Spontaneous Activity of Isolated Dopaminergic Periglomerular Cells of the Main Olfactory Bulb

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Puopolo, Michelino, Bruce P. Bean, and Elio Raviola. Spontaneous activity of isolated dopaminergic periglomerular cells of the main olfactory bulb. J Neurophysiol 94: 3618–3627, 2005. First published July 20, 2005; doi:10.1152/jn.00225.2005. We examined the electrophysiological properties of a population of identified dopaminergic periglomerular cells of the main olfactory bulb using transgenic mice in which catecholaminergic neurons expressed human placental alkaline phosphatase (PLAP) on the outer surface of the plasma membrane. After acute dissociation, living dopaminergic periglomerular cells were identified by a fluorescently labeled monoclonal antibody to PLAP. In current-clamp mode, dopaminergic periglomerular cells spontaneously generated action potentials in a rhythmic fashion with an average frequency of 8 Hz. The hyperpolarization-activated cation current (Ih) did not seem important for pacemaking because blocking the current with ZD 7288 or Cs+ had little effect on spontaneous firing. To investigate what ionic currents do drive pacemaking, we performed action-potential-clamp experiments using records of pacemaking as voltage command in voltage-clamp experiments. We found that substantial TTX-sensitive Na+ current flowed during the interspike interval, and blocking Ca2+ current hyperpolarized the neurons and stopped spontaneous firing. These results show that dopaminergic periglomerular cells have intrinsic pacemaking activity, supporting the possibility that they can maintain a tonic release of dopamine to modulate the sensitivity of the olfactory system during odor detection. Calcium entry into these neurons provides electrical drive for pacemaking as well as triggering transmitter release.

INTRODUCTION

The glomeruli of the olfactory bulb represent a crucial site for processing of olfactory information. In addition to containing synapses between axonal arbors of olfactory receptor neurons and the dendritic tufts of mitral and tufted cells, glomeruli contain processes from a shell of juxtaglomerular neurons that include periglomerular, external tufted, and short axon cells. Periglomerular cells send their dendrites into the glomerulus where they form dendrodendritic, reciprocal synapses with the dendrites of mitral, tufted and other periglomerular cells. The axons of periglomerular cells are in turn presynaptic to other cells of the same type and to the primary dendrites of mitral and tufted cells of neighboring glomeruli (Halasz 1990; Pinching and Powell 1971a,b; Shepherd and Greer 1998).

The electrical properties and functional roles of periglomerular cells are not well understood. Periglomerular cells represent a heterogeneous class of neurons, and at least three distinct groups can be recognized on the basis of their immunoreactivity for glutamic acid decarboxylase (GAD), calbindin, and calretinin (Kosaka et al. 1995, 1998). Periglomerular cells are also likely to be functionally heterogeneous because they exhibit different K+ conductances (Puopolo and Belluzzi 1998a) as well as different firing behaviors (Hayar et al. 2004; McQuiston and Katz 2001). Various groups of periglomerular cells target different domains within the glomerulus with only some of them receiving synaptic input from the axons of the olfactory nerve (Kasowski et al. 1999; Kosaka et al. 1998; Toida et al. 2000).

Understanding the functional role of periglomerular cells depends on the ability to reliably distinguish individual populations of the neurons. Among the periglomerular cells that contain GAD or GABA, some also express tyrosine hydroxylase (Gall et al. 1987; Halasz et al. 1981; Kosaka K et al. 1995; Kosaka T et al. 1985), the rate-limiting enzyme for the synthesis of catecholamines, and thus most likely release dopamine as a modulatory transmitter. By using a transgenic line of mice in which catecholaminergic (CA) neurons in the CNS express human placental alkaline phosphatase (PLAP) under the control of the promoter of the tyrosine hydroxylase gene (Gustincich et al. 1997), we were able to identify a population of dopaminergic periglomerular cells after acute dissociation of the main olfactory bulb. Current-clamp recordings showed that these neurons spontaneously generate action potentials in a rhythmic fashion. Voltage-clamp recordings then allowed us to explore the ionic currents responsible for their pacemaker activity.

METHODS

Cell isolation and identification of dopaminergic periglomerular cells

Transgenic mice were used in which CA neurons in the CNS express human PLAP on the outer surface of the cell membrane (Gustincich et al. 1997). Mice were 12–17 days old; development, migration, and synapse formation by periglomerular cells appear essentially complete at these ages (Brunjes and Frazier 1986; McLean and Shipley 1988). Anesthesia was carried out by intraperitoneal injection of 0.1 ml of a solution containing 5% ketamine HCl (Ketaset; Fort Dodge Laboratories, Fort Dodge, IA) and 1% xylazine (Rompun; Bayer, Shawnee Mission, KS). The procedure to release isolated dopaminergic periglomerular cells was adapted from methods used previously for other types of central neurons (Do and Bean 2003; Raman and Bean 1999). After decapitation, the brain was removed and placed in ice-cold dissociation medium (DM) containing (in mM) 82 Na2SO4, 30 K2SO4, 10 HEPES, 5 MgCl2, 10 glucose, and 0.001% glutamate. After injection of 0.1 ml of a solution containing 5% ketamine HCl (Ketaset; Fort Dodge Laboratories, Fort Dodge, IA) and 1% xylazine (Rompun; Bayer, Shawnee Mission, KS), the brain was removed and placed in ice-cold dissociation medium (DM) containing (in mM) 82 Na2SO4, 30 K2SO4, 10 HEPES, 5 MgCl2, 10 glucose, and 0.001% glutamate. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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phenol red indicator; pH was adjusted to 7.4 with NaOH, and the solution was continuously bubbled with 100% O₂. The olfactory bulbs were removed from the brain and cut into 400-μm horizontal slices with a vibrating tissue slicer (DTK-1000, DSK, Dosaka, Japan). Slices containing the glomeruli were incubated in DM containing 3 mg/ml protease type XXIII (Sigma, St. Louis, MO) for 30–40 min at 35°C. After enzymatic digestion, slices were washed with DM containing 1 mg/ml bovine serum albumin (Sigma) and 1 mg/ml trypsin inhibitor (Sigma). The enzyme-treated slices were then gently triturated through a series of fine-bore, fire-polished Pasteur pipettes to generate a suspension of isolated neurons. The dissociated neurons were then plated on the glass bottom of concanavalin A-coated (1 mg/ml) recording chambers and kept at 37°C in 5% CO₂-95% O₂ for 1 h before commencement of recordings. Dopaminergic neurons were identified by labeling of their membrane with a monoclonal antibody to PLAP (De Groote et al. 1983) directly conjugated to the fluorochrome Cy3 (E6-Cy3). Techniques for labeling of tissue sections (Fig. 1) followed those of Gustincich et al. (1997).

Electrophysiology

Equipment and recording techniques were described previously (Puopolo et al. 2001). Briefly, E6-Cy3-stained dopaminergic neurons were identified by scanning the coverslip in epifluorescence. The remaining procedures were carried out in visible light with Nomarski optics. It is unlikely that neurons are damaged as a result of expression of PLAP, immunolabeling, or fluorescence because in other experiments, we have recorded from dopaminergic cells in the substantia nigra pars compacta using the same transgenic mouse and labeling procedure and saw no difference in electrophysiological properties or cell viability compared with extensive recordings from unlabeled dopaminergic neurons in wild-type mice.

Current and voltage traces were low-pass-filtered at 2 kHz using the internal Bessel filter of the amplifier (Axopatch 200B, Axon instruments, Foster City, CA). The sample frequency was 20–50 kHz for whole cell voltage-clamp recordings and 10 kHz for current-clamp recordings. The resistance of the patch pipettes was 3–5 MΩ after filling with the internal solution. In voltage-clamp experiments, the series resistance of the pipettes was 10–15 MΩ and could be compensated ≥80% after cancellation of capacitative transients. Current-clamp recordings were carried out in the fast current clamp mode to reduce distortion of action potentials (Magistretti et al. 1996, 1998).

Unless noted otherwise, the extracellular bath solution for current-clamp recordings contained (in mM) 137 NaCl, 5.4 KCl, 1.8 CaCl₂, 1 MgCl₂, 5 HEPES, and 10 glucose. The intracellular solution contained

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**FIG. 1.** Identification of dopaminergic periglomerular cells. In a horizontal section of the olfactory bulb, the glomerular layer is intensely stained by the histochemical reaction for alkaline phosphatase activity (A). At higher magnification, dopaminergic periglomerular cells (*) and few external tufted cells express both placental alkaline phosphatase (PLAP, B) and tyrosine hydroxylase (C). C arrowhead indicates a dopaminergic tufted cell. After dissociation of the olfactory bulb, the cell bodies of dopaminergic periglomerular cells were identified by staining with a monoclonal antibody directly conjugated to Cy3 (B, inset). Scale bar: 15 μm in B and C, 5 μm in the inset of B.

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(in mM) 120 K-gluconate, 6 NaCl, 0.5 CaCl₂, 4 MgCl₂, 2 Mg-ATP, 0.3 Na-GTP, 14 phosphocreatine, 5 EGTA, 10 HEPES and 10 glucose. Sodium and Ca²⁺ currents were obtained by subtraction: for the Na⁺ current, the extracellular solutions contained (in mM) 137 NaCl, 1.8 CoCl₂, 1 MgCl₂, 5.4 TEA-Cl, 10 4-aminopyridine, 5 HEPES and 10 glucose with and without 1 μM tetrodotoxin (TTX). For determining Ca²⁺ current, 1.8 CoCl₂ was substituted for CaCl₂ in an extracellular solution containing (in mM) 137 NaCl, 1.8 CaCl₂, 1 MgCl₂, 5.4 TEA-Cl, 10 4-aminopyridine, 5 HEPES, and 10 glucose and 1 μM TTX.

A stock solution of TTX (Research Biochemicals, Natick, MA) was made in 2 mM citric acid and stored at ~20°C. Drugs were diluted to final concentration in the extracellular solution before the experiment and applied to single cells in the external bath solution by gravity flow through an array of microcapillary tubes controlled by a rapid solution changer (RSC-200, Biologic, Claix, France). Data were acquired with pClamp 8 (Axon instruments), and data analysis was carried out using Clampfit 8 (Axon instruments) and IGORPro version 3.14 (WaveMetrics, Lake Oswego, OR). Reported voltages were corrected for the junction potential between the K-gluconate-based internal solution and the extracellular solution [−11 mV, measured using a flowing 3 M KCl electrode as described in Neher (1992)]. Data are presented as means ± SD. All experiments were carried out at room temperature (22–24°C).

RESULTS

Identification of dopaminergic periglomerular cells

To identify dopaminergic periglomerular cells, we used a transgenic mouse line (Gustincich et al. 1997) in which placental alkaline phosphatase (PLAP) is expressed under the control of the promoter sequence of the gene for tyrosine hydroxylase (Banerjee et al. 1992). Figure 1 illustrates the location in the main olfactory bulb of the neurons that express PLAP and tyrosine hydroxylase. The staining for both proteins was very intense in the glomerular layer (Fig. 1A). PLAP (Fig. 1B) and tyrosine hydroxylase (C) were both expressed in two different cell types. The vast majority of the positive neurons were periglomerular cells, 7–10 μm in diameter, situated at the periphery of the olfactory glomeruli. In addition, a small number of external tufted cells were stained in the external plexiform layer. Because PLAP resides on the outer surface of the cell membrane, living neurons that express tyrosine hydroxylase could be fluorescently labeled by a monoclonal antibody to PLAP directly conjugated to Cy3 after neurons were dissociated from the olfactory bulb (Fig. 1B, inset). After dissociation, labeled cells fell into two distinct populations. The majority had small perikarya (7–10 μm) and cell capacitances of 5–10 pF, whereas a minority had perikarya of 13–15 μm and capacitances of 15–20 pF. These populations of two different sizes correspond to what would be expected of dopaminergic periglomerular cells and dopaminergic external tufted cells, respectively (Hayar et al. 2004; Puopolo and Belluzzi 1998a,b; Pignatelli et al. 2005). Therefore to confine our study to periglomerular cells, we performed experiment exclusively on cells with cell body diameters <10 μm. Of the cells considered in this study, average diameter was 8.6 ± 1.0 μm (n = 20) and average capacitance was 7.8 ± 2.0 pF (n = 72).

Spontaneous firing in dopaminergic periglomerular cells

Solitary dopaminergic periglomerular cells were found to be spontaneously active. Spontaneous activity was evident as action currents on formation of seals using patch pipettes in the cell-attached mode (Fig. 2A), and firing was highly regular and rhythmic (B). After breaking the cell membrane and switching to whole cell current-clamp mode, the majority of cells (77%) continued to fire spontaneously (Fig. 2, C and D). Occasionally, neurons fired spontaneously in cell-attached configuration but were silent in whole cell-current clamp apparently due to depolarization block; these cells were not used for further analysis. The average rate of firing was 8.2 ± 4.6 Hz (n = 20). The spontaneous firing had full-blown action potentials with an average amplitude of 85 ± 12 mV (n = 20), measured from an average after-spike potential of −63.5 ± 5.9 mV (n = 20). The width of action potentials (measured at half-amplitude) was 2.1 ± 0.8 ms (n = 20). Cells had an average input resistance of 1.4 ± 0.5 GΩ (n = 26).

Role of Ih in spontaneous firing in dopaminergic periglomerular cells

The ability of isolated dopaminergic periglomerular cells to fire action potentials spontaneously demonstrates that such activity is intrinsic to the neurons and does not require synaptic inputs or modulatory transmitters or peptides. In this respect, dopaminergic periglomerular cells resemble midbrain dopaminergic neurons (Grace and Bunney 1984; Hainsworth et al. 1991) and retinal dopaminergic amacrine cells (Feigenspan et al. 1998). In some cells that are spontaneously active, including sinoatrial myocytes of the heart (DiFrancesco et al. 1986), thalamic relay neurons (McCormick and Pape 1990), and some...
midbrain dopaminergic neurons (Neuhoff et al. 2002), the hyperpolarization-activated cation current known as $I_h$ appears to play a primary role in driving the spontaneous depolarization that leads to spike generation. To test the possible contribution of $I_h$ to pacemaking, dopaminergic periglomerular cells were challenged with the blocking agent ZD 7288 (Gasparini and DiFrancesco 1997). Applied at a concentration of 30 μM, ZD 7288 had little effect on spontaneous activity (Fig. 3). In results collected from four dopaminergic periglomerular cells, the drug caused a small depolarizing shift of the membrane potential, from $-74 \pm 2$ to $-73 \pm 2$ mV (measured at the trough), and a small increase of the firing frequency, from $8.2 \pm 2.4$ to $9.0 \pm 2.4$ Hz. Both changes are the opposite of what one would expect from blocking the inward-directed $I_h$. These results suggest that $I_h$ plays little or no role in pacemaking. The modest depolarization and speeding of firing most likely result from small nonspecific blocking effects of ZD 7288 on $K^+$ currents. We also tested the effect of 1 mM Cs$,^+$, another blocker of $I_h$. Cesium also had little effect on spontaneous firing rate ($8.5 \pm 1.4$ Hz in control, $8.7 \pm 1.4$ Hz with 1 mM Cs$,^+$, $n = 5$) or on membrane potential ($-65 \pm 6$ mV in control, $-64 \pm 7$ mV in Cs$,^+$), again consistent with little activation of $I_h$.

**Role of Ca$^{2+}$ and Na$^+$ currents**

Another candidate for an inward ionic current driving spontaneous firing is voltage-activated Ca$^{2+}$ current. Blocking various voltage-dependent Ca$^{2+}$ currents halts or slows pacemaking in some catecholaminergic neurons, including dopaminergic neurons of the substantia nigra (Kang and Kitai 1993; Mercuri et al. 1994; Nedergaard et al. 1993). In midbrain dopaminergic neurons, a component of Ca$^{2+}$ current activates at potentials as negative as $-60$ to $-50$ mV, well-positioned for a role in pacemaking (Durante et al. 2004; Wilson and Callaway 2000). To test the possible contribution of Ca$^{2+}$ current to spontaneous firing, dopaminergic periglomerular cells were challenged with a solution in which Ca$^{2+}$ ions were replaced by equimolar (1.8 mM) Co$^{2+}$. This treatment stopped spontaneous firing, and the cessation of firing was accompanied by a hyperpolarization (Fig. 4A), a result consistent with block of an inward current. These results were obtained in each of five neurons tested, with cessation of rhythmic firing and hyperpolarization of the cell (from $-66 \pm 2$ mV in control to $-73 \pm 4$ mV with Co$^{2+}$, $n = 5$). The cessation of pacemaking after Co$^{2+}$ substitution for Ca$^{2+}$ was not due to a generalized or nonspecific loss of excitability because in some of the cells there were occasional, rare, sporadic action potentials of full height in the Co$^{2+}$ solution.

In some types of spontaneously active cells, Ca$^{2+}$-dependent oscillatory activity continues even when formation of Na$^+$ spikes is blocked by application of TTX (Pennartz et al. 2002; Williams et al. 1984). Such behavior is seen in some dopaminergic midbrain neurons, suggestive that pacemaking may be driven by an underlying electrical oscillation due to Ca$^{2+}$ current (Wilson and Callaway 2000). We tested for this behavior in dopaminergic periglomerular cells. In these neurons,
however, application of TTX most commonly (10 of 13 cells) produced complete quiescence accompanied by a net hyperpolarization (Fig. 4B). The effect of TTX was fully reversible, and spontaneous firing at the original frequency resumed after washing TTX for 50–60 s. The hyperpolarization that accompanies cessation of firing with TTX application suggests that in addition to supporting spikes, TTX-sensitive Na⁺ channels contribute subthreshold current that flows during the interspike interval. In 3 of 13 cells, there were small oscillations in membrane potential in TTX, reminiscent of the Ca²⁺-dependent oscillations in midbrain dopamine neurons (Wilson and Callaway 2000) but far smaller (4.2 ± 2.5 mV, n = 3) and less regular. Thus although blocking Ca²⁺ current stops spontaneous activity in dopaminergic periglomerular neurons, the mechanism of pacemaking seems different from in midbrain dopamine neurons, where Ca²⁺ currents appear to drive pacemaking by producing an underlying large-amplitude voltage oscillation that persists when spikes are blocked by TTX (Wilson and Callaway 2000). In dopaminergic periglomerular neurons, both Ca²⁺ current and TTX-sensitive current are necessary for oscillatory pacemaking activity, and loss of either one produces complete quiescence in most neurons.

Analysis of currents flowing between spikes

These experiments suggest that both Na⁺ and Ca²⁺ currents play important roles in driving pacemaking activity. To better quantify the ionic currents that flow during the spontaneous depolarization between spikes, we used the action-potential-clamp method. In each cell, we recorded first a 5-s segment of spontaneous activity under current clamp. Then the amplifier was switched to voltage-clamp mode, and the recorded segment was used as command waveform (Taddeese and Bean 2002; Zaza et al. 1997). With this method, voltage-dependent channels are exposed to the same potential in voltage clamp as in current clamp, and ionic currents from purely voltage-dependent channels should be identical in both modes. Thus individual ionic currents flowing during and between spikes can be isolated pharmacologically or by solution exchange in the voltage-clamp experiments. Based on the current-clamp results, we focused on voltage-dependent Na⁺ and Ca²⁺ currents.

Figure 5A illustrates the brief segment of spontaneous activity recorded in current-clamp mode and administered to the same neuron as a voltage command. Figure 5B shows current carried by voltage-dependent Na⁺ channels in response to the command obtained by subtraction after TTX treatment (red trace). To measure Ca²⁺ current (blue trace), we adopted a strategy of replacing external Ca²⁺ ions by equimolar Co²⁺, which blocks current in all varieties of voltage-dependent Ca²⁺ channels known to be expressed in mammalian neurons. This subtraction was carried out with TEA present in both solutions to block Ca²⁺-activated K⁺ currents. As will be discussed, the replacement of Ca²⁺ by Co²⁺ could affect currents through other channels in addition to voltage-dependent Ca²⁺ channels, including nonvoltage-dependent Ca²⁺-permeant channels and Ca²⁺-activated nonselective cation channels. For simplicity we will refer to the current obtained by subtraction with Co²⁺ substitution as “Ca²⁺ current”, while recognizing the possibility that currents carried by multiple types of Ca²⁺-sensitive channels may contribute to it.

**Currents were signal-averaged over multiple cycles of spontaneous firing to improve the signal-to-noise ratio, thus allowing a better resolution of the relatively small currents flowing in between spikes. As expected, Na⁺ current was maximal during the upstroke of the action potential, whereas Ca²⁺ current was largest during the falling phase of the action potential (Fig. 5B, inset). Although both types of current reached their peaks during the action potential, both also contributed measurable currents at all times during the interspike interval. In the cell the results of which are shown in Fig. 5C, Na⁺ current and Ca²⁺ current each carried about 1.5–2.5 pA of inward current during the first half of the interspike interval at the voltage increased from about −60 to about −50 mV. In the later part of the interspike interval, as the voltage depolarized past −50 mV, both currents became increasingly large, with Na⁺ current always larger than Ca²⁺ current.

In results collected from 11 cells, the amplitude of the Na⁺ current (Fig. 6A, red symbols) flowing during the interspike interval was measured at four different voltages; the average values were: −1.8 ± 1.1 pA at −60 mV, −2.9 ± 1.7 pA at −55 mV, −4.9 ± 2.8 pA at −50 mV, and −7.6 ± 3.6 pA at −45 mV. In the same 11 cells, the average values for the interspike Ca²⁺ current (Fig. 6A, blue symbols) were: −1.3 ± 1.2 pA at −60 mV, −1.4 ± 1.3 pA at −55 mV, −1.7 ± 1.3 pA at −50 mV, and −2.2 ± 1.7 pA at −45 mV. Thus Na⁺ current was on average larger in magnitude than Ca²⁺ current at all
can also be compared with the net inward charge necessary to depolarize the cell from the afterhyperpolarization to \(-50\) mV, equal to cell capacitance \((7.8 \pm 2.1 \mu F, n = 72)\) times the change in voltage (average of \(12.1\) mV). The net inward charge amounted to \(94.4\) fC, and the integrated interspike \(Na^+\) and \(Ca^{2+}\) charges amounted to \(176 \pm 78\) and \(122 \pm 102\)% respectively, of the net inward charge necessary to depolarize the cell from the afterhyperpolarization to \(-50\) mV. This suggests that there are also substantial outward \(K^+\) currents during the interspike interval that partially counterbalance the inward charge carried by \(Na^+\) and \(Ca^{2+}\) current.

**Voltage dependence of \(Na^+\) and \(Ca^{2+}\) current**

To examine the voltage-dependence of \(Na^+\) and \(Ca^{2+}\) currents, we delivered voltage ramps \((-80\) to \(-20\) mV at \(170\) mV/s). Sodium and \(Ca^{2+}\) currents were isolated using TTX application and Co2+ substitution, as for interspike currents. Figure 7A shows the ramp-elicited currents before and after the application of TTX under ionic conditions designed to block \(Ca^{2+}\) currents and reduce \(K^+\) currents (external solution with 1.8 mM CoCl2 and 5.4 mM TEA-Cl). The current blocked by TTX began to activate near \(-60\) mV and reached a peak near times during the interspike interval and also increased more steeply with voltages over the range of \(-60\) to \(-45\) mV.

The relative contribution of \(Na^+\) and \(Ca^{2+}\) current to the spontaneous interspike depolarization was also assessed by integrating each current from the time of the afterhyperpolarization to the time at which voltage reached \(-50\) mV during the interspike interval. Charge carried by TTX- and Co2+-sensitive channels were plotted for each of the 11 cells tested. Cells were arranged by their firing rate. There was no evident correlation between the relative contribution of TTX- and Co2+-sensitive current to the spontaneous interspike depolarization and the firing rate. Bars are stacked. C: in collected results from the 11 cells examined, the mean charge carried by TTX-sensitive channels was bigger than the mean charge carried by Co2+-sensitive channels. Currents were isolated by subtraction as described in Fig. 5. Error bars show SD.

**FIG. 6.** Contribution of \(Na^+\) and \(Ca^{2+}\) currents to the spontaneous interspike depolarization. A: in 11 cells, TTX-sensitive (red dots) and Co2+-sensitive (blue dots) currents were measured at 4 different voltages \((-60, -55, -50,\) and \(-45\) mV) during the interspike interval. At all voltages, mean TTX-sensitive was bigger than Co2+-sensitive current. B: relative contribution of TTX-sensitive (red bars) and Co2+-sensitive (blue bars) current to the spontaneous interspike depolarization was evaluated also by integrating each current from the time of the afterhyperpolarization to the time at which voltage reached \(-50\) mV during the interspike interval. Charge carried by TTX- and Co2+-sensitive channels were plotted for each of the 11 cells tested. Cells were arranged by their firing rate. There was no evident correlation between the relative contribution of TTX- and Co2+-sensitive current to the spontaneous interspike depolarization and the firing rate. Bars are stacked. C: in collected results from the 11 cells examined, the mean charge carried by TTX-sensitive channels was bigger than the mean charge carried by Co2+-sensitive channels. Currents were isolated by subtraction as described in Fig. 5. Error bars show SD.

**FIG. 7.** Voltage dependence of TTX- and Co2+-sensitive currents. TTX- and Co2+-sensitive currents were elicited by a voltage ramp protocol \((-80\) to \(-20\) mV at \(170\) mV/s). A: TTX-sensitive current (red trace) was elicited using a solution in which \(Ca^{2+}\) ions were replaced by equimolar Co2+. The current was roughly linear from \(-80\) to \(-60\) mV and became inward positive to \(-60\) mV. The inward component was completely abolished by application of 1 \(\mu M\) TTX (black trace). B: cobalt-sensitive current (blue trace) was elicited using a solution supplemented with 1 \(\mu M\) TTX. The current was linear in the range from \(-80\) to \(-50\) mV and became inward positive to \(-50\) mV. The inward component was completely abolished when \(Ca^{2+}\) ions were replaced by equimolar \(Co^{2+}\) (black trace). C: subtracted TTX-sensitive (red trace) and Co2+-sensitive (blue trace) currents are presented together for comparison.
ionic machinery for generating pacemaking is located in the cell body and can be fruitfully studied in voltage-clamp experiments using dissociated neurons.

Unlike pacemaking in some other types of excitable cells, spontaneous activity in dopaminergic periglomerular neurons does not appear to involve \( I_h \) because neither 30 \( \mu \)M ZD 7288 nor 1 mM Cs\(^+\) slowed firing. In principle, \( I_h \) might play a larger role in intact cells than dissociated cells if it was present in dendrites, as firing of intact cells is slower than for dissociated cells (Pignatelli et al. 2005), perhaps due to the capacitative load of the dendritic tree. Also, Pignatelli and colleagues found that even intact dopaminergic periglomerular cells studied in brain slice do not have detectable \( I_h \), fitting well with our results. In this respect, dopaminergic periglomerular cells appear to differ from another (still undefined) population of periglomerular cells that do have \( I_h \) (Cadetti and Belluzzi 2001). Although pacemaking activity has often been associated with \( I_h \), this current is not required in many types of spontaneously active neurons, including cerebellar Purkinje cells (Raman and Bean 1999) and neurons of the suprachiasmatic nucleus (Llinas and Alonso 1992; Utseshev et al. 1995). In the case of midbrain dopaminergic neurons, neurochemically and topographically distinct populations can be recognized, and \( I_h \) is actively involved in pacemaking only in a subset of them (Raman and Bean 1999; Seutin et al. 2001). Dopaminergic amacrine cells in the retina show pacemaking activity that does not involve \( I_h \) (Feigenspan et al. 1998), in this way resembling dopaminergic periglomerular cells of the main olfactory bulb.

A “persistent” \( Na^+ \) current flowing at interspike voltages is important in driving the pacemaking activity of dopaminergic periglomerular cells. Our data suggest that this is the major current that drives pacemaking. In this respect, these neurons are similar to a number of other central neurons showing spontaneous firing, including those of the tuberomammillary (Llinas and Alonso 1992; Taddeese and Bean 2002; Utseshev et al. 1995), pedunculopontine (Takakusaki and Kitai 1997), subthalamic nucleus (Bevan and Wilson 1999; Do and Bean 2003), and tuberomammillary nucleus (Llinas and Alonso 1992; Uteshev et al. 1995). In the case of midbrain dopaminergic neurons, neurochemically and topographically distinct populations can be recognized, and \( I_h \) is actively involved in pacemaking only in a subset of them (Neuhoff et al. 2002; Seutin et al. 2001). Dopaminergic amacrine cells in the retina show pacemaking activity that does not involve \( I_h \) (Feigenspan et al. 1998), in this way resembling dopaminergic periglomerular cells of the main olfactory bulb.

The results presented here show that dopaminergic periglomerular neurons have intrinsic pacemaker activity and give some idea of the underlying ionic mechanisms. Spontaneous firing was observed in 115 of 150 (77%) acutely isolated dopaminergic periglomerular cells studied. The pacemaking of acutely isolated neurons is unlikely to be an artifact of damage during isolation because Pignatelli et al. (2005) found that a very similar percentage (~80%) of dopaminergic periglomerular neurons studied in brain slice were spontaneously active. Their studies used a different transgenic mouse strain in which dopaminergic neurons were labeled with green fluorescent protein (GFP) driven by the tyrosine hydroxylase promoter, and the virtually identical results in the two different mouse strains argues against artifacts arising from overexpression of GFP (which is cytoplasmic) or PLAP (which is extracellular). The similarity of our results to those of Pignatelli et al. also argues against the possibility that the presence of spontaneous activity is critically dependent on the precise ionic composition of the external solution, which was somewhat different in our experiments ([in mM] 1.8 CaCl\(_2\), 1 MgCl\(_2\), 5.4 KCl, 10 glucose) than in theirs ([in mM] 2.0 CaCl\(_2\), 1 MgCl\(_2\), 2.5 KCl, 15 glucose). When studied after dissociation, the neurons studied by Pignatelli et al. (2005) fired somewhat faster (average ~14 Hz) than in our experiments (~8 Hz), a difference that might be accounted for by the different ages (adult vs. P12–P17, respectively). Pignatelli et al. found that the neurons actually fired faster after dissociation (~14 Hz) than in brain slice (~7–8 Hz), consistent with the idea that the fundamental
Wilson 1999). Frequently, suppression of Ca\(^{2+}\) current produces a net depolarization, as if the dominant electrical effect is from blocking Ca\(^{2+}\)-activated K\(^+\) currents (Jackson et al. 2004; Penmartz et al. 2002; Raman et al. 2000). However, in dopaminergic periglomerular cells, block of Ca\(^{2+}\) current consistently resulted in net hyperpolarization and cessation of firing, suggesting that flow of Ca\(^{2+}\) current during the pacemaking cycle is not counterbalanced by an equal or greater Ca\(^{2+}\)-activated K\(^+\) current. When measured with the action potential clamp, interspike Ca\(^{2+}\) current amounted to ~70% of interspike Na\(^+\) current, consistent with a substantial role in pacemaking. Pignatelli and colleagues (2005) found that pacemaking was stopped by mibefradil or by 0.1 mM Ni\(^{2+}\), suggesting the particular importance of T-type Ca\(^{2+}\) channels, while blockers of L-, N-, and P-type channels did not stop firing. Interestingly, this is apparently different from dopaminergic neurons of the substantia nigra where L-type Ca\(^{2+}\) current appears to play a major role in pacemaking (Durante et al. 2004; Kang and Kitai 1993; Mercuri et al. 1994; Wilson and Callaway 2000).

Our experimental measurements of the ionic currents during the pacemaking cycle can be compared with the predictions of such currents made by Pignatelli et al. (2005) using a Hodgkin-Huxley-like mathematical model. Considering the small size of the currents that generate the spontaneous depolarization, and the consequent difficulty in either measuring or accurately predicting them, the experimental measurements and the model are remarkably similar. In both experiments and model, Na\(^+\) currents and Ca\(^{2+}\) currents are of comparable size (2–3 pA) in the mid-region of the interspike interval, with Na\(^+\) current somewhat larger and showing a greater degree of increase during the approach to spike firing. The experiments using trains of action potentials as command waveforms consistently showed a small but measurable inward current sensitive to Ca\(^{2+}\) replacement by Co\(^{2+}\) that flowed at the most negative voltages during the interspike interval, near ~70 mV. This is more negative than the voltage at which clear Ca\(^{2+}\) current flowed in most experiments using voltage ramps (approximately ~60 mV). The reason for this difference is not clear, but the current at more negative voltages can constitute a substantial part of the total current during the interspike interval that is sensitive to Ca\(^{2+}\) replacement by Co\(^{2+}\). One possibility is that this component of current during the pacemaking cycle represents “tail current” from T-type Ca\(^{2+}\) channels activated by the preceding spike, as predicted by the model of Pignatelli and colleagues (2005). Another interesting possibility is that some of the current at the most negative voltages represents current from the operation of the Na\(^+\)/Ca\(^{2+}\) exchanger, which would generate steady inward current at these voltages while extruding the Ca\(^{2+}\) that enters during the overall pacemaking cycle (mostly during the spikes). A role for Na\(^+\)/Ca\(^{2+}\) exchanger current in helping to drive pacemaking has been proposed in the case of cardiac pacemaking cells (Bogdanov et al. 2001; Kurata et al. 2002).

Previously, recordings from juxtaglomerular cells of undeфинес transmitter properties have shown a subset of cells that show spontaneous activity characterized by irregular bursting activity (Hayar et al. 2004; McQuiston and Katz 2001; Puopolo and Belluzzi 2001). This seems clearly different from the rhythmic pacemaking activity of dopaminergic periglomerular neurons and most likely represents a different cell type (or types) that remain to be identified. It is not surprising that cells characterized by regular pacemaking were not obvious in previous recordings from unlabeled cells given the small fraction (~10%) of dopaminergic neurons present in the glomerular layer compared with the overall population of juxtaglomerular cells.

Pacemaking activity of dopaminergic periglomerular neurons suggests that there may be tonic release of dopamine into glomeruli at least under some circumstances. A variety of experiments show that dopamine plays a major role in modulating the sensitivity of the olfactory system to odors. Olfactory deprivation by olfactory nerve transaction or occlusion of the nostril reduces the content of dopamine and the expression of tyrosine hydroxylase in the ipsilateral olfactory bulb by as much as 75% (Baker 1990; Baker et al. 1983). As a result, mitral cells exhibit enhanced responsiveness to odors (Guthrie et al. 1990). A similar effect is observed on administration of the D2 antagonist spiperone (Wilson and Sullivan 1995), whereas the D2 agonist quinpirole reduces the responsiveness of mitral cells to odors (Doty and Rissey 1989).

Mechanistically, dopamine depresses synaptic transmission between olfactory receptor neurons and mitral cells by acting at presynaptic D2 receptors (Berkowitz and Trombley 2000; Coronas et al. 1997; Ennis et al. 2001; Hsia et al. 1999; Koster et al. 1999; Mansour et al. 1990), reduces the excitability of mitral cells (Nowycky et al. 1983), and interferes with the invasion of mitral cell dendrites by somatic action potentials (Davila et al. 2003; Davison et al. 2004; Duchamp-Viret et al. 1997). Overall, dopamine has an important function in setting the gain for odor discrimination, with increasing levels of dopamine raising the threshold for odorant detection and reduced levels increasing the sensitivity of the olfactory system. This is analogous to dopamine’s role in the retina where it sets the gain of the retinal networks for vision in bright light (Witkovsky and Dearry 1991). The spontaneous activity of dopaminergic periglomerular cells is well-suited for maintaining a tonic or basal concentration of dopamine in the olfactory glomeruli and outer plexiform layer. This hypothesis is supported by the observation that D2 receptor blockers reduce the spontaneous oscillations in the electrical activity of the olfactory bulb (Davison et al. 2004). In other spontaneously active dopaminergic neurons, such as those of the substantia nigra and retina, dopamine release is influenced by the firing rate of the cell and can be modulated by neurotransmitters that increase or decrease the frequency of spontaneous firing (Jaffe et al. 1998; Puopolo et al. 2001). A similar control of the firing rate of dopaminergic neurons in the olfactory bulb represents a plausible mechanism for up or down regulation of the sensitivity of the neural networks responsible for odor detection and discrimination.

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Innovative Methodology


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