

Seager, D. Craig

The Role of AP-1 and Related Inhibitors in Cortisol Secretion and Inflammation

Faculty Mentor: Allan Judd, Physiology and Developmental Biology

Interleukin-6 (IL-6) increases the secretion of cortisol from bovine adrenal zona fasciculata (ZF) and human adrenal tumor H295R cells. However, the biochemical mechanisms involved in IL-6-stimulated cortisol secretion are not well understood. In previous studies, it has been determined that IL-6 increases the phosphorylation of Janus kinases (JAK) and signal transducer and activators of transcription (STATs). In many tissues, the JAK-STAT pathway and the activator protein-1 (AP-1) pathways interact. AP-1 is composed of homodimers and heterodimers of the proteins c-Fos, c-Jun, JunB, and JunD. Therefore, in this study we determined the effects of IL-6 on the cellular content of the mRNAs for c-Fos, c-Jun, JunB, and JunD.

In order to study the effects of IL-6 on the cellular content of the given proteins, bovine adrenal tissue and H295R cells (a human adrenal tumor cell line) were used. Bovine adrenal glands were obtained from an abattoir and the ZF was isolated. ZF tissue was treated with ACTH or IL-6 and the mRNA extracted. H295R cells were cultured in the normal manner and exposed to IL-6 and/or dibutyryl cAMP (dbcAMP) and the mRNA extracted. The c-Fos, c-Jun, JunB, JunD and 18s mRNA was measured with RT-PCR. The mRNA bands in the gels were visualized with ethidium bromide and the data expressed as a ratio of the density of the mRNA band divided by density of the 18s band.

For both c-Fos and c-Jun, a dramatic increase of cellular content was detected when exposed to IL-6. IL-6 (1000 pg/ml) and ACTH (10 nM) (40 min exposure) increased c-Fos mRNA in bovine ZF tissue. The effects of IL-6 and ACTH on c-Fos mRNA appear to be additive. Similar effects are apparent for c-Jun mRNA. IL-6 (1000 pg/ml) (40 min exposure) increases c-Jun mRNA content of H295R cells. Although dbcAMP (1 mM) had no effect on c-Jun mRNA by itself, the nucleotide enhanced the effect of IL-6 on c-Jun mRNA content. Similar effects of IL-6 and dbcAMP are apparent with c-Fos mRNA.

In order to strengthen these findings, we performed a time course for the AP-1 proteins. It was shown that IL-6 increases the cellular content of the mRNAs for c-Fos, JunB, and JunD in H295R cells, and furthermore, these effects of IL-6 were demonstrated to be rapid (within 20 min). However, the cellular mRNA content for the AP-1 proteins returned to pre-stimulation levels within 60 to 120 min.

Even though we have shown that IL-6 increases the components of AP-1 and it has been shown in previous studies in that IL-6 increases cortisol release (Judd 2000), future studies need to be conducted to demonstrate causation between an increase in AP-1-component expression and increased cortisol release, rather than the current correlation that has been shown. Known dominant-negative mutants of JAK and Stat will also be used in subsequent studies in order to inhibit this pathway (Peraldi 2001). Additionally, the AP-1 inhibitors that will be used will be

dominant negative inhibitors of the AP-1 components, c-Fos and c-Jun. A-Fos is known to be a dominant negative inhibitor of c-Fos (He 2000), and TAM-67 is a dominant negative inhibitor of c-Jun (Nakayama 2001).

Overall, we concluded that IL-6 increases the cellular content of the mRNAs for the proteins c-Fos, c-Jun, JunB, and JunD. Because these proteins make up the nuclear factor AP-1, this effect of IL-6 may be involved in mediating the actions of IL-6 to increase cortisol release. This hypothesis is particularly attractive because AP-1 proteins have recently been demonstrated by other investigators to regulate the mRNA synthesis of steroidogenic acute regulatory protein (StAR) (W. Shea-Eaton *et al.* *Mol . Cell. Endocrinol.* 188 (2002) 161- 170).

References

1. Judd, A.M., J.D. Bell, R.A. Heckman *et al.* 2000. *Endocrine*. **13(3)**: 369-377.1.
2. He H, McColl K, Distelhorst CW. *Oncogene*. **19(51)**: 5936-43.
3. Nakayama K, Furusu A, *et al.* *Journal of Immunology*. **167(3)**: 1145-50.
4. Peraldi, P, C.E. Filloux *et al.* *The Journal of Biological Chemistry*. **276(27)**: 24614-24620.