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Gary J Hodges

Dwayne N Jackson

Louis Mattar

John M Johnson

J Kevin Shoemaker

Western University, kshoemak@uwo.ca

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Neuropeptide Y and neurovascular control in skeletal muscle and skin

Gary J. Hodges,¹ Dwayne N. Jackson,² Louis Mattar,¹ John M. Johnson,³ and J. Kevin Shoemaker^{1,4}

¹School of Kinesiology, ²Department of Medical Biophysics, ⁴Department of Physiology and Pharmacology, The University of Western Ontario, London, Ontario; and ³Department of Physiology, The University of Texas Health Science Center, San Antonio, Texas

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Hodges GJ, Jackson DN, Mattar L, Johnson JM, Shoemaker JK. Neuropeptide Y and neurovascular control in skeletal muscle and skin. *Am J Physiol Regul Integr Comp Physiol* 297: R546–R555, 2009. First published July 1, 2009; doi:10.1152/ajpregu.00157.2009.—Neuropeptide Y (NPY) is a ubiquitous peptide with multiple effects on energy metabolism, reproduction, neurogenesis, and emotion. In addition, NPY is an important sympathetic neurotransmitter involved in neurovascular regulation. Although early studies suggested that the vasoactive effects of NPY were limited to periods of high stress, there is growing evidence for the involvement of NPY on baseline vasomotor tone and sympathetically evoked vasoconstriction in vivo in both skeletal muscle and the cutaneous circulation. In Sprague-Dawley rat skeletal muscle, Y₁-receptor activation appears to play an important role in the regulation of basal vascular conductance, and this effect is similar in magnitude to the α_1 -receptor contribution. Furthermore, under baseline conditions, agonist and receptor-based mechanisms for Y₁-receptor-dependent control of vascular conductance in skeletal muscle are greater in male than female rats. In skin, there is Y₁-receptor-mediated vasoconstriction during whole body, but not local, cooling. As with the NPY system in muscle, this neural effect in skin differs between males and females and in addition, declines with aging. Intriguingly, skin vasodilation to local heating also requires NPY and is currently thought to be acting via a nitric oxide pathway. These studies are establishing further interest in the role of NPY as an important vasoactive agent in muscle and skin, adding to the complexity of neurovascular regulation in these tissues. In this review, we focus on the role of NPY on baseline vasomotor tone in skeletal muscle and skin and how NPY modulates vasomotor tone in response to stress, with the aim of compiling what is currently known, while highlighting some of the more pertinent questions yet to be answered.

skin blood flow; skeletal muscle blood flow; blood flow control; BIBP3226

SKELETAL MUSCLE AND SKIN REPRESENT TWO organs that are under strong sympathetic neurogenic vasomotor control, a neural mechanism that contributes to the large range of vascular conductance in these tissues. Historically, emphasis has been placed on the purinergic and adrenergic (norepinephrine; NE) neurotransmitters and receptor mechanisms mediating neurovascular control in these beds. However, advances in the pharmacology of neuropeptide Y (NPY) and the Y-receptor family over the past two decades have enabled direct examination of the role of Y-receptor mechanisms influencing sympathetic neurogenic vasomotor control. NPY and Y-receptors are ubiquitous and involved in many neurogenic functions ranging from cortical actions in satiety and emotion, to blood flow distribution in the periphery. Determining a role of this neurotransmitter in vasomotor control has been challenging, as its effects appear to vary from tissue to tissue and with sex hormones. NPY and its receptors have been reported to be present in all the major tissues of the body and implicated in numerous processes; however, in this review, we limit our

discussion to 1) NPY and the Y₁-receptor (Y₁R) and Y₂-receptors (Y₂R) involved in vasomotor control, 2) the concept of frequency-dependent neuronal release of NPY and/or Y₁R activation, 3) sexual dimorphism in the NPY-Y₁R control system, and 4) recent evidence regarding a role for this system in the control of blood flow in skeletal muscle and skin.

Neuropeptide Y Structure and Synthesis

NPY, first isolated from the porcine brain (98), is a 36-amino acid residue member of a family of peptides that includes pancreatic polypeptide and peptide YY. Its name derives from the tyrosine residues located at both the NH₂- and COOH-terminal ends, as well as the relatively high tyrosine content of the complete NPY_{1–36} molecule. Biologically active NPY is derived from a 97-amino acid precursor, preproneuropeptide Y. NPY is formed following four posttranslational enzymatic reactions (42), the first of which results in the 69 amino acid residue proNPY after the removal of the signal sequence by signal peptidase. Subsequently, proNPY is broken down by prohormone convertase PC2 and/or PC1/3 at the paired basic site Lys₃₈-Arg₃₉, in turn, releasing the 30 amino acid C-flanking peptide of NPY and NPY_{1–39} (77). A carboxypeptidase-like enzyme further processes NPY_{1–39}, resulting in

Address for reprint requests and other correspondence: G. J. Hodges, Neurovascular Research Laboratory, School of Kinesiology, Thames Hall, Rm. 2122A, London, ON N6A 3K7 (e-mail: ghodges@uwo.ca).

NPY₁₋₃₇, which becomes amidated at its COOH-terminal end by peptidyl-glycine- α -amidating monooxygenase that cleaves another amino acid. The resulting fragment, NPY₁₋₃₆, is referred to as biologically active NPY or simply NPY.

NPY is coreleased with NE from within peripheral sympathetic nerves that supply blood vessels. Specifically, it is proposed that NE is contained and released from small dense cored vesicles, whereas NPY is contained within large dense cored vesicles. This hypothesis predicts that NE primarily controls vascular smooth muscle tone during basal conditions, whereas NPY should not; its release only occurs during periods of high stress. Although some debate remains, pharmacological and physiological studies support the differential release of NPY and NE (56, 62). The discovery of the physiological potential of NPY in blood vessels originated from experiments on the feline submandibular gland where, after sympathetic nerve stimulation, slow and persisting α -adrenergic independent vasoconstriction was observed (65). Anatomical evidence confirms that, in peripheral tissues, nerve fibers that contain NPY are more abundant around resistance vessels with increasing density as vessel size decreases (97).

Neuropeptide Y Y₁ Receptor

The NPY Y₁R, first of the Y-receptors cloned, was originally recognized in the rat brain (31), and it was cloned soon after from human transfected cells (41, 58). Y₁R are abundant throughout mammalian systems. Peripherally, Y₁R are expressed mainly in arteries and veins, where they are associated with vasoconstriction and potentiation of other vasoconstrictors of neurogenic origin (39). Although limited, there is evidence of prejunctional Y₁R inhibition of neurotransmitter release (24). Nonetheless, the NPY Y₁R is primarily located postjunctionally on vascular smooth muscle cells. The Y₁R is a G protein-coupled receptor. When activated, uncoupled G_i α and G $\beta\gamma$ subunits lead to the inhibition of adenylyl cyclase with subsequent reductions in the production of cAMP and increased phospholipase C activation. This sequence results in increased intracellular Ca²⁺ and potent, long-lasting vasoconstriction (29, 67, 115).

The complete NPY₁₋₃₆ molecule is necessary for NPY binding to Y₁R. Any proteolytic processes leading to alterations in the NH₂-terminal domain essentially abolishes the ability of NPY to bind to Y₁R. Therefore, NH₂-terminally truncated NPY fragments such as NPY₂₋₃₆, or NPY₃₋₃₆ have little or no affinity for the Y₁R (e.g., 7, 26). However, these fragments exhibit increased binding affinity for the Y₂R (discussed below). Modification of COOH-terminal residues does not affect agonist binding. Thus, it has been established that the NH₂ terminus is essential for NPY to activate Y₁R (73).

Neuropeptide Y Y₂ Receptor

The NPY Y₂R was first cloned in 1995 from SMS-KAN cells and later from human brain and neuroblastoma cells (34). Recent evidence has illustrated that endogenous, neuronally released NPY activates prejunctional Y₂R to exert auto-inhibition of its own release, as well as that of NE (69, 70, 105); this effect has also been exhibited using exogenous NPY and/or its truncated analogs (69). The concept of prejunctional autoinhibitory NPY receptors was first introduced by Lundberg and colleagues (66). Soon after, prejunctional auto-inhibitory

NPY effects were suggested to be mediated by Y₂R (105). The auto-inhibitory effect of Y₂R binding is manifested through inhibition of neuronal adenylyl cyclase and voltage-dependent (N-Type) Ca²⁺ channels. Recently, the development of a highly selective Y₂R antagonist, BIIE0246 (25) has led to the discovery of a vascular role for Y₂R in a limited number of species and tissues. Y₂R have been shown to regulate the release of NPY in porcine kidney (69, 70), porcine spleen (68, 70, 78), and rat vas deferens (92). Recent work by Gradin and coworkers (37) indicates that both Y₁R and Y₂R are involved in vasoconstriction of mesenteric arteries of spontaneously hypertensive rats (SHR).

Interaction Between Neuropeptide Y and Norepinephrine

Sympathetic postganglionic neurons in rat tail artery and guinea pig vas deferens release NE, NPY, and ATP (11, 52), with release of these contents being more prevalent at high nerve stimulation frequencies (11, 61). ATP and NPY provide significant contributions to rat tail vascular tone, especially at higher impulse frequencies (11). This cocktail of neurochemical messengers enables a number of biological effects and interactions (e.g., slow, intermediary, and rapid signaling) (61). For example, Lundberg and Tatemoto (65) describe the following vasoconstrictor potencies of sympathetic neurotransmitters: NPY produces a slow acting, potent, and persistent increase in vascular contractile state; NE-induced vasoconstriction develops and dissipates more quickly; ATP-induced vasoconstriction has a rapid onset but short duration. It has been postulated that the duration of the effects for each transmitter relies on the unique mode of deactivation/removal. For example, vascular effects of NE are removed quickly via NE reuptake, or rapid degradation by catechol-*O*-methyl transferase, whereas the prolonged duration of NPY-induced vasoconstriction is mediated by slower enzymatic degradation of "free" NPY (61).

NPY potentiates α -adrenergic vasoconstriction in both in vivo (17, 59, 88) and in vitro (11, 27, 28, 36, 105) preparations. The synergistic interaction between NPY and NE is receptor mediated. Thus, NPY enhances the vasoconstrictor response to both sympathetic nerve stimulation (11, 17) and phenylephrine- (specific α_1 R agonist) induced vasoconstriction (17). The likely mechanism responsible for the synergistic effects of NPY and NE is the convergence of second messenger signaling pathways acting through phospholipase C, resulting in protein kinase C activation (108). Also NPY transiently increases myosin light-chain phosphorylation (60), an effect that would augment contraction. This is consistent with the known ability of NPY to potentiate NE-mediated vasoconstriction and inhibition of vasorelaxation. Thus, the interactive effects must be due to consequent modification of specific receptor properties and/or second messengers (33).

Role of Neuropeptide Y in the Regulation of Vasomotor Tone

General background. A major issue regarding the involvement of NPY in neurovascular regulation in muscle has been the uncertainty regarding a role of this neurotransmitter under baseline (resting) conditions. These concerns are particularly important in regard to the controversies surrounding any impact of NPY on baseline blood pressure, as well as with the hypotheses regarding the mechanistic role of sympathetic neu-

rotransmitters in cardiovascular tissue damage during periods of chronically heightened sympathetic discharge with advancing age and disease. These issues are complicated further by variations of NPY composition that may heighten any detrimental aspects of this neurotransmitter.

NPY, blood pressure, and sympathetic discharge. As mentioned above, the role of NPY in blood pressure regulation is debated. Evidence from Y_1R knockout mice that display normal basal blood pressure (85) suggests that NPY does not play a critical role in blood pressure maintenance. Moreover, infusion of the Y_1R antagonist BIBP3226 does not affect basal blood pressure in normotensive or in SHR (which have elevated plasma NPY levels) (110). Consequently, a role for NPY as a regulator of baseline vascular control has been discounted. Nonetheless, circulating NPY levels rise during circulatory shock, chronic stress (e.g., sepsis, hemorrhage, cold stress) (87, 114), and even heavy exercise (63, 74). Thus, it may be that NPY release is related to discharge frequencies of the postganglionic sympathetic neurons and play a more important role in blood pressure regulation in times of significant stress.

Additional evidence in support of the idea that NPY is released primarily during periods of high sympathetic outflow comes from studies of sympathetic activity and vascular disease. Although the sympathetic nervous system is critical for maintaining blood flow/pressure homeostasis during acute stress (e.g., during exercise, cold exposure, or orthostatic stress), several investigations indicate that this nervous system is also involved in long-term vascular control and/or morphological changes in vascular structure. For example, a relationship is described between essential hypertension and chronically augmented sympathetic nerve activity and its association with increased vascular resistance (71), intimal wall thickness (23), and damage to cardiovascular tissues (30, 53, 84, 109). These deleterious effects of sympathetic outflow appear to include the dual actions of the sympathetic neurotransmitters NE (e.g., 38) and NPY (57) acting on postjunctional α - and Y-receptors, respectively. In this regard, NPY has been implicated in hypertension (76), as there are high circulating levels of NPY present in both men and women with the disease (106). Moreover, the mitogenic potential of NPY has been established (86, 90, 111, 112). These data suggest that NPY release is dependent upon the magnitude of nerve discharge frequency; thus, elevated sympathetic nerve activity may lead to increased mitogenesis via the actions of NPY. Nonetheless, Y_1R activation must occur chronically under baseline conditions if it is to factor importantly in long-term blood pressure regulation, and/or mitogenic effects. Chronic basal Y_1R activation, however, has not been demonstrated with certainty as outlined above.

The evidence that NPY does not affect baseline blood pressure but is released during periods of high stress suggests that NE and NPY are differentially released (4, 19, 62) with NE released at lower nerve activity and NPY released only under high neuronal stimulation frequencies. This hypothesis predicts that NE primarily controls vascular smooth muscle tone during basal conditions, whereas NPY does not; its release only occurring during periods of high stress. Pharmacological and physiological studies support the differential release of NPY and NE (56, 62).

Conversely, data produced by De Potter and coworkers (20–22) suggested that both NE and NPY contribute to base-

line vascular tone. The premise of this research was the analysis of proteins from small and large dense cored vesicles in sympathetic varicosities. Differential release of NE and NPY might occur if they are stored in different presynaptic vesicles. Analysis of the ratios of proteins from these different vesicles was reasoned to provide a means to assess the differential release hypothesis. In their studies, De Potter and colleagues (20–22) tested a number of different vascular beds innervated by sympathetic nerves with differing ratios of large dense cored vesicles and small dense cored vesicles. These vascular beds included the isolated perfused sheep spleen, dog spleen, and rat vas deferens (the latter containing primarily small dense cored vesicles). Using a range of nerve stimulation frequencies from 2 to 20 Hz, they observed that the ratio of proteins reflecting large and small dense cored vesicular release remained constant. Thus, from these data, it could be predicted that both NPY and NE are released concurrently and should contribute to baseline vascular tone. This issue of the influence of NPY on peripheral vasomotor control has been addressed recently in studies of Y_1R antagonism in skeletal muscle and skin and is discussed below.

NPY and vasomotor control in skeletal muscle. Skeletal muscle represents 30–40% of body mass and is under considerable neurogenic and metabolic control that enables blood flow changes that are rapid and large in magnitude, ranging from $5 \text{ ml} \cdot 100 \text{ ml}^{-1} \cdot \text{min}^{-1}$ to at least $250 \text{ ml} \cdot 100 \text{ ml}^{-1} \cdot \text{min}^{-1}$ (1). This translates to skeletal muscle receiving up to 85 to 90% of maximal cardiac output during extended periods of exercise (3, 18). Even under baseline conditions, sympathetic neurogenic inputs account for ~50% of the tonic contractile state. Thus, this tissue offers the ability to study NPY-induced vasomotor control under various conditions.

The first evidence that the NPY Y_1R caused an NPY-induced vasoconstriction was observed in cat skeletal muscle (29). More recently, Jackson and colleagues (46) reported an increase in baseline hindlimb blood flow and vascular conductance after Y_1R blockade with BIBP3226 in the Sprague-Dawley rat. In mongrel dogs, Buckwalter and colleagues (13) reported that intra-arterial infusion of BIBP3226 led to substantial increases in limb blood flow, without changes in systemic hemodynamics or contralateral limb blood flow. Furthermore, this latter group showed the contribution of Y_1R to blood flow, and vascular conductance was similar to that of α_1R (Fig. 1). These findings supported previous work by De Potter et al. (20, 21) that NPY and NE are coreleased from peripheral sympathetic nerves, even under baseline conditions.

By contrast, Coney and Marshall (16) observed no effect of Y_1R antagonism on baseline femoral vascular resistance. The authors (16) suggested that perhaps the different anesthetic regimes used offered a potential explanation for these opposing findings. For example, Jackson and colleagues (46, 47) used the barbiturate thiobutabarbital sodium (Inactin) anesthetic, whereas, Coney and Marshall (16) used halothane anesthesia at surgical depth (absence of withdrawal reflex) for their studies. Barbiturate anesthesia, including Inactin, has been shown to elevate baseline sympathetic outflow, relative to α -chloralose (nonbarbiturate) (101) with increases in hindlimb vascular resistance (99). Other studies have indicated that barbiturates have little impact on sympathetic levels or baseline vascular conductance (10, 72). Thus, it remains possible that a higher average muscle sympathetic nerve activity existed under the

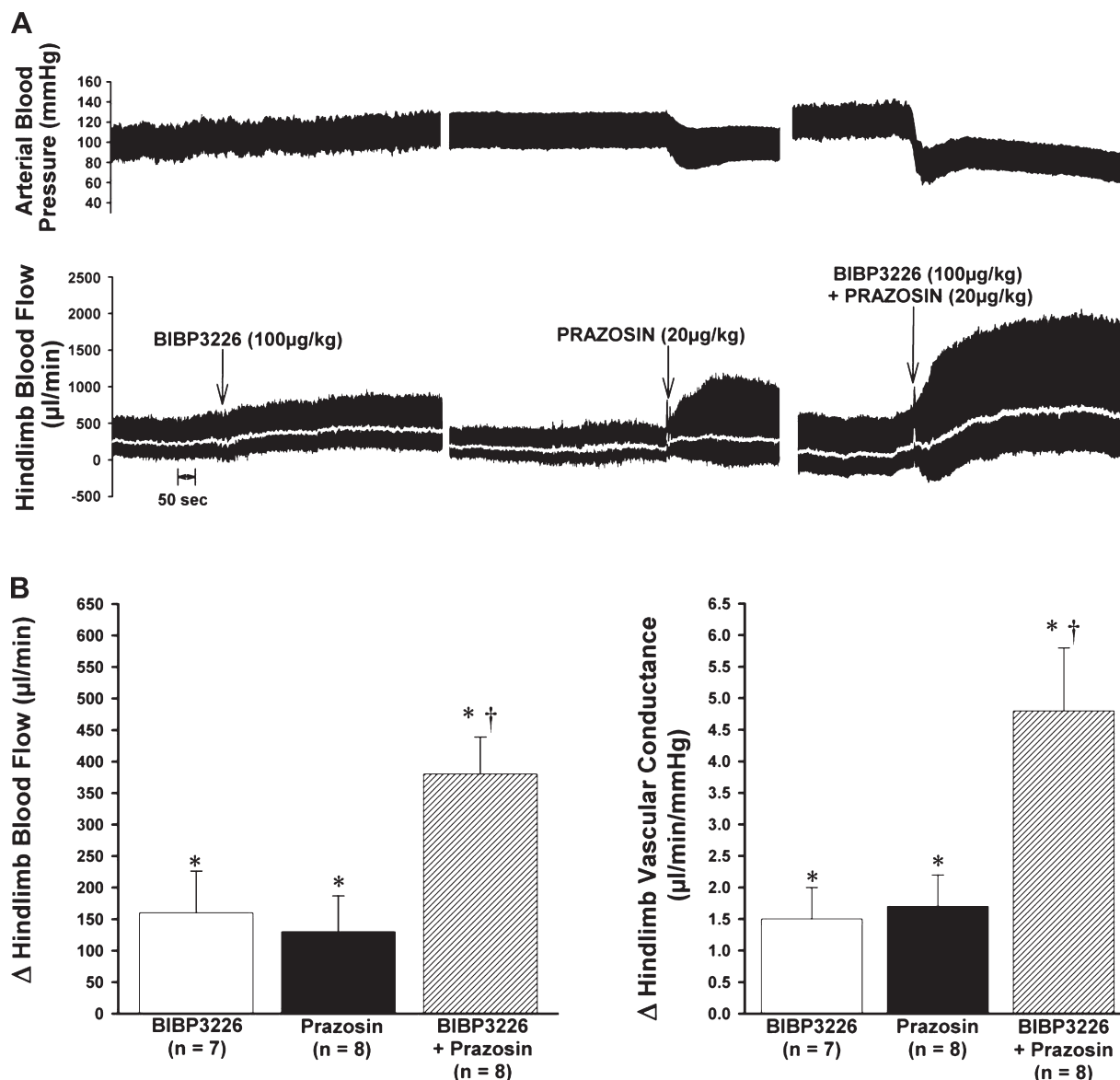


Fig. 1. A: representative responses of arterial blood pressure and hindlimb blood flow for each of the N^2 -(diphenylacetyl)- N -[(4-hydroxyphenyl)methyl]- D -arginine amide (BIBP3226), prazosin, and BIBP3226 + prazosin treatments. The bold arrow represents the time at which infusion of the respective drug (indicated above arrow) was given. The white trace indicates mean hindlimb blood flow for each treatment. Note the increase in diastolic blood flow only when BIBP3226 was present. B: mean changes (Δ) from baseline for hindlimb blood flow (left) and vascular conductance (right) with each treatment. Values represent means \pm SE. * $P < 0.05$, significant difference from baseline; † $P < 0.05$, significant difference from BIBP3226 and prazosin conditions. [From Jackson et al. (46).]

conditions of Jackson and coworkers (46) compared with Coney and Marshall (16). But, just how any such changes fit into the concept of frequency-dependent NPY release remains to be established. A less likely, but nonetheless testable, alternative explanation for these apparently conflicting results, is that neither NPY receptors nor the control of systemic blood pressure are homogeneously distributed among strains of species, as Jackson and colleagues (46) studied Sprague-Dawley rats, whereas Coney and Marshall (16) examined Wistar rats.

Sex-dependent NPY neurovascular control in skeletal muscle. Several lines of evidence support the conclusion that sex affects neurovascular control. In human studies, females have, on average, lower levels of baseline (51, 83) and reflex-mediated changes in muscle sympathetic nerve activity (91).

Similar studies on baseline sympathetic levels in rodents have not been in agreement, as sympathetic nerve activity is not reported in the same way. However, if the release of NPY is frequency dependent, then females might be expected to elicit less NPY Y_1 R vascular control, compared with males. The few investigations that have addressed sex differences in the effects of NPY on the cardiovascular system have produced contrasting conclusions. In support of the postulate that NPY release is affected by one's sex, Morris et al. (81) and Zukowska-Grojec. (11) observed a greater increase in blood pressure, heart rate, and mesenteric vasoconstriction in males compared with females during cold stress, an effect that was directly associated with increases of plasma NPY immunoreactivity. In contrast, Glenn et al. (35) found that NPY-induced vasoconstriction of

rat tail artery was greater in female compared with male rats. Other studies suggest that the blood pressure response to NPY infusion was similar between intact conscious male and female rats (113). The complexity of sex-dependent differences in NPY-induced cardiovascular control is advanced by studies of cortical Y_1R expression and sensitivity. Michel et al. (75) examined the cerebral cortex of Wistar-Kyoto rats and SHR and noted that although the number of Y_1R was similar between the breeds, females had half as many Y_1R compared with males. However, binding affinity of these Y_1R in females was twice that of males (75).

In skeletal muscle, there appears to be strong influence of sex hormones on the neurogenic regulation of baseline vascular contractile state. Jackson and et al. (48) provided the first account that female skeletal muscle contained greater overall Y_2R expression compared with males. Also, only males exhibited Y_1R modulation of vasoconstriction (with similar anesthetic regimes), illustrating sexual dimorphism in the control and maintenance of vascular tone in skeletal muscle. Although there was no observable difference in baseline blood flow between males and females, baseline vascular tone in males appears to be established by a synergy between Y_1R and α_1R . This interaction was unmasked with simultaneous antagonism of Y_1R and α_1R in the rat hindlimb. These findings support similar observations of synergism in studies of blood pressure (45) and vessel tone in human omental arteries, guinea pig uterine arteries, and rabbit gastroepiploic, pulmonary, and femoral arteries (6, 27, 32, 105). Thus, this Y_1R - α_1R "cross-talk" mechanism appears to exist in several tissue types, at least in males. In contrast, females appear to compensate for the lower NPY control through the up-regulation of α_1R modulation on hindlimb vascular conductance (47).

The functional data indicating reduced Y_1R activation in females vs. males are supported by evidence (47) that males exhibited greater Y_1R expression and NPY concentration in whole muscle tissue homogenate compared with females. These data are congruent with, and provide the mechanistic basis for, earlier findings of 1) greater and sustained pressor responses and NPY increases during stress in male vs. female rats, and 2) greater pressor responses to exogenous NPY in areflexive pithed male vs. female rats (113).

The mechanistic basis of gender differences in NPY and Y_1R -mediated vasomotor control is not fully understood. The mechanisms could include differences in postganglionic sympathetic neural activity, as discussed briefly above. Unfortunately, the relationship between sympathetic nerve discharge patterns and NPY release is not known. In addition, sex differences in the modulation of prejunctional control of NPY release and/or its metabolism may exist. For example, in skeletal muscle, Y_2R blockade with BIIE0246 resulted in a decrease in hindlimb vascular conductance in both female and male Sprague-Dawley rats (48). However, the BIIE0246-induced decrease in vascular conductance was Y_1R dependent in females, but not males. In addition, compared with baseline, BIIE0246 infusion resulted in increased plasma NPY concentration in females, while there was no observable change in males. These studies indicate that, although female rats do not appear to use a Y_1R vasomotor control system during baseline conditions in skeletal muscle, the molecular and neurotransmitter machinery exists for such a control mechanism to occur. Parenthetically, the ability of Y_2R blockade to increase vascular tone in

male animals by a non- Y_1R mechanism suggests that prejunctional control of NE release is also modulated by Y_2R .

In addition to prejunctional auto-inhibitory control, postrelease metabolism of NPY by junctional peptidases is an important mechanism regulating the effect of NPY. Glenn et al. (35) concluded that NPY-converting enzymes (peptidases) may be more active in females compared with males. This effect may reduce NPY availability for Y_1R binding and enhance Y_2R activation. Such differences in NPY metabolism may also explain some of the confusion regarding sex differences in sensitivity to exogenous NPY (9, 35, 113). Our group demonstrated that systemic inhibition of proteolytic enzymes dipeptidylpeptidase IV (via 500 nM diprotin A) and aminopeptidase P (via 180 nM 2-mercaptoethanol) elicited a Y_1R -dependent decrease in hindlimb vascular conductance in females (47). Therefore, NPY bioavailability is an important regulated mechanism in rat skeletal muscle, particularly in female animals. The role of prejunctional and peptidase modification of NPY bioavailability in muscle of other species is not known. Moreover, it is not yet clear if ovarian hormones are an important factor in sex-dependent differences in Y_1R activation within skeletal muscle.

Involvement of NPY in the Regulation of Skin Blood Flow

General background. Skin blood flow (SkBF) in humans is controlled through two branches of the sympathetic nervous system: a vasoconstrictor system and an active vasodilator system of uncertain neurotransmitter (49). The vasodilator system is not tonically active but is engaged during periods of increased internal temperature (49). Previous studies suggest this system to be cholinergic and to involve a cotransmitter, possibly vasoactive intestinal peptide (5, 55). In contrast, the vasoconstrictor system is tonically active, mediating the subtle changes in SkBF required to maintain internal temperature in normothermia (12) and the cutaneous vasoconstriction during periods of cold exposure (8, 12).

The sympathetic nature of the vasoconstrictor nerves was demonstrated by their sensitivity to bretylium tosylate, which can completely abolish reflex cutaneous vasoconstriction through inhibiting transmitter release from nerve endings (54). This reflex system accounts for ~50% of the cutaneous vasoconstrictor response to local cooling of skin (44, 107). There is mounting evidence that NPY is an important neurotransmitter in skin vascular conductance. Morris (79) demonstrated a vasoconstrictor effect of exogenously administered NPY in subcutaneous arteries of the ear in guinea pigs. Nilsson et al. (82) demonstrated a vasoconstrictor effect of NPY in human subcutaneous arteries that had been dissected out of the abdominal regions from patients who underwent nonvascular disease surgeries (e.g., hernia). Using electrical neural stimulation approaches that mimicked high physiologic stress, they noted that NPY released from sympathetic nerves played a significant role in the regulation of the rat cutaneous microcirculation. Heath and colleagues (40) demonstrated that exogenous NPY, administered at physiological levels, consistently invoked a dose-dependent decrease in rat tail SkBF. In mice, Chu et al. (14) concluded that NPY decreased cutaneous blood flow via Y_1R , with evidence for the additional involvement of postjunctional Y_2R . This ability of NPY and Y_1R to affect skin vascular conductance varies in accordance with relative inner-

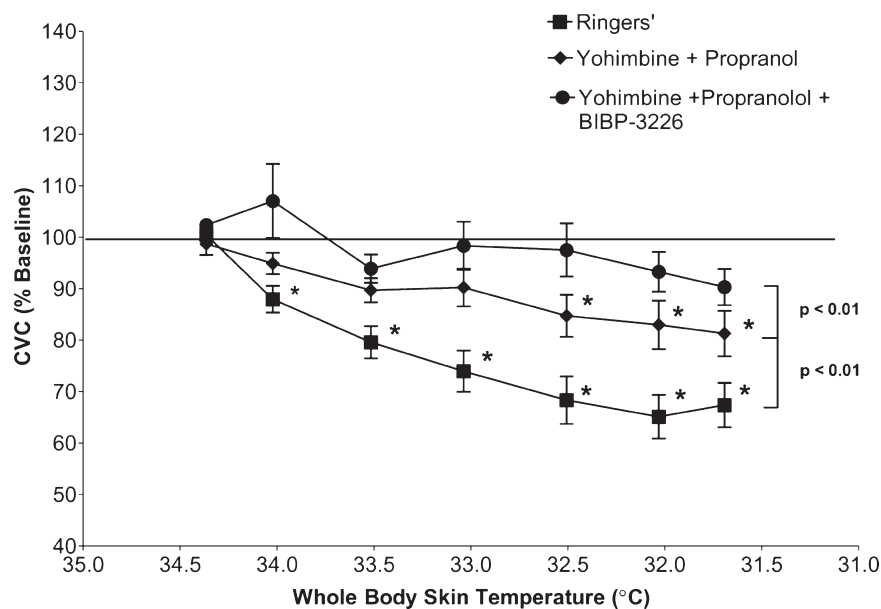


Fig. 2. Average responses in cutaneous vascular conductance (CVC) as a percentage of baseline (means \pm SE) from skin sites treated with Ringer solution; yohimbine plus propranolol; or yohimbine, propranolol, and BIBP-3226 during whole body cooling in eight healthy men. *Significant reduction from baseline ($P < 0.05$). At sites treated with yohimbine, propranolol, and BIBP-3226, CVC was not significantly reduced at any point in this cooling protocol ($P > 0.05$). These data indicate the vasomotor response to whole body cooling is mediated largely, if not entirely, by norepinephrine and NPY. P values indicate significant differences in the response between Ringer solution and yohimbine plus propranolol ($P < 0.001$) and between yohimbine plus propranolol plus BIBP-3226 and the other two sites ($P < 0.01$). [From Stephens et al. (96).]

variations at specific sites (80). Thus, conclusions about the role of NPY in skin microvascular control may depend on where you look and, more specifically, how deep you look.

In addition to the effects of NPY infusions, fundamental evidence that Y_1R activation affects vasomotor behavior is derived from blockade of the Y_1R . Following early studies with the BIBP3226 blockade approach in pigs (64), this ap-

proach was used. The Y_1R antagonist was then introduced in human studies (89), which led to explorations of the Y_1R cutaneous control in humans. With respect to human skin, Stephens and coworkers (94, 95) identified nonnoradrenergic reflex control in men and women by showing a persistent vasoconstriction to whole body cooling after pharmacological blockade of the effects of NE. Inasmuch as reflex cutaneous vasocon-

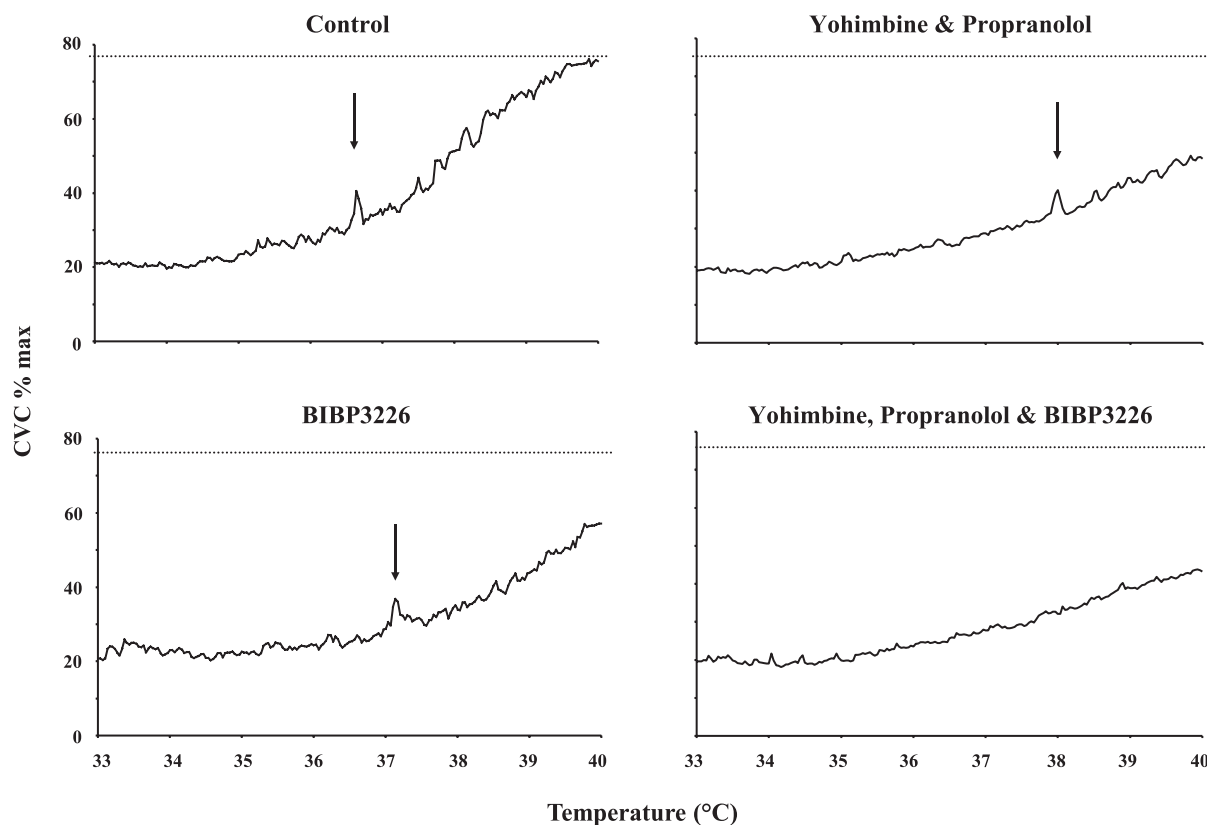


Fig. 3. Data from a representative subject showing axon reflexes were either offset to a higher local temperature, as in this case, or were abolished at the YOH/PRO and BIBP-3226 sites. They were uniformly abolished at sites treated with the combination of YOH, PRO, and BIBP-3226. Importantly, note the reduced responses in CVC at all treated sites. Arrows indicate the presence of an axon reflex. [From Hodges et al. (43).]

striction is blocked by the sympathetic presynaptic antagonist bretylium tosylate, but only partially inhibited by noradrenergic receptor antagonists, these nonnoradrenergic mechanisms are likely mediated by sympathetic cotransmitters. In a follow-up study, Stephens et al. (96) and colleagues used Y_1R antagonism with BIBP3226 and demonstrated the nonnoradrenergic constriction was due to Y_1R activation (Fig. 2). These data support the notion that, at least in skin under these particular conditions, NPY acts independently of NE to elicit vasoconstriction. In contrast to the above studies, using whole body cooling, Johnson et al. (50) observed that the involvement of NPY in the vasoconstrictor response to local cooling was minimal. Thus, it appears that NPY-induced cutaneous vasoconstriction requires a stress-induced increase in sympathetic nerve activity that would occur with systemic cooling but not local cooling.

NPY and vasomotor control in the cutaneous circulation. In human tissues, RT-PCR and immunocytochemistry studies have been used to determine the distribution of the Y_1R and Y_2R . Y_2R mRNA was detected weakly in subcutaneous arteries in the peripheral circulation (102), particularly when compared with Y_1R expression levels, suggesting that Y_1R are the primary receptors in human cutaneous circulation, with Y_2R playing little, if any, role in the regulation of vascular tone. This finding offers further support to the findings that local nonnoradrenergic mechanisms are entirely Y_1R based. Furthermore, it was also noted that this nonnoradrenergic mechanism of vasoconstriction was affected by reproductive hormone status, either being modulated directly by female reproductive hormones or indirectly by pathways sensitive to female reproductive hormones. When both estrogen and the progesterone are elevated (e.g., luteal phase), a significant, persistent vasoconstrictor response in cutaneous vascular conductance was observed at sites after α - and β -receptor antagonism (95). These findings are similar to the earlier observation in men (94), but in women, the nonadrenergic portion appears to play a measurable role only when estrogen and progesterone are high. During the time when both estrogen and progesterone are low (e.g., follicular phase), pharmacological blockade of α - and β -receptors essentially abolished reflex vasoconstriction. The interactive nature among ovarian hormones, adrenergic receptor sensitivity, and Y_1R -mediated cutaneous vasomotor control remains to be determined. Furthermore, there may be differences in those actions between exogenous (oral contraceptives) and endogenous hormones, as Thompson and Kenney (100) did not see the loss of apparent cotransmitter function in the follicular phase of normally menstruating women. Also, the role of nonnoradrenergic vasoconstriction is age dependent (100). Thompson and Kenney (100) noted that blockade of adrenergic receptors removed about 60% of cooling-induced vasoconstriction in younger subjects, similar to the results from Stephens and colleagues (94–96), but such blockade completely inhibited reflex cutaneous vasoconstriction in older subjects (100). Those findings indicate that the role of NPY (or other cotransmitters) in the reflex control of skin blood flow becomes less important with increasing age.

The role of NPY in skin vascular control is not limited to vasoconstriction during cooling. Intriguingly, recent work has shown that NPY is necessary for a complete vasodilator response in human skin to direct skin warming. Hodges and coworkers (43) found that inhibition of Y_1R with BIBP3226 or antagonism of α -receptors and β -receptors, with yohimbine

and propranolol, respectively, caused a delay in the onset of vasodilation and significantly reduced the cutaneous vasodilator response (Fig. 3). It is also noteworthy that the combination of Y_1R , α -receptor, and β -receptor antagonism did not cause a further depression of the vasodilator response (Fig. 3). Although it is somewhat counter-intuitive that adrenergic nerve transmitters should promote the vasodilator response, there are data that show NE and NPY bind to α_2R and Y_1R on endothelial cells and stimulate endothelial nitric oxide synthase, leading to the production of nitric oxide (2, 15, 103). In keeping with this possibility, we also tested whether the effects of NE and NPY on cutaneous vasodilation were dependent on nitric oxide synthase function (43). We observed that the heat-induced vasodilation was abolished with both presynaptic sympathetic blockade with bretylium tosylate and/or with local applications of L-nitro-arginine methyl ester (L-NAME) without evidence of any synergistic effect (43). Therefore, the neurogenic support of vasodilation appears to require the serial production of nitric oxide. The role of this vasodilatory pathway in constraining cutaneous vasoconstriction during whole body cooling has not been investigated.

Perspectives and Significance

NPY is now understood to be an important neurotransmitter in the control and regulation of skeletal muscle vasculature tone. In addition, cutaneous vasoconstrictor and vasodilator responses require NPY for a complete response with antagonism of Y_1R removing 20 to 30% of the reflex cutaneous vasoconstrictor response and 40 to 60% of the vasodilator response to local heating. However, many questions remain regarding this system. For example, is sympathetic nerve discharge related to NPY release and if so, does this control feature vary between “rest” and stress? Furthermore, the mechanism(s) by which ovarian hormones affect the NPY- Y_1R system remain unknown. Sex-based dimorphism is present in the NPY-supported vasomotor system for both muscle and skin, whereby the Y_1R control over vascular control appears to be minimized by ovarian hormones. The mechanistic basis of this sex-hormone effect is not known and may include alterations in postganglionic nerve activity, prejunctional auto-inhibition, junctional NPY metabolism by peptidases, and even alterations in receptor sensitivities.

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