BRAIN RESEARCH 1149 (2007) 118–126

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Characterization of brainstem preproglucagon projections to the paraventricular and dorsomedial hypothalamic nuclei

Niels Vrang[a,b,*], *Mikkel Hansen*[b], *Philip Just Larsen*[a,b], *Mads Tang-Christensen*[a,b]

[a]*Rheoscience, Glerupvej 2, 2610 Rødovre, Denmark*
[b]*Dept Anatomy, University of Copenhagen, Denmark*

ARTICLE INFO

Article history:
Accepted 7 February 2007
Available online 27 February 2007

Keywords:
Nucleus of the solitary tract
Preproglucagon
Appetite regulation
Paraventricular nucleus of the hypothalamus
Dorsomedial nucleus of the hypothalamus

ABSTRACT

In the brain preproglucagon expression is limited to a cluster of neurons in the caudal part of the nucleus of the solitary tract (NTS) as well as a smaller number of neurons that extend laterally from the NTS through the dorsal reticular area into the A1 area. These neurons process preproglucagon to glucagon-like peptide-1 (GLP-1), GLP-2, oxyntomodulin and glicentin. The neurons project mainly to the hypothalamus, where especially two nuclei involved in appetite regulation – the paraventricular (PVN) and dorsomedial (DMH) hypothalamic nuclei – are heavily endowed with GLP-immunoreactive nerve fibres. To gain further insight into this neurocircuitry, we injected the retrograde tracers cholera toxin, subunit B (ChB) and Fluorogold (FG) into the PVN and the DMH, respectively. Of thirty-five injected rats, six had successful injections that predominantly restricted within the boundaries of the PVN and DMH. Hindbrain sections from these rats were triple labelled for ChB, FG and GLP-2. A total of 24 ± 1% of the PVN-projecting NTS-neurons contained GLP-2-ir whereas 67 ± 4% of the DMH-projecting neurons were also stained for GLP-2, suggesting that the NTS-projections to the DMH arise mainly from preproglucagon neurons. Approximately 20% of backfilled cells in the NTS contained both retrograde tracers, therefore presumably representing neurons projecting to both the PVN and the DMH. The results of the present study demonstrate that the majority of the preproglucagon-expressing neurons in the NTS project in a target-specific manner to the hypothalamus. It is therefore possible that individual subgroups of GLP-containing neurons can mediate different physiological responses.

1. Introduction

The nucleus tractus solitarius (NTS) receives visceral afferent information from the cardiovascular, respiratory, gastrointestinal and taste systems in which it plays an important role as both a relay and integrative center (Blessing, 1997). The NTS is topographically organised as different sensory modalities are received and processed at different rostro-caudal levels. The complexity of the nucleus is highlighted by the numerous neurotransmitters synthesized by neurons in the NTS (Maley, 1996). Over the past decade, the function of a small population of preproglucagon-expressing neurons in the caudal "gastrointestinal" part of the NTS has been scrutinized. The processing of preproglucagon within these neurons is similar to the processing in intestinal L-cells giving rise to mainly glucagon-like peptide-1 (GLP-1) and -2 (GLP-2) and smaller amounts of

* *Corresponding author*. Rheoscience, Glerupvej 2, 2610 Rødovre, Denmark. Fax: +45 4450 1962.
E-mail address: nv@rheoscience.com (N. Vrang).

0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2007.02.043

glicentin and oxyntomodulin (Bell et al., 1983; Holst et al., 1994; Larsen et al., 1997). Since preproglucagon expression in the brain is limited to the NTS, it has been possible to investigate the projections of these neurons by immunocytochemistry using antibodies raised against preproglucagon or preproglucagon-derived peptides (Jin et al., 1988; Larsen et al., 1997; Rinaman, 1999). These studies have collectively shown that the major target of the brainstem preproglucagon neurons is the hypothalamus. In the hypothalamus, the densest innervation has been observed in two nuclei implicated in the control of food intake and bodyweight, namely the paraventricular (PVN) and dorsomedial (DMH) hypothalamic nuclei. In line with the preproglucagon projection pattern intracerebroventricular (i.c.v.) administration of either GLP-1, GLP-2 or oxyntomodulin has been shown to inhibit food intake in rats (Dakin et al., 2001; Tang-Christensen et al., 1996, 1998, 2000; Turton et al., 1996). Whereas oxyntomodulin presumably acts via the GLP-1 receptor, GLP-2 specifically activates the GLP-2 receptor (Druce and Bloom, 2006; Munroe et al., 1999). Recent studies have revealed an intriguing complexity of the brainstem–hypothalamic preproglucagon system. Whereas the GLP-1 receptor mRNA is expressed in all hypothalamic areas receiving GLP-immunoreactive fibers (Merchenthaler et al., 1999), the GLP-2 receptor expression in the hypothalamus is confined to the compact part of the DMH (Tang-Christensen et al., 2000). The differential distribution of GLP-1 and GLP-2 receptors indeed suggest that GLP-1 and GLP-2 play different roles in appetite regulation despite the fact that the peptides are produced in the same neurons and are derived from the same precursor peptide. To better understand the anatomy of the brainstem–hypothalamic preproglucagon system we employed double retrograde tracing from the PVN and DMH in combination with GLP-2 immunohistochemistry. Using this approach we sought to topographically characterize preproglucagon projections and determine whether the preproglucagon neurons project in a site-specific fashion to the hypothalamus, or whether single GLP-containing neurons target multiple sites.

2. Results

A total of six rats had injections that were centered in both the PVN and the DMH. The center of the ChB and FG injection sites are shown in Fig. 1. Animals with ChB injections spreading caudally in the DMH or with FG injections spreading rostrally in the PVN were not included in the analysis. As seen in Fig. 1, rats T2140, T2065 and T2114 had ChB injection sites that were centered in the caudal part of the PVN (Plate #27, Swanson, 1998) whereas rat numbers T2126, T2116 and T2138 were centered at the level of the posterior magnocellular PVN (Plate #26, Swanson, 1998). Only animals with FG injections involving the ventral subnucleus of the DMH – that receives the densest GLP-ergic innervation (Larsen et al., 1997) – were included in the analysis. Animals T2140, T2126, T2114 and T2116 were centered in the mid-DMH (Plate # 30, Swanson, 1998), the remaining (T2065, T2138) at a slightly more caudal level (Plate

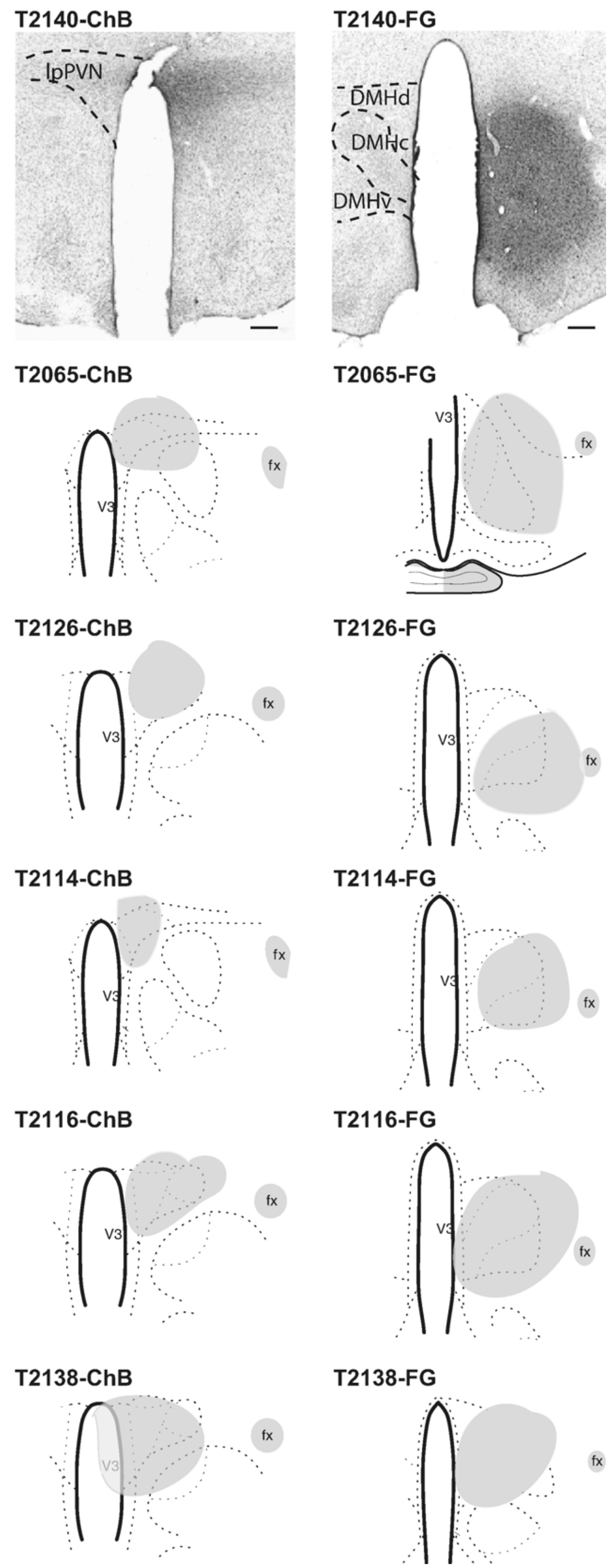

Fig. 1 – Location of ChB and Fluorogold injections in the rats selected for further analysis. A and B are medium power photomicrographs showing the size and location of the PVN (A) and DMH (B) injections (single stained using DAB as chromagen) from rat T2138. The extent and location of the remaining 5 rats have been plotted onto Adobe Illustrator maps from the rat atlas by Swanson (1998). In general, ChB injections are smaller and more clearly demarcated than FG injections (compare A and B). Scale bars in A, B = 100 μm.