NPY-SAP Selectively Disrupts Hypothalamic Controls of Food Intake

(continued from page 1)

area in the NPY-SAP injected rats (Fig. 4), indicating that NPY-SAP is not internalized by NPY terminals or that, once internalized, there is no mechanism for retrograde transport of the conjugate. Supporting these findings, we also showed that NPY terminals in the area of cell body loss, though initially reduced, were not obliterated, as they would be if all NPY neurons innervating that area had been retrogradely destroyed.

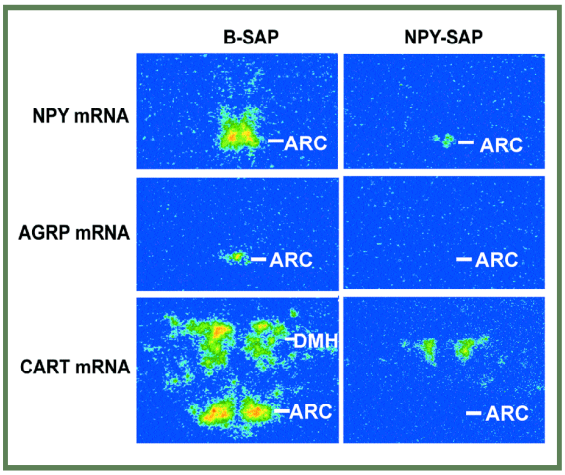

Figure 3. Pseudocolor representation of OD of NPY, AGRP and CART mRNA expression in the arcuate nucleus after bilateral injection of Blank-SAP (B-SAP, left) or NPY-SAP (right) into the arcuate nucleus. NPY-SAP significantly reduced NPY, AGRP and CART hybridization signal at the injection site.

In previous work, we used anti-DBH-SAP to examine the importance of hindbrain catecholamine neurons that innervated the hypothalamus for glucoregulation (7-10). We found that these neurons (many of which co-express NPY) are required for a number of glucoregulatory responses, including feeding, corticosterone secretion and suppression of estrous cycles in response to glucose deficit. In addition, we found that catecholamine neurons with projections to the spinal cord, which are distinct from those that project to the hypothalamus, are required for the adrenal medullary hyperglycemic response to glucoprivation. The goal of our work with NPY-SAP (1) was to determine whether the ARC NPY neurons, which co-express agouti-related protein (AGRP), are required for systemic glucoregulation. Gene knockout studies indicate that the NPY gene is required for glucoprivic feeding (11). However, there are multiple, presumably functionally heterogeneous, NPY populations in the brain. Furthermore, in the medial hypothalamus, the terminals of ARC NPY neurons are co-extensive with those of the hindbrain catecholamine neurons, making it difficult to distinguish the separate functions of these two NPY cell populations. NPY-SAP was useful in addressing this question. To date, we have examined feeding (Fig. 5), hyperglycemic and corticosterone responses to glucoprivation. None of these responses were impaired by ARC NPY-SAP injections that destroyed NPY receptor-expressing neurons, including the NPY/AGRP neurons, in the ARC and basomedial hypothalamus. However, these same lesions severely reduced feeding and body weight responses to leptin and feeding responses to ghrelin, which are known to depend upon ARC NPY receptor-expressing neurons. Thus, using anti-DBH-SAP and NPY-SAP we have been able to functionally differentiate the hindbrain NPY/catecholamine and the ARC NPY/AGRP co-expressing neuronal populations and to establish the primacy of the hindbrain NPY/catecholamine neurons for elicitation of systemic glucoregulatory responses.

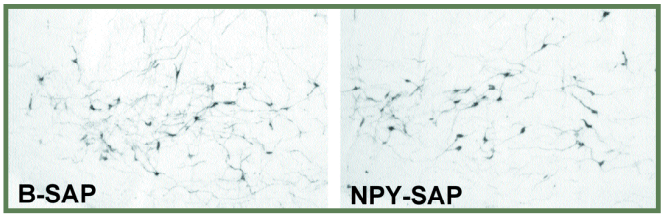

Figure 4. Coronal sections showing tyrosine hydroxylase-ir in the ventrolateral medullary catecholamine cell column ventral to the area postrema in rats injected into the arcuate nucleus with Blank-SAP (B-SAP, control) or NPY-SAP. Nearly all cells in this area co-express NPY and project to the hypothalamus. Arcuate NPY-SAP injections did not cause retrograde destruction of the hindbrain catecholamine/NPY neurons.

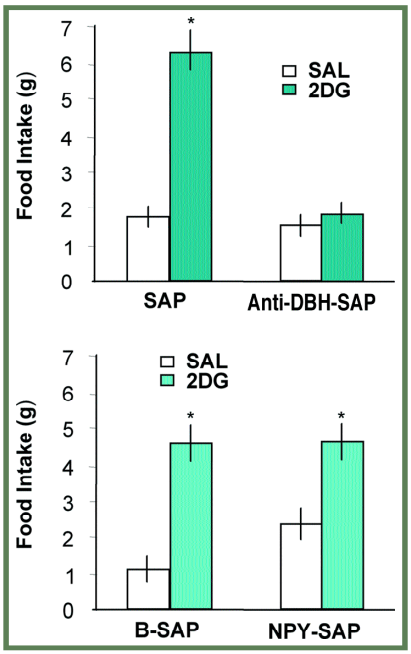

Figure 5. Food intake in response to glucoprivation induced by the antiglycolytic agent, 2-deoxy-D-glucose (2DG, 200 mg/kg, s.c.) in rats injected into the medial hypothalamus with anti-DBH-SAP (top) or NPY-SAP (bottom) or their respective control solutions (unconjugated saporin and Blank-SAP, respectively). Anti-DBH-SAP, but not NPY-SAP, caused impairment of the glucoprivic feeding response. *2DG vs saline for the same group, P<.001

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