Morphological and Membrane Properties of Rat Magnocellular Basal Forebrain Neurons Maintained in Culture

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Sim, J. A. and T.G.J. Allen. Morphological and membrane properties of rat magnocellular basal forebrain neurons maintained in culture. J. Neurophysiol. 80: 1653–1669, 1998. Morphological and electrophysiological characteristics of magnocellular neurons from basal forebrain nuclei of postnatal rats (11–14 days old) were examined in dissociated cell culture. Neurons were maintained in culture for periods of 5–27 days, and 95% of magnocellular (>23 µm diam) neurons stained positive with acetylcholinesterase histochemistry. With the use of phase contrast microscopy, four morphological subtypes of magnocellular neurons could be distinguished according to the shape of their soma and pattern of dendritic branching. Corresponding passive and active membrane properties were investigated with the use of whole cell configuration of the patch-clamp technique. Neurons of all cell types displayed a prominent (6–39 mV; 6.7–50 ms duration) spike afterdepolarization (ADP), which in some cells reached firing threshold. The ADP was voltage dependent, increasing in amplitude and decreasing in duration with membrane hyperpolarization with an apparent reversal potential of −59 ± 2.3 (SE) mV. Elevating [Ca²⁺], (2.5–5.0 mM) or prolonging spike repolarization with 10 mM tetraethylammonium (TEA) or 1 mM 4-aminopyridine (4-AP), potentiated the ADP while it was inhibited by reducing [Ca²⁺], (2.5–1 mM) or superfusion with Cd²⁺ (100 µM). The ADP was selectively inhibited by amiloride (0.1–0.3 mM or Ni²⁺ (10 µM) but unaffected by nifedipine (3 µM), ω-conotoxin GVIA (100 nM) or ω-agatoxin IVA (200 nM), indicating that Ca²⁺ entry was through T-type Ca²⁺ channels. After inhibition of the ADP with amiloride (300 µM), depolarization to less than −65 mV revealed a spike afterhyperpolarization (AHP) with both fast and slow components that could be inhibited by 4-AP (1 mM) and Cd²⁺ (100 µM), respectively. In all cell types, current-voltage relationships exhibited inward rectification at hyperpolarized potentials ≥Ek (approximately −90 mV). Application of Cs⁺ (0.1–1 mM) or Ba²⁺ (1–10 µM) selectively inhibited inward rectification but had no effect on resting potential or cell excitability. At higher concentrations, Ba²⁺ (>10 µM) also inhibited an outward current tonically active at resting potential (Vh −70 mV), which under current-clamp conditions resulted in small membrane depolarization (3–10 nV) and an increase in resting excitability. Depolarizing voltage commands from prepulse potential of −90 mV (Vh −70 mV) in the presence of tetrodotoxin (0.5 µM) and Cd²⁺ (100 µM) to potentials between −40 and +40 mV cause voltage activation of both transient A-type and sustained delayed rectifier-type outward currents, which could be selectively inhibited by 4-AP (0.3–3 mM) and TEA (1–3 mM), respectively. These results suggest that, although acetylcholinesterase-positive magnocellular basal forebrain neurons exhibit considerable morphological heterogeneity, they have very similar and characteristic electrophysiological properties.

INTRODUCTION

Magnocellular cholinergic neurons within basal forebrain nuclei provide the main source of ascending cholinergic innervation to cortical and subcortical regions of the mammalian brain (see Koliatsos and Price 1991). Their selective degeneration has been closely associated with the neuropathology of Alzheimer’s disease in man (Whitehouse et al. 1982). Early pioneering studies using acetylcholinesterase (AChE) histochemical techniques have provided invaluable information about the topographical organization of the cholinergic projections within the mammalian brain (see Fibiger 1982; Woolf 1991). However, subsequent studies have revealed that other noncholinergic cell groups within the brain also stained positive for AChE. Notwithstanding, combined studies using choline acetyltransferase (ChAT) immunochemistry, a more reliable cholinergic marker, and AChE histochemistry have revealed good correlation between projecting neurons immunoreactive for ChAT and those stained positive for AChE in the horizontal limb of the diagonal band (HDBB) and substantia innominata-nucleus basalis (SI-nB) of the rat brain (Dinopoulos et al. 1988; Eckenstein and Sofroniew 1983; Levey et al. 1983; Saper 1984; Woolf et al. 1984).

Information regarding the intrinsic membrane properties of neurons within basal forebrain nuclei have been obtained in studies using either in vitro brain slice preparations (Alvarez de Toledo and López-Barneo 1988; Castellano and López-Barneo 1991; Gorelova and Reiner 1996; Griffith 1988; Griffith and Matthews 1986; Griffith et al. 1991; Khateb et al. 1992; López-Barneo et al. 1985; Matthews and Lee 1991) or using in vitro culture preparations (Nakajima et al. 1985; Segal 1986; Stanfield et al. 1990; Suszkiw et al. 1992). Although in these studies, some authors used concurrent intracellular labeling and recording, followed by verification of the cholinergic nature of recorded neurons with either ChAT immunohistochemistry or AChE histochemistry, the frequency of recording from cholinergic neurons was not high. Given the importance of these neurons in neurodegenerative diseases, it is crucial to develop an in vitro model in which cholinergic neurons can be easily visualized and reliably identified for physiological, pathological, and molecular biological studies. The study was undertaken in two parts: first, the cholinergic population of neurons maintained in in vitro dispersed cell cultures was identified and verified with AChE histochemistry, and, second, whole cell current- and voltage-clamp recordings were made from visually identified neurons of comparable morphology, to examine their firing characteristics and the properties of the underlying membrane conductances. Our results demonstrate that the approach of dispersed cultures provides a viable and amenable in vitro model to characterize the properties of magnocellular basal forebrain neurons. Preliminary findings have been communicated previously in abstract form (Sim 1994).
METHODS

Tissue preparation and culture procedures

Sprague-Dawley (11- to 14-day-old) rats were killed with excess anesthesia, decapitated, and their brains rapidly removed. The brain was then hemisected, and coronal slices (400 μm in thickness) were prepared using a McIlwain chopper. The slices were collected in cold (4°C) Gey’s balanced salts solutions (GBSS, Life Technologies) containing 0.6% d-glucose and 8 mM MgCl₂. With the aid of a dissecting microscope, regions of basal forebrain nuclei [namely the vertical and horizontal limb of diagonal band of Broca (VDBB and HDBB, respectively), and Si-nb] were carefully dissected out using a small (1 mm) fragment of a razor blade held by a pair of hemostatic forceps. This approach allows for a more accurate and clean dissection of discrete brain regions over micro-punching. Details of the enzymatic incubation and dissociating methods are similar to those described previously (see Allen et al. 1993). The neurons were routinely maintained for days to several weeks at 37°C in an atmosphere of 5% CO₂ in air, and were fed twice weekly for the first week, and then once weekly thereafter.

Histological studies

Basal forebrain cultures were stained for AChE using the modified histochemical technique described by Hedreen et al. (1985) and Hefti et al. (1985). Briefly, the cultures were incubated in a fixative solution containing 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for no longer than 1 h. After several rinses with 0.1 M sodium acetate buffer (pH 6.8), the cells were incubated in 2 ml of staining solution of the following composition: 50 mg acetylthiocholine iodide, 35.5 mg glycine, 3.6 mg ethopropazine, 25 mg copper sulfate, 205 mg sodium acetate, and made up to 50 ml H₂O and its pH adjusted to 5.0 with 0.1 M acetic acid. Incubation was carried out either at room temperature (20–23°C) for no longer than 30 min or at 4°C for several hours. After several rinses in 0.1 M acetate buffer, the cells were treated with 1.25% ammonium sulfide for 1 min, followed by several washes in 0.1 M sodium nitrate. Cells were then briefly (1 min) exposed to 0.1% silver nitrate, followed by several washes in 0.1 M sodium nitrate and then H₂O, before mounting in glycerol. Camera lucida drawings of AChE-positive neurons in culture were made with a drawing tube attached to a microscope at the magnification of ×400. The diameter of the cells was defined as a geometric mean (M̅m), where M is the major and m is the minor soma diameter. The values are presented as means ± SE. However, due to the nature of the AChE staining, the fine swellings and dendritic spines were not readily visualized. Thus, in a few experiments, some of the magnocellular neurons were intracellularly labeled with the fluorescent dye, Lucifer yellow (0.5%) by its inclusion in the internal solution, to permit more direct verification of morphological structures of visually selected neurons under study. However, a major disadvantage of the use of a patch electrode for intracellular staining was the high frequency of cell loss on removal of the patch. Therefore conventional ‘‘sharp’’ intracellular electrodes were used because these could be retracted from cells with minimum damage to the overall morphology of the stained cells. Sharp electrodes were pulled on a Brown/Flaming horizontal puller (Sutter Instruments) and were filled with 2% Lucifer yellow (in 1 M LiCl), then back-filled with 4 M K-acetate, and had resistances of 140–200 MΩ. Staining was achieved by injecting hyperpolarizing currents of 0.5 nA and 200 ms in duration at intervals of 500 ms for 10–20 min. Cells were visualized and photographed, before fixation and AChE histochemistry, because in our hands the chemical reaction of AChE staining appeared to interfere with the fluorescence of Lucifer yellow. (Note: no electrophysiological data were obtained with Lucifer yellow–filled electrodes.)

Electrophysiological studies

The dissociated cultures of basal forebrain neurons were routinely used after 5–27 days in culture. The culture dishes were mounted onto the stage of a conventional microscope (Microtec-2A, Oxford Instruments) fitted with Hoffman modulation optics or onto the stage of an inverted microscope (TMS, Nikon) fitted with phase-contrast optics. The preparation was then superfused at a rate of 8 ml/min with Krebs solution of the following composition (in mM): 118 NaCl, 3 KCl, 1.2 MgCl₂, 2.5 CaCl₂, 25 NaHCO₃, 5 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), and 11 d-glucose, adjusted to pH 7.4 (where necessary) and gassed with 95% O₂-5% CO₂. Whole cell recordings (Hamill et al. 1981) were made using an Axoclamp-2A (Axon Instruments) using either conventional bridge or discontinuous single-electrode voltage-clamp recording. Patch-clamp electrodes were pulled from 1.5-mm-OD thin-walled borosilicate glass capillaries (Clark Electromedical Instruments) using a Kopf (model 720) electrode puller. Recording electrodes were coated to within 100 μm of their tip with silicone elastomer (Sylgard) 184 (Dow Corning) and were fire-polished using a microforge (Nikon MF-9). Electrodes had resistances ranging from 6 to 9 MΩ when filled with internal solution of the following composition (in mM): 102.4 KAcetate, 15.6 KCl, 1 MgCl₂, 0.822 CaCl₂, 40 HEPES, 3 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’-tetraacetic acid (EGTA), 4 MgATP, and 0.1 NaGTP. The free calcium concentration of the internal solution was calculated and adjusted to 30 nM (see Allen et al. 1993).

TABLE 1. Some intrinsic and active properties of the four morphologically identified magnocellular basal forebrain neurons maintained in dispersed cell culture

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell diameter (M̅m), μm</td>
<td>26.07 ± 0.26</td>
<td>25.93 ± 0.17</td>
<td>25.70 ± 0.26</td>
<td>27.44 ± 0.23</td>
</tr>
<tr>
<td>(21.45 to 28.98)</td>
<td>(21.45 to 28.84)</td>
<td>(22.80 to 27.45)</td>
<td>(22.8 to 30.00)</td>
<td></td>
</tr>
<tr>
<td>Resting membrane, mV</td>
<td>−71.82 ± 0.48</td>
<td>−71.39 ± 0.40</td>
<td>−70.97 ± 0.63</td>
<td>−70.42 ± 0.55</td>
</tr>
<tr>
<td>(−65 to −79)</td>
<td>(−65 to −78)</td>
<td>(−65 to −78)</td>
<td>(−65 to −77)</td>
<td></td>
</tr>
<tr>
<td>Overshoot of action potential, mV</td>
<td>41.97 ± 1.12</td>
<td>39.61 ± 1.18</td>
<td>39.18 ± 1.85</td>
<td>40.57 ± 1.75</td>
</tr>
<tr>
<td>(24 to 54)</td>
<td>(22 to 52)</td>
<td>(26 to 57)</td>
<td>(33 to 55)</td>
<td></td>
</tr>
<tr>
<td>Width of action potential, ms</td>
<td>1.14 ± 0.05</td>
<td>1.11 ± 0.04</td>
<td>1.27 ± 0.09</td>
<td>1.11 ± 0.08</td>
</tr>
<tr>
<td>(0.76 to 2.0)</td>
<td>(0.60 to 1.88)</td>
<td>(0.68 to 1.88)</td>
<td>(0.72 to 1.52)</td>
<td></td>
</tr>
<tr>
<td>Time constants, ms</td>
<td>3.37 ± 0.19</td>
<td>3.25 ± 0.17</td>
<td>3.62 ± 0.25</td>
<td>2.86 ± 0.34</td>
</tr>
<tr>
<td>(0.88 to 4.98)</td>
<td>(0.74 to 5.38)</td>
<td>(0.95 to 4.87)</td>
<td>(0.91 to 5.57)</td>
<td></td>
</tr>
<tr>
<td>% Cells with afterdepolarization</td>
<td>90</td>
<td>91</td>
<td>89</td>
<td>92</td>
</tr>
<tr>
<td>% Cells with inward rectifier</td>
<td>90</td>
<td>92</td>
<td>89</td>
<td>93</td>
</tr>
</tbody>
</table>

Values are means ± SE with ranges in parentheses. Number of cells for type I was 55, for type II was 88, for type III was 30, and for type IV was 45.
al. 1993). The solution was then adjusted to a pH of 7.2 with NaOH and had a final osmolarity of 295 mosmol/l.

Phase bright neurons of comparable morphology to AChE-positive neurons were visually identified and classified before being studied electrophysiologically. In all experiments, magnocellular basal forebrain neurons were held near their resting membrane of −70 mV (see Table 1) with DC current injection. The reference electrode was a glass bridge containing agar-saline with one end in the recording chamber and the other end in a side chamber filled with 3 M KCl and connected to ground, via an Ag-AgCl pellet.

Data acquisition and analysis

Current and voltage were simultaneously generated and sampled on-line using a Labmaster (TL-1) DMA interface (Axon Instruments) connected to an IBM PC/AT clone (Dell 325D) computer. Signals were filtered (0.3–10 kHz, Bessel filter of Axoclamp-2A) before digitizing. Acquisition and subsequent analysis of the acquired data were carried out using the pClamp suite of software (Axon Instruments). In addition, the current and voltage signals were also recorded onto a chart recorder (Gould 2400S) and simultaneously stored on an analog 4-channel FM tape recorder (Racal store 4DS). Voltage-current (V-I) curves were plotted using Graphpad Inplot computer software (Graphpad California). Input resistance of the cells was estimated from small (0.1–0.2 nA; 100 ms in duration) hyperpolarization steps from a potential of −70 mV. Action potentials were generated by passing 3- to 5-ms DC current pulses through the recording electrode, and parameters such as spike amplitude and duration were measured. Spike duration was measured at half-amplitude baseline to peak. All data are expressed as means ± SE.

Drugs

All drugs were applied via the superfusing Krebs solution. Drugs used were 4-aminopyridine (4-AP, Sigma); barium chloride

![FIG. 1. Basal forebrain neurons of postnatal rats maintained in culture. A–C: photographs of magnocellular (somatic diameter >23 μm) basal forebrain (MBF) neurons viewed under phase contrast, after 5–7 days in culture. D and E: photographs of MBF neurons stained with acetylcholinesterase and viewed with bright-field microscopy. Note the extensive arborization of the processes of the neurons after 12 days in culture shown in D, G and E: morphological characteristics as revealed by cells that have been intracellularly stained with Lucifer yellow. The scale bar is 50 μm, except in A and D, where it is 100 μm.](image-url)
(BaCl₂, Sigma); (-)-bicuculline methochloride (Cookson, UK); cadmium chloride (CdCl₂, BDH); 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Cookson, UK); kynurenic acid (Sigma); Lucifer yellow (di-sodium salt, CH₂Cl₂, Sigma); nickel chloride (NiCl₂, Sigma); niflumic acid (Sigma); tetrathyamine chloride (TEA-Cl, Lancaster synthesis, UK); tetrodotoxin (TTX, Calbiochem-Novabiochem).

RESULTS

Morphological properties

The dissociated preparation of basal forebrain maintained in culture yielded a subpopulation of magnocellular neurons with somatic diameters of >23 µm (26.24 ± 0.12 µm; mean ± SE, n = 218 cells) and extensive processes. These cells could be readily and reliably visualized using phase-contrast microscopy (Fig. 1, A-C) and exhibited similar morphological features to cholinergic magnocellular basal forebrain (MBF) neurons previously described in the intact brain, being either fusiform, ovoid, or triangular in shape. Of these cells, 95% (279/294) stained positive for AChE (see examples in Fig. 1, D and E).

The AChE staining pattern consisted of a dark brown to black granular reaction product within cell soma and proximal dendrites, which became more diffuse toward the distal ends of the dendrites. The characteristic ‘large’ nucleus (Dinopoulos et al. 1988) of MBF neurons was readily distinguished by its light staining for AChE and after a period of ≥4 days in culture, the dendrites of most AChE-positive neurons extended for distances in excess of 1 mm from the cell soma (cf. Fig. 1D). Figure 2 illustrates camera-lucida drawings of representative examples of each of the main morphological phenotypes of AChE-positive cultured MBF neurons. For convenience of cell identification during electrophysiological recording, neurons were divided into four cell types on the basis of the shape of their soma and the pattern of their dendritic branching: type I had a triangular-shaped soma; type II had a fusiform or roughly pear-shaped soma, and had one major process bifurcated ~25–50 µm from the soma and more than two-third of the processes emanating from the soma; type III had a similar shaped somata as type II, but one or more of the major processes bifurcating close to the base of their soma and had fewer dendrites emanating from the base of their soma; and type IV were multipolar with rounded soma, and more than four dendrites emanating from the soma. Type I, II, and III cells had large (1–2 µm) primary dendrites that left the soma at its angular portions before ramifying distally (see Fig. 2). Characteristic dendritic spines have been described on neurons in intact preparations (Sembä et al. 1987) (see DISCUSSION). Consistent with this, staining of MBF neurons with the intracellular marker Lucifer yellow revealed numerous dendritic spines and fine varicose swellings along the processes of all the cells (n = 37 cells).

It should be noted that in addition to magnocellular neurons, the culture preparation also contained a population of smaller AChE-positive neurons (<20 µm diam). However, because these could not be clearly distinguished from other similar sized neurons using simple phase contrast microscopy, they were not studied.

Properties of the different cell types

The active and passive membrane properties of the four morphologically distinct types of MBF neurons were examined with the use of the whole cell variant of the patch-clamp technique. The electrophysiological characterization of the neurons was based on their responses to intracellular current stimulation (Table 1). Cells were judged to be suitable for study only if they had a membrane potential of at least −65 mV and an action potential with an overshoot of greater than +20 mV. Out of a total of 218 visually identified MBF neurons studied, 25% (55) had type I morphology, 40% (88) had type II, 14% (30) had type III, and 21% (45) had type IV. Although there were some differences between the four subtypes, these were not restricted to any particular subtype, and as summarized in Table 1, the electrophysiological characteristics of these magnocellular neurons were indistinguishable. Figure 2 illustrates representative examples of the membrane characteristics of each of the four morphological cell types. Figure 2, Aa–Da, shows responses to 100-ms depolarizing and hyperpolarizing current pulses elicited from a holding potential of −70 mV. Hyperpolarization of the membrane revealed the presence of strong inward rectification in all cell types. In most cells, threshold depolarizing current stimulation elicited only a single action potential (Fig. 2, Aa, Ba, and Ca), although in a few cells (of all morphological types) a spike doublet was occasionally observed (see Fig. 2Da). Following a single action potential elicited with brief (3–5 ms) depolarizing current pulse, all cell types displayed a pronounced afterdepolarization (see Fig. 2, Ab–Db).

In most cells, including those initially firing a doublet spike, further increases in stimulus intensity evoked brief trains of action-potential discharge of increasing frequency. In all cells studied, the pattern of spike discharge was found to be dependent on the initial potential at which the cell was held. When the cell was held close to its resting potential (−70 mV), a pronounced delay, characteristic of activation of a transient A-type current, was observed before discharge of the first action potential (see Fig. 3). Consistent with this, shifting the holding potential to more depolarized potentials...
FIG. 3. Effect of membrane potential on the firing characteristics of MBF neurons in culture. The recordings illustrated were made from a type II neuron. From a membrane potential of −70 mV (−10 pA, DC injection), gradual increases in the intensity of 100-ms depolarizing current pulse to threshold level, revealed a “hump” in the voltage transient, followed by a small delay before evoking an action potential. With increases in current intensity beyond threshold, the cell begins to fire trains of action potentials; the frequency of firing increasing with increasing current stimulation. Depolarizing the cell to membrane potentials of −60 and −50 mV (+40 pA and +180 pA DC injection, respectively) abolished the hump in the voltage transient close to threshold and reduced the current required to elicit tonic spike discharge.

thereby inactivating the A-current abolished the spike delay and reduced the stimulus current required to facilitate the switch from phasic to tonic firing behavior. However, in 18–35% of cells of all morphological types, only a single action potential could be evoked (within the 100-ms command period), irrespective of the stimulus intensity or holding potential.

Spike afterpotentials

A prominent feature of most MBF neurons in culture was the presence of a spike afterdepolarization (ADP; Table 1 and Fig. 2, Ab, Bb, and Cb). Typically this consisted of a single nonsynaptic component (Fig. 2, Aa–Ca), although occasionally multicomponent ADPs were observed (see Fig. 2Db). Where multiple components were observed, these could be inhibited by either a combination of the non-N-methyl-D-aspartate (NMDA) receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10–30 μM) and the NMDA receptor antagonist kynurenic acid (1 mM) or the γ-amino butyric acid-A (GABA_A) antagonist bicuculline (1–10 μM), indicating that they resulted from activation of recurrent synaptic inputs. In the presence of 4-AP to block the A-current, peak activation of this conductance was observed immediately after a single action potential. With repetitive stimulation, the amplitude of the individual ADP declined rapidly after the first few stimuli and required a recovery period of between 10 and 20 s to return to control. The amplitude and duration of the nonsynaptic spike ADP varied considerably between cells. However, no significant differences were observed between the ADP elicited in the different cell types and so for analysis, data were pooled. The mean amplitude and duration of the ADP evoked from cells held at resting potential was 15.5 ± 1.0 mV (range, 6–39 mV) and 26.4 ± 1.48 ms (range, 6.7–50 ms; n = 110), respectively. In a number of cells, the amplitude of the ADP was sufficiently large to reach threshold and evoke a second spike (data not illustrated). In such cells, this was almost invariably associated with discharge of a spike doublet in response to DC injection. In all cells, the ADP was found to be strongly voltage dependent, increasing in amplitude and decreasing in duration with membrane hyperpolarization from resting potential (Fig. 4A) and had an apparent reversal potential of −59 ± 2.3 mV (n = 10; see DISCUSSION).
**Fig. 4.** Characteristics of the ADP in MBF neurons. A: effect of membrane potential on the afterdepolarizing potential evoked by a single action potential in MBF neuron. Action potentials were evoked with 3 ms duration depolarizing current pulse, the intensity (0.2–2.1 nA) of which decreased with membrane depolarization. The amplitude of the spike ADP increased with membrane hyperpolarization from its resting level of $0.67 \text{ mV}$ to a negative potential of $0.99 \text{ mV}$. Note that the duration of the spike ADP was decreased with membrane hyperpolarization. Spike ADP appeared to reverse around membrane depolarization to $0.59 \text{ mV}$.

B: reversible inhibition of the ADP by the Ca$^{2+}$/channel blocker, cadmium (Cd$^{2+}$, 100 nM) in the same cell ($V_m = 70 \text{ mV}$). Bd: this effect on an expanded time base.

C: the Ca$^{2+}$ dependence of the spike ADP in a MBF neuron. ADPs were evoked by eliciting action potentials in response to 4 ms (0.7–1 nA), depolarizing current pulses in the presence of 1.0, 2.5, and 5 mM [Ca$^{2+}$]$_o$ and after block with Cd$^{2+}$ (0.1 mM; $V_m = 72 \text{ mV}$). Note that as well as inhibiting the ADP, lowering [Ca$^{2+}$], or addition of Cd$^{2+}$ ions also reduced the negative-going potential that preceded it.

### Ca$^{2+}$ dependence of the spike ADP

Raising [Ca$^{2+}$]$_o$ from 2.5 to 5 mM increased the amplitude of the ADP by $47.5 \pm 11.9\%$ ($n = 10$), whereas lowering [Ca$^{2+}$], from 2.5 to 1 mM reduced its amplitude by $41.6 \pm 4.9\%$ ($n = 7$; see Fig. 4C). Increasing Ca$^{2+}$ entry by delaying spike repolarization using the K$^+$ channel blockers TEA (10 mM) or 4-AP (1 mM) increased the amplitude of the ADP by $87.7 \pm 19.4\%$ ($n = 5$) and $114.6 \pm 18.7\%$ ($n = 7$), respectively (Fig. 5A). The ADP could also be reversibly inhibited by blocking voltage-sensitive Ca$^{2+}$ channels using cadmium (Cd$^{2+}$, 100 µM; $n = 12$, Fig. 4D). The effect of Cd$^{2+}$ ions on the rising phase of the ADP can be clearly seen on an expanded time course (as shown in Fig. 4, Bd and C). The Ca$^{2+}$ channel subtypes underlying Ca$^{2+}$ entry and generation of the ADP were investigated.
Spike afterhyperpolarization (AHP)

Following the block of the ADP with amiloride (300 μM), depolarization of MBF neurons to potentials greater than −65 mV revealed the presence of a spike AHP. Application of 4-AP (1 mM) to block the A-current inhibited the fast component of the AHP. The remaining AHP was inhibited by blocking voltage-sensitive Ca²⁺ channels using Cd²⁺ (100 μM; n = 4; see Fig. 5C), indicating that MBF neurons also express Ca²⁺-activated K⁺ channels, but that under normal conditions the effects of their activation are largely masked by the larger Ca²⁺-dependent ADP. Finally, it is worth noting that under control conditions, block of the A-current with 4-AP resulted in inhibition of the small negative-going potential that was observed to precede the ADP in many cells (see Fig. 5A). Thus the slow time-to-peak and apparent reversal potential of the ADP may simply be due to the interplay between the A-current and the Ca²⁺-dependent AHP current, coupled with reduced activation of the ADP as a result of voltage-dependent inactivation of T-type Ca²⁺ channels, rather than the intrinsic kinetics of the ADP.

Ca²⁺ dependence of the action potential

Action potentials in MBF neurons were blocked by TTX (0.5 μM). Furthermore, in contrast to the clear effects of Ca²⁺ on the ADP and AHP, neither the amplitude or duration of the action potential was strongly affected by block of voltage-sensitive Ca²⁺ channels with Cd²⁺ (Fig. 5C). When evoked from potentials close to V₉⁰, the repolarization phase of the action potential was also found to be relatively insensitive to K⁺ channel blocking compounds, requiring quite high concentrations of 4-AP (≥3 mM) or TEA (≥10 mM) before any significant broadening could be observed (data not shown). Under similar recording conditions, we have previously shown that MBF neurons exhibit a robust Ca²⁺ current of the order of 3–5 nA (Allen et al. 1993). However, MBF neurons rarely exhibit a Ca²⁺-dependent plateau phase to their action potentials (Figs. 1 and 5A). The reason for this may be the very large size (≥10 nA) of the outward A-current and delayed rectifier current in these cells (see Fig. 9), which may have such a strong repolarizing effect on the spike that they negate any effect that Ca²⁺ channels may have in slowing spike repolarization. Consistent with this, we observed that spikes evoked from depolarized holding potentials (−55 to −60 mV) frequently exhibited a plateau potential on their repolarization phase, especially in the presence of low concentrations of 4-AP or TEA.

Inward rectification

All MBF neurons studied exhibited strong inward rectification when hyperpolarized from resting potential. Examination of the voltage transients recorded in response to the injection of hyperpolarizing current steps revealed a number of distinct features. Small current steps that hyperpolarized the cell to potentials of between −80 and −90 mV produced voltage transients with a largely exponential rising phase that reached a plateau with the first 10–20 ms (see Fig. 2, Aa, Ba, Ca, and Da). With larger negative current steps the voltage responses exhibited an initial transient peak that was reached within the first 3–6 ms before declining within ~3–5 ms to a steady plateau potential (see Fig. 2, Aa, Ba, Ca,
FIG. 6. Inward rectification in a MBF neuron. 

Aa: using simple ‘bridge’ voltage recording mode, families of potentials in response to hyperpolarizing current pulses were evoked in a MBF neuron and the corresponding peak (●) and steady-state voltage (○) current-voltage (I-V) relationship plotted in Ab (V_{rest} = −68 mV). Note the ‘negative spike’ elicited with large hyperpolarization. 

Ba: the same cell under voltage clamp (V_{h} = 70 mV). Families of currents in response to 300-ms depolarizing and hyperpolarizing command pulses. Bb: corresponding peak (●) and steady-state I-V relationships. Note the I-V relationship shows a region of negative slope conductance at potentials in excess of −130 mV.

and Da, and Fig. 6A). Further increases in the amplitude of the current steps, which resulted in hyperpolarization of the cell to potentials in excess of −110 mV revealed a region of negative slope conductance in the current-voltage (I-V) relationship (Figs. 6Bb and 8B). In this region, evoked membrane voltage transients instead of declining from the initial peak potential slowly continued to increase in amplitude throughout the duration of the current step, and in many cells this resulted in ‘runaway’ of the voltage transient and the generation of a negative-going spike (Fig. 6A). To characterize this inward rectification further, MBF neurons were voltage clamped close to their resting potentials (−70 mV), and a series of 300-ms depolarizing and hyperpolarizing pulses (10-mV increments) were applied every 10 s (Fig. 6B). Figure 6Ba shows a typical I-V relationship for the ‘instantaneous’ (I_{in}) current responses (merging with capacity transients) and the plateau or ‘steady-state’ (I_{ss}) current. With small (<20 mV) positive and negative voltage steps, the membrane behaved essentially ‘ohmically.’ However, with successively larger hyperpolarizing voltage steps to potentials in excess of −110 mV, the current responses began to exhibit progressively greater time-dependent inactivation that, at strongly hyperpolarized potentials, resulted in upward curvature of the steady-state I-V curve (see Fig. 6Bb).

Cs\(^+\) and Ba\(^{2+}\) sensitivity

One of the defining features of inward rectifier currents in various cell types is their relative sensitivity to barium (Ba\(^{2+}\)) and cesium (Cs\(^+\)) ions (Gay and Stanfield 1977; Hagiwara et al. 1976, 1978; Standen and Stanfield 1978). In cholinergic basal forebrain neurons, substance P (SP) has been reported to inhibit an inwardly rectifying conductance and increase cellular excitability (Stanfield et al. 1990). Furthermore, both Cs\(^+\) and Ba\(^{2+}\) ions were found to block the SP-sensitive current (Yamaguchi et al. 1990). Therefore the effects of externally applied Cs\(^+\) and Ba\(^{2+}\) on the cellular excitability and inward rectification in MBF neurons were investigated. Figure 7 illustrates the effects of bath-applied Cs\(^+\) and Ba\(^{2+}\) on the active properties of MBF neurons held at −70 mV. Cs\(^+\) (1 mM) had no effect on resting membrane potential (n = 5 cells) or cell excitability (Fig. 7A). In contrast, bath application of Ba\(^{2+}\) (1 mM) produced a large membrane depolarization (offset by the passage of hyperpolarizing current through the electrode) and a large increase in cell excitability (Fig. 7B). A number of studies have reported inwardly rectifying conductances that can be selectively inhibited by low concentrations of extracellular Cs\(^+\) and Ba\(^{2+}\) (see DISCUSSION). Therefore the concentration dependence of the effect of these ions on the inward rectifier was investigated (Fig. 8). Cells were voltage clamped at −70 mV, and a series of 300-ms voltage steps to potentials of −50 to −160 mV were applied to the cells under control conditions and in the presence of Cs\(^+\) (0.01−1 mM; Fig. 8A) and Ba\(^{2+}\) (0.1−100 μM; Fig. 8D). The inhibition produced by extracellular Cs\(^+\) was concentration and voltage dependent and was largely confined to a block of an inwardly rectifying con-
ductance activated at hyperpolarized potentials in excess of −90 mV with little or no observable block of membrane conductances at more depolarized potentials (Fig. 8A). At low concentrations (up to 3 μM) the block produced by Ba²⁺ was similar to that of Cs⁺ (100 μM) in that it selectively inhibited inward rectification activated at hyperpolarized potentials greater than −90 mV (Fig. 8B). However, at higher concentrations (>10 μM), Ba²⁺ (unlike Cs⁺) also produced a concentration-dependent decrease in membrane conductance at depolarizing as well as hyperpolarized potentials (Fig. 8C). In an unclamped cell held at resting potential, concentrations of Ba²⁺ in excess of 10 μM produced a concentration-dependent depolarization of MBF neurons and a large increase in cell excitability. Neither Cs⁺ or Ba²⁺ at concentrations up to 1 mM inhibited the current relaxations associated with region of negative slope conductance at strongly hyperpolarized potentials.

Outward currents

Outward potassium currents have previously been described in basal forebrain neurons using a brain slice preparation (Griffith and Sim 1990), although the cholinergic nature of the individual cells was not directly determined. In the present study, the properties of the outward K⁺ currents in visually identified MBF neurons were examined under voltage clamp in the presence of TTX (0.5 μM) and Cd²⁺ (100 μM). Cells were held at either −70 or −80 mV, then following a prepulse to −90 mV (for 150 ms), a series of depolarizing voltage steps (200 ms duration) to potentials up to +40 mV were used to activate voltage-sensitive K⁺ channels. Both transient and sustained components of outward current were observed (see Fig. 9). The sustained component of outward current was selectively inhibited by application of TEA (3 mM; Fig. 9B) but remained unaffected by 4-AP (1 mM). The characteristic kinetics of the TEA-sensitive delayed rectifier current (I_{DK}) was revealed by subtracting currents elicited in TEA from their respective control (Fig. 9C). TEA depressed I_{A} and I_{DK} at higher concentrations (10 ± 30 mM). Unlike TEA or 4-AP, Ba²⁺ (0.1 ± 3 mM) nonselectively inhibited both I_A and I_{DK}, and its effects were not readily reversible on wash out (Fig. 9C). The voltage dependence of activation and inactivation of I_A channels in MBF neurons was studied in the presence of 3 mM TEA (Fig. 9B). Unfortunately, because of the large amplitude of
FIG. 8. Effects of Cs\(^+\) and Ba\(^{2+}\) on inward rectification in MBF neurons studied under voltage clamp. Families of currents were evoked in response to 300-ms depolarizing and hyperpolarizing commands from a holding potential of \(-70\) mV, in 2 separate cells. A: Cs\(^+\) was applied cumulatively at 100 \(\mu\)M and 1 mM and was readily reversed on wash out. B: the corresponding steady-state I-V were plotted in the absence (○), and in the presence of 100 \(\mu\)M (●) and 1 mM (▼) Cs\(^+\), reveal a voltage-dependent blocking by Cs\(^+\). C: in another MBF neuron, Ba\(^{2+}\) was cumulatively applied at 1, 10, and 100 \(\mu\)M. D: the corresponding steady-state I-V relationships obtained in the absence (○) and in the presence of 1 \(\mu\)M (■), 3 \(\mu\)M (▲, not shown in C), 10 \(\mu\)M (▼), and 100 \(\mu\)M (●) Ba\(^{2+}\). Note, at low concentration (±3 \(\mu\)M), Ba\(^{2+}\) blocks the rectifier in a voltage-dependent manner similar to Cs\(^+\)
these outward currents, which frequently exceeded the output of the amplifier headstage (10 nA), we were unable to construct complete activation curves for either $I_A$ or $I_{DK}$. It was, however, possible to examine the voltage dependence of steady-state inactivation (see Fig. 9A): this was described by a Boltzmann curve with a half-inactivation potential ($V_{1/2}$) of $-67.9 \pm 1.9$ mV and a slope factor ($k$) of $11.5 \pm 0.6 \ (n = 11)$. Although the relative amplitudes of $I_A$ and $I_{DK}$ and the time constant of decay of $I_A$ varied slightly between neurons, their pharmacological profiles were similar in all the MBF neurons studied, and no consistent specific characteristics of the outward currents were observed that could reliably discriminate between the different morphological subtypes of neuron.
Spontaneous activity

An interesting feature of MBF neurons maintained in culture was the presence of spontaneous synaptic activity. After ≈6 days in culture, MBF neurons began to exhibit increasing levels of spontaneous synaptic activity. The identity of these spontaneous events was studied using the glutamate receptor antagonist, CNQX, and the GABA<sub>A</sub> receptor antagonist, bicuculline. In the presence of CNQX (5 μM), the frequency of the spontaneous synaptic activity was only slightly reduced (Fig. 10A). The effect of CNQX was reversible on wash out. In the presence of 10 μM bicuculline, the frequency of the spontaneous synaptic responses was greatly reduced (80–90%; see Fig. 10B), and the remaining bicuculline-insensitive synaptic activity could be blocked by CNQX. [Note: because the chloride equilibrium potential was set at approximately −50 mV and the cells were held at −80 mV (using DC current injection), both GABA<sub>A</sub> and glutamate-mediated synaptic responses would result in membrane depolarization.] These results indicate that MBF neurons in culture are synaptically active, exhibiting both inhibitory (GABA<sub>ergic</sub>) and excitatory (glutamatergic) spontaneous synapses.

Discussion

The main aim of the present study was to develop a simple cell culture preparation for maintaining cholinergic basal forebrain neurons in vitro that would preserve much of the in vivo properties of these cells and to use this preparation to study the electrophysiological characteristics of the different morphologically identified subtypes of projecting magnocellular neurons.

Postnatal neurons in culture

The basic tissue culture methods employed were similar to those previously described for isolating and culturing cholinergic neurons from septum and diagonal band of Broca (Hartikka and Hefti 1988; Hatanaka et al. 1988; Hefti et al. 1985; Köller et al. 1990; López-Barneo et al. 1985; Segal 1986; Suszkiw et al. 1992). A wide range of different enzymatic dissociation procedures was tested. Although some enzymes such as papain were found to be more effective than trypsin at dispersing the brain tissue and in some cases give a higher initial cell yield, these advantages were offset by other problems such as poor long-term cell survival and damage to receptors. In our hands, trypsin was found to provide the best trade-off between the different conflicting considerations. The large basal forebrain neurons were also found to be particularly susceptible to mechanical damage during trituration, and, to obtain a good yield of these cells, we found it was essential to minimize disruption during this stage of the dissociation procedure. A variety of different substrate matrices were tested. Basal forebrain neurons adhered very poorly to untreated glass or plastic and other substrates such as laminin, whereas supporting cell adhesion and growth resulted in cells with morphological characteris-
tics that were markedly different from those reported in vivo. Of all the substrates tested, poly-D-lysine was found to maintain a cellular morphology that was most like that observed in vivo. As reported for other basal forebrain neurons, the inclusion of nerve growth factor (NGF, 25 ng/ml) in the growth medium was required for successful long-term maintenance of magnocellular neurons, and its continuing presence in long-term cultures is likely to contribute to the maintenance of the morphological and cholinergic properties of these neurons in vitro (Knuesel et al. 1994). As an alternative strategy, Nakajima et al. (1985) have reported that cholinergic basal forebrain neurons when grown on a glial cell feeder layer could be maintained in culture for 2–3 wk in the absence of NGF.

Cholinergic nature of magnocellular neurons?

ChAT staining is now considered to be a more reliable marker for cholinergic neurons than staining for AChE. However, within the basal forebrain, there is a very high correlation between cells labeled with ChAT and those staining positively for AChE (Eckenstein and Sofroniew 1983; Levey et al. 1983; Nakajima et al. 1985). Thus within the basal forebrain, neurons that are AChE positive can reliably be considered to be cholinergic. As reported by other groups studying basal forebrain neurons (Nakajima et al. 1985; Suszkiw et al. 1992), the vast majority (~95%) of the large neurons (>23 μm) in our cultures stained positive for AChE. This value is slightly higher than the 75% reported by Nakajima et al. (1985). However, this difference may reflect, in part, to our use of NGF in the culture medium, which increases survival of cholinergic neurons in culture. Finally, as observed in vivo, our cultures also contained a population of smaller AChE-positive neurons. However, because these could not be readily distinguished morphologically from other small neurons of comparable morphology, we did not attempt to investigate their properties in the present study.

Notwithstanding, the question of the reliability of AChE as a cholinergic marker in our study cannot be ignored. Our choice to use AChE in the present study was based on the reliability and ease of application of this technique to numerous cultures. However, the fact that magnocellular neurons in the present study stained positive with AChE histochemistry alone cannot be regarded as unequivocally proof of their cholinergic nature. In parallel studies, we have shown that basal forebrain neurons of comparable size and morphology consistently release acetylcholine in response to somatic stimulation (Allen and Brown 1996). These findings are not conclusive demonstration of the cholinergic nature of magnocellular neurons, but they do provide substantial support that our magnocellular neurons might be cholinergic in nature.

Morphology

Basal forebrain nuclei neurons have previously been classified into four main cell types on the basis of their somatic shape and pattern of dendritic branching in Golgi-impregnated neurons in dog (Leontovich and Zhukova 1963) and in human (Arendt et al. 1986, 1995). In our dispersed culture preparation, MBF neurons were grown in the presence of NGF but in the absence of target neurons. This inevitably limited our ability to differentiate dendrites from axons using phase-contrast microscopy. Therefore a detailed morphological study of MBF neurons in culture was not attempted. Nevertheless, when maintained on a poly-D-lysine–treated substrate, neonatal rat (11–14 day old) magnocellular neurons in vitro displayed similar heterogeneity. Like their in vivo counterparts (Dinopoulos et al. 1988; Semba et al. 1987; Woolf 1991; Woolf et al. 1984), they have a large (23–50 μm) soma and ranged in shape being either fusiform, triangular, or multipolar, and displayed characteristic “dendritic” outgrowth.

In addition, one consistent observation in all cell types was the presence of varicosities on the dendrites. Similar varicosities have been described along the length of the dendrites of basal forebrain neurons in the intact brain, and it has been suggested that these may be sites of en passant transmitter release (Houser et al. 1985). Although the structure of these varicosities in our cultures has yet to be examined at the ultrastructural level, we have demonstrated ACh release from such varicosities, following stimulation by a single action potential to the cell soma (Allen and Brown 1996).

Electrophysiological properties

In the present study, we examined the electrophysiological characteristics of AChE-positive MBF neurons. In addition, we attempted to determine whether there was any consistent correlation between the cell’s morphology and its electrophysiological characteristics. However, although some differences were observed in the firing characteristics of individual cells, these were not consistently attributable to any one of the four morphological cell types. For example, in certain neurons a depolarizing current pulse evoked a spike doublet rather than a single spike. This type of behavior was more frequently observed, but not limited to, neurons in cell types I and IV. Thus, in agreement with other studies on the intrinsic membrane properties of septal neurons (Suszkiw et al. 1992), MBF neurons of the four cell types described in the present study were qualitatively indistinguishable (see Table 1).

Spike afterpotentials

After action-potential discharge, MBF neurons exhibited a characteristic afterpotential consisting of a brief (5–8 ms) initial negative-going component superimposed on a generally much larger depolarization. The initial negative-going component could be abolished by 4-AP at concentrations that were found to selectively inhibit the transient A-current. Furthermore, as has been observed, for example, in CA1 hippocampal pyramidal cells (Storm 1987) blocking the A-current with 4-AP inhibited the initial fast AHP and slightly delayed repolarization of the action potential, indicating that it activates sufficiently rapidly to contribute to spike repolarization. In the vast majority (>90%) of magnocellular MBF neurons, the action potential also activated a larger and more prolonged (20–50 ms) depolarizing afterpotential (ADP). In some cases this resulted in discharge of a spike doublet as has been reported in other studies of basal forebrain neurons.
The ADP was calcium dependent and resulted from Ca\(^{2+}\) influx through T-type Ca\(^{2+}\) channels. Low-voltage–activated Ca\(^{2+}\) channels have been shown to be involved in the generation of phasic burst spike discharges in a number of central neurons (Llinás and Yarom 1981; Suzuki and Rogawski 1989) and to act as the trigger for activation of a variety of different Ca\(^{2+}\)–dependent depolarizing afterpotentials (Hasuo and Gallagher 1990; Mayer 1985; Tatsumi and Katayama 1994; Umemiya and Berger 1994; Viana et al. 1993). In a number of studies where ADP has been found to result from activation of Ca\(^{2+}\)–activated chloride channels, niflumic acid has been shown to selectively inhibit this response (Scott et al. 1995). However, when applied to MBF neurons, niflumic acid also inhibited a number of other conductances, and at least at concentrations (up to 30 \(\mu M\)) where it did not block these conductances, its effect on the ADP was inconclusive. Notwithstanding, the fact that the apparent reversal potential of the ADP was close to \(E_C\) does not necessarily indicate the involvement of a chloride current. Rather, it may simply reflect reduced activation of the ADP as a consequence of voltage-dependent inactivation of T-type Ca\(^{2+}\) channels, coupled with depolarization-induced prolongation of the spike, resulting in an increase influx of Ca\(^{2+}\), thus unmasking an AHP conductance (Fig. 4A).

The presence of the ADP appeared to coincide with maturation of the cultures and neurite outgrowth. When we attempted to study the voltage dependence of this current using acutely dissociated cells with no processes, we were unable to evoke an ADP. However, within the first few days of placing the cell into culture during which time the cells had developed extensive neurite outgrowth, an ADP became apparent. This suggests that the site of generation of the ADP may lie in the proximal regions of the dendrites or axon. However, it has been reported that axotomising rat sympathetic neurons can induce a Ca\(^{2+}\)–dependent chloride current and associated ADP that is absent under normal conditions (Sánchez-Vives and Gallego 1994). It is thus possible, that placing MBF neurons in culture has a similar effect on these cells. However, the duration of ADP that develops in sympathetic cells was >10 times longer than the one exhibited by MBF neurons, and, unlike the ADP in MBF neurons, the one in axotomised sympathetic neurons was facilitated by rather than inhibited by repetitive stimulation. Whether magnocellular rat basal forebrain exhibits such a conductance under normal condition has yet to be determined. However, although there have been no detailed studies made, several reports do show examples of current-evoked spike discharge where action potentials are followed by depolarizing afterpotentials of similar duration and magnitude to that reported in the present study (Khatheb et al. 1992; Segal 1986). However, the ADP reported by Segal (1986) in rat medial septal neurons was Cd\(^{2+}\) insensitive.

Finally, previous investigations using slice preparations and conventional intracellular recordings have reported that the most prominent features distinguishing magnocellular neurons from noncholinergic neurons was the presence of a slow AHP following a single or train of action potentials and, in the guinea pig, a distinct plateau on the repolarizing phase of the action potential (Alvarez de Toledo and López-Barneo 1988; Griffith 1988; Griffith and Matthews 1986; Markram and Segal 1990; Matthews and Lee 1991; Sim and Griffith 1991). Although the MBF neurons in our cultures did exhibit Ca\(^{2+}\)–dependent AHPs, these were quite short (<100 ms) and largely masked by the much more dominant spike ADP. Further, these cells only exhibited a plateau phase on their action potentials when held at relatively depolarized potentials. The reason for the difference in the action–potential profile between guinea pig and rat cells is not immediately obvious because rat MBF neurons in culture have large and robust Ca\(^{2+}\) currents (Allen et al. 1993). Notwithstanding, the amplitude of the K\(^{+}\) currents in these rat neurons is very large (see RESULTS) and may act to accelerate spike repolarization. Furthermore, the brief duration of action potentials in rat MBF neurons may act to limit Ca\(^{2+}\) entry and activation of the slow AHP. Another possible explanation for the small size of the AHP in the present study could also be the relatively high level of intracellular Ca\(^{2+}\) buffering we employed (3 mM EGTA), because other workers have been able to record slow AHP in basal forebrain neurons using the whole cell recording technique, provided that low levels of Ca\(^{2+}\) buffering was used (0.1 mM EGTA) (Gorelova and Reiner 1996) or 10 \(\mu M\) Fura (Tatsumi and Katayama 1994). Preliminary studies using the perforated rather than whole cell recording technique, to prevent dialysis of the cell interior, have revealed the presence, in some cells, of a slow AHP, which sums with repetitive stimulation and persists for several seconds following spike discharge in a number of these cells (unpublished observation).

**Inward rectification**

The other consistent feature of MBF neurons in culture was the presence of a pronounced inward rectifier current that displayed voltage-dependent activation with membrane hyperpolarization from resting potential. Similar findings have been reported in a number of previous studies of basal forebrain neurons (Griffith 1988; Griffith and Matthews 1986; Nakajima et al. 1985; Stanfield et al. 1990) and also in a variety of other tissues (Constanti and Galvan 1983; Katz 1949; Leech and Stanfield 1981; Standen and Stanfield 1978). This conductance is characterized by its negative activation range and its sensitivity to Cs\(^{+}\) and to Ba\(^{2+}\) (Constanti and Galvan 1983; Gay and Stanfield 1977; Hagiwara et al. 1976; Standen and Stanfield 1978). However, although this current has previously been shown to be Ba\(^{2+}\) sensitive, the present study reveals the extent of this sensitivity, with significant inhibition being observed at Ba\(^{2+}\) concentrations as low as 0.3–1 \(\mu M\). The findings of the present study show that, although Cs\(^{+}\) and Ba\(^{2+}\) are both effective blockers of inward rectification in MBF neurons, only Ba\(^{2+}\) caused membrane depolarization from resting potential and a concomitant increase in excitability. Indeed Ba\(^{2+}\) also blocks many other K\(^{+}\) currents, and at the Ba\(^{2+}\) concentrations that were observed to induce membrane depolarization in MBF neurons, we observed clear inhibition of outward K\(^{+}\) currents.

**Outward K\(^{+}\) currents**

Our study revealed the presence of large outward currents in MBF neurons, with transient and sustained components.
similar to $I_A$ and $I_{DK}$ previously found in other neuronal preparations (cf. Rogawski 1985; Rudy 1988). $I_A$, which appears to be the dominant transient component and probably functions to facilitate spike repolarization. From its fast activation kinetics (time-to-peak, $\sim 10$ ms), the transient component resembled a high-threshold $I_A$ subtype, as described by Surmeier et al. (1989) in rat neostriatum. In addition to 4-AP- and TEA-sensitive outward currents, a Cd$^{2+}$-sensitive outward current has also been reported in septal neurons from young postnatal (1–7 day) rats in culture (Suszkiw et al. 1992). Although the A-current was very dominant, examination of the action potential revealed the presence of a small Cd$^{2+}$-sensitive Ca$^{2+}$-activated conductance in MBF neurons at depolarized potentials after inhibition of the ADP with amiloride. Furthermore, inhibition of this conductance by Cd$^{2+}$ was found to delay spike repolarization and inhibit the fast AHP.

**Spontaneous activity**

As reported in intact slice preparation of basal forebrain nuclei (Segal 1986), in our in vitro conditions, MBF neurons exhibited spontaneous excitatory and inhibitory synaptic activity. Similar spontaneous synaptic activity has also been reported by other workers studying basal forebrain neurons in culture (Köller et al. 1990; Suszkiw et al. 1992). However, Nakajima et al. (1985) failed to detect any spontaneously active neurons in their cultures of basal forebrain neurons. The reason for this is not clear; however, it may reflect their omission of NGF in their culture media. However, in keeping with the observation of Köller et al. (1990), our spontaneous activity was irregular and did not exhibit rhythmic firing patterns (Suszkiw et al. 1992).

In conclusion, the findings of the present study show that MBF neurons isolated from postnatal (11–14 day old) rats maintain much of their in vivo morphological heterogeneity and can be reliably identified both morphologically and electrophysiologically in dissociated culture.

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