M70 SECRETION OF OUABAIN-LIKE SUBSTANCE (OLS) BY BOVINE ADRENOCORTICAL CELLS IN CULTURE

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Recent evidence suggests the presence of an endogenous sodium transport inhibitor in various mammalian tissues and biological fluids. It has been suggested that the inhibitor(s) is ouabain or an isomer of ouabain. We have previously demonstrated using a radioimmunoassay (RIA) that in both animals and humans serum concentration and urinary excretion of OLS were increased by high sodium intake. Highest content of OLS was found in the rat adrenal gland compared to other tissues analysed. In the present investigation, we have examined the secretion of OLS by primary cultures of bovine adrenocortical cells and the effect of ACTH and angiotensin II (AII). The outer cortex of fresh adrenal tissue was cut into 2 mm thick slices and treated with collagenase to liberate zona glomerulosa and zona fasciculata cells. Approximately 1 x 10^6 cells/well were plated in culture medium (Dulbecco's Modified Eagle's Medium) and incubated at 37°C for 2 days. Stimulation studies were conducted over 2 hours by replacing DMEM with 5 ml of secretion media (Krebs-Ringer solution supplemented with glucose) containing either AII (10 nM) or ACTH (10 nM) with appropriate control wells. OLS was measured by an RIA developed in our lab, cortisol was measured using a commercial assay and cell protein was determined by the Lowry method. Adrenal cells in culture secreted cortisol and OLS and ACTH significantly increased the secretion of cortisol ($P<0.01$) and OLS ($P<0.05$) AII increased OLS ($P<0.05$) but not cortisol. The results are summarised (mean ± SEM) in the table below:

	Cortisol (pmol/mg protein/2 h)	OLS (fmol/mg protein/2h)	
Control	8.9 ± 7.1	41.0 ± 8.1	
ACTH	124.0 ± 41.2**	62.4 ± 2.2*	*p<0.05
AII	17.4 ± 9.3	58.5 ± 5.1*	**p<0.01

Our results suggest that the adrenal gland secretes OLS, the secretion of which appears to be influenced by the secretogogues ACTH and AII by an, as yet unknown, mechanism.

M72 THE DIVERGENT EFFECTS OF CENTRAL AND PERIPHERAL LEPTIN INJECTIONS ON FEEDING AND HYPOTHALAMIC NEUROPEPTIDE Y (NPY) mRNA IN LEAN AND FATTY ZUCKER RATS

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Leptin inhibits feeding and decreases body weight. It may act partly by inhibiting hypothalamic neurons that express NPY, a powerful inducer of feeding and obesity. These NPY neurons express the OB-Rb leptin receptor and are overactive in the fatty (*fa/fa*) Zucker rat. The *fa* mutation affects the extracellular domain of the leptin receptor, but its impact on leptin action and NPY neuronal activity is not fully known. We compared the effects of intracerebroventricular (ICV) and intraperitoneal (IP) injection of 3 doses of leptin on food intake and hypothalamic NPY mRNA, in lean and fatty Zucker rats.

In lean rats, 4-hr food intake was significantly reduced ($p<0.01$) in a dose-related fashion by up to 40%, by leptin doses of 2, 10 and 20 µg given ICV and by IP doses of 100 and 200 µg. Hypothalamic NPY mRNA levels after the highest ICV and IP dosages were reduced by 28% and 21% respectively (both $p<0.01$). In fatty rats, ICV injection of 20 µg reduced food intake by 22% ($p<0.01$), but lower ICV dosages and all IP dosages had no effects. Hypothalamic NPY mRNA levels were 100% higher in fatty rats than in lean animals, and were reduced by 18% ($p<0.01$) after 20 µg leptin ICV, but were unaffected by 200 µg IP.

The *fa/fa* Zucker rat is therefore less sensitive to leptin given ICV and particularly IP, suggesting that the *fa* mutation interferes both with leptin's direct effects on neurones and its transport into the CNS. Obesity in the *fa/fa* Zucker rat may be partly due to the inability of leptin to inhibit hypothalamic NPY neurones.

Erratum

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Plasma volume did not differ between dietary groups at day 14 of gestation ($P=0.117$).