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ELECTRICAL PROPERTIES OF PERIGLOMERULAR CELLS IN THE FROG OLFACTORY BULB

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INTRODUCTION

The inhibitory interneurones of the olfactory bulb (OB) play an important role in the signal processing of sensory input. The periglomerular cells (PG) and the granule cells, located at the input and output of the bulb, respectively, are interneurones mediating the horizontal flow of information, in an arrangement closely resembling that of the horizontal and amacrine cells in the retina (6). In the OB, however, the role of interneurones is not understood so well, especially for the PG cells, the object of this study. Understanding the basic properties of these cells holds the key for understanding synaptic interactions in the olfactory bulb and, in particular, for the comprehension of signal processing taking place at the synaptic triad between olfactory nerve (ON), mitral/tufted (M/T) cells and PG cells (12, 13).

To date only a few reports are available on intracellular activity in identified PG cells (4, 18), and these diverge even in the description of such basic features as the presence or absence of voltage-dependent Na^+-channels and the consequent capability of spiking or lack thereof. These uncertainties about their properties are most likely due to the small size (6-8 μm) of the PG cells, among the smallest in the brain (5). The difficulties in accessing these cells can now be overcome by applying the technique of patch-clamp recordings in thin slices (8), which makes it possible to access visually identified adult neurons retaining their functional cytoarchitecture. Furthermore, the small size of these cells becomes an advantage for whole-cell recordings since good voltage control is possible.

In the present study the slice technique was applied in combination with whole-cell patch-clamp recordings in the frog olfactory bulb. We describe the membrane properties of intact PG cells and the kinetics and pharmacological properties of the main voltage-dependent currents, i.e. I_{Na}, I_{Ca} and I_A. We believe that the present study, extending the knowledge on interneurone membrane properties, is a necessary prerequisite for studies on synaptic transmission in the olfactory bulb.

METHODS

After anaesthesia with MS222 (Sigma), adult frogs (*Rana esculenta*, 40-60g) were decapitated and pithed. The head, pinned onto a Petri dish, was submerged in chilled Ringer solution and the skull was then opened using a ventral approach. The OB was pasted to the stage of a vibroslicer (Campden) with cyanoacrylate glue, and sliced on a coronal plane at a thickness of

150-200 µm. Slices were then mounted in the observation bath (1 ml) and transferred onto the microscope stage (Zeiss Axioscope, with 63w objective and Nomarski optics). During recordings the slices were perfused at a rate of 2 ml/min. Electrical stimulation was achieved either with a pair of teflon-insulated Ag wires (0.2 mm, Clark Instr.) or with suction electrodes. The stimulus width was 1-1.5 ms and amplitude ranged from 2 to 9 V.

Solutions

The standard saline used had the following composition (mM): 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 10 HEPES, and 5 glucose. The pH and osmolarity were adjusted to 7.8 (9, 14) and 240 mOsm respectively. Glycine 1 µM was usually present. As a rule the cell was accessed in standard saline then, if the cell responded to stimulation, 10 µM bicuculline was added and maintained throughout the experiment in order to block the $GABA_A$ inhibition PG cells exert on MT cells through the dendro-dendritic synapses (7).

All drugs were dissolved in standard bath solution and perfused the entire preparation. The drugs were obtained from Sigma Chemical Co., St. Louis, MO.

The pipettes were back-filled with a solution of the following composition (mM): 110 KCl, 0.5 $CaCl_2$, 5 K-EGTA, 10 K-HEPES, 2 Mg-ATP, 8 glucose. pH was set to 7.8 with KOH, and osmolarity was adjusted to 235 mOsm with glucose.

Electrophysiological recordings

The tight-seal whole-cell recording technique was used. The cells were not cleaned before patching. Seal resistances were usually greater than 5 GΩ. Patch pipettes were made from borosilicate glass capillary tubing (OD 1.5 mm, ID 0.87 mm) with internal filament. The pipettes, not fire-polished, had tip resistances of 5-8 MΩ when filled with a standard internal solution. The series resistance in these experiments was 19.1 ± 0.37 MΩ (n=285).

Histological processing

During whole-cell recording the PG cells were labelled with Lucifer Yellow, dipotassium salt (Sigma), which was added to the pipette solution at a concentration of 4 mg/ml. After fixation with formaldehyde, the slices were mounted with glycerol and observed within a week.

Data storage and analysis

PG cell recordings were routinely stored on VHS VCR tape for off-line analysis. Stimulation and data acquisition were controlled by a PC-486 computer using 12 bit A/D - D/A converters (Digidata 1200, Axon Instruments). Prior to acquisition, the signals were filtered at 5 KHz by a low-pass 4-pole Bessel filter and digitised with sample times ranging from 10 to 100 µs. Data are given as mean ± S.E.M.

RESULTS

1. *Cell identification.* - Under direct visual control, cells were identified and accessed with the recording electrodes. A total of 203 periglomerular cells was recorded, and identified by their location deep within the glomerular layer. At this level, only PG cells are described in the frog (15). Moreover, 34 cells were dye filled with Lucifer Yellow to correlate physiological and morphological features. Typically these cells were characterised by a small cell body (5-6 µm), located outside the glomeruli, and by well developed dendritic tufts, with numerous spines, finely arborised within nearby glomeruli. A well discernible axon could occasionally be seen. The general aspect of these cells is similar to that described by Cajal (1904) and confirmed by more modern studies (12, 13, 16). In line with a recent

report in the rabbit (4), most of the cells did not send a unique dendrite arborising into a single glomerulus, but usually originated two or more dendrites arborising in different adjacent glomeruli or a single dendritic process branching into multiple tufts.

2. *Membrane properties.* - Membrane properties of PG cells were tested in both current- and voltage-clamp modes. Membrane capacity averaged 6.0 ± 0.13 pF (n=203) and resting membrane potential was about -40/-45 mV. Most of the cells had rich spontaneous activity at rest and were capable of firing fast, TTX sensitive action potentials.

Input resistance, calculated from the slope of the I-V relationship at hyperpolarised potentials, where no active currents could be elicited, averaged 913 ± 33 MΩ (n=92), consistent with the value measured in PG cells of the neonatal rabbit (4).

All cells displayed a pronounced outward rectification in their current-voltage (I-V) relationship.

Sustained depolarising currents greater than 20 pA evoked overshooting action potentials in all cells. In most of the cells, further depolarisation resulted in a nearly regular train of action potentials, whose frequency increased with the amplitude of the injected current up to about 50 spike/s. However, in approximately 25% of the cases, the cells responded with a single action potential to steady depolarisations of different amplitudes. Neither the anatomy nor the cable properties of these cells were significantly different from those of the other PG cells.

The voltage trajectories in response to hyperpolarising pulses were further analysed following the "peeling off" procedure (3) and (10) to determine the electrotonic compactness of the structure: in nearly all the cells studied (n=82) the trajectories accurately followed a single exponential, and only in two cases could more than one exponential be separated. According to the model, the behaviour exhibited suggests that the neurone under study behaved as a single, nearly isopotential compartment.

For the sake of comparison, in this preparation mitral cells had a membrane capacity of 13.2 ± 0.77 pF (n=36), an input resistance of 457 ± 51 MΩ (n=9), and were capable of sustaining repetitive firing at frequencies up to 90 spikes/s (personal unpublished observation).

3. I_{Na}. - The I_{Na} (Fig. 1A) has been studied in isolation preventing Ca^{2+} inflow with Cd^{2+} 100 µM and the K^+ currents by equimolar ionic substitution of K^+ ions with Cs^+ in the intracellular solution. The Na^+ currents were routinely generated by a series of depolarising steps extending from -30 to +50 mV from a holding potential of -70 mV. Under these ionic conditions any active outward current is completely suppressed. The I_{Na} is activated at membrane potentials more positive than -20 mV; its peak amplitude increases steeply with voltage becoming maximum for test pulses at 0 mV, and then declines approaching the Na^+ equilibrium potential.

This current is completely and reversibly blocked by TTX 0.3 μM, and no evidence has been found for a TTX-resistant component (11). The I_{Na} *I-V* relationship for an eight-neurone sample over a range of voltage pulses extending from -50 to +50 mV had a maximum of about 700 pA at 0 mV. Peak Na^+ conductance was calculated from the mean current values using the equation $g_{Na}(V) = g_{Na\ max} / (V-E_{Na})$, where V is the membrane potential and E_{Na} the Na^+ reversal potential as calculated by extrapolating the *I/V* relationship on the abscissa axis.

g_{Na} has a sigmoidal dependence on membrane potential; it exhibits a threshold at about -40 mV, then rises sharply with small changes in membrane potential increasing e-fold per 6.75 mV of potential, and saturates around +20 mV. On average, the maximal somatic open-channel conductance, $g_{Na\ max}$, was 9.7 nS and the midpoint was observed at -17.4 mV.

The rising phase of the current could be best fitted with a third-order exponential at most of the voltages tested. The activation time constant proved to be remarkably rapid: for our seven-neurone sample τ_a ranged from 259 μs at -20 mV to 47 μs at +50 mV. Once activated the I_{Na} declines to zero following a single exponential with a time constant of about 1 ms, scarcely dependent on voltage in the range of potentials tested.

The steady-state voltage-dependence of Na^+ inactivation, h_∞ was studied by evaluating the non-inactivating fraction of the Na^+ current as a function of membrane potential. The I_{Na} was measured in each experiment at a constant test voltage of 0 mV after 250 ms preconditioning to various potentials and plotted after normalisation to the maximum current evoked with hyperpolarisation. The steady-state inactivation curve $h_\infty(V)$ thus obtained has a sigmoidal dependence on voltage, which can be fitted by the equation:

$$h_\infty = (1+exp[(V+ 56)/8.1])^{-1}$$

The non-inactivated fraction of I_{Na} falls virtually to zero at -30 mV and is maximal at about-90 mV.

4. I_{Ca}. - A distinct inward current could be observed under conditions where the Na^+ inflow was prevented by 0.3 μM TTX and the K^+ currents were blocked with TEA 20 mM. Identified as Ca^{2+}-current on the basis of its kinetic and pharmacological profile, this current (Fig. 1B) has been studied both from the flow of Ca^{2+} ions and after ion substitution of Ca^{2+} with Ba^{2+}. The I_{Ca} I-V relationship over a range of voltage pulses extending from -40 to +40 mV peaked at -20 mV. The neurones were bathed in 3 mM external Ca solution, maintained at a holding potential of -50 mV, and depolarised in 10 mV steps from -40 to +40 mV.

Both calcium and barium ions were effective as charge carriers eliciting this inward current, but the amplitude of the currents and the *I/V* relationships after ion substitution were significantly different. Substitution of equimolar Ca^{2+} with Ba^{2+}

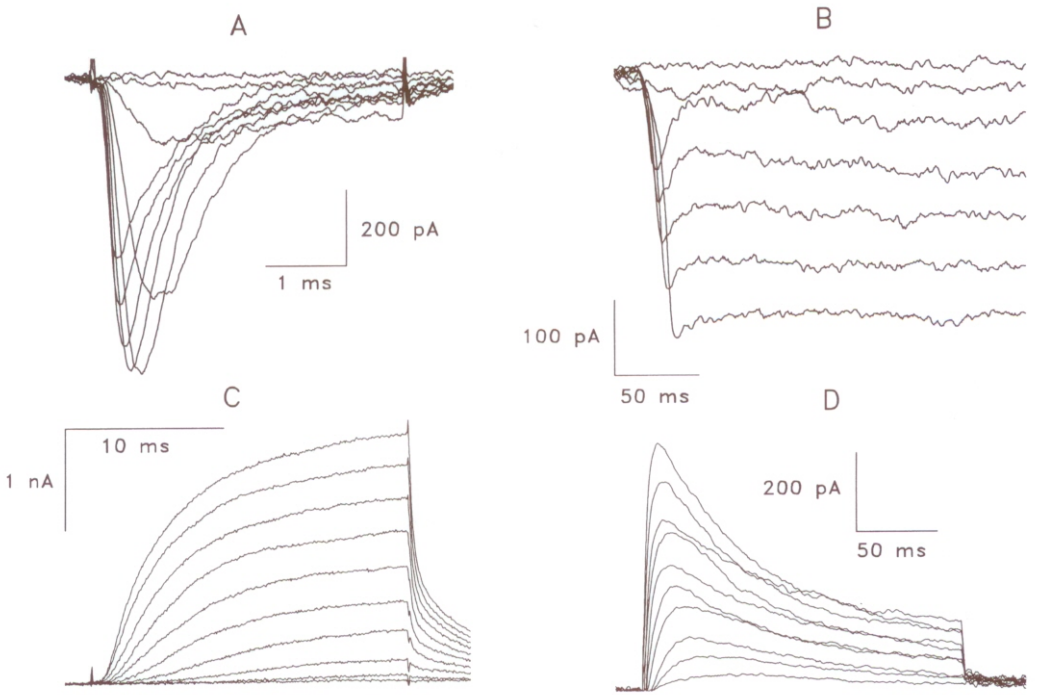

Fig. 1. - *Voltage-dependent currents found in periglomerular cells of the frog olfactory bulb.*

A, fast sodium current; family of tracings evoked by depolarising steps to potentials ranging from -50 to +40 mV after 200 ms preconditioning at -130 mV. *B*, calcium current; tracings evoked by depolarising steps to potentials from -50 to +30 mV from resting membrane potential (-60 mV). *C*, delayed rectifier potassium current; tracings obtained by depolarisations ranging from 40 to +50 mV after 200 ms preconditioning at -50 mV. *D*, fast transient outward current (I_A); tracings evoked by depolarising steps to potentials ranging from -40 to +50 mV after 250 ms preconditioning at -130 mV.

increased the amplitude of the inward currents and shifted *I/V* curves by 10-15 mV towards more negative potentials.

The I_{Ca} was blocked by a low concentration of Cd^{2+} ions (100 μM) and by nifedipine 5 μM. The potency of the dihydropyridine was greatly increased by a preconditioning depolarising pulse: 5 μM nifedipine nearly halved the amplitude of the I_{Ca}, but the same concentration completely blocked the Ca^{2+} current when, under the same conditions and in response to the same protocol, the membrane was manually preconditioned to -10 mV for 30 s. Thr nifedipine block developed slowly after the Ca channels were fully open (τ_{on} of block 100 ms) and reached almost steady-state conditions at the end of a 300 ms pulse. The DHP block had no significant effects on I_{Ca} activation and was incompete even during pulses lasting 800 ms. Complete recovery of the Ca-current required about 2 min wash-out.

5. *I_A*. - The I_A (Fig. 1D) was isolated by making a point-by-point subtraction of the tracings obtained by depolarizing the membrane directly from a potential at which the A channels are inactivated (-50 mV) from the tracings obtained at the same test voltage after removal of A inactivation with a preconditioning pulse. Pharmacological separation of the I_A, obtained by using the delayed K^+ channel blocker TEA, was also possible: TEA proved very effective and selective in suppressing the delayed outward currents, thus allowing the separation of virtually pure A-current tracings. The 4-amino-pyridine (4 mM) had a relatively specific inhibitory effect on the I_A, contrary to what has been demonstrated in other preparations, where the 4-AP equally affected fast transient and delayed outward currents (1, 2).

The A-current activates at potentials more positive than -50 mV and gives a mean maximal amplitude of 370 pA at 0 mV and of 900 pA at +40 mV.

The order of the I_A activation kinetics was computed by fitting the onset of the current with exponentials raised to different integer powers; the lowest power allowing satisfactory fits over the entire voltage range was 3, and therefore a third-order activation kinetics was adopted.

The A-current peaks in 10-15 ms; the activation time constant, τ_a, at different voltages was extracted by fitting a third-order exponential to the rising phase of the current. Its dependence upon voltage has been studied in the range of potentials from -30 to +40 mV.

The steady-state inactivation of the A-current was studied by depolarising the membrane to a fixed test potential (+10 mV) after 250 ms preconditioning at potentials ranging from -130 to -40 mV; the A-current was isolated with the subtraction procedure. Peak currents relative to their greatest measured value were plotted *versus* the conditioning voltage level. The steady-state inactivation process thus obtained could be described by a sigmoid curve derived from the Boltzmann distribution, with a midpoint at -78.8 ± 1.3 mV and slope factor of 8.4 ± 0.14 mV for an e-fold potential change.

The inactivation time constant of the A-current, τ_{ha}, measured from the decay of the current, showed little or no voltage-dependence in the voltage range from -30 to +40 mV and had a mean value of 60 ms.

6. *I_{KV}*. - The delayed potassium current (Fig. 1C) was isolated by depolarising the membrane from a holding potential of -50 mV, where the fast transient current is fully inactivated. The current activated following a second-order kinetics at potentials more positive than -40 mV, and was much larger at the end of a 25 ms depolarising pulse than the A-current at the corresponding potential: we have measured a mean value of 500 pA at 0 mV and 1600 pA at +40 mV (n=12).

The activation time constant was voltage-dependent and proved to be relatively fast for this type of current: 8.3 ms at -20 mV and 3 ms above +40 mV.

DISCUSSION

The objective of this work was to gather a series of data concerning the basic properties of periglomerular cells, interneurones which are likely to play a key role in the early stages of elaboration of olfactory information. Knowledge of the basic membrane and electrophysiological properties of these cells is an essential prerequisite for understanding how sensory information is processed in the olfactory bulb.

A first observation is that these cells are capable of generating TTX-sensitive action potentials that can be elicited at rest by the spontaneous synaptic activity or, under current-clamp conditions, by steady depolarising pulses. A well-developed somatic Na^+ current has been isolated and kinetically characterised and, one must, therefore, conclude that frog PG cells do have a prominent population of Na^+ channels. The presence of Na^+-channels, and more generally the capability of PG cells to sustain Na-action potentials, has recently been questioned by researchers working on neonatal rabbit OB (4). This point has a certain relevance since if these cells do not fire, it must be assumed that they transmit signals only electrotonically, as do the horizontal cells of the retina to which they have been compared (17). However, the presence of Na^+ -channels, and more specifically the capability of these cells to generate conventional action potentials, suggests that the role of these inhibitory interneurons, located at the inlet of the sensory information, cannot easily be assimilated to that of horizontal cells in the retina.

The properties of the potassium currents reveal a dominance of the delayed rectifier potassium current over the I_A. In fact, not only is the absolute amplitude of I_{KV} larger than that of the trasient outward current, but its activation time constants also compete with those of the A-current, relegating the latter to a marginal role.

SUMMARY

Whole-cell patch clamp recording techniques were applied to periglomerular (PG) cells in slices of the frog olfactory bulb (OB) preparation to study the basic electrical properties of these inhibitory interneurons. The cells were intracellularly stained with Lucifer Yellow for precise identification. Under current-clamp conditions PG cells showed rich spontaneous excitatory synaptic activity at rest, usually leading to overshooting, TTX-sensitive action potentials. The passive cable properties of the cell membrane have been carefully characterised. Depolarisation of this neurone under voltage-clamp conditions activated a complex pattern of current flow, that has been dissected into its main components. The currents have been isolated resorting to their different kinetic and pharmacological properties. Four main voltage dependent ionic currents have been isolated, two inward currents, I_{Na} and I_{Ca}, and two outward currents carried by potassium ions, one fast

transient, I_A-type and another similar to the delayed rectifier type. These currents have been characterised kinetically and pharmacologically. The functional implications of their properties are discussed.

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REFERENCES

1. Bardoni, R. and Belluzzi, O. Kinetic study and numerical reconstruction of A-type current in granule cells of rat cerebellar slices. *J. Neurophysiol.*, **69**: 2222-2231, 1993.
2. Belluzzi, O., Sacchi, O. and Wanke, E. A fast transient outward current in the rat sympathetic neurone studied under voltage-clamp conditions. *J. Physiol., Lond.*, **358**: 91-108, 1985.
3. Brown, T. H., Perkel, D. H., Norris, J. C. and Peacock, J. H. Electrotonic structure and specific membrane properties of mouse dorsal root ganglion neurons. *J. Neurophysiol.*, **45**: 1-15, 1981.
4. Bufler, J., Zufall, F., Franke, C. and Hatt, H. Patch-clamp recordings of spiking and nonspiking interneurons from rabbit olfactory bulb slices: Membrane properties and ionic currents. *J. Comp. Physiol. A*, **170**: 145-152, 1992
5. Cajal, S., Ramon, Y. *La Textura del Sistema Nervioso del Hombre y los Vertebrados*, Madrid, Moya, 1904.
6. DeVries, S. H. And Baylor, D. A. Synaptic circuitry of the retina and olfactory bulb. *Cell*, **72**: 139-149, 1993.
7. Duchamp-Viret, P., Duchamp, A. and Chaput, M. GABAergic control of odor-induced activity in the frog olfactory bulb: electrophysiological study with picrotoxin and bicuculline. *Neuroscience*, **53**: 111-120, 1993.
8. Edwards, F. A., Konnerth, A., Sakmann, B. and Takahashi, T. A thin slice preparation for patch clamp recordings from neurones of the mammalian central nervous system. *Pflügers Arch., Eur. J. Phisiol.*, **414**: 600-612, 1989
9. Howell, B. J., Baumgardner, F. W., Bondi, K. and Rahn, H. Acid-base balance in cold-blooded vertebrates as a function of body temperature. *Amer. J. Physiol.*, **218**: 600-606, 1970.
10. Johnston, D. Passive cable properties of hippocampal CA3 pyramidal neurons. *Cell. Mol. Neurobiol.*, **1**: 41-55, 1981.
11. Kostyuk, P. G., Veselovsky, N. S. and Tsyndrenko, A. Y. Ionic currents in the somatic membrane of rat dorsal root ganglion neurons I. Sodium currents. *Neuroscience*, **6**: 2423-2430, 1981.
12. Pinching, A. J. and Powell, T. P. S. The neuron types of the glomerular layer of the olfactory bulb. *J. Cell Sci.*, **9**: 305-345, 1971.
13. Pinching, A. J. and Powell, T. P. S. The neuropil of the glomeruli of the olfactory bulb. *J. Cell Sci.*, **9**: 347-377, 1971.
14. Reeves, R. B. Role of body temperature in determining the acid-base state in vertebrates. *Fed. Proc.*, **28**: 1204-1208,1969.
15. Scalia, F., Gallosius, G. and Roca, S. A note on the organization of the amphibian olfactory bulb. *J. Comp. Neurol.*, **305**: 435-442, 1991.

16. SCHNEIDER, S. P. and MACRIDES, F. Laminar distributions of interneurons in the main olfactory bulb of the adult hamster. *Brain Res. Bull.*, **3**: 73-82, 1978.
17. SHEPHERD, G. M. and KOCH, C. Introduction to synaptic circuits. Pp. 3-31. In: SHEPHERD, G. M. (Ed.), *The Synaptic Organization of the Brain*, Oxford, University Press, 1990.
18. WELLIS, D. P. and SCOTT, J. W. Intracellular responses of identified rat olfactory bulb interneurons to electrical and odor stimulation. *J. Neurophysiol.*, **64**: 932-947,1990.