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Targeting Trends

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Basomedial hypothalamic Injections of Neuropeptide Y Conjugated to Saporin Selectively Disrupt Hypothalamic Controls of Food Intake

This article is a summary of data presented in reference #1. Figures 1-4 are taken from that article. This work was funded by NS045520 and DK40498 to S. Ritter.

Neuropeptide Y (NPY) conjugated to saporin (SAP), a ribosomal toxin, is a compound designed to selectively target and lesion NPY receptor-expressing cells. We conducted competitive binding studies using I^{125}-NPY to evaluate the binding of NPY-SAP to rat forebrain homogenates (1). Results indicate that NPY-SAP binds to and has a higher binding affinity than NPY for the NPY receptor (Fig. 1). The binding results, in combination with previous studies demonstrating agonist-driven NPY receptor internalization (2, 3), indicated that this peptide-saporin conjugate would produce effective lesions of NPY receptor-expressing neurons. Accordingly, when we injected NPY-SAP (48 ng in 100 nl) bilaterally into the arcuate nucleus (ARC) of the hypothalamus, we found a profound reduction of NPY Y1 receptor-immunoreactivity (-ir) in the ARC (Fig. 2). We also found a nearly complete loss of NPY, AGRP and CART mRNA expression and α-MSH-ir in the ARC and mediobasal hypothalamus, showing that these NPY receptor-expressing neurons were lesioned by NPY-SAP (Fig. 3).

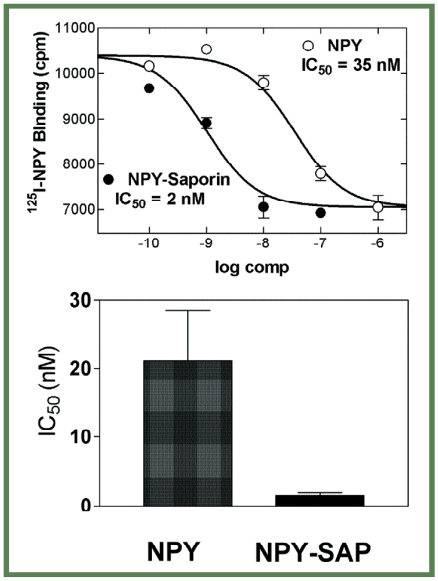

Figure 1. *Top*: Competitive binding of NPY and NPY-SAP with I^{125}-NPY in rat forebrain tissue homogenates. Duplicate determinations were made for each concentration. *Bottom*: Bars show IC50 for NPY and NPY reduced NPY-SAP binding. Data show that NPY-SAP has a binding affinity for NPY receptors that is equal to or greater than NPY at the concentrations examined.

To date, there is no evidence that any of the available peptide-saporin conjugates are retrogradely transported. To determine whether NPY-SAP is retrogradely transported, we injected the conjugate into the ARC and examined catecholamine cell bodies in the A1/C1 region of the ventrolateral medulla. Nearly all of the catecholamine neurons in this area co-express NPY and project to the medial hypothalamus. A1/C1 neurons are almost completely destroyed by medial hypothalamic injections of the retrogradely transported immunotoxin, anti-dopamine-beta-hydroxylase-saporin (anti-DBH-SAP) (4-6). However, there was no loss of cells in this

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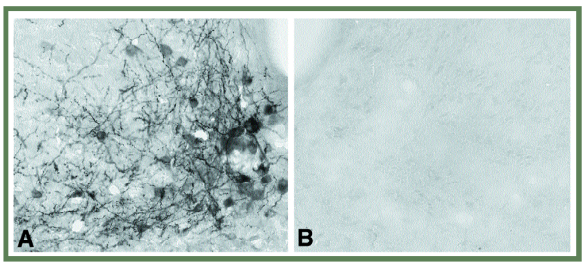

Figure 2. Coronal sections through the arcuate nucleus of the hypothalamus showing effects of Blank-SAP (B-SAP) control (A) and NPY-SAP (B) injections into the arcuate nucleus on NPY-Y1 receptor immunoreactivity.

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Denise Higgins, Editor

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