ureotelic metabolism can occur even among embryos developing in fully cleidoic eggs, which do not take up supplemental water from the environment. Retention of ureotely in this case cannot be explained by suggesting that embryonic softshell turtles lack the biochemical preadaptations necessary for uricotely (*18*) because they have the capacity to form urate (Fig. 1). Thus, our results raise the possibility that uricotely is not a necessary outcome of natural selection for mechanisms to conserve water during embryonic development (*19*) and indicate a need to reassess the adaptive significance of uricotely among embryos of other terrestrial vertebrates.

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7. Eggs were collected on 23 and 24 June 1982 from natural nests located on sandbars in the South Platte River, near Crook, Logan County, Colorado. White spots had not formed on the uppermost surfaces of the eggs, indicating that nests were only 1 to 3 days old at the time eggs were collected.
8. Half the eggs were incubated on a wet substrate (water potential of −150 kPa), and the rest were incubated on a dry substrate (−800 kPa). See G. C. Packard *et al.* [*Physiol. Zool.* **54**, 165 (1981)] for full details on methods. Because analyses of variance indicated that different substrates for incubating eggs had no statistically significant effect on amounts of nitrogen excreted in the form of ammonia ($P > 0.81$ for main effect and interaction), urea ($P > 0.57$), and soluble urate ($P > 0.25$), data for the two treatments were pooled for presentation here.
9. Ammonia-nitrogen and urea-nitrogen were measured by a colorimetric procedure based on the methods of J. K. Fawcett and J. E. Scott [*J. Clin. Pathol.* **13**, 156 (1960)] and of A. L. Chaney and E. P. Marbach [*Clin. Chem. (Winston-Salem, N.C.)* **8**, 130 (1962)], as detailed in *Sigma Tech. Bull. 640* (Sigma Chemical, St. Louis, revised October 1974). Readings of absorbance were taken at 570 nm with a Bausch & Lomb Spectronic 21 digital spectrophotometer.
10. Uric acid was measured by a colorimetric procedure based on the methods of M. B. Blauch and F. C. Koch [*J. Biol. Chem.* **130**, 443 (1939)] and of R. J. Henry *et al.* [*Am. J. Clin. Pathol.* **28**, 152 (1957)], as detailed in *Sigma Tech. Bull. 680* (Sigma Chemical, St. Louis, revised June 1974). The procedure entails digestion by uricase to correct for interfering substances. Absorbances were read at 650 nm.
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Burst Discharge in Mammalian Neuroendocrine Cells Involves an Intrinsic Regenerative Mechanism

Abstract. *Intracellular recordings from mammalian neuroendocrine cells showed that steady, injected currents can modify and block periodic spike bursts previously associated with increased neurohormone release. Spike afterpotentials could sum to form plateau potentials, which generated bursts and did not depend on axonal conduction or chemical synapses. Therefore, bursting involves a spike-dependent, positive-feedback mechanism endogenous to single neuroendocrine cells.*

Neuroendocrine cells, whose electrical activity causes secretion of peptides into the nervous and circulatory systems, are vital in the regulation of such diverse bodily functions as reproduction (*1*), endocrine control (*2*), and maintaining salt and water balance. The cells that secrete vasopressin and oxytocin have been the primary model, particularly in mammals, for electrophysiological (*1*) and biochemical (*3*) studies of neuroendocrine systems. In vivo extracellular

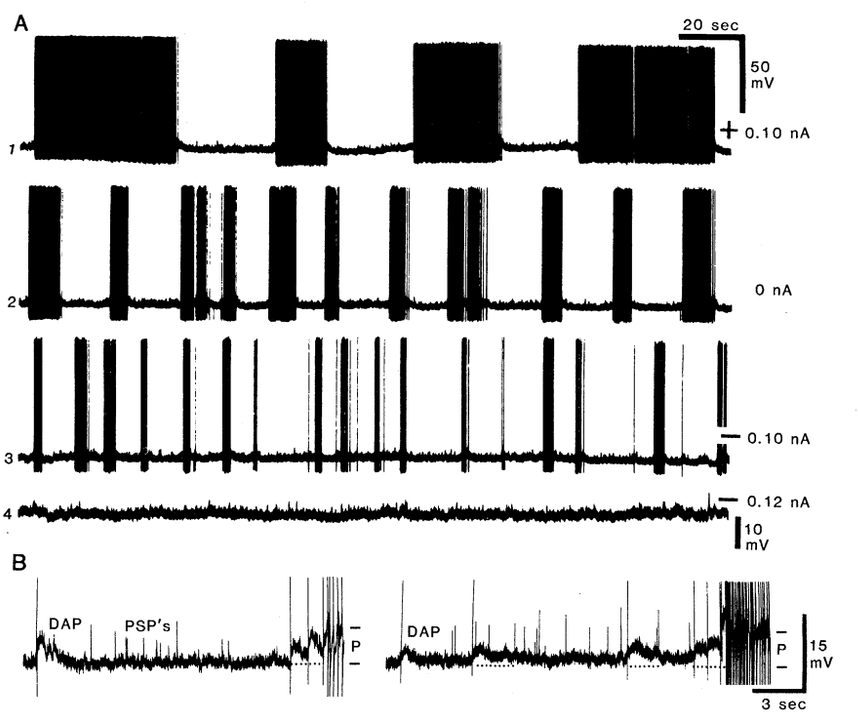

Fig. 1. (A) Alteration of burst length in a phasic burster by steady changes in membrane potential from injected current. Membrane depolarization (trace 1) promoted longer bursts with higher spike frequency than observed at resting potential (trace 2). Conversely, membrane hyperpolarization caused shorter bursts with lower intraburst spike frequency (trace 3). Stronger hyperpolarizing currents (trace 4) blocked the slow underlying depolarization and revealed postsynaptic potentials (*PSP's*) that appeared random. Neuronal input resistance was ~ 120 megohms. (B) Depolarizing afterpotentials and plateau potentials in two phasic bursters. A DAP followed each action potential during the period between bursts. The DAP's could summate, leading to a plateau potential (*P*) and an overriding spike burst. Spike peaks are not shown. Input resistance was ~ 190 megohms (left) and ~ 200 megohms (right). Small depolarizations are excitatory postsynaptic potentials.

recordings from single magnocellular neuroendocrine cells (MNC's) in the rat supraoptic and paraventricular nuclei have shown that, under conditions requiring water conservation, phasic bursts of action potentials lead to vasopressin release (*4*). A separate population of MNC's bursts synchronously to release oxytocin during lactation (*5*). Intracellular recordings, which are required for direct study of the mechanisms underlying such bursting, have been nearly impossible to make from mammalian MNC's in vivo. Some molluscan and crustacean neurons, however, are known to undergo slow changes in ionic conductances responsible for cyclic bursting (*6–9*).

We recorded intracellularly from MNC's in rat hypothalamic slices and found that single action potentials generate slow depolarizing afterpotentials (DAP's), which can sum to promote a long burst. Our data support the long-standing hypothesis, arising from studies of invertebrates (*6–9*), that endogenous mechanisms generate the spike bursts in mammalian MNC's, particularly the periodic bursting pattern associated with vasopressin release.

Extracellular recordings from single MNC's have shown that phasic bursting can occur in hypothalamic slices (*10, 11*). In seven phasic cells we confirmed this result with intracellular recording (trace 2 in Fig. 1A) (*12, 13*). Phasic bursts, comparable to those seen in vivo during the release of vasopressin (*4*), were superimposed on slow depolarizations. When steady depolarizing current was injected through the intracellular electrode, bursts became longer and intraburst spike frequency increased (trace 1 in Fig. 1A). Steady hyperpolarizing current resulted in shorter bursts of spikes with a lower intraburst firing frequency (trace 3 in Fig. 1A). Stronger hyperpolarizing currents blocked spontaneous action potentials, eliminated the slow fluctuations in membrane potential that normally underlie phasic bursting, and revealed that chemical postsynaptic potentials occurred randomly (trace 4 in Fig. 1A). Therefore, changes in membrane potential could modify and even block bursting. Phasic synaptic potentials did not appear to cause the phasic firing pattern of these seven cells (*11*). Instead, action potentials provided a regenerative drive for further firing (*6, 7*). A slow DAP, which was most obvious during the period between bursts, followed each spike (Fig. 1B). The DAP's could promote burst initiation by summing to form a plateau potential. In our recordings DAP's and plateau potentials were seen only in impalements of the highest quality (*12*).

An important observation made previously in mammalian MNC's in vivo was that antidromic spikes can trigger a single burst (*14*). This suggested an endogenous mechanism for burst firing because weaker stimuli that activated other neurons did not cause a burst in the recorded cell. We directly corroborated this result in four silent MNC's by finding that injection of a brief depolarizing pulse to elicit spiking could repeatedly evoke an afterdischarge that lasted up to 30 seconds and was superimposed on a sustained plateau potential (Fig. 2A). The long duration of such afterdischarges is remarkable and probably results from a positive feedback mechanism. That is, DAP's sum to form a plateau potential, thus increasing the likelihood of further spikes and their DAP's, which in turn add to the plateau potential and prolong the burst. In two silent cells failure of this mechanism to terminate after a brief intracellular current pulse resulted in fast continuous firing.

Recurrent excitation from axon collaterals may exist in vertebrate hypothalamic systems (*15*), and this mechanism could contribute to the plateau potential. However, bath application of tetrodotoxin, which blocked the fast, presumably Na^+-dependent spikes, had no substantial effect on the plateau potential after a current-evoked burst (Fig. 2B). Typically, a tetrodotoxin-insensitive afterhyperpolarization transiently interrupted the plateau potential. Because axonal spikes are generally thought to be Na^+-dependent (*16*), these data for tetrodotoxin-treated cells are evidence that recurrent synaptic inputs from axon collaterals do not generate the plateau potential. Furthermore, the plateau potential could still be evoked in a concentration of Cd^{2+} high enough to block evoked chemical synaptic potentials (Fig. 2C) but too low to affect action potential duration or amplitude. Therefore the plateau potential does not arise from chemical synaptic transmission but results from endogenous mechanisms that are activated in the neuron by previous action potentials (*17*).

Phasic burst firing could result from chemical synaptic input (*18*) or an endogenous pacemaker potential (*19*). However, our experiments demonstrate the presence of an intrinsic, positive-feedback mechanism that involves progressive summation of DAP's to form a plateau potential that sustains the burst. An endogenous inhibitory mechanism, probably including a Ca^{2+}-activated K^+ conductance, may contribute to burst termination (*20*). Alternation between these activity-dependent changes in excitability appear intrinsic to the MNC and can shape periodic spike bursts.

Additional intracellular recordings are required to elucidate fully the electrophysiological properties responsible for bursting, which is associated with enhanced release of hypothalamic neuropeptides. The phasic bursters in this

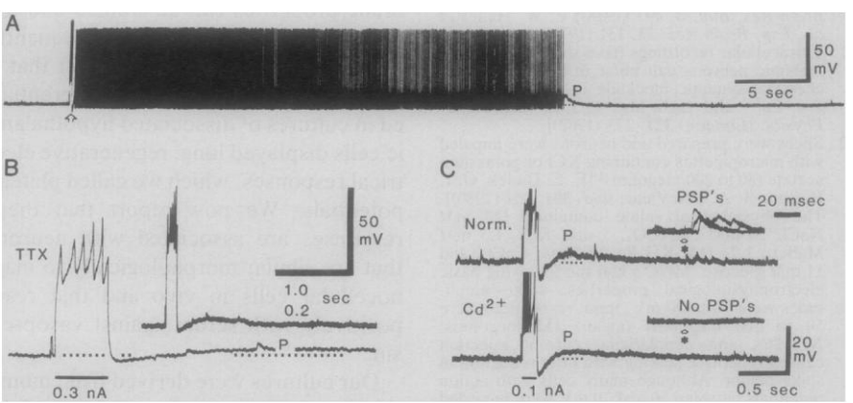

Fig. 2. (A) Burst from a brief injected current pulse. This silent cell fired a 30-second afterdischarge after injection of a brief (150 msec) depolarizing current pulse that was suprathreshold (arrow). Action potentials rode on a plateau potential (*P* indicates end of plateau potential). Input resistance was ~ 130 megohms. (B) Persistence of plateau potentials in tetrodotoxin (*TTX*). Despite elimination of presumed fast Na^+ spikes during an evoked spike train, tetrodotoxin did not block the plateau potential. Inset at upper right shows the same response at a slower time scale. Input resistance was ~ 200 megohms. (C) Persistence of plateau potentials during blockade of chemical synaptic transmission with Cd^{2+}. The recorded cell was orthodromically stimulated 1 mm dorsal to the supraoptic nucleus. A low concentration of Cd^{2+}, which did not alter the action potential wave form, blocked evoked postsynaptic potentials (*PSP's*) even when stimulus strength was doubled (insets on right). However, the plateau potential was unaffected. Spike peaks are not shown. Input resistance was ~ 120 megohms. Spontaneous postsynaptic potentials in Cd^{2+} and tetrodotoxin probably represented miniature postsynaptic potentials.

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.

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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.
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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.
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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-