EFFECTS OF ESTRADIOL ON MEMBRANE PROPERTIES OF CULTURED GOLDFISH (CARASSIUS AURATUS) NEURONS.

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It is generally agreed that estradiol-17 (E2) acts via nuclear receptors to regulate gene expression in its target tissues; however, several lines of evidence indicate that non-genomic mechanisms may operate in parallel with conventional genomic actions in neural tissues. For example, Nabekura et al. (Science 253: 227-233, 1986), using intracellular recording techniques, found that E2 modulates excitability of neurons in rat medial amygdala slices. Although some cells in this region have nuclear E2 receptors, the response had a latency (msec) too short to be explained by an effect on mRNA transcription and was unaffected by inhibitors of protein synthesis. Further support for the view that E2 functions as a neuromodulator comes from studies showing that the brain is capable of synthesizing E2 in situ. Because aromatase (estrogen synthetase) activity in goldfish brain is 100-1000-fold greater than in mammals and is present in neural regions devoid of conventional nuclear E2 receptors (Pasmanik and Callard, Gen. Comp. Endocrinol. 60: 244-251, 1985), we reasoned that goldfish neurons may be especially sensitive to the non-genomic actions of E2. Recently, we developed a primary monolayer system for studying estradiol synthesis and action in cultured goldfish brain cells (Callard et al., Proc. 19th Ann. Mtg. Soc. Study of Reprod., 1986), and report here short latency effects of E2 on neuronal currents as recorded by whole cell patch clamp techniques.

Cells were obtained by trypsin-dispersion of adult goldfish forebrain, the region with the highest aromatase activity. Approximately 25 fish of mixed sex were used for a single culture preparation. Cells were resuspended in Liebowitz L-15 containing 10% fetal bovine serum (dextran charcoal adsorbed to remove endogenous steroids), 10 mM HEPES, penicillin (1 i.u./ml) and streptomycin sulfate (1 ug/ml). They were seeded in poly-l-lysine coated 15 mm wells of a Multiwell plate (Falcon) (Approx. 50 ug protein/500 ul); and cultured in air at 22°C in a humidified chamber. Cells were primarily neuronal in morphology during the first 7 days of culture, although non-neuronal cell types progressively increased thereafter. In 30-day cultures used in the present study, neurons were recognizable by large, phase-bright cell bodies with extensive neurite outgrowth and were often found in aggregates of 10 or more cells (Fig. 1A). Immediately before testing, the spent medium was replaced with 500 ul Liebowitz L-15 without supplements. Using an inverted, phase contrast microscope (Nikon), a reference Ag/AgCl electrode was placed near the edge of the well. A seal was obtained with little or no suction when the patch clamp electrodes (double pull, double number 39-41, WPI 1B150F-4, filament, not annealed) were brought near the cell body. The patch clamp amplifier (Dagan) was used with a head stage incorporating a 0.1 G-ohm feedback resistor. Recordings were made before or immediately after (5-20 min) the addition of E2 (1 uM, final concentration in L-15).

In few of the examined cells a transient inward current lasting 1-2 ms developed with depolarizing pulses more positive than -50 mV (Fig. 1B). The

inward current inactivated with steady state depolarizing potentials in the range of -80 to -40 mV. The time course, the voltage range of activation, and inactivation for this current makes it highly likely that the fast Na^+ channel carries the inward current in these cells. In most cells we also observed a second much slower (about 20 ms) and smaller inward current (Fig. 1C). The resting conductance in most of the cells examined was low, but did show inward rectification (Fig. 1E). Large non-inactivating outward currents were activated at positive potentials (Fig. 1E). Although ionic substitution experiments were not carried out, the kinetics and the voltage-dependence of the outward current were similar to those of K^+ channel in other neuronal tissues. The graph quantifies the outward (at 30 ms) and the inward currents of these cells. The inward current component was measured as the difference between a linear approximation to the slowly changing part of the current trace and its early minumum (see inset).

The addition of 1 uM E2 strongly and rapidly (2-3 min) decreased the resting membrane conductance (compare Fig. 1C & D). This effect of E2 was consistently seen (3 experiments) at all membrane potentials negative to -20 mV (filled circles, Fig. 1E). Thus E2 suppressed an outward current component and shifted the reversal potential of this current toward more negative values. The two fold decrease in the inwardly rectifying K^+ current caused by E2 (Fig. 1D), would have a marked effect on the resting potential of the cell. The drug had no significant effect on either the Na^+ channel nor on the K^+ current at potentials positive to -10 mV.

Our results therefore suggest that a cell type in these neuronal cultures is highly and rapidly responsive to addition of estradiol. The decrease in the inward current at potentials negative to -40 mV is consistent with the previously reported hyperpolarizing effect of this drug in intact rat brain slices (Nabekura et al., Science 253: 227-233).

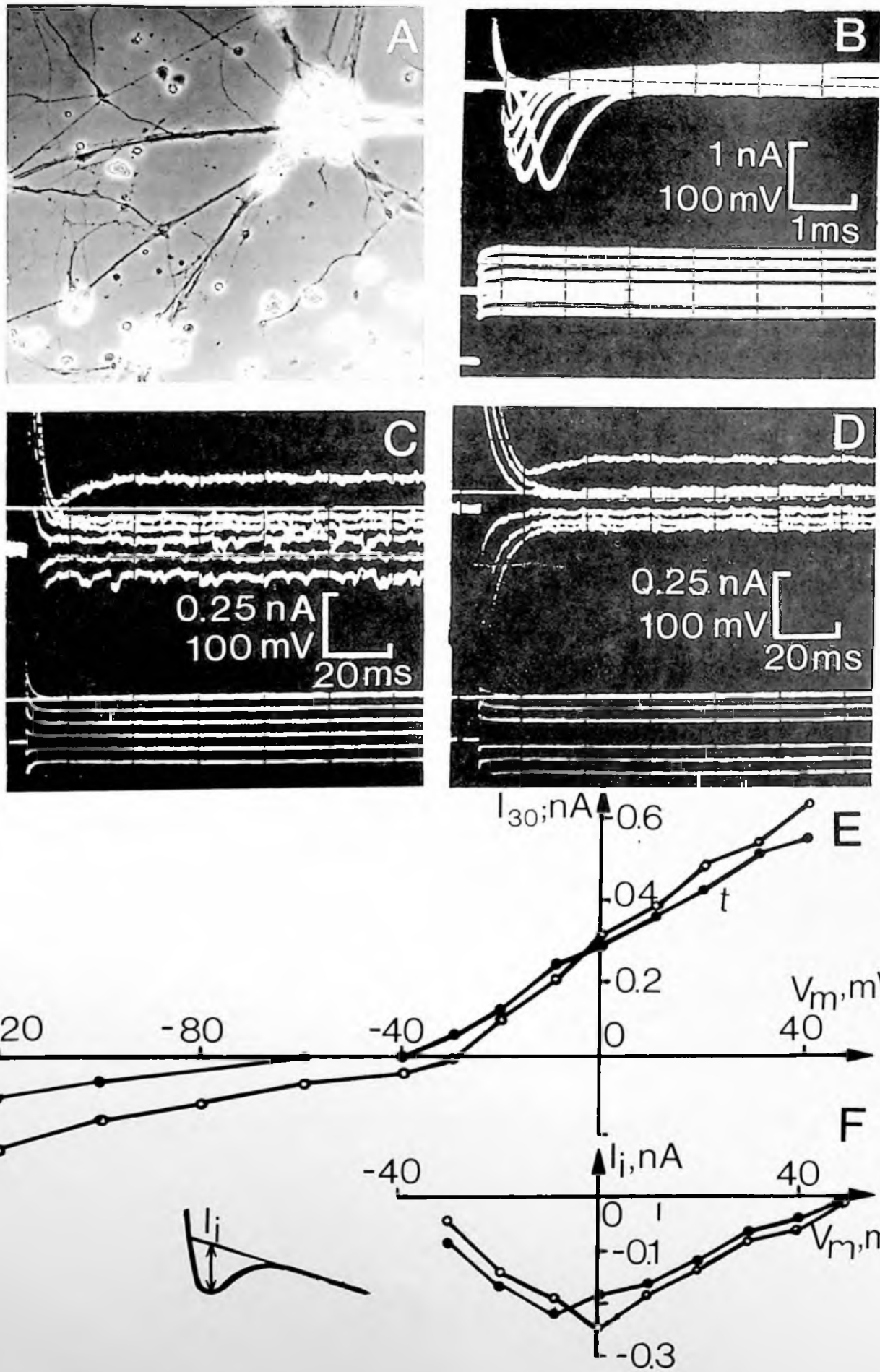
Our results are not consistent with the conclusion of these authors, however, who suggest an increased K^+ conductance as the cause for hyperpolarization, since the K^+ conductance was strongly suppressed around resting potential by E2 (compare Figs. 1C & D). An alternate explanation which would be consistent with observed hyperpolarization, and the decreased K^+ current would be that E2 as it blocks K^+ current also stimulates the Na^+ pump which would tend to hyperpolarize the membrane and reduce the evoked potentials and spike activity.

We conclude that cultured goldfish neurons under patch clamp conditions may be used as models for the study of hormonal effects of estradiol in the mammalian neurons. The results suggest a new intriguing possibility that estradiol effects on neuronal cells may be in part mediated by direct action of these agents on the cell membrane.

(Supported by NSF DCB 85-19739 and NIH RO1-HL16152)

Legends

Figure 1. Cultured amygdala neurons from the goldfish brain and the effect of estradiol on voltage clamp currents. Panel A: Micrograph of a cluster of cultured cells. Panel B: rare example of large inward current spikes measured in a patch clamped neuron. Panels B, C and D show current traces at the top, voltage clamp pulses at the bottom and calibration brackets in between. Panel C: Voltage clamp records from a more typical cell with an inward rectifying resting conductance and a rudimentary inward current. Panel D: Same cell as in panel C after addition of 1 μM 17-beta-estradiol. Panel E: 20 msec current voltage relations, I_{30} vs. Vm, measured in the absence (o) and presence (•) of estradiol. Panel F: The current voltage relation for the inward current, Ii vs. Vm, in the absence (o) and the presence (•) of estradiol. The current voltage relations in panels E and F are based in part on the records in panels C and D.


A
B
1 nA
100 mV
1ms
C
0.25 nA
100 mV
20ms
D
0.25 nA
100 mV
20ms
E
I_{30};nA
0.6
0.4
t
0.2
V_m,mV
-120
-80
-40
0
40
F
I_i,nA
-40
40
0
-0.1
-0.3
V_m,mV
I_i