DIFFERENTIAL REGULATION OF INHIBIN BINDING VIA BETAGLYCAN EXPRESSION IN SEVERAL MOUSE CELL LINES

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Inhibin A, a member of the transforming growth factor (TGF)-β superfamily, binds to mouse adrenocortical (AC), Leydig (TM3) and Sertoli (TM4) cell lines with high affinity via at least eight membrane protein species, two of which are forms of betaglycan. Inhibin A has been proposed to inhibit the actions of activin and BMP by sequestering their type II receptors in high affinity complexes with betaglycan (1). We previously found that BMPs appear to counteract inhibin action in AC cells by selectively suppressing the expression of endogenous betaglycan, consequently reducing inhibin binding. In the present studies, we have examined how factors that stimulate betaglycan expression in other systems modify the binding of radiolabelled inhibin A at the surface of AC, TM3 and TM4 cells.

AC, TM3 and TM4 cells were treated overnight with glucocorticoid, membrane-permeable cAMP analogue or retinoic acid, after which the levels of betaglycan mRNA, corrected for GAPDH content, were measured using real-time RT-PCR, and [^{125}I]inhibin A binding was determined. Treatment of AC cell cultures with 8Br-cAMP (1 mM), glucocorticoid (RU28362, 100 nM) or retinoic acid (30 mM) increased betaglycan mRNA levels 120-150%, and increased subsequent inhibin A binding to 146 ± 12, 132 ± 13 and 125 ± 18% of control (mean ± SD, n=6–12). The glucocorticoid and cAMP treatments also increased inhibin binding to TM3 and TM4 cells by similar amounts, but retinoic acid was less effective. Affinity labelled protein species of deduced sizes 115 and >170 kDa, consistent in size with betaglycan forms, were the primary target for stimulation by these agents, whereas species of 65 and 75 kDa were selectively increased by retinoic acid in the AC cells.

In summary, glucocorticoids, retinoids and hormones that stimulate cAMP production may increase the expression of betaglycan in inhibin target cells, increase their binding of inhibin, and thereby promote inhibin action. These studies confirm that betaglycan is a primary determinant of inhibin binding and action. The protein species other than betaglycan that are selectively upregulated by retinoic acid in AC cells are yet to be identified. *Funded by the NH&MRC of Australia (RegKey 241000 & 198705).*

(1) Wiater & Vale (2003) *J. Biol. Chem.* **278**, 7934.

10.1071/SRB04Ab125