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LUTEINIZING HORMONE-RELEASING FACTOR AND MUSCARINIC AGONISTS ACT ON THE SAME VOLTAGE-SENSITIVE K^+-CURRENT IN BULLFROG SYMPATHETIC NEURONES

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The effect of bath-applied luteinizing hormone-releasing factor (LHRF) was recorded in voltage-clamped bullfrog lumbar sympathetic neurones. At a holding potential of −30 mV, LHRF induced a steady inward (depolarizing) current and reduced membrane conductance; at −60 mV LHRF produced negligible inward current and much less conductance change. The effects of LHRF were accompanied by a reduced amplitude of the slow current relaxations following hyperpolarizing and depolarizing commands, without prominent change in their time-course. This suggests a selective depression of the voltage-sensitive outward K^+-current which is suppressed by muscarinic agonists.

Introduction Brief repetitive stimulation of the preganglionic input to bullfrog lumbar sympathetic ganglia produces a sequence of three different excitatory postsynaptic potentials (e.p.s.ps): 'fast', 'slow' and 'late slow' (see Nishi, 1974). The fast and slow e.p.s.ps are both generated by acetylcholine, acting on nicotinic and muscarinic receptors respectively; the late slow e.p.s.p. is insensitive to atropine (Nishi & Koketsu, 1968) and is probably generated through the release of a peptide closely resembling luteinizing hormone-releasing factor (LHRF: Jan, Jan & Kuffler, 1979).

We have recently found that the slow muscarinic depolarization of these neurones results from the selective depression of a voltage-sensitive K^+-current, which we have termed the 'M-current' (Brown & Adams, 1979). This current is progressively activated between −60 and −10 mV. Since it does not show time-dependent inactivation, it contributes an increasing proportion of the total membrane current from −60 to −25 mV, at which point the conventional delayed rectifier current makes its appearance.

We now find that exogenous LHRF also inhibits the M-current.

Methods Experiments were performed under voltage-clamp on neurones in the ninth and tenth lumbar sympathetic ganglia of bullfrogs (*Rana catesbiana*) maintained *in vitro* in flowing Ringer solution at 22°C. The connective tissue sheath covering the ganglion cells was removed with the aid of trypsin (1% for 5 to 10 min) and cells impaled with two microelectrodes filled with 3 M KCl (20 to 50 MΩ resistance), one for recording voltage and one for passing or recording current. The Ringer solution contained (mM): KCl 2.5, $CaCl_2$ 2, Tris-base 2 (buffered to pH 7.2 with HCl) and $MgCl_2$ 10, to improve electrode sealing. LHRF was obtained from Peninsular Biochemicals. Trypsin (type III) was obtained from Sigma Ltd.

Results The essential action of LHRF observed under voltage-clamp, confirmed in several cells, is illustrated in Figure 1. The cell was initially held at −30 mV, a membrane potential at which a substantial fraction of the M-channels are open, and subjected to 700 ms hyperpolarizing commands to −60 mV. The current response to these voltage commands consisted of an instantaneous ohmic step followed by a slow inward relaxation. The latter results from the slow closure of the M-channels (time-constant about 60 ms at the command potential of −60 mV). On repolarizing the cell to −30 mV the M-channels reopen, yielding a slow outward relaxation (time-constant 110 ms at −30 mV). The difference between the ohmic steps at the beginning and end of the hyperpolarizing command reflect the fall in membrane conductance associated with the closure of the M-channels, about 45% in this case.

Addition of LHRF (4 μM) produced a net inward (depolarizing) current of about 1.7 nA at the holding potential of −30 mV but did not change the steady-state current at −60 mV. The membrane conductance at −30 mV (represented by the inward ohmic step) was reduced by 38%, whereas the conductance at −60 mV (represented by the outward ohmic step) was much less affected (−18%); This latter might reflect incomplete M-channel closure at −60mV. Thus, both the net inward current and the fall in membrane conductance produced by LHRF are compatible with a selective depression of the M-current. The semi-

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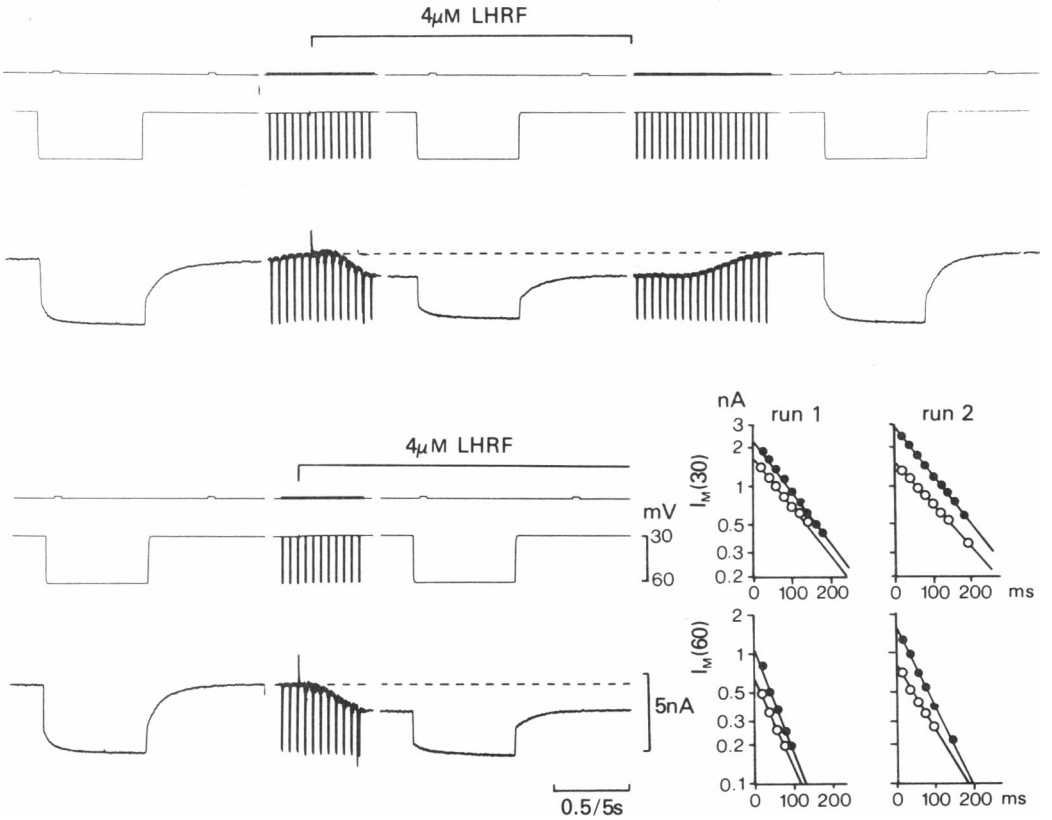

Figure 1 Responses of a voltage-clamped bullfrog ganglion cell to two successive applications of 4 μM luteinizing hormone-releasing factor (LHRF). The upper and lower chart records run continuously. Upper trace, time in seconds (with periodic tenfold acceleration); middle trace, membrane potential (holding potential −30 mV, command potential −60 mV); lower trace, current responses (inward current downwards). The graphs in the lower right quadrant are semi-logarithmic plots of the amplitude of the slow current relaxations (I_M = M-current, see text) against time in ms, after stepping from −30 to −60 mV (lower graphs, $I_M(60)$) and back from −60 to −30 mV (upper graphs, $I_M(30)$) measured before (●) and during (○) the two LHRF applications. (Increasing leak currents precluded an adequate recovery record after the second application (○) LHRF.)

logarithmic plots of the M-current time-course in Figure 1 suggest that this depression occurs without appreciable modification of the voltage sensitivity of the M-channel opening and closing rates. The latter may be gauged by the ratio of the time-constants at −30 and −60 mV (between 1.7 and 2.1 in the experiment illustrated): this ratio was unchanged in the presence of LHRF, although the individual time-constants showed some variation.

The action of LHRF was not blocked by 1 μM atropine.

Discussion The action of LHRF described above is identical to that of the muscarinic agonist, (±)-muscarine reported previously (Brown & Adams, 1979): both drugs produce a voltage-sensitive inward current and conductance decrease, and both effects may be largely or entirely attributed to the suppression of the M-current. Since LHRF was not antagonized by atropine (whereas muscarine was), its action cannot be due to the release of acetylcholine. Instead, it seems that the two transmitter-mimetics have precisely the same postsynaptic action.

Interestingly, exogenous muscarine and LHRF act with equal speed. Although largely governed by the flow rate, the onset rate of bath-applied LHRF is, in fact, rather faster than that of the late slow e.p.s.p. described by Jan *et al.* (1979). This suggests that the different time courses of the two postsynaptic re-

sponses following preganglionic stimulation might be due to differences in the rate of release of acetylcholine and LHRF.

In rat sympathetic ganglia another peptide, angiotensin, imitates the voltage-sensitive action of muscarinic agonists (Brown, Constanti & Marsh, unpublished observations). Thus, a shared end-point of muscarinic cholinergic and peptidergic transmission may be quite common.

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