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doi: 10.1152/nips.01437.2003

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Neuropeptide Y: Neurotransmitter or Trophic Factor in the Heart?

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Neuropeptide Y (NPY) is released from sympathetic neurons and exerts short-term (acute) effects on prejunctional nerve terminals and postjunctional cardiac ion channels. However, NPY also exerts long-term (trophic) effects on angiogenesis, cardiac hypertrophy, autonomic signaling, and cardiac ion channels, including effects on L-type Ca2+ and pacemaker channels.

The influence of the sympathetic nervous system on both normal physiology and pathophysiology of the heart extends beyond the beat-to-beat regulation of rate and contractile force arising from acute exposure to neurally released norepinephrine (NE). For example, postinfarction arrhythmias have been ascribed to nerve sprouting and excess sympathetic innervation (7). In addition, congenital arrhythmias in a German shepherd model of sudden death are associated with an abnormal distribution and density of sympathetic innervation as well as excess responsiveness to β1-adrenergic stimulation (11). These results suggest a long-term influence of innervation to modify the autonomic sensitivity of the heart and/or the ionic channels that are the target of those autonomic agonists. Here we define long-term, or trophic, actions as those appearing only after hours or days of exposure, as opposed to typical neurotransmitter-like (short-term or acute) actions that occur over a period of seconds or minutes.

Sympathetic innervation and neuropeptide Y

Abundant data support the general concept that innervation influences phenotypic expression of target tissue. Examples include denervation supersensitivity of blood vessels, correlation of cardiac autonomic sensitivity with the time course of innervation, and induction by motor neuron innervation of a Na+ channel isoform switch in skeletal muscle. Although there are multiple trophic factor(s) and signaling cascades through which innervation acts, in at least some cases neurally released peptides such as neuropeptide Y (NPY) are involved. Several studies have identified a trophic role for NPY in the nervous (6) and vascular (20) systems and in cardiac hypertrophy (2, 9). In addition, we and others (16) have provided evidence that a number of developmental changes in cardiac ion channel function and autonomic signaling are dependent on the proper progression of sympathetic innervation. Our results indicate that neurally released NPY serves as the trophic factor in some of these cases.

NPY is a 36-amino acid peptide that acts through a family of G protein-coupled receptors and has been associated with numerous physiological processes, including feeding, memory, circadian rhythms, and regulation of blood pressure. NPY is present in, and released from, peripheral sympathetic nerve terminals. Five NPY receptors have been cloned (Y1, Y2, Y4, Y5, Y6) and at least one other (Y3) has been suggested on the basis of pharmacological evidence. Data indicate the presence of multiple NPY receptors in the heart, including Y1, Y2, Y4, Y5, and Y6. To some extent, these subtypes can be distinguished pharmacologically by the use of peptide fragments of NPY and peptide analogs, which exhibit varying preferential activity at the different receptor subtypes. More recently, both peptide and nonpeptide subtype-selective antagonists have become available (1).

Acute cardiac actions of NPY

To investigate the long-term effects of NPY, one must first identify and eliminate from consideration the acute or rapid-onset actions. NPY exhibits a variety of acute effects on cardiac performance, and these involve both prejunctional and postjunctional sites of action. Prejunctional effects, which largely relate to reducing the release of both sympathetic and vagal neurotransmitters, are not further considered here. Postjunctional inotropic and chronotropic effects of NPY, on whole heart or strips of tissues, vary with species and preparation. These effects can be contaminated by prejunctional effects or by slow diffusion of NPY and related compounds toward the site of action. For this reason, experiments on isolated cardiac myocytes are more informative, and these have reported acute actions of NPY on transient outward current (Ito), L-type Ca2+ current (ICa,L), and pacemaker current (If) (3, 4, 10).

NPY (100 nM) enhances the fast component of Ito by 65% in adult rat ventricular myocytes and abolishes the positive inotropic response of these cells to β-adrenergic stimulation by isoproterenol (10). Blocking Ito with 4-aminopyridine (0.5 mM) prevents the antiadrenergic inotropic effect of NPY (10). In the same study, NPY increased peak slow inward current.
in contractility but does not reduce the positive inotropic response evoked by isoproterenol. It should be noted, however, that in other studies (3) on guinea pig ventricular myocytes, I_{Ca,L} was reduced by 100 nM NPY; this effect is sensitive to pertussis toxin (PTX) and nonhydrolyzable GTP analogs. The two opposing inotropic effects of NPY in adult rat cardiac myocytes are mediated by different NPY receptor subtypes: positive effects by Y_1 and negative effects by Y_2 receptors. Moreover, different intracellular signaling pathways appear to be involved because only the negative effect is PTX sensitive.

The effect of NPY on I_f was studied in isolated canine Purkinje fibers. NPY (200 nM) reduces the current, and this effect is inhibited by the NPY antagonist NPY-18–36. NPY acts by shifting I_f activation to more negative potentials. These results indicate that NPY may affect heart rate by decreasing I_f. They also are interesting because, as with prejunctional actions of NPY, the effect would be to mitigate the action of coreleased NE. However, there are no published data on the effect of NPY on the primary pacemaker (sinus node) of the heart.

The best-characterized signaling pathway for the acute effects of NPY in cardiac myocytes is inhibition of adenyl cyclase by stimulation of PTX-sensitive G protein(s) and reduction of cAMP level (basal or β-adrenergic stimulated). An alternative pathway has been proposed (18) that includes interaction with Y_1 or Y_2 receptors, inhibition of PTX-insensitive G_q protein, and reduction of inositol 1,4,5-trisphosphate formation.

### NPY and cell growth

NPY exerts an angiogenic effect (20). This has been demonstrated both in vitro and in vivo, and evidence suggests that the mechanism is largely Y_2 mediated, although Y_1 receptors also have been implicated. NPY promotes vessel sprouting and capillary tube formation by endothelial cells.

NPY also is associated with cardiac hypertrophy. Evidence for hypertrophic effects of NPY comes from studies of adult rat cardiac myocytes in culture (2, 9). Twenty-four-hour incubation of 7-day cultures with 10 nM NPY increases total cell protein by 45%, and both decreased protein degradation and increased synthesis contribute to the hypertrophy. In 1-day cultures only protein degradation is influenced, and the total protein increases just 10%. However, in 1-day cultures of cells obtained from adult spontaneously hypertensive rats, 24-h incubation with NPY produces hypertrophy by increasing protein synthesis. The effect is age dependent, with a maximum (20% increase of de novo protein synthesis) observed in cells from 16-wk-old animals. This dependence of the hypertrophic effect of NPY on the "history" of the pathological condition and the finding of an increased level of NPY in patients with cardiac hypertrophy and heart failure indicate that NPY may contribute to development of hypertrophy in the course of some heart diseases. In addition, NPY can potentiate the effect of NE to produce hypertrophy in adult and neonatal cardiac myocytes in culture.

The hypertrophic effect of NPY is accompanied by increased activity of cytosolic creatine kinase (CK) and completely or partially abolished by PTX (5, 9, 19). Short-term exposure (15 min) to NPY induces activation of PKC and PKC-dependent activation of MAPK in adult and neonatal myocyte cultures and activation of phosphoinositol 3-kinase (PI3K) in adult cultures. In adult cultures, inhibition of PI3K or inhibition of p70^s6k (a downstream target of PI3K) prevents NPY-induced hypertrophy. At the same time, NPY-induced MAPK activation in these cells is not affected by PTX. These findings suggest that the PI3K/p70^s6k pathway, rather than the PKC/MAPK/CK pathway, participates in the hypertrophic effect of NPY. On the other hand, PMA abolishes the effect of NPY in neonatal cardiac cultures, indicating that in this model PKC and MAPK may be involved.

Experiments using selective NPY agonists and antagonists suggest that the hypertrophic effect of NPY in cardiac myocytes isolated from spontaneously hypertensive rats is mediated by Y_5 receptors (2).

### NPY and cardiac autonomic signaling

Prolonged NPY incubation has been reported to increase β-adrenergic receptor density via a PTX-sensitive pathway in neonatal rat ventricle cultures (see Ref. 14). However, this is not associated with increased sensitivity to β-adrenergic agonists.

Other evidence demonstrates that neurally released NPY exerts a trophic effect on cardiac α-adrenergic signaling and in particular on its developmental regulation (16). Neonatal canine cardiac Purkinje fibers preferentially exhibit a monophasic positive chronotropic response to α-adrenergic agonists. In comparison, fibers from adult animals exhibit a biphasic chronotropic response, negative at low doses and positive at higher doses. Other evidence suggests a temporal association between onset of the negative chronotropic response and the ontogeny of cardiac sympathetic innervation (16). A similar phenomenon is observed in a rat ventricular septal preparation, which provides additional in vivo evidence of the link between sympathetic innervation and a negative chronotropic α-adrenergic response. Ventricular septal preparations were studied on postnatal day 10 following daily injection of NGF or NGF antibody to accelerate or delay the onset of sympathetic innervation, respectively. Relative to a vehicle group, the percentage of preparations exhibiting a negative chronotropic α-adrenergic response is smaller in the NGF

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“NPY is also associated with cardiac hypertrophy.”
Neonatal rat ventricular cells maintained in primary culture are a convenient preparation for studying the role of sympathetic innervation because the rat ventricle is not sympathetically innervated before birth and neonatal myocytes are readily maintained in short-term cell culture, beat spontaneously in culture, and can be innervated in vitro by neurons dissociated from the paravertebral sympathetic chain (16). Noninnervated neonatal rat ventricular myocytes in culture exhibit an exclusively positive chronotropic response to α-adrenergic agonists, whereas approximately two-thirds of innervated cultures exhibit a negative chronotropic response. The negative chronotropic response observed after in vitro innervation is lost when the cultures are treated with PTX, suggesting that the positive and negative chronotropic responses in rat ventricle are associated with distinct α-adrenergic receptor subtypes.

This cell culture system was used to identify NPY as the trophic signal released by the sympathetic nerves. The effect of sympathetic innervation is not reproduced by growing myocytes in the presence of nerve-conditioned medium, suggesting that any neurally released trophic factor is not present in the bulk medium in significant quantity. However, the effect of innervation is reproduced by maintaining the neonatal myocytes in culture for several days in the sustained presence of NPY (Fig. 1). NPY-treated cultures exhibit a predominantly negative chronotropic response to the α-adrenergic agonist phenylephrine, whereas control cultures exhibit a positive chronotropic response. There is no effect of short-term NPY exposure on the percentage of cultures exhibiting a positive or negative chronotropic response to a fixed concentration (10^{-6} M) of phenylephrine, but 96-h NPY exposure fully mimics the effect of innervation. In addition, long-term but not short-term exposure of innervated cultures to an NPY antagonist prevents the effect of innervation on α-adrenergic chronotropy (17). The α1β-adrenergic receptor is the subtype responsible for the negative chronotropic response in NPY-treated rat ventricle cultures, the same subtype previously associated with negative chronotropy in the intact rat ventricle (16). Data generated by using a series of NPY peptide analogs suggest that the NPY Y_2 receptor is the subtype likely mediating the trophic effect of NPY on α-adrenergic signaling in the neonatal rat ventricle. This same study, by using a series of NPY peptide analogs, provided data suggesting that the NPY Y_2 receptor is the subtype likely mediating the trophic effect of NPY on α-adrenergic signaling in the neonatal rat ventricle.

The evidence indicates that, during postnatal development, sustained release of NPY from sympathetic nerve terminals acts on NPY Y_2 receptors to modify α-adrenergic signaling. This results in the functional availability of a PTX-sensitive α_1β-adrenergic signaling cascade. Both the α_1β-adrenergic receptor and PTX-sensitive G proteins are present in the neonatal rat heart but are not well coupled before innervation.

**FIGURE 1.** Innervation or neuropeptide Y (NPY) alters α-adrenergic chronotropic response in neonatal rat ventricle cultures. Top: incubation of myocytes for several days results in reappearance of a negative chronotropic response to the α-adrenergic agonist phenylephrine. Bottom left: with increasing incubation time in NPY, a progressively greater percentage of noninnervated muscle (M) cultures exhibit a negative chronotropic response to phenylephrine. Bottom right: innervated (NM) cultures also exhibit a negative chronotropic response to phenylephrine, which is prevented if the cultures are incubated with an NPY antagonist [Ac-(3-2,6-dichlorobenzyl)-Tyr^{27-36(2-Thr)}]NPY-(27-36); PYX] for 96 h but not when the incubation is only 0.5 h. Adapted from Ref. 17.

**NPY and I_{Ca,L}**

Another developmental change that can be reproduced by sympathetic innervation of neonatal ventricle myocytes in culture involves I_{Ca,L}. Current density and channel protein level increase postnatally, and current density is greater in sympathetically innervated neonatal ventricle cells in culture than in noninnervated myocytes (12, 14). α-Adrenergic receptor activation increases dihydropyridine binding (an index of channel density) and transcription of the α_{1C}-subunit of the L-type Ca^{2+} channel (8), consistent with the effect of in vitro innervation being mediated by NE acting at α-adrenergic receptors. However, sustained incubation of neonatal myocytes with NPY in culture fully mimics the effect of sympathetic innervation on I_{Ca,L} density. Furthermore, sustained incubation of innervated cultures with an NPY antagonist fully prevents the effect of innervation (Fig. 2) (14). These data argue that NPY, rather than NE, is the relevant trophic factor released from sympathetic neurons in vitro.

As in the case of α-adrenergic signaling, the effects of innervation and NPY in cell culture are intriguing but require in vivo confirmation of physiological significance. Such evidence has recently been obtained by taking advantage of a transgenic mouse in which the gene for NPY has been disrupted (13). Ani-
mals that are homozygous for the disruption lack endogenous NPY. When ventricular myocytes are isolated and studied from these animals and strain-matched control animals, there is no postnatal increase in $I_{\text{Ca,L}}$ density in NPY-deficient animals. Adult myocytes from these mice exhibit $I_{\text{Ca,L}}$ density that is not only less than that of the control cells but identical to current density in neonatal cells of both the control and NPY-deficient mice. This indicates that the influence of NPY on $I_{\text{Ca,L}}$ in vivo is entirely postnatal (since newborn control and NPY-deficient I_{Ca,L} current-voltage curves are identical) and that NPY is the only contributor to the postnatal increase in $I_{\text{Ca,L}}$ density (since newborn and adult current density in the NPY-deficient myocytes are identical). Furthermore, the 68% increase in $I_{\text{Ca,L}}$ density of control vs. NPY-deficient cells in this in vivo mouse model is remarkably similar to the effect observed in vitro in rat myocytes, either as a result of sympathetic innervation or sustained exposure to NPY (see Fig. 2).

**NPY and $I_f$**

$I_f$ is present throughout the heart and exhibits marked variation in voltage dependence with cardiac region, age, and disease. Activation threshold varies by >80 mV between sinoatrial node, where activation is relatively positive, and ventricle. Adult ventricle activation threshold occurs negative to the cell's resting potential, so that the channel is physiologically silent. However, with cardiac disease ventricular activation is observed within the physiological range, where it may contribute to arrhythmogenesis. It thus becomes important to understand the regulatory processes controlling voltage dependence of $I_f$ and since the channel also activates at less-negative potentials in neonatal ventricle, clues may be found in studies of development.

Using the cell culture model of innervation described earlier, it was found that sympathetic innervation shifts $I_f$ activation negative by ~20 mV. This effect of innervation cannot be reproduced by incubating noninnervated cells in either NPY or NE. However, it can be reproduced by incubating noninnervated cells in the combined presence of both NPY and NE (15). Experiments with various pharmacological agents suggest that the NE action is $\alpha$-adrenergically mediated and that both the NPY receptor subtype and $\alpha$-adrenergic receptor subtype are the same as those implicated in developmental maturation of the negative chronotropic $\alpha$-adrenergic response.

This led to the hypothesis that the requirement for NPY and NE is actually sequential rather than simultaneous. The reasoning is that chronic exposure to NPY, most likely acting via the Y_2 receptor, results in the functional availability of the $\alpha_{1B}$-adrenergic cascade, which is then chronically activated by NE. Therefore, sequential exposure to NPY followed by NE should mimic the effect of innervation, but sequential exposure to NE followed by NPY should be ineffective. As pre-
dicted, when neonatal myocytes are incubated in culture with NPY for 2–3 days, followed by removal of the NPY and subsequent 2- to 3-day incubation with NE, the If activation curve shifts negative and is comparable with that observed after in vitro innervation (Fig. 3). When the order of incubation with NPY and NE is reversed, there is no effect. The signaling pathway involved in this developmental regulation of If, as well as that for the earlier-described regulation of ICa,L, are schematically illustrated in Fig. 4.

Conclusion

The role of innervation to regulate heart rate and force on a beat-to-beat basis is well established. More recently, attention has focused on the long-term influence of innervation and its disruption in the setting of cardiac disease. In this context, it becomes important to understand both the short- and long-term effects of not only the traditional neurotransmitters but also the other substances present in and released from nerve terminals within the heart. NPY is the major neuropeptide in sympathetic nerve terminals, and it clearly possesses trophic actions on cell growth (angiogenesis and cardiac hypertrophy), autonomic signaling cascades, and cardiac ion channel function. At least some of these effects contribute to the normal phenotypic development of the heart, but their contribution to pathological cardiac remodeling remains to be determined. The details of the signaling cascade(s) contributing to the trophic actions of NPY also remain to be determined, but in most cases studied to date the Y2 or Y5 NPY receptor appears to be involved.

References