Deletion of Catecholaminergic Neurons by Anti-DBH-Saporin

(continued from page 1)

CRH neuroendocrine neurons under these conditions is deduced, in part, by observing changes in the expression of various cellular markers within these neurons in control vs. stress conditions in laboratory rats. For example, we have shown that systemic insulin or 2-DG injection rapidly elevates levels of the phosphorylated forms of MAP kinases ERK1 and/or ERK2[1,2]. The phospho-ERK1/2 staining appears to be induced selectively by these challenges, as little to no phospho-ERK1/2 appears in the CRH neuroendocrine neurons under basal conditions (Fig. 1).

We employed the use of anti-DBH-SAP (Cat. #IT-03) injections to determine whether this phospho-ERK1/2 response requires intact catecholaminergic afferents originating in the hindbrain. Rats given PVH microinjections of anti-dopamine-beta-hydroxylase-saporin conjugate (anti-DBH-SAP) or mouse IgG-saporin control conjugate (Cat. #IT-18) received either normal 0.9% saline vehicle or insulin (2 U/kg, i.v.) and received lethal doses of intravenous anesthesia 30 min later. Brains were processed for DBH and phospho-ERK1/2 immunocytochemistry. Relative to rats receiving sham lesions (injections of control conjugate), anti-DBH-SAP-treated animals displayed a marked loss of DBH immunostaining, indicative of a pronounced loss of catecholaminergic fibers (Fig. 2). This loss was accompanied by loss of the phospho-ERK1/2 response to insulin or 2-DG within these neurons, as evident from the marked reductions of phospho-ERK1/2 immunostaining observed in these animals (Fig. 2, page 1). Additionally, hindbrain cell groups in lesioned rats displayed frank loss of DBH staining, indicative of true cell loss in these hindbrain groups as a result of the saporin lesions (Fig. 3).

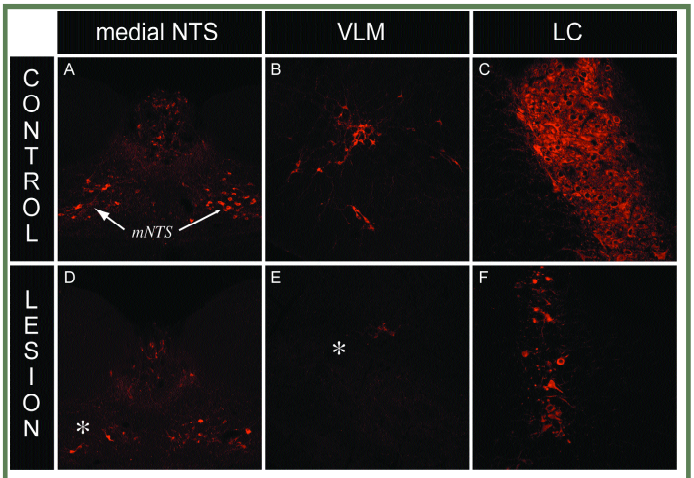

Fig 3. Confirmation of lesion efficacy: The loss of DBH staining in the paraventricular hypothalamus was accompanied by cell loss in hindbrain catecholaminergic cell groups expressing DBH (including the medial subnucleus of the nucleus of the solitary tract (mNTS), the ventrolateral medulla (VLM), and the locus coeruleus (LC); compare D, E and F with A, B, and C, respectively), indicating that the lost DBH+ inputs were from these neurons. Asterisks indicate areas showing cell loss as compared to corresponding regions in control animals.

A characteristic response of CRH neuroendocrine neurons to various homeostatic challenges is the initiation of CRH gene transcription, which is thought to be controlled, in part, by the activation (phosphorylation) of the transcription factor, CREB (cyclic AMP response element binding protein). Indeed, we observed phospho-CREB levels to increase markedly in response to insulin and 2-DG in PVH neurons. To ascertain what happens to CREB activation in CRH neuroendocrine neurons in rats receiving anti-DBH-SAP lesions, we performed dual immunocytochemistry for both markers in lesioned rats. As shown in Fig. 4, lesioned animals displayed marked reductions in phospho-CREB, in line with the reductions observed in phospho-ERK1/2 levels.

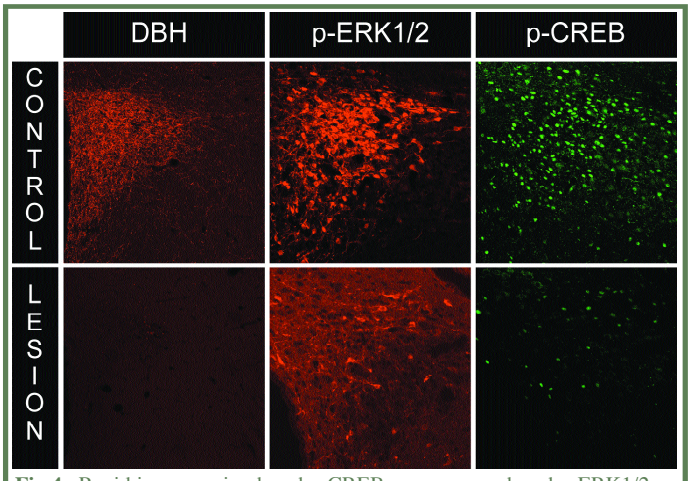

Fig 4. Rapid increases in phospho-CREB accompany phospho-ERK1/2 elevations in the paraventricular hypothalamus of intact (non-lesioned) animals receiving 2-deoxy-D-glucose (2-DG). Loss of DBH is associated with disruptions in both 2-DG-induced phospho-ERK1/2 and phospho-CREB signaling, demonstrating their dependence on catecholaminergic input.

Collectively, our data demonstrate that rats mount CRH neuroendocrine responses to glycemic challenges in a manner that requires intact catecholaminergic afferents from the hindbrain. Both phospho-ERK1/2 and phospho-CREB signals appear to rely on signals propagating along these catecholaminergic pathways. The targeting of this afferent system using saporin-based immunotoxin conjugates has proven to be a valuable technique for probing the afferent circuitry of this important physiological system.

References:

1. Khan AM, Watts AG. 2004. Intravenous 2-deoxy-D-glucose injection rapidly elevates levels of the phosphorylated forms of p44/42 mitogen-activated protein kinases (extracellularly regulated kinases 1/2) in rat hypothalamic parvicellular paraventricular neurons. *Endocrinology* 145(1):351-359.
2. Khan AM, Ponzio TA, Sanchez-Watts G, Stanley BG, Hatton GI, Watts AG. 2007. Catecholaminergic control of mitogen-activated protein kinase signaling in paraventricular neuroendocrine neurons *in vivo* and *in vitro*: A proposed role during glycemic challenges. *The Journal of Neuroscience* 27(27):7344-7360.