Comparison of the Distributions of Neuropeptide Y-, Tyrosine Hydroxylase-, and Tryptophan Hydroxylase-Expressing Neurons in the Hypothalamic Arcuate Nucleus

SAMPSA VANHATALO*  

Department of Anatomy, Institute of Biomedicine, University of Helsinki, Finland; Unit of Child Neurology, Hospital for Children and Adolescents, University Hospital of Helsinki, Finland

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Several levels of interactions between serotonin and neuropeptide Y (NPY) have been proposed in the hypothalamic control of food intake. This study aimed at elucidating the anatomical relationship between the NPY-expressing neurons and the newly characterized neuronal population of tryptophan hydroxylase (Tph)-expressing (serotonin synthesizing enzyme) neurons in the hypothalamic arcuate nucleus. In addition, their distribution was compared to that of tyrosine hydroxylase (TH), the dopamin synthesizing enzyme. No co-localization of NPY and Tph, or NPY and TH was found in the arcuate nucleus either in intact or in colchicine-treated animals. These results suggest that there is likely no functional co-transmission between these transmitter systems in an intact arcuate nucleus.

Keywords: Serotonin, Neuropeptide Y, Tyrosine hydroxylase, Tryptophan hydroxylase, Hypothalamus, Arcuate nucleus

INTRODUCTION

The current major theoretical framework in the research of central dietary control is that circulating leptin acts on the hypothalamic neurons, mainly in the arcuate nucleus (ARC), and activates the neuropeptide Y (NPY)-expressing neurons (Kalra et al., 1999; Elmquist et al., 1999). This idea has been substantiated by a detailed analysis of the hypothalamic distribution of the leptin receptors (Håkansson et al., 1998; Elmquist et al., 1999; Baskin et al., 1999), its co-expression with NPY and various other transmitters, and by repeated demonstration of the leptin-mediated regulation of NPY expression in the ARC (Elmquist et al., 1999). In addition to NPY and leptin, a large number of other neurotransmitters have been implicated in the hypothalamic feeding circuitry (Leibowitz, 1992; Kalra et al., 1999). Serotonin is clinically an intriguing transmitter in the sense

*Address for correspondence: Department of Anatomy, Institute of Biomedicine, P.O. Box 9, 00014 University of Helsinki, Helsinki, Finland. Fax: +358 9 191 8499. E-mail: svanhata@helsinki.fi.
that disturbances in serotonin metabolism and therapeutic potential of drugs acting through serotoninergic mechanisms have been acknowledged for a longer time (Leibowitz, 1990; Wolfe et al., 1997). A number of studies have demonstrated an interaction of serotoninergic and NPY-ergic mechanisms in the control of feeding behaviour (Grundemar and Håkanson, 1994). In addition, immunohistochemical and ultrastructural studies have repeatedly visualized close appositions between the serotoninergic terminals (raphe nuclei projections) and the NPY-expressing neurons in the ARC (Jahng et al., 1998; Guy et al., 1987: 1988).

In addition to NPY-ergic neurons, a large population of the ARC neurons are known to comprise the dopaminergic A12 cell group (Björklund and Lindvall, 1984), which are often visualized by their immunoreactivity for tyrosine hydroxylase (TH), the dopamine synthesizing enzyme. In addition, an inducible expression of tryptophan hydroxylase (TPH), the serotonin-synthesizing enzyme, has been recently demonstrated in the ARC neurons (Weissmann et al., 1987; Vanhatalo and Soinila, 1995a), and the TPH expression is probably strictly confined to a subset of neurons expressing also TH (Vanhatalo and Soinila, 1999). These neurons display some characteristics of authentic serotoninergic neurons (expression of TPH and serotonin binding protein), but still they do not synthesize serotonin under physiological conditions (Vanhatalo and Soinila, 1999; Vanhatalo et al., 1995). They comprise a part of the hypothalamic serotoninergic system, the control and function of which is as yet poorly understood (Ugrumov, 1997).

While several lines of studies have suggested a close interaction between the hypothalamic NPY-ergic and serotoninergic mechanisms, the present theoretical framework is based on the ascending serotoninergic projections from the raphe nuclei. However, a number of recent studies have provided evidence that hypothalamic ARC, periventricular and dorsomedial nuclei may possess many characteristics of serotoninergic neurons (Ugrumov, 1997; Vanhatalo and Soinila, 1998b; 1999). For a better understanding of the serotonin–NPY interaction the distribution of the NPY was compared to the distribution patterns of TPH and TH.

MATERIALS AND METHODS

Adult male Wistar rats (n = 23, weighing 200–300 g), obtained from the animal house of the institute, were used. During the experiment the animals were kept in a constant light–dark cycle of 12:12 h, and they were given water and food ad libitum. The study was approved by the ethical committee of the University of Helsinki. In order to raise neuropeptide levels in the neuronal somata (Liu et al., 1991), some of the animals received an intracerebroventricular (i.c.v.) injection of colchicine (100 μg/10 μl, Sigma), one day before sacrifice, under anaesthesia with a combination of Hypnorm® (0.8 ml/kg, Janssen Pharmaceuticals) and Dormicum® (0.8 mg/kg, Roche). Animals were euthanized under anaesthesia by intracardiac perfusion with saline followed by 4% paraformaldehyde in the phosphate buffer system (PBS, pH 7.4). Tissues were postfixed for 1–2 h and immersed in 20% sucrose for cryoprotection.

For immunohistochemistry, 10–15 μm cryostat sections were cut and subsequently incubated first with normal swine serum, then overnight with the primary antibody, followed by 1 h incubation with the secondary antibody. The glasses were coverslipped using glycerol-Na-veronal mounting medium. The sections were examined with a Leitz Vario-Orthomat fluorescence microscope using Leitz rhodamine (N2.1, 515–560, 580 nm) and fluorescein (13, 450–490, 510–520 nm) filters. Antibody dilutions were 1:500 for the polyclonal rabbit anti-NPY (Amersham), 1:400–500 for the polyclonal sheep anti-TPH (Weissmann et al., 1987; Cash et al., 1985; Vanhatalo and Soinila, 1995a) (Chemicon Inc., CA, USA, Lot. nrs.AB148), 1:1000–2000 for the polyclonal TH antibody (Vanhatalo et al., 1995),
and 1:50–100 for the monoclonal TH antibody (Boehringer Mannheim, Germany, Clone 9B9, Cat.No. 1017 381). Secondary antibodies were rhodamine- or fluorescein-conjugated goat anti-rabbit IgG (1:200, Jackson ImmunoRes. Lab., Inc., West Grove, PA, USA) for the polyclonal NPY and TH antibodies, fluorescein-conjugated donkey anti-sheep (Chemicon Inc., CA, USA) for the Tph1 antibody, and fluorescein-conjugated goat anti-mouse (1:300, Jackson ImmunoRes. Lab., Inc., West Grove, PA, USA) for the monoclonal TH antibody. Cross-reactions between the present antibodies have been previously carefully ruled out (Vanhatalo and Soinila, 1996a; 1999).

RESULTS

The hypothalamic distribution of neuronal cell bodies and fibres immunoreactive for NPY, TH, and Tph1 corresponded well to the previously described patterns of expression for each protein. Colchicine treatment was required for a proper visualization of the immunoreactivity for NPY. Most of the intensively staining hypothalamic NPY-IR cell bodies were found in the ARC, mainly in the rostral and ventral parts of the nucleus, some of the NPY-IR neurons being close to the external zone of the median eminence (Figure 1). Numerous NPY-IR nerve fibres were seen passing to the median eminence and further to the pituitary stalk. Arcuate nucleus neurons immunoreactive for TH were found throughout the rostrocaudal extent of the nucleus, as has been previously described in great detail (Björklund and Lindvall, 1984; Kawano and Daikoku, 1987; Chan-Palay et al., 1984). As compared to the distribution of the NPY-IR neuronal somata, the TH-IR neuronal cluster was oriented from the rostroventral to the dorsocaudal parts of the ARC, while the NPY-IR neurons were mostly located in the ventrocaudal areas of the ARC (Figure 1). Distribution of the Tph-IR neurons closely corresponded to that of TH-IR, and their number markedly increased after colchicine treatment (Vanhatalo and Soinila, 1995a; 1999). As the Tph1-IR neurons form a subpopulation of the TH-IR neurons (Vanhatalo and Soinila, 1999), they did also locate mainly rostrally or dorsally to the NPY-IR neurons (Figure 1).

Double staining for NPY and TH confirmed the completely discrete localization of these two transmitters (Figure 2). Likewise, none of the neurons exhibiting Tph1-IR were found to be immunoreactive for NPY (Figure 3).

DISCUSSION

The present results are in agreement with the large number of previous studies demonstrating the distributions of NPY (Hooi et al., 1989; Horvath et al., 1992; Everitt et al., 1984; Chronwall et al., 1985), TH (Björklund and Lindvall, 1984; Chan-Palay et al., 1984; van den Pol et al., 1984), and Tph1 (Weissmann et al., 1987; Vanhatalo and Soinila, 1999) in the ARC. The NPY-ergic neurons were shown to locate ventromedially to the catecholaminergic (TH) cell group, which confirms the previous reports that neurons expressing NPY in the ARC do not normally exhibit co-localization with the catecholamines (Everitt et al., 1984; Pelletier, 1996). The present results extend the previous knowledge by comparing the distribution patterns of the neurons expressing NPY and Tph1.

Hypothalamic ARC neurons with Tph1 expression have been shown to lack synthesis capacity for serotonin, while they may use serotonin as a false transmitter (Vanhatalo and Soinila, 1998a; Jaim-Etcheverry, 1994) in their axon terminals, at least in the pituitary intermediate lobe (Vanhatalo and Soinila, 1995b). Expression of gene products without their natural function, i.e. superfluous expression, has been reported in several locations in the nervous system (Jaeger et al., 1983; Mathieu et al., 1994; Grizzana and Corte, 1978; Ross et al., 1984; Vanhatalo and Soinila, 1996b; 1999), and it has been considered to result from optimizing the energy costs between suppression of a gene
FIGURES 1 AND 2. This pair of photomicrographs shows immunoreactivity for NPY (1A, 2A), TH (1B), and TpH (2B) in the arcuate nucleus in sections double stained for NPY and TH (1A, B) or NPY and TpH (2A, B). Neurons immunoreactive for NPY (arrow heads) were never immunostained for TH (1B) or TpH (2B). Likewise, TH-IR (1B, solid arrow) or TpH-IR (2B, solid arrow) neurons were not found to exhibit NPY-IR (open arrows in 1A and 2A, respectively) (3V = third ventricle, scale bar is 100µm).
expression and production of small amounts of a number of useless proteins (Bowers, 1994). However, despite the apparent lack of serotonin synthesis in the ARC under the conditions studied so far the possibility remains that these neurons may synthesize serotonin under some as yet unknown condition.

An inducible co-expression of TH and NPY has been shown to occur during lactation (Ciofi et al., 1991), which raises the possibility that these two transmitter systems may be dynamically overlapping. It also raises the question as to whether the TpH-expressing ARC catecholaminergic neurons may exhibit co-localization with NPY. In the present study the expressions of NPY and TpH were studied in intact and in colchicine-treated animals, and no co-localization was found. There is still a possibility that these systems may overlap under some other conditions, like was shown to occur during lactation for TH. This possibility deserves further experiments subjecting animals to various other challenges. Interestingly, a recent study reported a sprouting of the ascending serotonergic nerve terminals around NPY neurons in ARC in anorectic mice (Jahng et al., 1998), which suggests that the serotonin–NPY interactions in the hypothalamic dietary control may even involve morphological plasticity.

Recent studies have demonstrated that the majority of leptin receptor-expressing neurons do also express NPY (Baskin et al., 1999; Elmquist et al., 1999). Some of the ARC neurons, however, possess leptin receptors and no NPY-expression (Baskin et al., 1999), and their distribution pattern would suggest that they might be TH-expressing and/or TpH-expressing ARC neurons. Indeed some TH-IR neurons have been shown to co-express leptin receptors in the dorsal ARC (Håkansson et al., 1998), which raise a possibility of TpH-NPY co-expression in a subpopulation of ARC neurons. Taken together, the present results exclude the possibility of NPY–serotonin interaction by co-transmission (Kupfermann, 1991) in the ARC under normal circumstances, but the possibility still remains that the ARC TpH-expressing neurons may be themselves as targets of leptin and thereby be involved in the regulation of feeding.

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References

