EPITHEORESE KLINIKES FARMAKOLOGIAS
KAI FARMAKOKINETIKES, INTERNATIONAL
EDITION 13: 136-137 (1999)


# Prodynorphin-derived k-Opioid Peptides are Expressed in Normal and Tumoral Cells of Human Endometrium: Paracrine Effects on Transforming Growth Factor beta 1

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## AIM

Human endometrium expresses endogenous opioid precursors proopiomelanocortin (POMC) and proenkephalin (PENK) and their end products. In parallel, a panel of opioid binding sites has been characterized in the same site, the κ-opioid receptor ($\kappa_1$, $\kappa_2$, $\kappa_3$) being the predominant type. However, no information is available on the expression of endogenous κ-opioid receptor ligands, the dynorphins. Aim of the present study was: (a) to identify in this tissue the transcript and protein product of the prodynorphin (PDYN) gene, and (b) to examine the possible autocrine/paracrine effects of endometrial dynorphins.

Northern blot analysis revealed the presence of PDYN mRNA transcripts in biopsies of normal human endometrium as well as in Ishikawa human endometrial adenocarcinoma cells, the size of which was 2.4 kb, i.e. identical to the pituitary PDYN mRNA. The immunoreactive product of the precursor processing belongs mainly to the 8kDa type of bioactive dynorphin, as shown by gel filtration chromatography. Immunofluoresence localization of PDYN-derived peptides revealed the presence of cytoplasmic secretory granules in both normal and tumoral human endometrial cells.

## METHODS AND RESULTS

To *investigate* a possible involvement of dynorphins in autocrine/paracrine phenomena in endometrium, we tested the effect of a synthetic κ-opioid receptor agonist U69593 on the expression of transforming growth factor beta 1 (TGF-β1). TGF-β1 holds a central role in the regulation of all cellular and molecular processes of endometrial cell apoptosis and proliferation associated with menstruation, decidualization, pregnancy maintenance and parturition. Ishikawa cells were treated with U69593 and TGF-β1 content was measured in the culture media. A significant decrease in the secretion of TGF-β1 was observed (52.02±5.26% of non-treated controls, $p<0.05$, on the second day of treatment), that was statistically significant over a period of 6 days treatment. The dose-response curve of this effect had a biphasic profile, reflecting the presence of multiple binding sites. The opioid antagonist diprenorphine reversed the suppression by U69593, indicating that the inhibition observed is an opioid receptor-mediated effect. This effect on TGF-β1 release was confirmed in a series of primary endometrial cell monolayers from proliferative and secretory, normal and tumoral endometrial biopsies, showing that it is a generalized response of all types of endometrial cells to κ-opioids. In order

to define the molecular mechanisms of the effect of k-opioids on endometrial TGF-β1 we conducted transfection experiments using Ishikawa cells transiently transfected with the TGF-β1 promoter linked to the luciferase reporter gene. Treatment of the transfectants with U69593 had no effect on luciferase expression by these cells. However, it appears that opioids may regulate TGF-β1 post-transcriptionally. A stem-loop formed in the 5'-untranslated region of the transcript represses translation by binding to a cytosolic factor, and is probably the target of this regulation.

## CONCLUSIONS

*In summary*, we report here the expression of the PDYN gene by human endometrium and also the synthesis and secretion of immunoreactive dynorphins by these cells. Following, we show a strong inhibitory effect of κ-opioid agonist U69593 on endometrial TGF-β1. To our knowledge the κ-opioid-induced TGF-β1 reduction is the first description of a direct receptor-mediated effect of opioids on human endometrium. Our results underline the significance of these neuropeptides in endometrial physiology, since they implicate their activity with the expression of a key growth factor. We postulate that cross-talk between the opiate system and endometrial growth factors may regulate hormone responses and in turn, influence normal and aberrant cellular phenomena, such as cell proliferation or apoptosis.