study were probably vasopressinergic, although confirmation requires immunohistochemical identification (*21*). Other types of hypothalamic neuroendocrine cells that control secretion from the adenohypophysis (*2*) and have not been amenable to intracellular recordings may show properties similar to those seen here in MNC's. Whether burst initiation originates endogenously or from excitatory synaptic input, our intracellular recordings strongly support the hypothesis that the ability to promote and sustain bursting can lie within the mammalian neuroendocrine cell itself.

R. DAVID ANDREW*
F. EDWARD DUDEK
Department of Physiology,
Tulane University School of Medicine,
New Orleans, Louisiana 70112

References and Notes

1. D. A. Poulain and J. B. Wakerley, *Neuroscience* **7**, 773 (1982).
2. A. V. Schally, *Science* **202**, 18 (1978); R. Guillemin, *ibid.*, p. 390.
3. M. J. Brownstein, J. T. Russell, H. Gainer, *ibid.* **207**, 373 (1980).
4. M. J. Brimble and R. E. J. Dyball, *J. Physiol. (London)* **271**, 253 (1979); A. Dutton and R. E. J. Dyball, *ibid.* **290**, 433 (1979); J. D. Vincent, D. A. Poulain, E. Arnauld, in *Abnormal Neuronal Discharges*, N. Chalazonitis and M. Boisson, Eds. (Raven, New York, 1978), pp. 103–110; R. J. Bicknell and G. Leng, *Neuroendocrinology* **33**, 295 (1981).
5. J. B. Wakerley and D. W. Lincoln, *J. Endocrinol.* **57**, 477 (1973); D. W. Lincoln and J. B. Wakerley, *J. Physiol. (London)* **242**, 533 (1974).
6. S. H. Thompson and S. J. Smith, *J. Neurophysiol.* **39**, 153 (1976).
7. D. F. Russell and D. K. Hartline, *Science* **200**, 453 (1978).
8. K. Tazaki and I. M. Cooke, *J. Neurophysiol.* **42**, 1000 (1979).
9. A. L. F. Gorman, A. Hermann, M. V. Thomas, in *Molluscan Nerve Cells: From Biophysics to Behavior*, J. Koester and J. H. Bryne, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1980), pp. 169–180.
10. G. I. Hatton, W. E. Armstrong, W. A. Gregory, *Brain Res. Bull.* **3**, 497 (1978); E. W. Haller *et al.*, *Exp. Brain Res.* **33**, 131 (1978).
11. Extracellular recordings have shown that hypothalamic neurons can burst in the slice during chemical synaptic blockade in a solution with low Ca^{2+} and high Mg^{2+} [G. I. Hatton, *J. Physiol. (London)* **327**, 273 (1982)].
12. Slices were prepared and neurons were impaled with micropipettes containing KCl or potassium acetate (80 to 200 megohms) [F. E. Dudek, G. I. Hatton, B. A. MacVicar, *ibid.* **301**, 101 (1980)]. The physiological saline contained 124 m*M* NaCl, 26 m*M* $NaHCO_3$, 5 m*M* KCl, 1.3 m*M* $MgSO_4$, 1.24 m*M* KH_2Po_4, 2.4 m*M* $CaCl_2$, and 11 m*M* glucose. MNC's had the following basic electrophysiological properties: spike amplitudes were 70 to 90 mV, input resistances were 50 to 200 megohms (mean, 155 megohms; $N = 26$), and depolarizing current injection caused repetitive spiking with little reduction in spike height. Although many cells with action potentials between 50 and 70 mV were recorded during this study, none showed the DAP's and plateau potentials seen in the recordings of higher quality. A perfusion bath (1 ml/min) beneath the slice was used to slowly introduce tetrodotoxin (100 μ*M*) or $CdCl_2$ (250 μ*M* in saline without sulfate or phosphate). Not all spontaneous postsynaptic potentials were blocked in tetrodotoxin or $CdCl_2$. Because of the high input resistance of these cells, these events were probably miniature synaptic potentials.
13. Previous injections of Lucifer yellow into numerous neurons recorded in the supraoptic nucleus [R. D. Andrew, B. A. MacVicar, F. E. Dudek, G. I. Hatton, *Science* **211**, 1187 (1981); B. A. MacVicar, R. D. Andrew, F. E. Dudek, G. I. Hatton, *Brain Res. Bull.* **8**, 87 (1982); W. T. Mason, *J. Physiol. (London)* **327**, 44P (1982)] stained only magnocellular neurons (15 to 30 μm in diameter), which terminate primarily in the neurohypophysis [L. W. Swanson and P. E. Sawchenko, *Neuroendocrinology* **31**, 410 (1980)]. Thus, we considered all recorded neurons to be MNC's. The added difficulty of maintaining high-quality impalements (*12*) with Lucifer yellow–filled micropipettes precluded immunohistochemical identification.
14. J. J. Dreifuss, E. Tribollet, A. J. Baertschi, D. W. Lincoln, *Neurosci. Lett.* **3**, 281 (1976).
15. K. Koizumi, T. Ishikawa, C. M. Brooks, *Brain Res.* **63**, 408 (1973).
16. J. Koester, in *Principles of Neural Science*, E. R. Kandel and J. H. Schwartz, Eds. (Elsevier/North-Holland, Amsterdam, 1981), pp. 53–62.
17. Comparable events occur in some molluscan (*6*) and crustacean (*7*) neurons and in mammalian brain slices [R. K. S. Wong and D. A. Prince, *J. Neurophysiol.* **45**, 86 (1981); R. Llinás and M. Sugimori, *J. Physiol. (London)* **305**, 171 (1980)].
18. As seen in neurons cultured from rat supraoptic nucleus [B. H. Gähwiler and J. J. Dreifuss, *Brain Res.* **177**, 95 (1979)] and in one MNC in our slice preparation, periodic input from chemical synapses appears to drive some phasic MNC's.
19. A classic "pacemaker" potential, revealed as a sinusoidal oscillation in membrane potential that increases in frequency during steady depolarization (*9*), does not appear to underlie phasic firing by MNC's because the phasic bursting decreased in frequency during steady depolarization.
20. R. D. Andrew and F. E. Dudek, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 1354 (1982).
21. Some well-impaled hypothalamic neurons cultured from fetal mice display periodic plateau potentials. Preliminary immunohistochemical evidence suggests they are vasopressinergic. Their inability to spike repetitively during a plateau may reflect immature electrical properties [P. Legendre, I. M. Cooke, J.-D. Vincent, *J. Neurophysiol.* **48**, 1121 (1982)].
22. We thank N. R. Kreisman, R. W. Snow, and C. P. Taylor for constructive comments on the manuscript. Supported by a Canadian Medical Research Council fellowship to R.D.A. and by PHS grant NS 16877 to F.E.D.
* Present address: Department of Anatomy, Queen's University, Kingston, Ontario, Canada K7L 3N6.

16 December 1982; revised 29 March 1983

Immunocytochemically Identified Vasopressin Neurons in Culture Show Slow, Calcium-Dependent Electrical Responses

Abstract. *From morphological characterization and intracellular recordings, monolayer cultures derived from fetal mouse hypothalami were found to include functionally differentiated peptide neurons, a number of which appear to contain vasopressin. These cells exhibited particular patterns of slow, calcium-dependent membrane depolarizations, resembling in their periodicity and duration the phasic activity of vasopressin neurons recorded extracellularly in vivo.*

The phasic pattern of electrical activity of vasopressin neurons consists of periodic bursts of action potentials (*1*). Whether this periodicity is synaptically driven or is an intrinsic property of the neurosecretory cell may be determined from intracellular recordings from neurons growing in monolayer culture. With such recording, intrinsic neuronal membrane properties can be studied and the neurons visualized and subsequently identified. We have reported (*2*) that a number of neurons that had differentiated in cultures of dissociated hypothalamic cells displayed long, regenerative electrical responses, which we called plateau potentials. We now report that these responses are associated with neurons that are similar morphologically to magnocellular cells in vivo and that react positively with serum against vasopressin.

Our cultures were derived from mouse (IOPS/OFI) fetuses (13 to 15 days old) by a procedure adapted from Benda *et al.* (*3*). Phase microscopy showed that, after 4 weeks, the cultures consisted of a continuous basal layer of flat cells on which birefringent cells were growing singly or in clusters. Intracellular recordings (*4*) identified the overlying cells as neurons since they displayed overshooting action potentials and postsynaptic potentials similar to those recorded from other cultured neurons derived from various areas of the central nervous system (*5*). They exhibited resting potentials of −40 to −60 mV and input resistances of 50 to 200 megohms. Certain of the largest neurons exhibited, in addition to the usual neuronal activity, plateau potentials. The characteristics of such depolarizations have already been described in detail (*2*).

Plateau potentials usually arose in response to depolarizing postsynaptic potentials (Fig. 1A) but could also be evoked by brief depolarizing current pulses injected through the recording electrode (Fig. 1B). They required 50 to 100 msec to reach an absolute value of −20 mV, which was maintained for 20 to 90 seconds. The duration of the plateau was relatively constant for a given neuron, though it varied from one neuron to another. Plateau depolarizations were observed only when membrane potential was greater than −50 mV; in some cases, it was necessary to pass a steady holding current (< 0.1 nA) to maintain such a value. The plateau corresponds to a high conductance state of the neuronal membrane for the input resistance was about 15 percent of that at rest (Fig. 1A). Presumably the main depolarizing and repolarizing ions are Ca^{2+} and K^{2+}, respectively: plateau generation was inhibited by Co^{2+} and Cd^{2+} but not by tetrodotoxin, and the depolarization and its duration were augmented by tetraethyl-

ammonium chloride (*2*). Plateau potentials probably represent an endogenous property of the neurons since they could be evoked by depolarizing current pulses even in the presence of tetrodotoxin, which blocks action potentials and postsynaptic potentials (*2*).

During the refractory period between successive plateau responses, another type of slow regenerative potential was at times apparent (Fig. 1, A and B). These potentials reached a maximum value of −30 mV and had a short duration (0.2 to 4 seconds). They were blocked by agents that interfere with calcium channels (*2*). The application of tetraethylammonium chloride converted them to plateau potentials lasting more than 20 seconds, but no responses of intermediate duration were observed. In some cases, we could only record slow potentials lasting up to 4 seconds but no associated plateau potential. Whether they should be regarded as partially inactivated plateau responses or as an independent form of response is not clear.

After electrophysiological characterization, a number of neurons were injected with Lucifer yellow or horseradish peroxidase, tracers that diffuse throughout the cells and permit their total visualization (*6*). The neurons varied in their morphology, ranging from small, bipolar forms (cell body diameter, 5 to 10 μm) to large multipolar elements. Of the latter, one type was easily distinguishable by its large, oval cell body (diameter, 15 to 25 μm), two or three tapering dendritic-like processes, and long, thin untapering axon-like process which was occasionally branched and often showed dilatations (Fig. 2A). The neurons from which plateau potentials were recorded were invariably of this type and had a striking resemblance to adult magnocellular cells in vivo revealed by immunocytochemistry (*7*) or by Golgi impregnation (*8*). In addition, cultures incubated for equivalent periods of time as those used for recording were found to contain many neurons that displayed ultrastructural features typical of neurosecretory cells (Fig. 2, D and E). We therefore stained our cultures immunocytochemically (*9*) using rabbit antisera to vasopressin and oxytocin.

Positively immunoreactive cells were found in each of the cultures treated with serum against vasopressin (N = 20). No immunoreactive cells were found in cultures treated with serum against oxytocin (N = 10), or in cultures treated with preimmune or adsorbed serum. All immunoreactive cells had a similar form (Fig. 2, B and C) that closely corresponded to that of the large multipolar neurons rendered totally visible by dye-filling (Fig. 2A) and from which the plateau potentials were recorded (Fig. 1, A and B). Moreover, in three out of five cultures, the same neuron that displayed a plateau potential showed positive immunoreactivity to antiserum to vasopressin (Fig. 2C).

The long duration regenerative potentials observed in our cultured neurosecretory cells may represent mechanisms underlying the phasic activity of magnocellular neurons in vivo. The duration of the plateaus and their variability correspond roughly to the duration of the bursts of firing in vivo, and the refractory

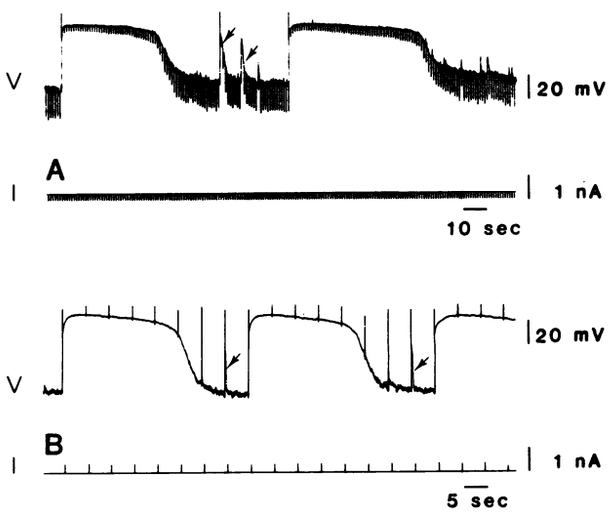

Fig. 1. Penwriter recordings of plateau potentials observed in dissociated hypothalamic neurons in culture for 6 weeks. (A) Spontaneous plateau potentials (*V*) that recurred periodically. During these depolarizations, there is a dramatic drop in the input resistance (85 percent) which was visualized by passing hyperpolarizing pulses of current (*I*) (50 msec, 0.3 nA). Slow potentials of shorter duration (arrows) are apparent between the two consecutive plateau potentials. (B) Plateau potentials (*V*) evoked periodically by repetitive depolarizing pulses (*I*) (50 msec, 0.4 nA). After each plateau, there is a refractory period during which only slow potentials of short duration (arrows) could be ellicited.

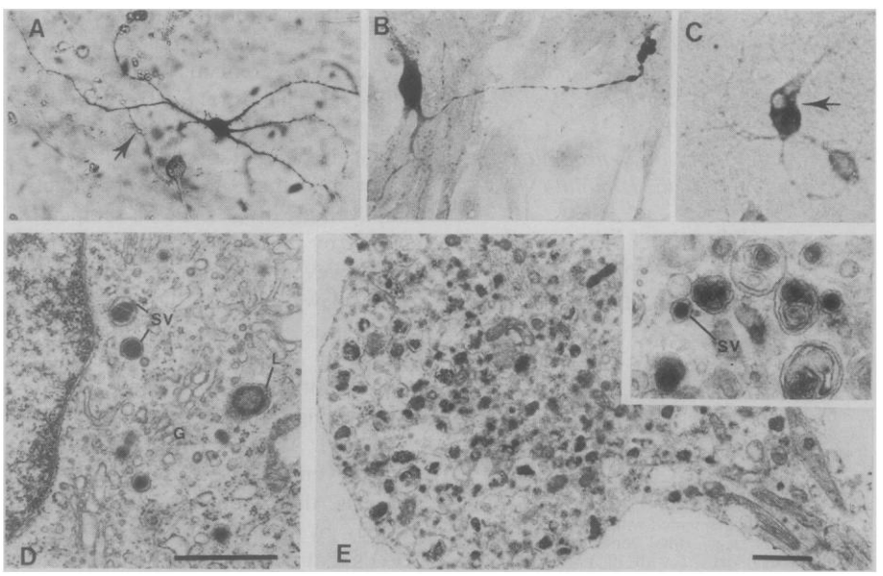

Fig. 2. (A) Example of a dissociated hypothalamic neuron that displayed slow responses (Fig. 1) and was then injected with horseradish peroxidase (Sigma, type 6). Note that numerous spines cover the cell body and dendritic processes but not the thin axonal process that emerges from one of the dendrites (arrow) (×200). (B and C) Hypothalamic cultures treated with serum against vasopressin. The immunoprecipitate, made evident by peroxidase, was localized in cell bodies and axonal-like processes and their swellings. The darkly stained neuron in (C) (arrow) had displayed electrical responses similar to those described in Fig. 1. Cells of the basal layer and adjacent neurons showed no or very faint immunoreactivity (×250 and ×350, respectively). (D and E) Electron micrographs of horizontal sections through 30-day old hypothalamic cultures. Fixation by 2.5 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer followed by 1 percent osmium tetroxide and embedding in epon were done directly in culture dishes. In (D), dense-cored secretory vesicles (120 to 180 nm) (*sv*), lysosomes (*L*), and well-developed Golgi complexes (*G*) identify the cell body as neurosecretory. In (E), a large dilatation is filled with lysosomes and secretory vesicles (*sv*), shown at higher magnification in the inset. Such dilatations, similar to Herring bodies in the adult neurohypophysis, presumably correspond to the axonal swellings in (B). Scale bar, 1μm.

period following the plateaus corresponds to the silent periods separating the bursts (*1*). The frequency of recurrence of the plateaus and of the bursts also shows parallel behavior. It is thus tempting to speculate that the phasic pattern of firing recorded extracellularly from vasopressin-secreting cells in vivo may be triggered by endogenous plateau potentials. Indeed, such potentials are of appropriate form and magnitude to serve as driver potentials supplying depolarizing current to an axonal impulse-initiating zone (*10*). Nevertheless, in contrast to in vivo experiments where action potentials are recorded from the somata in vitro, we observed no action potentials superimposed on the plateau depolarizations. This is not surprising since action potential generation would be inactivated by a depolarization and fall in the input resistance of the magnitude recorded in our cells. If plateau potentials do exist in vivo, they must be of smaller magnitude to permit somatic impulse generation. The magnitude of the depolarizations recorded in our cultured neurons may be due to the in vitro conditions in which the cells are raised, to their stage of maturation, or to both.

D. T. THEODOSIS
P. LEGENDRE
J. D. VINCENT
Unité de Recherches de Neurobiologie des Comportements, INSERM, U. 176, Bordeaux, France
I. COOKE
Bekesy Laboratory of Neurobiology, University of Hawaii, Honolulu 96822

References and Notes

1. D. A. Poulain and J. B. Wakerley, *Neuroscience* **7**, 773 (1982).
2. P. Legendre, I. Cooke, J. D. Vincent, *J. Neurophysiol.* **48**, 1121 (1982).
3. P. Benda, F. de Vitry, R. Picart, A. Tixier-Vidal, *Exp. Brain Res.* **23**, 29 (1975); see also (*2*).
4. The recordings were performed with KCl (3*M*)-filled glass micropipettes (50 to 70 megohms). A conventional bridge circuit (Dagan 8500) permitted simultaneous voltage recording and injection of current through the same electrode. The preparations, maintained at 30°C, were continuously perfused with balanced salt solution (Hanks), supplemented with 0.6 percent glucose and 2 m*M* Ca^{2+} (final concentration, 4 m*M*), buffered at *p*H 7.2 with 10 m*M* Hepes. Drug applications were made with micropipettes with tip diameters of 8 to 10 μm; the ejections were controlled by a gas pressure system with a micropump (Medical System, BH2).
5. See P. G. Nelson, E. A. Neale, R. L. MacDonald, in *Excitable Cells in Tissue Culture*, P. G. Nelson and M. Liebermann, Eds. (Plenum, New York, 1981), pp. 39–80.
6. W. W. Stewart, *Cell* **14**, 741 (1978); E. A. Neale, R. L. MacDonald, P. G. Nelson, *Brain Res.* **152**, 265 (1978). For more detail on injection of these tracers into the hypothalamic neurons, see also (*2*).
7. M. V. Sofroniew and W. Glasmann, *Neuroscience* **6**, 619 (1981).
8. W. E. Armstrong, J. Scholer, T. H. McNeill, *ibid.* **7**, 679 (1982); I. J. Luqui and C. A. Fox, *J. Comp. Neurol.* **168**, 7 (1976).
9. Immunohistochemical staining was performed directly in the culture dishes. After rinsing in phosphate-buffered saline (PBS, Dulbecco's, Mg^{2+}- and Ca^{2+}-free), the cultures were fixed with 4 percent paraformaldehyde in PBS for 2 hours at room temperature, rinsed well again, and exposed to 0.1 percent Triton X-100 in PBS for 10 minutes. They were then incubated with 1 percent human serum albumen in PBS to prevent nonspecific binding of antibody and immediately incubated with the antibodies, diluted 1:1000 in PBS for 24 hours at 4°C. Certain cultures were then reacted with fluorescein-conjugated sheep antiserum to immunoglobulin G (IgG; Institut Pasteur) at a dilution of 1:10. Other cultures were first reacted with sheep serum against rabbit IgG, diluted 1:30 for 30 minutes, and then with a peroxidase-antiperoxidase complex (Dakopats) diluted 1:75 for 1 hour; peroxidase activity was visualized with diaminobenzidine. Control staining was performed either with rabbit preimmune serum or with the antibody solutions to which the respective peptides were added, free or coupled to Sepharose beads.
10. K. Tazaki and I. M. Cooke, *J. Neurophysiol.* **42**, 1000 (1979).
11. We thank D. Poulain for his help with the manuscript, R. Bonhomme and B. Dupouy for technical assistance, and M. A. Delaage for the sera against vasopressin and oxytocin. Supported by grant DGRST 82 EO 26.

20 October 1982; revised 14 March 1983

Differential Effects of Classical and Atypical Antipsychotic Drugs on A9 and A10 Dopamine Neurons

Abstract. *Prolonged treatment with classical antipsychotic drugs decreased the number of spontaneously active dopamine neurons in both the substantia nigra (A9) and the ventral tegmental area (A10) of the rat brain. In contrast, treatment with atypical antipsychotic drugs selectively decreased the number of A10 dopamine neurons. Related drugs lacking antipsychotic efficacy failed to decrease dopamine activity. These findings suggest that the inability of atypical antipsychotic drugs to decrease A9 dopamine neuronal activity may be related to their lower potential for causing tardive dyskinesia and that the inactivation of A10 neurons may be involved in the delayed onset of therapeutic effects during treatment.*

The dopamine (DA) hypothesis of schizophrenia postulates hyperactive DA neurotransmission as an etiological factor in schizophrenic symptomatology (*1*). Despite continued efforts to provide direct support for DA overactivity in unmedicated schizophrenics, the available evidence remains largely uncompelling (*2*). Therefore, the DA hypothesis continues to rest primarily on a pharmacological foundation, which has as its cornerstone the evidence that effective antipsychotic drugs (APD's) dampen DA activity by blocking brain DA receptors (*3*). However, this cornerstone is structurally flawed because DA receptor antagonism is an almost immediate consequence of APD administration whereas antipsychotic efficacy becomes manifest only during prolonged treatment with APD's (*4*). Although this flaw could lead to the eventual collapse of the DA hypothesis, the foundation might be reinforced by demonstrating effects of APD's that develop only as a secondary effect of long-term DA receptor blockade and, therefore, may be causally related to the delayed onset of therapeutic efficacy. However, most reports have suggested that the efficacy of DA receptor antagonism diminishes during treatment with APD's (*5*), a finding that seems incompatible with the slowly developing onset of therapeutic efficacy.

Recently, we confirmed (*6*) the previous finding (*7*) that prolonged haloperidol (HAL) treatment decreases the number of spontaneously active DA-containing neurons in the rat substantia nigra zona compacta (A9) and extended this finding to DA-containing neurons in the ventral tegmental area (A10) (*6*). The nigrostriatal A9 DA system is thought to be involved in APD-induced extrapyramidal side effects such as tardive dyskinesia (TD) (*8*), whereas the mesolimbic and mesocortical A10 DA systems have been implicated in the therapeutic actions of APD's (*1*). Our experiments demonstrated that the decline of spontaneously active DA neurons during HAL treatment is a slowly developing process that occurs earlier and to a greater extent in A10 than in A9. Since the time courses for the decline of A10 and A9 DA activity correspond to the fact that the therapeutic effects of APD treatment precede the onset of TD, we proposed that the inactivation of A10 and A9 DA neurons may be related to the delayed onset of pharmacotherapy and TD, respectively. If this is the case, then it would be expected that "atypical" APD's, which possess a low potential for causing extrapyramidal side effects and TD, would preferentially inactivate A10 DA neurons. This hypothesis was tested in the present experiments by comparing the effects of prolonged treatment with various classical and atypical APD's on the number of spontaneously active A9 and A10 DA neurons as determined using extracellular single-unit recording techniques. We report that atypical APD's differ from classical APD's in that they selectively inactivate A10 DA neurons (*9*).

In these experiments we investigated