A Novel Nongenomic Action of Estrogens

The Regulation of Exocytotic Kinetics

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The nongenomic actions of estrogens have received renewed interest. It is now becoming clear that the rapid onset of cellular responses upon drug application, together with its insensitivity to blockers of transcription or translation, cannot be attributed to genomic activity. Several molecules with estrogen activity have been widely studied in a variety of tissues, including chromaffin cells.[1–3] Also, a number of drugs exhibit agonist effects in some tissues, whereas they behave as antagonists in others. This observation has motivated the coining of the term "estrogen modulator" for tamoxifen and related compounds: that is, raloxifene or LY117018, which are currently under investigation.

We have studied the role of nongenomic action of estrogens on elicited catecholamine secretion from perfused rat adrenals[4] and the kinetics of exocytosis on single cultured bovine chromaffin cells.[5]

17β-Estradiol, as well as the estrogen receptor modulators raloxifene and LY117018, but not 17α-estradiol, inhibited the catecholamine output elicited by acetylcholine or high potassium only at micromolar concentrations. However, these agents failed to modify the secretion elicited by high Ca^{2+} in glands treated with the ionophore A-23187, suggesting that estrogens acted directly on the cell membrane, not on the secretory machinery.

At the single-cell level, estrogens modified the kinetics of exocytosis at nanomolar range (see FIGS. 1 and 2). All of the drugs tested, except 17α-estradiol, produced a profound slowing of the exocytosis as measured by amperometry.[6] 17β-Estradiol reduced the intracellular free Ca^{2+}, measured with fura-2, but only at micromolar

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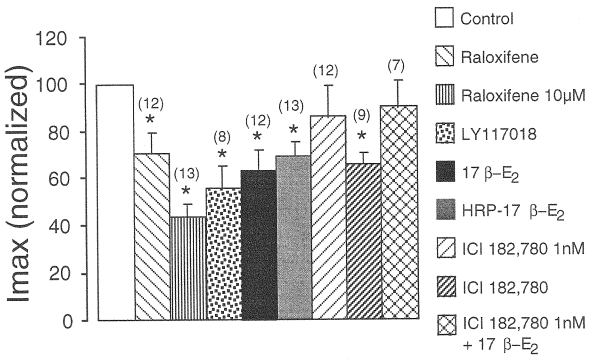

FIGURE 1. The effects of various estrogens on the secretory spikes height. Unless specified, drugs are used at 10 nM. Normalized data (mean ± SEM) are compared with their own control group of untreated cells ($n = 12–16$); $P < .05$ (Mann-Whitney test). The number of secretory spikes computed ranged from 345 to 1232. (From Machado *et al.*[7] Reproduced by permission.)

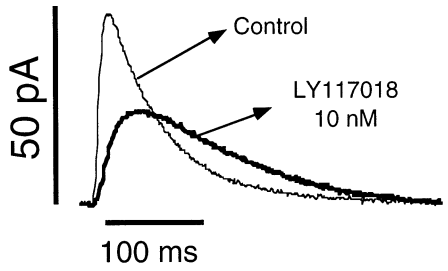

FIGURE 2. The estrogen modulator LY117018 slows the time-course of exocytosis down. Spikes are plotted incorporating the average values of the kinetic parameters obtained from real data. Note the different ascending slope, the increase in the spike width, and the time to reach the spike maximum. The catecholamine concentration reaching the electrode was greatly reduced. Net granule charge was maintained, however. (From Machado *et al.*[7] Reproduced by permission.)

concentrations. However, nanomolar concentrations of estradiol increased the cAMP levels.

These effects have been reproduced with the nonpermeable drug 17β-estradiol-HRP and antagonized with nanomolar concentrations of the antiestrogen ICI 182,780.

Our data suggest the presence of a membrane site that regulates both the exocytotic phenomenon and the total catecholamine release with high and low affinity, respectively. The effects of estrogens on the kinetics of exocytosis appear to be mediated, at least partially, by cAMP.

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