Electrophysiological Properties of Cholinergic and Noncholinergic Neurons in the Ventral Pallidal Region of the Nucleus Basalis in Rat Brain Slices

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Bengtson, C. Peter and Peregrine B. Osborne. Electrophysiological properties of cholinergic- and noncholinergic neurons in the ventral pallidal region of the nucleus basalis in rat brain slices. J. Neurophysiol. 83: 2649–2660, 2000. The ventral pallidum is a major source of output for ventral corticobasal ganglia circuits that function in translating motivationally relevant stimuli into adaptive behavioral responses. In this study, whole cell patch-clamp recordings were made from ventral pallidal neurons in brain slices from 6- to 18-day-old rats. Intracellular filling with biocytin was used to correlate the electrophysiological and morphological properties of cholinergic and non-cholinergic neurons identified by choline acetyltransferase immunohistochemistry. Most cholinergic neurons had a large whole cell conductance and exhibited marked fast (i.e., anomalous) inward rectification. These cells typically did not fire spontaneously, had a hyperpolarized resting membrane potential, and also exhibited a prominent spike afterhyperpolarization (AHP) and strong spike accommodation. Noncholinergic neurons had a smaller whole cell conductance, and the majority of these cells exhibited marked time-dependent inward rectification that was due to an h-current. This current activated slowly over several hundred milliseconds at potentials more negative than ~80 mV. Noncholinergic neurons fired tonically in regular or intermittent patterns, and two-thirds of the cells fired spontaneously. Depolarizing current injection in current clamp did not cause spike accommodation but markedly increased the firing frequency and in some cells also altered the pattern of firing. Spontaneous tetrodotoxin-sensitive GABAA- mediated inhibitory postsynaptic currents (IPSCs) were frequently recorded in noncholinergic neurons. These results show that cholinergic pallidal neurons have prominent spike afterhyperpolarization (AHP) and strong spike accommodation. Noncholinergic ventral pallidal neurons have large h-currents that could have a physiological role in determining the rate or pattern of firing of these cells.

INTRODUCTION

The ventral pallidum was first identified in the rat as a subcommissural extension of the globus pallidus (Heimer and Wilson 1975). By virtue of the dense projection it receives from the nucleus accumbens (Heimer et al. 1991; Nauta et al. 1978; Zahm and Heimer 1990), the ventral pallidum is a major source of output for ventral corticobasal ganglia circuits that are organized in parallel to dorsal basal ganglia-thalamocortical pathways involved in sensorimotor and associative functions (Alexander and Crutcher 1990; Alexander et al. 1986; Groenewegen et al. 1990; Parent and Hazrati 1995; Smith et al. 1998). In contrast to their dorsal counterparts, ventral striatopallidal pathways receive “limbic” afferent input from the cortex, hippocampus, and amygdala and are considered to play a role in translating motivationally relevant stimuli into adaptive behavioral responses (Kalivas 1993; Kalivas et al. 1999; LeMoal and Simon 1991; Mogenson et al. 1990).

The ventral pallidum in the rat contains two main populations of neurons. The majority are GABA neurons (i.e., immunoreactive for glutamic acid decarboxylase) (Gritti et al. 1993), which have a somatodendritic morphology that is shared with the main cell group found in the dorsal pallidum (Iwahori and Mizuno 1981; Millhouse 1986; Nambu and Llinás 1997; Pang et al. 1998; Park et al. 1982). Intracellular filling has separated these neurons into those with simple axons and few intranuclear branches, or those with complex axons and extensive intranuclear arborizations, which may correspond to projection and interneurons, respectively (Kita and Kita 1994; Pang et al. 1998). Targets supplied by ventral pallidal projection neurons include both structures that are traditionally included in the “extrapyramidal motor system” (entopeduncular nucleus, substantia nigra, and mesencephalic locomotor area) and “limbic” circuits (ventral tegmental area, mediodorsal nucleus of the thalamus, medial prefrontal cortex, basolateral amygdala, and entorhinal cortex) (Bevan et al. 1996; Groenewegen et al. 1993; Haber et al. 1985, 1993; Maurice et al. 1997; O’Donnell et al. 1997; Zahm and Heimer 1990; Zahm et al. 1996). About 23% of the cell population in the rat ventral pallidum are neurons that belong to the magnocellular forebrain cholinergic system (Gritti et al. 1993), which has been studied extensively in relation to its function in cognitive processing and putative role in disease processes such as schizophrenia and Alzheimer’s disease (e.g., Détari et al. 1999; Page and Sf orniev 1996). In the rat these choline acetyltransferase (ChAT) immunoreactive neurons intersperse with noncholinergic neurons through a contiguous volume of basal forebrain that extends through the mediodorsal septum, nucleus of the diagonal band of Broca, substantia innominata, and the pallidal complex (Gritti et al. 1993; Grove 1988; Mesulam et al. 1983; Rye et al. 1984; Schwaber et al. 1987). The cholinergic neurons in the ventral pallidum also receive synaptic input from the amygdala (Zaborszky et al. 1984) and nucleus accumbens (Bolam et al. 1986; Zaborszky and Cullman 1992) and project to the cerebral cortex, amygdala, and lateral habenula (Carlson et al. 1985; Ingham et al. 1985; McKinney et al. 1983; Mesulam et al. 1983; Parent 1979).
Three types of neuron have been identified in the ventral pallidum by intracellular microelectrode recording in vivo (Lavin and Grace 1996). These were distinguished by electrophysiological criteria relating to action potential discharge patterns and responses to afferent fiber stimulation. Although no anatomic characterization was done in this study, two of these types (A and B) were tentatively identified as noncholinergic neurons based on electrophysiological similarities to neurons that have been morphologically characterized in the globus pallidus (Kita and Kitai 1994; Nambu and Llinás 1997) and entopeduncular nucleus (Nakanishi et al. 1991). More recently, ventral pallidal neurons with similar patterns of firing identified by extracellular recording in vivo, have been shown to be noncholinergic by using juxtanuclear staining in combination with immunohistochemistry for ChAT (Pang et al. 1998). The third type of neuron (type C) identified in ventral pallidum may be cholinergic. The strongest line of evidence supporting this conclusion is that these neurons fire in bursts that are associated with a low-threshold calcium spike. This pattern of firing is characteristic of magnocellular cholinergic neurons as shown by many studies that have recorded from immunohistochemically identified cells in other forebrain areas (rat: Gorelova and Reiner 1996; Markram and Segal 1990; Sim and Allen 1998; guinea-pig: Alonso et al. 1996; Griffith and Matthews 1986; Khateb et al. 1992, 1993, 1995).

Our aim in this study was to integrate the findings of previous studies of pallidal neurons by correlating the electrophysiological and morphological properties of identified noncholinergic and cholinergic neurons. To do this, patch-clamp techniques were used to record from ventral pallidal neurons in brain slices. The cells were intracellularly filled and later immunolabeled for ChAT. Voltage-clamp recording was used to characterize the expression of inward rectification, which has been found to be diagnostic for cholinergic neurons situating elsewhere in the rat magnocellular forebrain complex (Gorelova and Reiner 1996; Markram and Segal 1989; Sim and Allen 1998).大脑切片的准备和记录

Transverse brain slices (200–250 μm thick) containing the ventral pallidum were prepared from 6- to 18-day-old Wistar rats that had been anesthetized by halothane inhalation and decapitated. The slices were submerged in ice-cold artificial cerebrospinal fluid (ACSF, containing in mM: 125 NaCl, 2.5 KCl, 2 CaCl2, 1.25 NaH2PO4, 1 MgCl2, 25 glucose, and 25 NaHCO3) equilibrated with 95% O2-5% CO2, and cut with a tissue slicer (Campden Instruments). The slices were kept for recording, in a holding chamber, submerged in ACSF at RT (25°C).

Electrophysiological recordings were made from slices continuously superfused with ACSF (32°C) that were placed in a chamber (0.75 ml volume) mounted on a fixed-stage upright microscope (Zeiss Axioskop). Differential interference contrast/infrared (DIC/IR) optics and a charge-coupled device camera (Javelin) enabled neurons to be seen on a video monitor. Patch-clamp recordings were made in the whole cell configuration using an Axopatch 1D amplifier (Axon Instruments). The electrodes (4–8 MΩ) were filled with a potassium methylsulphate based solution (containing, in mM, 135 KCl, 5 NaCl, 10 HEPES, 2 MgATP, and 0.25 NaGTP) that was used to minimize changes in the action potential waveform with current-clamp recording (Sah and Isaacson 1995; Velumian et al. 1997). The solution also included 0.3% bovine serum albumin (Sigma). The solution was adjusted to a pH of 7.4 with KOH, and an osmolarity of 270–290 mosmol/l. Corrections were made for a calculated liquid junction potential of the solution of –10 mV (Barry 1994). All data were obtained from recordings where the series resistance was maintained below 20 MΩ, which was monitored at regular intervals throughout each experiment.

Intracellular filling and immunohistochemistry

Brain slices containing biocytin-filled neurons were fixed overnight (at 4°C) in 4% paraformaldehyde in phosphate buffer (PB: 0.1 M, pH 7.4), rinsed in PB, and placed for 4 days in 0.3% triton X-100 and 0.01% sodium azide in PB. After being rinsed in PB and placed for 60 min in 10% normal horse serum and 0.1% triton X-100 in PB, the slices were then incubated for 24 h at RT in a primary antiserum raised in goat against choline acetyltransferase (ChAT, 1:500, Chemicon). The slices were rinsed in PB and incubated for 1 h at RT in either 1) Cy3-conjugated donkey anti-goat IgG (1:1,000, Jackson Immunoresearch Laboratories) and FITC-conjugated streptavidin (1:1,000, Sigma Aldrich) or 2) FITC-conjugated rabbit anti-goat IgG (1:400, Sigma Aldrich) and Cy3-conjugated streptavidin (1:2,000, Sigma Aldrich). All antisera and streptavidin-conjugates were diluted in 1% normal horse serum and 0.3% triton X-100 in PB. An additional group of biocytin-filled neurons in 10 brain slices were single-stained using a Vectastain Elite ABC kit (Vector Laboratories) using 3’3’-diaminobenzidine as the chromagen.

Drugs used

Barium chloride, bicuculline, cesium chloride (Sigma); tetrodotoxin citrate (Tocris); and ZD7288 (Research Biochemicals) were made up as stock solutions in deionized water. Picrotoxin (Research Biochemicals) was made up as a stock solution in DMSO. All drugs were diluted in artificial cerebrospinal fluid (ACSF) and applied by superfusion. The final concentration of DMSO in the superfuse was ≤0.1%, which had no direct effects.

RESULTS

Identification and morphological characteristics of cholinergic and noncholinergic ventral pallidal neurons

In this study we successfully recovered 49 biocytin-filled cells that had previously been electrophysiologically characterized in brain slices. Thirty-one of these were also subjected to choline acetyltransferase (ChAT) immunohistochemistry, which identified 13 ChAT-immunopositive and 18 ChAT-immunonegative neurons. Another eight small cells were filled with a group of biocytin-filled neurons in 10 brain slices were single-stained using a Vectastain Elite ABC kit (Vector Laboratories) using 3’3’-diaminobenzidine as the chromagen.
Measurements of soma size (made from the video monitor at the time of recording) were used to construct the frequency histogram in Fig. 3, which shows that cholinergic neurons had the largest somata (29 ± 1.5 µm, mean ± SE, n = 12) of the neurons we recorded from. The morphology of a representative ChAT-positive neuron is shown by the camera lucida drawing on the left of Fig. 4. These neurons typically had multipolar somata with four to seven thick, tapering primary dendrites that extended <100 µm before branching. In six fills, spines were visible on the soma, as well as forming a moderate covering on primary and higher order dendrites. In five cases, filled axons projected 200–1,000 µm from the soma in a ventral (n = 3) or medial (n = 2) direction, one of which had a short axon branch.

Three examples of filled ChAT-immunonegative neurons are shown by the pairs of photomicrographs in Fig. 1B. Recordings were made from these noncholinergic neurons at all rostrocaudal levels of the ventral pallidum (Fig. 2). These cells had smaller somata than cholinergic neurons (Fig. 3; 14 ± 1.4 µm; range: 5–24 µm, n = 18; P < 0.001, Mann-Whitney U, n = 18, 12). A camera lucida drawing of a representative noncholinergic neuron is shown on the right of Fig. 4. These neurons typically had triangular somata with two to four thick tapering primary dendrites that extended less than 100 µm before branching. Spines were visible on both primary, and higher order dendrites in 13 of the fills. In eight cases, axons originating from primary dendrites that extended 200–1,200 µm within the 200-µm-thick brain slices. In three cases, axon branches were observed. The orientation of the projecting axons was ventral (n = 2), dorsomedial (n = 3), or both dorsal and ventral after bifurcating close to the soma (n = 2).

Eight cells were filled that had characteristics more typical of glial cells (Fig. 4), particularly oligodendrocytes (e.g., Verkadakis and Roots 1995). They had small somata (5.9 ± 0.5 µm; range: 4–8 µm) and multiple stubby primary processes, which branched to form an extensive tertiary arborization that failed to extend beyond 100 µm of the soma. Consistent with this identification, we were unable to evoke action potentials in these cells in current clamp.
Cholinergic and noncholinergic ventral pallidal neurons express different inwardly rectifying currents

Only two of the ventral pallidal neurons we filled did not exhibit inward rectification in voltage clamp in response to hyperpolarizing voltage steps from a holding potential of −60 mV. Representative recordings from a cholinergic and a noncholinergic neuron are shown in Fig. 5, along with the corresponding current-voltage (I-V) relationships for the quasi-instantaneous and steady-state currents measured respectively at the time points shown by the open and closed circles. In cholinergic neurons the major inwardly rectifying current had a time constant of 0.5–1 ms, whereas in noncholinergic neurons the major current activated slowly over several hundred milliseconds (τ = 600 ± 230 ms at −100 mV, and 290 ± 80 ms at −120 mV; Fig. 5B).

The values for G_{HOLD} (slope conductance at −60 to −80 mV), G_{INST} (“instantaneous” conductance at −100 to −120 mV), and G_{SS} (“steady-state” conductance at −100 to −120 mV) were calculated from I-V relationships and are summarized in Table 1. In cholinergic neurons (n = 13) the conductance of the fast inward rectifier current (equal to G_{INST}−G_{HOLD}) was 4.5 ± 0.8 nS, and the conductance of the time-dependent inward rectifier current (equal to G_{SS}−G_{INST}) was 0.6 ± 0.2 nS. In noncholinergic neurons (n = 18), the instantaneous inward rectifier conductance was smaller (0.7 ± 0.2 nS; Mann-Whitney U, P < 0.001, n = 13, 18), but the time-dependent current was larger (2.1 ± 0.4 nS, P < 0.001, n = 13, 18) and contributed to a greater proportion of the total conductance (41% cf. 5% of G_{SS}). These data are summarized in Fig. 6 in which the instantaneous and time-dependent inward rectifier conductances are plotted against the resting conductance, G_{HOLD}. Figure 6A shows that in 10 of the 13 cholinergic neurons the large conductances of the fast inward rectifier and holding currents separate these neurons from the remainder of the population. One noncholinergic neuron also fell in this cluster, which based on its size (20 μm) and pattern of firing in current clamp (see next section) could represent an immunohistochemical false-negative. Figure 6B shows that in 14 of 18 noncholinergic neurons the conductance of the time-dependent inward rectifier was equal to or greater than the conductance of the holding current. Only one cholinergic neuron fell into this group. Two noncholinergic neurons (□, Fig. 6) did not exhibit rectification.

In separate experiments, we tested the effects of channel blocking drugs applied in the extracellular solution. Fast inward rectifier currents were completely blocked by 200 μM barium (n = 4; Fig. 7A). Time-dependent inward rectifier currents were not affected by barium (n = 6) but were completely blocked by cesium ions (n = 7; Fig. 7B) or by 30 μM ZD 7288 (n = 2), which has been reported to be a selective blocker of h-current channels (BoSmith et al. 1993).

Large, frequent spontaneous postsynaptic currents (PSCs) were frequently observed in neurons that had strong time-dependent rectification (Fig. 8). These appeared to be caused by the activity of other neurons in the slices as they were abolished by 1 μM tetrodotoxin (n = 7). In most cases the PSCs were blocked by 60 μM bicuculline (n = 11) or 100 μM picrotoxin (n = 8) and reversed polarity between −50 and −60 mV. However, in three neurons, spontaneous PSCs were recorded that were not blocked by picrotoxin and reversed polarity at potentials negative to −80 mV. In a further three
neurons, apparent excitatory postsynaptic currents (EPSCs) were recorded that reversed polarity at potentials positive to −30 mV. Spontaneous PSCs were also observed in cholinergic neurons but were less frequent and were not analyzed in this study.

Cholinergic and noncholinergic neurons show different patterns of action potential firing

Previous in vivo and in vitro studies have used intracellular microelectrodes to make record membrane potential recordings in dorsal and ventral pallidal neurons (Kita and Kitai 1994; Lavin and Grace 1996; Nambu and Llinás 1994, 1997) To further characterize the properties of ventral pallidal neurons and facilitate comparison with these earlier studies, current-clamp recordings were made from eight cholinergic neurons and nine noncholinergic neurons.

Cell-attached recording identified spontaneous firing in only 2 of 13 cholinergic neurons. Immediately after establishing whole cell recording, these cells had a mean resting membrane potential of −65 ± 2.7 mV (n = 13). In current-clamp, hyperpolarizing current injection produced inward rectification, as shown in Fig. 9A. Depolarizing current injection evoked one to two action potentials near to threshold of −41 ± 1 mV (n = 8), which accommodated during 1- to 1.4-s pulses (Fig. 9A and C). The spikes typically arose off a depolarizing plateau potential and had a mean amplitude of 60 ± 9 mV and a half-width of 1.3 ± 0.1 ms. They were followed by a large afterhyperpolarization (AHP) that was 23 ± 1.7 mV in amplitude and 288 ± 24 ms in duration. Further depolarizing current injection increased the number of action potentials from 1–2 spikes/s to 2–7 spikes/s, and the instantaneous frequency (between the 1st spike pair) to maxima of 6–13 Hz. Spike frequency accommodation was maintained during a 1-s pulse in seven of eight neurons. One ChAT-immunonegative neuron exhibited a pattern of action potential firing that was indistinguishable from the ChAT immunopositive neurons. This cell had a large h-current (5.2 nS) but had resting and inward rectifier conductances (8.7 and 3.5 nS, respectively) that were similar to cholinergic neurons.

Spontaneous firing was seen in 10 of 18 noncholinergic neurons with cell-attached recording. Immediately after establishing whole cell recording, the resting membrane potential of the cells was −53 ± 2.8 mV and less polarized than in cholinergic neurons (Mann-Whitney U, P = 0.006, n = 13, 18). Current-clamp recordings were made from eight neurons, four of which fired spontaneous action potentials in continuous or irregular patterns. Hyperpolarizing current injection produced a depolarizing sag that developed slowly during a 1-s pulse at potentials negative to −80 mV, as shown in Fig. 9B. Depolarizing current injection evoked action potentials that showed little or no spike frequency adaptation. Action potentials measured near threshold (−45 ± 5 mV, n = 9) had a mean amplitude of 70 ± 3.5 mV and half-width of 1.2 ± 0.2 ms and were followed by a prominent AHP that was −18 ± 2.6 mV in amplitude (n = 8) and 133 ± 11 ms (n = 8) in duration. Graded increases in the depolarizing currents increased the spike number from around 1–6 spikes/s to 7–29 spikes/s and the instantaneous frequency of the first spike pair from 1–2 Hz to rates over 15 Hz (Fig. 9D).
**DISCUSSION**

To our knowledge this is the first electrophysiological study of the ventral pallidum to be performed in vitro. Patch-clamp recordings were made from noncholinergic and cholinergic neurons, which were identified by intracellular filling and immunolabeling for ChAT. This allowed us to use both voltage- and current-clamp recording to define the electrophysiological properties of these two neuron types.

**Inward rectification in pallidal neurons**

Prior to this study, voltage-clamp recording had not been used to characterize pallidal neurons. We found that cholinergic and noncholinergic neurons exhibited different types of inward rectification. In cholinergic neurons, there was a large inward rectifier current that had fast activation kinetics and was blocked by barium, which suggests that it was carried by inward rectifier potassium channels (Gay and Stanfield 1977; Standen and Stanfield 1978; Uchimura et al. 1989). In most noncholinergic neurons (13/18) there was a large h-current that caused time-dependent rectification and was blocked by cesium ions and the selective antagonist ZD7288 (BoSmith et al. 1993; Mayer and Westbrook 1983; Pape 1996). Although h-currents were present in some cholinergic neurons, they contributed to no more than 20% of the whole cell conductance, whereas in the main group of noncholinergic neurons, the h-current comprised 40–60% of the whole cell conductance. A group of six neurons (2 cholinergic and 4 noncholinergic) either did not rectify, or had inward rectifier currents with small conductances. Some of these could represent additional electrophysiological subtypes of pallidal neuron, or alternatively they could be cells damaged by brain slicing.

**TABLE 1. Slope conductance values of cholinergic and noncholinergic ventral pallidal neurons**

<table>
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<tr>
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<th>ChAT-Positive</th>
<th>ChAT-Negative</th>
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<tr>
<td>$G_{\text{hold}}$, nS</td>
<td>6.1 ± 0.7 (13)</td>
<td>2.4 ± 0.4 (18)</td>
</tr>
<tr>
<td>$G_{\text{inst}}$, nS</td>
<td>10.5 ± 1.3 (13)</td>
<td>3.1 ± 0.6 (18)</td>
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<tr>
<td>$G_{\text{ss}}$, nS</td>
<td>11.1 ± 1.2 (13)</td>
<td>5.2 ± 0.9 (18)</td>
</tr>
<tr>
<td>Resting membrane potential, mV</td>
<td>-65.3 ± 2.7 (13)</td>
<td>-52.5 ± 2.8 (18)</td>
</tr>
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Values are means ± SE with number of neurons given in parentheses. $G_{\text{hold}}$ and $G_{\text{ss}}$ were respectively measured between −60 and −80 mV, and −100 and −120 mV from current-voltage relationships of steady-state currents produced by voltage steps (1–1.2 s duration) from a holding potential of −60 mV. $G_{\text{inst}}$ was measured between −100 and −120 from the current-voltage relationship of the instantaneous current seen immediately after the capacitive transient.
filled noncholinergic neurons that did not exhibit inward rectification. The "steady-state" conductance at $G_{SS}$ was calculated from $I-V$ relationships. A: the conductance of the fast inward rectifier current ($G_{INST} - G_{HOLD}$) plotted against $G_{HOLD}$; B: the conductance of the time-dependent inward rectifier current ($G_{SS} - G_{HOLD}$) plotted against $G_{HOLD}$. 2 filled noncholinergic neurons that did not exhibit inward rectification.

FIG. 6. Cholinergic and noncholinergic neurons in the ventral pallidum have different conductances. Values for the slope conductance at $-60$ to $-80$ mV ($G_{HOLD}$), "instantaneous" conductance at $-100$ to $-120$ mV ($G_{INST}$) and the "steady-state" conductance at $-100$ to $-120$ mV ($G_{SS}$) were calculated from $I-V$ relationships. A: the conductance of the fast inward rectifier current ($G_{INST} - G_{HOLD}$) plotted against $G_{HOLD}$; B: the conductance of the time-dependent inward rectifier current ($G_{SS} - G_{HOLD}$) plotted against $G_{HOLD}$. 2 filled noncholinergic neurons that did not exhibit inward rectification.

Llinaés (1994). Although time-dependent inward rectification may disappear in adult ventral pallidal neurons (we used immature rats), we believe that the kinetics and voltage dependence of the h-current in these cells could make it difficult to identify using in vivo recording. In our recording conditions the h-current activated at potentials negative to $-80$ mV with an activation time constant of 600 ms at $-100$ mV, and 290 ms at $-120$ mV. Lavin and Grace (1996) used current pulses around 200 ms in duration that hyperpolarized the neurons to around $-90$ mV as it is difficult to deliver longer, larger pulses in vivo (A. Grace, personal communication). The depolarizing sag that is characteristic of the h-current would be difficult to resolve under these conditions (cf. Fig. 9B). It is interesting to note that one of the two mouse h-current channel proteins that have been recently cloned (Ludwig et al. 1998; Santoro et al. 1998) has slow activation kinetics ($\tau$ activation: 241 ms, cf. 98 ms), similar to the h-current in pallidal noncholinergic neurons.

Anomalous or fast inward rectification is an identifying characteristic of magnocellular cholinergic neurons in other areas of the forebrain cholinergic complex. This has been shown for histochemically identified neurons in the medial septum and nucleus diagonal band of Broca complex in rat (MS/DBB) (Gorelova and Reiner 1996; Markram and Segal 1990) and in more caudally located nucleus basalis neurons in guinea pig (Alonso et al. 1996; Griffith and Matthews 1986; Khatib et al. 1992). It is not clear why anomalous inward rectification was not exhibited by ventral pallidal neurons in vivo (Lavin and Grace 1996), although it is possible that recordings were not made from cholinergic neurons in this study. The difference in age could account for this result, although inward rectification is maintained in other magnocellular cholinergic neurons in both adult rat and guinea pig (Griffith 1988; Griffith and Matthews 1986; Khatib et al. 1992; Markram and Segal 1990). Inward rectification is also exhibited by all three types of globus pallidus neurons identified in adult guinea pig (Nambu and Llinaés 1994).

Electrophysiological properties of pallidal neurons identified by current-clamp recording

Noncholinergic pallidal neurons identified by ChAT-immunohistochemistry have been characterized by extracellular recording in vivo combined with juxtaglomerular labeling (Pang et al. 1998). This study identified two neuron types: cells with axons that had few or no axon branches and fired in random or regular patterns at around 13 Hz (type I), and cells with extensive axonal arborisations that fired at a faster rate of 36 Hz (type II). Ventral pallidal neurons have also been classified according to electrophysiological criteria by intracellular recording in vivo without reference to morphological or histochemical characteristics (Lavin and Grace 1996). Three types of neuron were distinguished according to action potential discharge patterns and responses to afferent fiber stimulation. Type A fired a tonic discharge at 8.7 spikes/s and had long duration action potentials with no AHPs. Type B fired at a rate of 14.5 spikes/s, and exhibited a slow ramp-like depolarization that preceded a short-duration spike and a prominent afterpolarization. Type C fired in couplets or bursts and showed accommodation. A similar scheme had previously been used to classify globus pallidus neurons in guinea pig brain slices (Nambu and Llinaés 1994, 1997). Although not completely identical, there is a general correspondence between the type A, B, and C classes in rat, and respectively, type III, II, and I classes in guinea pig (see Table 3 in Pang et al. 1998).

We found in rat brain slices that the main characteristics distinguishing cholinergic ventral pallidal neurons in current-clamp recordings were a more hyperpolarized resting membrane potential, the longer duration of the spike AHP, and strong spike accommodation in response to depolarizing current injection. Previous studies of cholinergic neurons in other parts of the magnocellular complex have shown that a slow AHP is a characteristic of these cells. However, strong spike accommodation is not normally seen, and instead depolarization from the resting membrane potential elicits continuous single spikes in a slow rhythmic pattern (Alonso et al. 1996; Gorelova and Reiner 1996; Griffith 1988; Griffith and Matthews 1986; Khatib et al. 1992, 1993, 1995; Markram and Segal 1990; Sim and Allen 1998). The accommodation exhibited in our recordings could result from the use of patch-clamp recording. In rat cholinergic neurons in the medial septum and diagonal band of Broca (MS/DBB), a slow AHP opposes spike frequency adaptation, which is rarely observed in these cells (Gorelova and Reiner 1996). However, spike frequency adaptation is revealed if the AHP is shortened by using channel blockers (e.g., cadmium or apamin), applying serotonin (5-
FIG. 7. Cesium and barium selectively block inward rectifier currents in ventral pallidal neurons. A: effect of 200 µM barium in the superfusate on the inward rectification exhibited by a cholinergic neuron in voltage clamp. The plot is of the current-voltage relationships of the instantaneous current measured before (●) and during (○) application of barium at the time point indicated by the circular markers. Note that barium at this concentration did not reduce the smaller time-dependent inward rectifier current that was also present in this neuron. B: 3 currents measured before, during, and after superfusing a noncholinergic neuron with 2 mM cesium. The plot is of the current-voltage relationships of the steady-state current measured in the same neurons before (●) and during (○) application of cesium. Note that extracellularly applied cesium at this concentration completely blocked the time-dependent inward rectifier current.

FIG. 8. Postsynaptic currents (PSCs) in a noncholinergic neuron are blocked by tetrodotoxin (TTX) or picrotoxin, and reverse polarity near −50 mV. Current traces are shown for a neuron voltage clamped at −80 mV and perfused with the solution indicated at the top of each of the 4 sets of 5 sweeps in the left of the figure. “Wash” refers to sweeps recorded after tetrodotoxin application just before picrotoxin application. Elevated PSC activity relative to the original control is due to an intervening serotonin application that strongly stimulates PSC activity (not shown). The 6 current sweeps at the right of the figure are from the same neuron and show the effect of changing the holding potential on the polarity of the PSCs.
HT), or increasing the internal calcium concentration by altering the buffering capacity of the electrode solution. Under these conditions the normal slow (2–4 Hz) regular pattern of firing is lost, resulting in high-frequency discharge in more complex patterns. In contrast, although we saw accommodation in cholinergic pallidal neurons, they maintained a slow regular rate of firing when multiple spikes were elicited by depolarizing current injection. Furthermore, although the AHP was shorter in the ventral pallidum than in the MS/DBB (mean: 228 ms, cf. 375 ms) it was larger in amplitude (23 mV, cf. 10 mV). The recording solution we used had no added EGTA or BAPTA and has been shown previously to minimally affect the measurement of AHPs in other cells (Sah and Isaacson 1995; Velumian et al. 1997).

The type C/type I pallidal neurons respectively identified in rat and guinea pig have been put forward as candidate cholinergic neurons. This is based in part on the morphology of the guinea pig neurons (Nambu and Llinás 1997) but more particularly on identification of high-frequency burst firing (Lavin and Grace 1996; Nambu and Llinás 1994, 1997). This pattern of firing is produced in some cholinergic neurons when depolarized by current injection from a hyperpolarized resting membrane potential and is related to the activity of a low-threshold calcium current (Alonso et al. 1996; Khateb et al. 1992). Although we did not see burst firing in pallidal cholinergic neurons, the effects of depolarization from hyperpolarized potentials was not rigorously tested. However, it also should be noted that burst firing is not necessarily a diagnostic characteristic for all cholinergic magnocellular neurons in the rat, because it is rarely observed in MS/DBB cholinergic neurons (Gorelova and Reiner 1996). In general the type C neurons identified in rat ventral pallidum in vivo correspond poorly with the cholinergic neurons we identified in vitro. They lack a prominent AHP, do not have a different input resistance to type A or B cells, and only two of four neurons showed spike accommodation (Lavin and Grace 1996). In contrast, type I neurons in the guinea pig globus pallidus do have an AHP (although it is relatively short in duration; mean: 93 ± 89 ms), show strong spike accommodation, and maintain a low frequency and number of spikes during tonic firing in response to increased current injection (Nambu and Llinás 1994). This class also has a low input resistance and has been morphologically identified by intracellular filling as large neurons that are similar in appearance to cholinergic neurons (Nambu and Llinás 1997).

We found that noncholinergic neurons had action potential waveforms in vitro that are most similar to the type B neurons of Lavin and Grace (1996), which have a short-duration spike
AHP and exhibit a depolarizing ramp preceding the spike. We did not observe neurons without an AHP, which is the identifying characteristic of type A neurons. This may be a feature only seen in vivo, because the type III globus pallidus neurons identified by in vitro recording in guinea pig have an AHP but are otherwise very similar to the type A cells (Nambu and Llinás 1994, 1997). In the rat, both type A and type B neurons can be antidromically activated by stimulation from the mediiodorsal nucleus of the thalamus, which is strong evidence that both types include GABA projection neurons. This is further supported by the morphological identification of the counterparts of these cell types in guinea pig (i.e., types II and III) (Nambu and Llinás 1997).

We recorded spontaneous inhibitory postsynaptic currents (IPSCs) in many noncholinergic neurons that were blocked by tetrodotoxin and GABA_A antagonists. The transverse (coronal) orientation of the slices used for this study would disconnect most extranuclear cell bodies from their pallidal terminals, especially those in the nucleus accumbens, which is the major source of inhibitory input to the ventral pallidum. Therefore it is highly likely that these tetrodotoxin-sensitive IPSCs involve synaptic transmission from the terminals of spontaneously firing GABA neurons within the pallidum. Golgi and intracellular staining studies in the pallidum have identified intranuclear axons that form en passant and terminal boutons (Iwashiro and Mizuno 1981; Millhouse 1986; Park et al. 1982). Two basic patterns of axon branching are seen, which may identify projection and local circuit neurons, respectively: axons with infrequent or no intranuclear branches and axons that form a more complex arborization that often does not extend outside the dendritic field (Kita and Kitai 1994; Pang et al. 1998). Stimulation of the mediiodorsal nucleus of the thalamus in vivo produces short-latency monosynaptic inhibition in type A and B pallidal neurons, which has been attributed to release from axon collaterals in response to antidromic activation of the soma (Lavin and Grace 1996).

Concluding remarks

We show in this study that cholinergic ventral pallidal neurons can be identified in brain slices by their large size in combination with strong inward rectification seen in voltage-clamp recording. Similarly, the major population of noncholinergic neurons can be distinguished by a large h-current, which contributes to a major proportion of the whole cell conductance. The ability to discriminate these two neuron types using electrophysiological criteria will simplify future studies of the synaptic physiology and pharmacological properties of these neurons. The electrophysiological properties of the magnocellular cholinergic neurons in the ventral pallidum largely conform to the patterns seen in cholinergic neurons located in other parts of the forebrain complex. The main outstanding feature shown by the pallidal neurons is strong spike accommodation, but it remains to be established if this is a feature that is maintained into adulthood. The presence of a large h-current in pallidal noncholinergic neurons is likely to be of major physiological significance. In addition to having a well-characterized role in pacemaking, h-currents can also participate in shaping more complex patterns of cell firing such as rhythmic bursting (see review by Luthi and McCormick 1998). Furthermore, these currents are modulated by intracellular cAMP and are a potential target for endogenous transmitters such as dopamine and opioids that couple to adenylyl cyclase signaling, have been localized to the pallidum, and have been shown to affect the firing of ventral pallidal neurons in vivo (e.g., Napier et al. 1991; Napier and Maslowski-Czubuzi 1994; Mitrovic and Napier 1995, 1996).

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