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NPY and its involvement in axon guidance, neurogenesis, and feeding

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Abstract

Objectives: The role of neuropeptides in nervous system function is still in many cases undefined. In the present study we examined a possible role of the 36–amino acid neuropeptide Y (NPY) with regard to three functions: axon guidance and attraction/repulsion, adult neurogenesis, and control of food intake.

Methods: Growth cones from embryonic dorsal root ganglion neurons were studied in culture during asymmetrical gradient application of NPY. Growth cones were monitored over a 60-min period, and final turning angle and growth rate were recorded. In the second part the NPY Y_1 and Y_2 receptors were studied in the subventricular zone, the rostral migratory stream, and the olfactory bulb in normal mice and mice with genetically deleted NPY Y_1 or Y_2 receptors. In the third part an anorectic mouse was analyzed with immunohistochemistry.

Results: 1) NPY elicited an attractive turning response and an increase in growth rate, effects exerted via the NPY Y_1 receptor. 2) The NPY Y_1 receptor was expressed in neuroblasts in the anterior rostral migratory stream. Mice deficient in the Y_1 or Y_2 receptor had fewer proliferating precursor cells and neuroblasts in the subventricular zone and rostral migratory stream and fewer neurons in the olfactory bulb expressing calbindin, calretinin or tyrosine hydroxylase. 3) In the anorectic mouse markers for microglia were strongly upregulated in the arcuate nucleus and in projection areas of the NPY/agouti gene-related protein arcuate system.

Conclusion: NPY participates in several mechanisms involved in the development of the nervous system and is of importance in the control of food intake.

Keywords: Anorexia; Dorsal root ganglia; Microglia; Neuropeptide; Trophism

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Introduction

Neuropeptide Y (NPY), a 36-amino acid peptide discovered by Tatemoto and collaborators [1,2], has been reported to be involved in a variety of neuronal functions, as presented e.g. in the Proceedings of the 8th International NPY Meeting in 2006 [3] and in a book edited by Zukowska and Feuerstein [4]. In the present report we briefly summarize the findings from our laboratories in three different areas: NPY's role as a trophic and guiding factor, its involvement in neurogenesis, and some new findings possibly related to anorexia.

Growth-promoting and guiding effects of NPY

Studies have shown that peripheral nerve injury induces a dramatic increase in NPY expression in adult dorsal root ganglion (DRG) neurons in the rat [5–8] and mouse [9]. These findings raised the possibility that NPY may be important for the survival and regenerative processes. In fact, White and Mansfield [10] and White [11] demonstrated that NPY promotes growth of DRG neurites in vivo and in vitro.

The observation that NPY is expressed in the ensheathing cells of the peripheral olfactory system in the rat [12] raised the possibility of a trophic action of these peptides in the olfactory system. Olfactory neurons are continuously renewed throughout life [13], and the ensheathing cells tightly surround and support the growing olfactory axons on their way from the olfactory mucosa to the glomeruli in the olfactory bulb [14]. In fact, Hansel et al. [15] demonstrated that NPY induces proliferation of neurons in the olfactory mucosa via the Y_1 receptor (Y1R).

In our laboratory we tested the hypothesis that NPY released from ensheathing cells could also be important for guiding the olfactory axons. For this purpose we crossed NPY-null mice [16] with mice in which a single olfactory receptor is genetically labeled with a Tau-LacZ construct [17]. When comparing the wild type with the resulting crossed mice, there was, disappointingly, no difference with regard to distribution of labeled glomeruli in the olfactory bulb [18]. Therefore, NPY does not seem to be essential for targeting of the olfactory axons to reach their proper termination in the olfactory system. Here, the key function of NPY apparently is the trophic effect shown by Hansel et al. [15].

More recently, we addressed the same question, but now in growth cones of DRGs and with an in vitro technique [19]. This approach is based on the knowledge that, during development, the advance of axons is controlled by molecules that affect the rate and direction of growth (see Tessier-Lavigne and Goodman [20]). Such molecules include nerve growth factor [21,22], brain-derived neurotrophic factor, netrin-1, and slit [23].

Individual growth cones of fetal rat DRG neurons were exposed to asymmetric gradients of NPY (10^{-9} M) and their final turning angles and growth rates were recorded. NPY elicited a strong attractive response (final turning angle of 15.1 ± 5.0 degrees, $P \leq 0.05$) and exerted a significant, but moderate, increase in growth rate (65.4 ± 5.2 μm/h, $P \leq 0.03$). A small-molecule non-peptide Y1R antagonist, H409/22 (at 10^{-7} M) [24–26], had no effect by itself on growth rate. However, when turning assays to NPY were performed in the presence of H409/22, NPY lost its ability to elicit an attractive turning response and an increase in rate of extension (final turning angle from 15.1 ± 5.0 to 3.0 ± 6.8 degrees, $P \leq 0.02$; growth rate from 65.4 ± 5.2 to 35.8 ± 8.4 μm/h, $P \leq 0.002$). The "sister" peptide galanin [27] had a significant effect on growth rate, which was somewhat stronger than that of NPY (85.5 ± 13.1 μm/h, $P \leq 0.008$). However, galanin had no effect on turning angle, that is, it caused neither an attractive nor a repulsive response. The effect on growth rate is in agreement with extensive studies [28–31], in particular by Hobson et al. [31] who showed that galanin promotes neurite outgrowth, an effect exerted via the galanin receptor-2.

Taken together, these findings indicate that NPY (and galanin) at the spinal level not only acts as a transmitter-like molecule but also can exert trophic actions, features similar to classic growth factors. Thus, NPY promotes growth and attracts growth cones of embryonic DRG neurons. In fact, its attractive effect is stronger than those by nerve growth factor or insulin-like growth factor-1, but less pronounced than hepatocyte growth factor [19]. The fact that the Y1R antagonist H409/22 completely blocked attraction and its effect on growth rate strongly suggests that the NPY effects are mediated via the Y1R, whereas the growth-promoting effect of galanin on DRG neurons shown in other studies is mediated via galanin receptor-2 (see above).

An interesting question is, under which circumstances, if any, are the growth and turning properties of NPY of physiological significance. Under normal circumstances NPY is not expressed in DRG neurons, but at embryonic day 16 the NPY-related peptide tyrosine tyrosine [2,32] is transiently expressed in DRGs [33], and this peptide has an affinity for the Y1R [34]. In fact, the Y1R has been detected in trigeminal neurons at embryonic day 16.5 [35], and one could hypothesize that during this window NPY-related peptide tyrosine tyrosine released from primary afferent neurons could influence the turning response and growth rate of growth cones. In contrast to DRG neurons, there is an abundant expression of NPY in the dorsal horn [36] and NPY is already present prenatally, at embryonic day 15 [37]. Thus, NPY released from dorsal horn interneurons could influence how the primary afferents, carrying Y1Rs, are guided and grow, once they have arrived in the superficial dorsal horn.

It has been shown that the Y1R is virtually always expressed in neurons that produce the neuropeptide calcitonin gene-related peptide [38,39], and at least some of these neurons are involved in pain processing. The marked upregulation of NPY in DRG neurons after peripheral nerve injury mainly occurs in large neurons associated with