism of ETCs. I_h may play a role in the excitability of PGCs and the rhythmic firing of JGC plateau potentials (Cadetti and Belluzzi, 2001; Zhou et al., 2006). Regarding the inward currents that create JGC plateau potentials, it is thought that they are low voltage-activated and not dependent on intracellular calcium (Zhou et al., 2006). Plateau potential persistence in extracellular TTX, sensitivity to 250 μM extracellular nickel, and insensitivity to 200 μM extracellular cadmium suggested a critical role for T-type calcium currents (Zhou et al., 2006; McQuiston and Katz, 2001). Such currents may be dendritically located (Zhou et al., 2006). This suggests that the plateau potential mechanism may be different than that of ETC bursts.

LVA calcium current has been studied previously in other JGC subtypes. GABAergic PGCs in rat OB possess LVA calcium current, but it is blocked by dihydropyridines and cadmium and not by nickel, suggesting that these PGCs differ from plateau potential-firing PGCs (Murphy et al., 2005). Dopaminergic PGCs in mouse OB possess a T-type current, but it is blocked by 100 μM nickel and 100 μM cadmium (Pignatelli et al., 2005). However, dopaminergic PGCs are not plateau potential-firing, and in this context T-type channels instead generates a subthreshold current that, along with $I_{Na,P}$, supports repetitive spiking in the absence of synaptic input (Puopolo et al., 2005). Therefore, it seems that plateau potential-firing neurons of the rat glomerulus possess a different LVA current to enable such prolonged depolarizations. This LVA current may be similar to that found in bursting mouse ETCs mentioned above, given that mouse ETC LVA current also promotes prolonged depolarizations and displays similar nickel sensitivity (Liu and Shipley, 2008).

In our accompanying study (Masurkar and Chen, 2011), we characterized the outward potassium currents of JGCs. Here, we pursued study of the calcium current of the JGCs with two purposes. First, we sought more specific evidence that JGCs possess a T-type calcium current that could mediate the plateau potential. Second, we created a single compartment computational model of plateau potential firing based on our characterizations. This enabled us to overcome pharmacological limitations and determine which inward currents (calcium, sodium) and potassium currents were critical for plateau potential generation.

EXPERIMENTAL PROCEDURES

Animal use and care

Animal experimentation protocols were reviewed and approved by the Yale University Animal Care and Use Committee. Sprague–Dawley (*Rattus norvegicus*) rats, 12–21 days old, were anesthetized with 1.2 g/kg urethane from Sigma, USA (i.p.) until unresponsive to tail pinch and decapitated according to aforementioned committee guidelines. The number of animals used was minimized.

Slice preparation and solutions

Olfactory bulbs were extracted in chilled (4 °C) and oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF), composed of 124 mM NaCl, 2 mM $CaCl_2$, 3 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 1.3 mM $MgSO_4$, and 10 mM dextrose at pH 7.4. Horizontal slices of 400 μm thickness were made with a rotorslicer (Dosaka, Japan) and immediately immersed in oxygenated ACSF at 34 °C for 15–20 min. Slices were maintained in oxygenated ACSF at room temperature for up to 8 h.

For Na^+ washout experiments, slice preparation ACSF was made using a HEPES buffer (140 mM NaCl, 3 mM KCl, 1.3 mM $MgSO_4$, 2 mM $CaCl_2$, 10 mM HEPES, 10 mM dextrose). Oxygenation was achieved with 100% O_2.

Extracellular drugs (cadmium, nickel, mibefradil, TTX) were dissolved in ddH_2O except for nimodipine, which was dissolved in 100% EtOH such that final ethanol concentration was 0.02%. These stock solutions were stored in refrigeration, except for TTX, which was stored at –20 °C. All chemical reagents in this study were from Sigma, USA.

Current clamp recording

JGCs were recorded in whole cell mode using a Kgluconate-EGTA internal solution (130 mM Kgluconate, 10 mM HEPES, 0.2 mM EGTA, 4 mM MgATP, 0.3 mM Na_3GTP, and 10 mM Na_2phosphocreatine), adjusted to pH 7.3-7.4 with KOH.

JGCs were visually identified via an infrared camera (Hamamatsu, Japan, C2400-07ER) on an upright microscope (Olympus BX50WI) employing differential interference contrast microscopy with a 40× water immersion objective. JGCs were identifiable by their large somata at the deep border of glomeruli. Based on their size, we felt that these cells were similar to the external tufted cells and larger periglomerular cells that have been morphologically identified and shown to exhibit plateau potentials (McQuiston and Katz, 2001; Zhou et al., 2006). In unpublished work, we have also demonstrated that some of these cells give glutamatergic output, and are therefore most likely external tufted cells (results not shown). Current clamp whole cell recordings were made with the Axoclamp 2A and 2B amplifiers (Axon Instruments, Union City, CA, USA) in bridge balance current clamp mode. Data were acquired with Pclamp 9.0 software at 10–20 kHz sampling rate. Pipettes of resistance 8–12 MΩ were pulled from borosilicate glass tube with filament (1.20 mm outer/0.69 mm inner diameter; Sutter Instruments, San Rafael, CA, USA) using a P-97 Flaming/Brown micropipette puller (Sutter). The recording chamber contained ACSF heated to 33–37 °C using a TC-344B temperature controller (Warner Instruments, New Haven, CT, USA).

For Na^+ washout experiments, HEPES-buffer control ACSF was replaced with an ACSF in which Na^+ was replaced with equimolar Tris (140 mM Tris–Cl, 3 mM KCl, 1.3 mM $MgSO_4$, 2 mM $CaCl_2$, 10 mM HEPES, and 10 mM dextrose).

Voltage clamp recording

For voltage clamp characterization of calcium current, the following intracellular solution was used: 100 mM CsMES, 10 mM TEA–Cl, 10 mM EGTA, 0.5 mM $CaCl_2$, 10 mM HEPES, 5 mM MgATP, 10 mM Na_2phosphocreatine, and 0.5 mM Na_3GTP at pH 7.4 (CsMES internal solution). The EGTA and $CaCl_2$ clamped internal calcium concentration to a known level of approximately 80 nM, resulting in a theoretical Ca^{2+} reversal potential of +134 mV. However, due to nonlinear properties of lipid membrane (Goldman, 1943; Hodgkin and Katz, 1949), the apparent reversal potential by linear extrapolation of IV curves was much less (+35 mV).

The following were added to ACSF for further calcium current isolation: 1 μM TTX to block fast Na^+ current, 3 mM CsCl to block