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## Adrenocorticotropic Hormone Causes Long-Lasting Potentiation of Transmitter Release from Frog Motor Nerve Terminals

Abstract. *Exposure of frog neuromuscular preparations to adrenocorticotropic hormone for several minutes increased both nerve-evoked and spontaneous transmitter release for several hours. No changes in postsynaptic sensitivity to transmitter were detected. The long-lasting potentiation shows little sensitivity to changes in extracellular calcium concentration and seems to be entirely presynaptic in origin.*

Recent studies suggest that adrenocorticotropic hormone (ACTH) has a broad distribution in the nervous system (*1*) and exerts a wide spectrum of physiological, behavioral, and biochemical actions (*2*), in addition to its well-known function in stimulating the adrenal cortex. In this report we show that ACTH and some closely related peptides produce a long-term increase in amplitudes of end-plate potentials (EPP's) and frequency of miniature end-plate potentials (mEPP's) in frog cutaneous pectoris and sartorius neuromuscular preparations. These effects appear to be entirely presynaptic in origin.

Purified porcine ACTH(1-39) (Sigma), synthetic melanocyte-stimulating hormone (α-MSH) (Sigma), synthetic ACTH(4-10) (Peninsula), and ACTH(1-24) (Organon) at concentrations of 0.2 to 2 m*M* in water were kept frozen until ready for use, then diluted into saline at 0.4 to 10 μ*M*. The normal Ringer solution contained 116 m*M* NaCl, 2 m*M* KCl, 1.8 m*M* $CaCl_2$, and 5 m*M* D-glucose, buffered at *p*H 7.2 with 5 m*M* Hepes. Nerve-evoked contractions were blocked by adding *d*-tubocurarine chloride (1 to 5 μg/ml) (Sigma) or by lowering $CaCl_2$ and adding $MgCl_2$, adjusting NaCl to maintain isosmolarity; $[Na^+]$ varied less than 5 percent, which was too little to affect release appreciably (*3*). Preparations were constantly superfused with Ringer solution at temperatures between 12° and 20°C (kept to within 1°C throughout each experiment). Conventional nerve stimulation and microelectrode techniques were used to study one or two muscle fibers at a time. The EPP's and mEPP's were recorded on tape and chart recorders and analyzed with the aid of a microcomputer. Tension was measured with a force transducer and preamplifier (Grass).

During and after a 15- to 30-minute exposure to ACTH, the amplitudes of the synaptic potentials increased slowly to a level sometimes exceeding twice the control value (Fig. 1A). Figure 1B shows representative EPP's from an experiment on a sartorius nerve-muscle preparation. This increase in amplitude was generally sustained: the preparation neither recovered from the effects of ACTH nor responded to a second trial of hormone for as long as 4 hours after the first exposure. The increase in amplitude ranged from 16 to 106 percent, depending on hormone concentration (Fig. 2), in 17 cutaneous pectoris muscle fibers from 12 frogs. Sartorius preparations varied more: in three of seven fibers we saw little or no increase in amplitude in response to ACTH (*4*).

The augmentation of synaptic potentials was observed also with α-MSH and ACTH(1-24), peptides closely related to ACTH, although slightly higher concentrations were necessary than with ACTH(1-39) (Fig. 2). ACTH(4-10) produced no discernible response.

The EPP amplitudes were analyzed for quantal content by the methods of failures and of coefficient of variation (*5*).

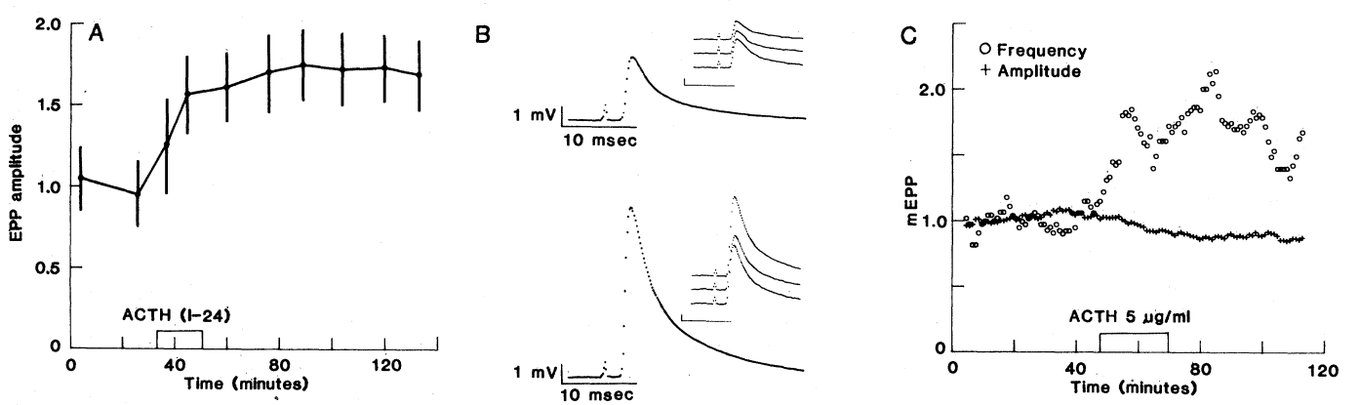


Fig. 1. (A) Effect of ACTH on EPP amplitudes. After a 30-minute control period, ACTH(1-24) was applied to a cutaneous pectoris preparation at a concentration of 1.3 μ*M* for 18 minutes ($[Ca^{2+}]$ = 0.9 m*M*, $[Mg^{2+}]$ = 6 m*M*). Each point is the average of 100 EPP's evoked by nerve stimulation at 0.33 Hz. Error bars are standard deviations; the standard error of the mean was less than 3 percent (about the size of the data marks). The ordinate is EPP amplitude normalized to the average during the control period. The ACTH-induced increase in this fiber was 69 percent and was stable until the penetration was lost. (B) Average of two groups of 100 EPP's recorded from a sartorius muscle fiber before (top) and 200 seconds after (bottom) 3.9 μ*M* ACTH(1-39) was added to the bathing medium (0.5 m*M* $[Ca^{2+}]$, 2.5 m*M* $[Mg^{2+}]$). Insets show individual EPP's from the same period. All calibration bars are 1 mV and 10 msec. (C) Effect of ACTH on mEPP frequency and amplitude. After a 45-minute control period, ACTH(1-39) was added for 20 minutes at a concentration of 1.3 μ*M* (5 μg/ml) ($[Ca^{2+}]$ = 1.8 m*M*, $[Mg^{2+}]$ = 0 m*M*). Values were normalized to averages obtained during the control period. Frequencies and amplitudes were calculated by counting and measuring the mEPP's in 1-minute time periods. Because of the very low mEPP frequency (0.1 to 0.2 Hz), frequency and amplitude data were subsequently smoothed with a moving average (bin width, 10 minutes; $\Delta t$ = 1 minute).

The rise in EPP amplitudes was always paralleled by an increase in calculated quantal content. Since there was little or no change in the calculated quantum size, we conclude that ACTH acts on the motor nerve to augment transmitter liberation.

ACTH also increased spontaneous transmitter release, as shown by a rise in mEPP frequency. This effect had a magnitude and time course similar to the EPP responses (Fig. 1C). As with EPP's, the increased level of release was maintained long after the hormone was washed from the preparation. Amplitudes of mEPP's did not change significantly. Since changes in muscle length and tension can affect evoked and spontaneous transmitter release (*6*), we looked for an ACTH-induced contracture but could detect none. Taken together, the increases in EPP quantal content and in mEPP frequency and the invariance of mean mEPP amplitude and EPP quantal size after exposure to ACTH provide strong evidence that its predominant effect is on motoneuron terminals to enhance transmitter release.

Since normal transmitter release depends on extracellular [$Ca^{2+}$], we checked whether the ACTH enhancement of release showed a similar dependence. We found that increases in mEPP frequency were similar in preparations bathed in normal saline, low $Ca^{2+}$–high $Mg^{2+}$ saline, or in $Ca^{2+}$-free saline (no $Ca^{2+}$ buffers). Moreover, EPP's recorded from preparations treated with *d*-tubocurarine in normal Ringer showed ACTH responses similar to those observed in low $Ca^{2+}$ experiments. Thus the ACTH augmentation of transmitter release seems insensitive to the concentrations of external $Ca^{2+}$.

Birnberger *et al.* (*7*) have reported that ACTH decreased transmitter release in rat phrenic nerve–diaphragm preparations. We are not certain of the reason for the discrepancy between their results and ours: a species difference (frog or rat) or the prolonged, high-frequency (30 Hz) stimulation they used could account for the differences. In addition, ACTH, in large doses, has been used with moderate success to treat severe myasthenia gravis (*8*). Animal models of this therapeutic regime revealed a growth or enlargement of motor nerve terminals (*9*).

Whatever underlies the ACTH-induced potentiation, its extended duration indicates that it is not readily reversed. Does this finding have physiological relevance? At neuromuscular junctions, various compensatory mechanisms exist for increasing EPP amplitude. Intrinsic to the nerve terminal are

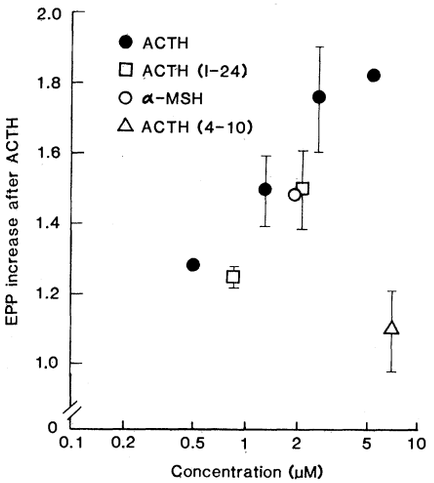


Fig. 2. Relative effectiveness of ACTH, ACTH fragments, and α-MSH. Ordinate is the ACTH-stimulated increase in EPP amplitude normalized to control values. Error bars show standard deviations. Symbols without error bars represent a single measurement.

processes of facilitation, augmentation, and potentiation in which repetitive stimulation generates an increased capability for transmitter release for periods of milliseconds to minutes (*10*). Exogenous agents also can influence synaptic efficacy. For example, norepinephrine released by sympathetic stimulation exerts a defatiguing action on vertebrate skeletal muscle preparations by enhancing transmitter release and postsynaptic responsiveness for several minutes (*11*). Peptide effects, like those reported here, may represent yet another, even longer-term modulation of transmitter release. This property may be shared with other peptides: brief exposures to substance P have a diphasic, apparently presynaptic, effect, culminating in a long-lasting increase in EPP amplitude and quantal content (*12*). The ineffectiveness of ACTH(4-10) should not be surprising, since it mimics some but not all of the actions of ACTH(1-39) in the nervous system (*2*), suggesting that multiple receptors mediate ACTH effects. The hormone concentrations we have used are two to three orders of magnitude greater than circulating concentrations in other vertebrates (*13*). This discrepancy may have several explanations. (i) The natural agonist for the potentiation may not be ACTH but another related compound; (ii) ACTH, α-MSH, or a similar peptide may be released from sympathetic or sensory endings near the endplate regions, resulting in a high local concentration; or (iii) the stress of capture probably stimulates ACTH release and may mask an effect at lower concentrations.

Although vertebrate neuromuscular junctions have been generally assumed to function with a large transmission safety factor, wide variations exist in the amount of transmitter liberated at different nerve endings in frog sartorius muscles (*14*). In fact, a substantial proportion of terminals fail to fire their muscle fibers with a single nerve stimulus. With repetitive stimulation, where synaptic depression would be active, still more fibers may fail to generate action potentials and contract. Thus, if motor inputs to vertebrate skeletal muscles ordinarily leave many muscle fibers just below their thresholds for firing, moderate increases in synaptic efficacy, like those demonstrated in this report, may produce large increases in muscle strength—a useful response in stressful situations.


MICHAEL F. JOHNSTON
EDWARD A. KRAVITZ
*Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115*
HALINA MEIRI
RAMI RAHAMIMOFF
*Department of Physiology, Hebrew University–Hadassah Medical School, Jerusalem, Israel*


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