Effect of Luteinizing Hormone-Releasing Hormone on the Release of Acetylcholine from Motor Nerve Terminals

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Received for publication December 17, 1982

Key words: LHRH — ACh-release — skeletal muscle end-plate — EPP — miniature EPP — ACh potential

Neurotransmitters change the resting membrane potential of the postsynaptic cell (postsynaptic potentials) and also appear to modulate the release of neurotransmitters (Eccles, 1964). For example, the amount of acetylcholine (ACh) released from the preganglionic nerve terminals is modulated by catecholamines (Christ and Nishi, 1971; Kuba et al. 1981), 5-hydroxytryptamine (Hirai and Koketsu, 1980), γ -aminobutyric acid (Kato and Kuba, 1980), ACh (Koketsu and Yamada, 1982) and adenosine triphosphate (Akasu et al. 1982).

Luteinizing hormone-releasing hormone (LHRH) is a neurotransmitter candidate for the late slow excitatory postsynaptic potential in bullfrog sympathetic ganglia (late slow EPSP) (Jan et al. 1979; Jan and Jan, 1982). In the present study, the effect of LHRH on ACh-release from the presynaptic nerve terminals of the frog neuromuscular junction was examined electrophysiologically. The results obtained suggest that LHRH at a low concentration ($4.6 \mu\text{M}$) increases the amount of ACh released from the motor nerve terminal of sciatic nerve-muscle preparations.

Sartorius muscles with intact sciatic

nerves were isolated from frogs (*Rana nigromaculata*). These preparations were continuously superfused with Ringer solution. Conventional microelectrode techniques were employed for recording end-plate potential (EPP) using microelectrodes filled with 3 M KCl and having a tip resistance of $20 \text{ M}\Omega$. The ionic composition of the Ringer solution was (mM): NaCl 112, KCl 2, CaCl_2 1.8 and NaHCO_3 2.4. EPPs were induced by stimulating the motor nerve at a rate of 0.2 Hz in low Ca^{2+} -high Mg^{2+} Ringer solutions (CaCl_2 0.6-1.1 mM, MgCl_2 4.3-7.0 mM) and in Ringer solution containing d-tubocurarine (d-TC) ($2 \mu\text{M}$).

Quantal content was calculated by the variance method (del Castillo and Katz, 1954) from the mean amplitude and its standard deviation of 60 EPPs (5 min) induced before, during and after the application of LHRH. Changes in the quantal content during the application of LHRH were expressed as a percentage of the mean quantal content. To obtain the ACh potentials, ACh was directly applied to the end-plate by microiontophoretic methods from a microelectrode filled with 1 M ACh with a tip resistance of $70 \text{ M}\Omega$.

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Fig. 1. Effects of LHRH (4.6 μM) on the amplitude (A) and mean quantal content (B) of the EPP, and on ACh potentials (C) recorded from sartorius muscle end-plates. A: EPPs were recorded by stimulation of the sciatic nerve at a rate of 0.2 Hz in Ringer solution containing 2 μM d-TC. LHRH (4.6 μM) was applied between the downward and upward arrows. B: EPPs were recorded in a low Ca^{2+} -high Mg^{2+} Ringer solution. The ordinate is the percent of the control quantal content of the EPP. The control mean quantal content (60 EPPs) is 100%. Each vertical bar indicates the S.D. (n=7). Downward and upward arrows indicate the period of application of LHRH. C: The upper traces are the currents used for iontophoresis of ACh. The lower traces are ACh potentials obtained by iontophoretic application of ACh to the end-plate region from a microelectrode filled with 1 M ACh.

All experiments were carried out at room temperature (20–24 °C). The drugs used were acetylcholine chloride (Wako), d-TC (SIGMA) and LHRH (Protein research, Osaka).

Fig. 1-A shows the effect of LHRH (4.6 μ M) on the end-plate potential (EPP) elicited by motor nerve stimulation at a rate of 0.2 Hz. The muscle was superfused with Ringer solution containing 2 μ M d-TC to suppress the generation of an action potential. When LHRH (4.6 μ M) was added to the superfusing solution, the resting membrane potential and resistance (input resistance) did not change, significantly. LHRH (4.6 μ M) increased the amplitude of the EPP to $149 \pm 8.8\%$ (mean $\pm$ S.D., $n=4$) of the control value. The augmentation of the EPP was sustained throughout the application of LHRH. After removal of LHRH from the superfusing solution, the EPP did not change for 5 min and then gradually returned to the control amplitude. More than 25 min were necessary for complete recovery from the effects of LHRH. The facilitatory effect of LHRH on EPP amplitude was concentration-dependent. The minimum effective concentration of LHRH was 500 nM.

The quantal content of the EPP of skeletal muscle was determined in a low Ca^{2+} -high Mg^{2+} Ringer solution. Fig. 1-B shows the effect of LHRH (4.6 μ M) on the quantal content of the EPPs from 7 preparations. The quantal content slowly increased during the application of LHRH and reached a maximum value within 15 min. When LHRH was removed from the superfusing solution, the augmentation of the quantal content was sustained for approximately 15 min. The quantal content calculated by the variance method was increased by LHRH (4.6 μ M) to $142 \pm 11.7\%$ of the control. Approximately 30 min after withdrawal of LHRH, the quantal content of the EPP returned to the control value. The facilitatory action of LHRH was dependent on the external Ca^{2+} concentration. In Ca^{2+} free Ringer

solution, the EPP was completely eliminated and LHRH (4.6 μ M) could not restore the EPP. This indicates that LHRH does not act on the presynaptic nerve terminals as a substitute for Ca^{2+} .

The possibility that the augmentation of the EPP by LHRH was due to an increase in the sensitivity of the nicotinic ACh receptor was examined. ACh potentials induced by microiontophoretic applications of ACh to the end-plate were reduced to approximately 73% ($n=5$) of the control amplitude by LHRH (4.6 μ M) (Fig. 1-C). The mean amplitude of miniature EPPs (MEPPs) was decreased to 82% of the control amplitude by LHRH (4.6 μ M). LHRH (4.6 μ M) did not alter the frequency of the MEPPs.

A presynaptic facilitatory action of LHRH was clearly demonstrated by these experiment. LHRH increased the amount of ACh released from motor nerve terminals of the frog sartorius muscle end-plate. These results also demonstrate that the sensitivity of the nicotinic ACh receptors at the skeletal muscle end-plates and bullfrog sympathetic ganglion cells are decreased by LHRH (cf. Akasu et al. 1983a).

LHRH at a low concentration depressed the after-hyperpolarization of action potentials and prolonged the duration of action potentials of bullfrog sympathetic ganglion cells (Akasu et al. 1983b). The duration of the Ca^{2+} spike (Minota and Koketsu, 1977) was also increased by LHRH (Akasu et al. 1983b). It was demonstrated with voltage-clamp experiments (Akasu et al. 1983b) that LHRH markedly depressed the outward K^+ currents, $\text{I}_{\text{k-1}}$ (delayed rectifier K^+ current) and $\text{I}_{\text{k-2}}$ (the M current, Brown and Adams, 1980). Furthermore, Akasu et al. (1983b) reported that LHRH at a low concentration (5 μ M) increased the amplitude of the slow inward current (I_{si}) carried by Ca^{2+} (Akasu and Koketsu, 1981). It is tentatively concluded on the basis of these experimental results that LHRH increases the release of ACh from motor nerve termi-

nals by increasing the Ca^{2+} current activated during the generation of action potentials in the presynaptic nerve terminals.

Acknowledgement: This study was supported in part by the Ministry of Education, Science and Culture of Japan.

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