Action potentials occur in cells of the normal anterior pituitary gland and are stimulated by the hypophysiotropic peptide thyrotropin-releasing hormone

(electrical excitability/sodium/calcium/tetrodotoxin/calcium blocker D600)

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ABSTRACT Electrical activity in the form of action potentials (spikes) was discovered in normal anterior pituitary cells obtained from rats by tissue dissociation and maintained in culture. Passage of outward current through the microsuction electrodes used for recording often increased spike frequency in spontaneously active cells or initiated spikes in cells previously electrically silent. Spiking persisted in the presence of tetrodotoxin and in the absence of sodium, but was inhibited by the calcium blockers D600 and lanthanum. Such spikes appear, therefore, to be calcium spikes, but contributions to spiking by other ions are not excluded. The stimulant hypophysiotropic peptide thyrotropin-releasing hormone elicited spiking in about ten percent of the cells on which it was tested. These cells are possibly thyrotrophs and mammotrophs, the physiological target cells for this hormone. These results, considered along with existing evidence that adenohypophysyal secretion requires calcium and is elicited by calcium ionophores, prompt the conclusion that action potentials involving calcium influx participate in stimulus-secretion coupling in the anterior pituitary. It may be by stimulating or modulating such electrical activity (with hypophysiotropic hormones) that the brain regulates anterior pituitary secretion.

The anterior pituitary gland is a composite endocrine organ made up of several cell types secreting different hormones. The secretory activity of the various cells is regulated by the brain through specific stimulant or inhibitory substances (hypophysiotropic hormones) that are elaborated by neurosecretory fibers in the hypothalamus and delivered into the blood supply that courses to the anterior pituitary gland. How the individual gland cells recognize, and respond to, the various hypophysiotropic hormones is a central question in endocrinology to which there are only fragmentary answers (1-3). This particular issue is, however, but one facet of the more general problem of stimulus-secretion coupling. Within this broader context a pattern can often be discerned in which a stimulus for secretion (secretagoge) acts on the plasma membrane of the cell to promote influx of calcium ions, which then mediate the secretory response (4, 5). Sometimes the calcium entry is a consequence of action potentials. Neurons and neurosecretory fibers are the most familiar and clear-cut examples (5, 6), but action potentials have also been detected in endocrine cells of the pancreas (7, 8) and adrenal medulla (9, 10). Moreover, the possibility of a wider involvement of such electrical activity in the normal control of endocrine function has recently been raised by electrophysiological studies on several transformed (neoplastic) cell types of supposed endocrine origin (11) including a pituitary adenomatous (GH3) cell line (12). Our purpose here is to report the discovery, in normal anterior pituitary cells, of electrical activity (action potentials, spikes) that is increased by an identified stimulant hypophysiotropic hormone, thyrotropin-releasing hormone (TRH), and to provide evidence that spikes in adenohypophysyal cells involve a calcium component and may participate in stimulus-secretion coupling.

METHODS AND MATERIALS

To prepare each batch of cells, anterior pituitaries from 5 to 10 male (350–450 g) or lactating female (330–400 g) rats were dissociated using the method of Hopkins and Farquhar (13) except that 25 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes)/NaOH buffer (pH 7.4) was used in place of bicarbonate buffer in the dissociation media. Portions containing approximately 106 cells were maintained in individual Falcon dishes (35-mm diameter) in 2 ml of Ham’s F-10 nutrient mixture (GIBCO) supplemented with 12.5% horse serum, 2.5% fetal calf serum, penicillin at 100 units/ml, and streptomycin at 100 μg/ml in a 5% CO2/air atmosphere at 37°. After 1 or more days the culture medium was replaced by recording solution and the dish was placed on the stage of an inverted microscope allowing phase contrast observation at 800X magnification. For the first day or so, the adenohypophysyal cells, although attached, were round and relatively easily recorded from with the suction electrode. Thereafter, they became increasingly flat and for this reason recording became increasingly difficult. Nevertheless, successful recordings were commonly obtained up to 8 days after dissociation and sometimes as long as 2 weeks after. Recording solution (30–34°C) contained (in mM): NaCl, 152; KCl, 5.6; CaCl2, 1.8; glucose, 5.5; Hepes buffer, 5 (pH 7.4); with bovine serum albumin at 1 mg/ml. Sodium-free solutions were prepared by replacing NaCl with an equiosmotic amount of mannitol (Hepes was here titrated with KOH) or by replacing NaCl and Hepes with Tris. Test solutions of TRH (Bachem), D600 (Knoll), the methoxy derivative of verapamil, or lanthanum (La) were made up in the recording solution and applied to individual cells by gravity flow from a micropipette whose tip (diameter 10–25 μm) could be rapidly positioned near the cell of interest. Because some mixing with the recording solution already in the bath may have occurred, the stated concentrations for TRH, D600, and La should be considered maximum rather than actual values. An indication of the efficacy and time course of drug delivery by this system was provided by intracellular recordings we have obtained (unpublished results) from a clonal line (GH3) of transformed adenohypophysyal cells. These recordings show that delivery of excess K medium (recording solution with 100

Abbreviations: TRH, thyrotropin-releasing hormone (thyrroliberin); Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.
FIG. 1. Action potentials (spikes) recorded extracellularly from normal adenohypophysial cells. (a–c) Records from three cells illustrating different patterns of spontaneous activity: random (a), regular (b), and bursting (c); vertical calibrations 1 mV. (d) Action potentials evoked in an electrically silent cell by passing outward current (5.5 nA, top trace) through the recording electrode. The small potential oscillation may be an aborted action potential. Note that sweep speed is faster than in records a–c. (e) A correspondingly fast sweep of a spontaneous action potential from another cell.

mM Na replaced by 100 mM K) through such a pipette always causes the cells to depolarize, and that the effect appears within a few seconds of positioning the pipette, is maintained, and subsides to control resting membrane potential within 15 sec or so of withdrawing the pipette.

Extracellular electrical recording of the normal pituitary cells was done with glass microsuction electrodes (10) with internal tip diameters of 3–6 μm and resistances of 1–10 MΩ (depending on shank shape and length) when filled with the recording solution. A bridge circuit (M4A, W-P Instruments) was used for passing current through the recording electrode. Potentials were displayed on the screen of a storage oscilloscope (RM564, Tektronix, ac coupled frequency range 1.6 Hz to 5 KHz) and a chart recorder (7402A, Hewlett-Packard).

RESULTS

Spontaneous action potentials were observed in about 20% of the more than 200 cells recorded from extracellularly. They occurred at random or fairly regular intervals or in bursts (Fig. 1 a–c). Their frequency ranged from 0.01 to 7 Hz. When outward current was passed through the recording electrode, the frequency of action potentials was often increased in spontaneously active cells and action potentials appeared in about one-third of the cells previously electrically silent (Fig. 1d). Spike amplitudes were usually 0.1–2 mV but sometimes exceeded 10 mV, the range probably reflecting variations inherent in recording with microsuction electrodes (see ref. 10).

Action potentials were recorded in the presence of tetrodotoxin (2 μM), the well-known blocker of sodium spikes (15–17), and also when all the sodium in the solution was replaced with Tris (Fig. 2) or mannitol. On the other hand, they were suppressed by D600 (10 μg/ml), a substance known to block calcium channels (18, 19). This drug reduced both the amplitude and frequency of the spikes, sometimes abolishing them, and its effects were wholly or partially reversible (Fig. 3). Lanthanum (1 mM), another blocker of calcium channels (19–21), also reversibly inhibited the action potentials.

In 12 cells (about 10% of those tested) TRH (5 or 50 nM) elicited action potentials, causing them to appear in previously silent cells (Fig. 4a) or to increase in frequency in cells spontaneously active (Fig. 4b). The TRH-induced action potentials typically appeared abruptly within a few seconds of positioning the delivery pipette, rapidly reached a maximum frequency which sometimes exceeded 10 Hz, and slowly returned to the control level when the delivery pipette was removed (Fig. 4). Such responses could be repeatedly elicited from the same cell, thus the record of Fig. 4a is the fourth of seven similar responses elicited successively from one cell; while the record of Fig. 4b is the fourth of five successive, and similar, responses from

FIG. 2. Action potentials recorded from adenohypophysial cells in the absence of extracellular sodium (Na replaced by Tris). (a) Spontaneous action potentials. (b) Evoked action potentials (different cell). Top trace signals duration of outward current (4 nA) pulse.

FIG. 3. Depressant effect of D600 on the frequency and amplitude of action potentials in adenohypophysial cells. D600 (10 μg/ml) was delivered to two spontaneously active cells for the times indicated by the bars above the records. (a) Continuous record. (b) Segments of a similar record from a second cell obtained before, during, and after D600 (center record begins 10 s after exposure to D600. Right-hand record begins 100 s after withdrawal of the D600 pipette).
another cell. Occasionally were encountered that yielded a somewhat different pattern of response to TRH: each application of TRH caused a much briefer ill-sustained burst of action potentials.

**DISCUSSION**

Our experiments provide two new pieces of evidence on the function of normal anterior pituitary cells: first, that these cells, or at least many of them, show electrical activity in the form of action potentials (spikes); and, second, that such action potentials can be elicited, or their frequency increased, by a known physiological stimulus to adenohypophyseal cells, TRH. Although endocrine cells as a class are not generally regarded as being electrically excitable, and previous electrophysiological studies on normal cells of the pituitary pars distalis have provided no evidence of action potentials (22, 23), spikes have been observed in several different neoplastic cell types of reported endocrine origin (11), including a line (GH3) from a pituitary adenoma, where spiking increased in response to TRH (12). The electrical activity observed in neoplastic cells could, of course, be a peculiarity resulting from differentiation and hence not representative of normal function. However, there is precedent for electrical activity in normal endocrine cells, including certain adenohypophyseal cells. Thus, Davis and Hadley (24) have observed potential oscillations in pars intermedia, which they suggest are "most probably depolarizations." And, there are demonstrations of spiking in β cells of the endocrine pancreas (7, 8) and chromaffin cells of the adrenal medulla (9, 10). Both these cell types, like the cells of the anterior pituitary, belong to Pearse's APUD (amine precursor uptake and decarboxylation) series, all of which he has argued arise from neuroectoderm (25). The fact that only about one in three of the cells we recorded from showed spontaneous or evoked action potentials does not necessarily mean that electrical activity and excitability is a property of only some adenohypophyseal cells: others might have lost excitability as a result of the conditions of culture or recording. Such factors might also have reduced the number of cells yielding action potentials in response to TRH. However, it should be borne in mind that TRH elicits secretion only from thyrotrophs and mammotrophs in cultures of the sort we have used (26) and not from any of the several other adenohypophyseal cell types present (3). It is not unlikely, therefore, that the TRH-induced action potentials reflect a selective stimulant effect of TRH on physiologically appropriate target cells. In pancreatic β cells and adrenal chromaffin cells, spiking increases in response to appropriate physiological se-

cretagogues, glucose and acetylcholine, respectively (7, 9, 10, 27). One reasonable inference, therefore, is that the TRH-induced spikes are involved in stimulus–secretion coupling. In line with this is our observation of a vigorous discharge of spikes with TRH at a concentration (5 nM) that provides a highly effective stimulus for secretion of TSH and prolactin from dispersed and cultured rat adenohypophyseal cells (28). Moreover, because the discharge of spikes begins within a few seconds of applying TRH this short latency could easily account for the promptest secretory responses recorded; timing of secretion over a period of seconds has not yet been achieved but it is known that secretion is detectable within minutes (4, 28). The view that such spikes participate in stimulus–secretion coupling is further encouraged by the evidence that they involve calcium ions. Calcium is essential for secretion of adenohypophyseal hormones and its entry into adenohypophyseal cells apparently provides an adequate stimulus (witness the effects of calcium ionophores, see refs. 3 and 28). Our conclusion that the spikes involve inward calcium currents rests on the observation of persistent spiking in tetrodotoxin-containing and sodium-free media and on the inhibitory effects of known blockers of calcium channels, D600 and lanthanum. This evidence, like that provided by Kidokoro (12), for GH3 cells, is admittedly indirect, but is buttressed by observations we have made on another clonal anterior pituitary cell line (GH4), which show that spike amplitude increases as the concentration of extracellular calcium (but not sodium) is raised (29). Whether or not ions other than calcium can contribute to spiking in adenohypophyseal cells remains to be tested. Although spikes in pancreatic β cells are calcium dependent (8), this is not true of chromaffin cells, where the major component of the action potential is sodium dependent (9, 10).

Calcium may have an important role in addition to charge carrying during individual spikes. The calcium channel blocker D600 did not simply reduce spike amplitude; it also reduced spike frequency. This suggests that calcium permeability is important for regulating the depolarizations that trigger spikes.

In light of evidence that calcium entry provides an adequate stimulus for release of adenohypophyseal hormones (3, 28), our observations suggest to us that action potentials, involving calcium ions as charge carriers, participate in stimulus–secretion coupling, and that it is by initiating or modulating action potentials that the brain, through the hypophysiotropic hormones, regulates secretion in the anterior pituitary.

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