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Neuropeptide Y and the cerebral circulation

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Introduction

Neuropeptide Y (NPY) is one of the most abundant neuropeptides in both the peripheral and the central nervous systems. In addition, NPY is found in blood and cerebrospinal fluid (approximately 100 pmoles/L). Immunohistochemical studies show NPY to be widely distributed in sympathetic nerves supplying the cardiovascular system including the cerebral arteries. In general [1, 2] perivascular NPY-containing fibers are more abundant around arteries than veins. Larger cortex pial arteries tend to be more densely innervated by NPY-positive sympathetic fibers than smaller arteries; and rostral arteries receive more NPY fibers than caudal vessels. NPY-positive nerve fibers also are closely associated with penetrating arterioles and intraparenchymal microvessels of the brain.

In sympathetic nerve terminals, NPY is packaged with norepinephrine in large, dense core synaptic vesicles, whereas adenosine 5' triphosphate (ATP) is co-stored with norepinephrine in both small and large dense core vesicles. Consistent with this general scheme, NPY immunogold labeling is associated with large granular vesicles in the varicosities of nerves innervating human brain vessels [3].

NPY receptor subtypes

NPY acts on a family of G-protein coupled receptors (Y_1–Y_6) [4]. So far, five distinct NPY receptors have been cloned, and a sixth receptor (Y_3) has been characterized pharmacologically. All of the subtypes appear to act through similar signaling pathways mediated by pertussis toxin-sensitive G proteins (G_i and G_o). The Y_1, Y_2, and Y_5 subtypes preferentially bind NPY and the related endogenous peptide YY (PYY). Y_2 receptors, which were first described as presynaptic receptors, are uniquely activated by C-terminal fragments of NPY, e.g., $NPY_{3–36}$, $NPY_{13–36}$ and $PYY_{13–36}$, which do not affect Y_1 receptors. Specific agonists that are more potent at Y_1 than Y_2 receptors have been synthesized, e.g., [Leu^{31}, Pro^{34}]-NPY and [Pro^{34}]-PYY, which contain a proline

residue instead of glutamine at position 34 of the molecule [4]. The Y_3 and Y_4 receptor subtypes preferentially bind NPY and another related peptide, pancreatic polypeptide (PP), while the pharmacological profile of the Y_6 subtype is still controversial. The cerebrovascular actions of NPY appear to involve primarily Y_1 and Y_2 receptors; there is no evidence at this time that Y_3, Y_4, Y_5 and Y_6 subtypes are present in cerebral blood vessels.

The Y_1 receptor, which is the predominant vascular receptor mediating vasoconstriction [5] is the best studied and understood of the NPY receptor subtypes. The presence of Y_1 receptor protein and mRNA has been demonstrated in human and rat cerebral arteries [6, 7]. Using reverse transcriptase-polymerase chain reaction (RT-PCR), mRNA for the Y_1 receptor is found in isolated human pial vessels and intracortical microvessels as well as cerebrovascular smooth muscle cells in culture. *In situ* hybridization localized Y_1 mRNA to the smooth muscle layer of pial vessels. Neither RT-PCR nor *in situ* hybridization techniques were able to detect mRNA for Y_2, Y_4 or Y_5 receptors, suggesting that these receptor subtypes are absent or expressed in very low concentrations in cerebral vessels. As discussed below, functional studies, however, indicate a role for both the Y_1 and Y_2 subtypes.

Using immunohistochemistry, Y_1 receptor protein can be visualized in smooth muscle cells of rat pial arteries and arterioles [8]. The intensity of immunostaining is highest in arterioles, especially those with a diameter of 15–30 μm, with fewer receptors observed in the arteries on the basal surface of the brain. Y_1 receptor immunostaining is found on all sides of the vascular smooth muscle cells with the highest concentration on the abluminal surface. It is somewhat surprising that, in the rat, NPY-positive fibers are mainly found around the larger arteries exhibiting low levels of Y_1 receptors, while few NPY-positive fibers are observed around Y_1 receptor-rich arterioles [8]. In contrast, other species such as cat and man exhibit dense NPY innervation around pial vessels of all sizes.

NPY-induced constriction

Exogeneous NPY produces a long-lasting response [1, 2, 7, 9], and it potently constricts cerebral arteries in all species that have been examined, including human. For example, in isolated rat cerebral arterioles, NPY causes a profound constriction, to 81% of control diameter; and the concentration of NPY eliciting a half-maximum response is 6×10^{-10} M [10]. In isolated cat middle cerebral artery and pial arteries, constriction to NPY is about half as strong as that produced by K^+ [11]. *In situ*, cat pial arteries constrict markedly following perivascular application of NPY at concentrations of 7 nM or more [12]. The maximum NPY-induced constriction of cat pial arteries *in situ* is about 35%, which is equivalent to or exceeds that of other cerebrovascular constrictor agents such as norepinephrine or angiotensin II. In contrast, the isolated basilar artery of the rabbit responds to NPY with weak contraction. In

general, the ability of NPY to induce arterial vasoconstriction increases as vascular diameter decreases with the greatest effects on small resistance vessels. Pial veins of the cat also respond to perivascular NPY, but much larger concentrations of the peptide are required to produce a significant reduction in diameter [12].

Vasoconstrictor mechanisms

In cerebral arteries, constrictor responses to NPY appear to be mediated by changes in smooth muscle membrane potential [11] and influx of extracellular calcium. In guinea pig basilar artery, NPY evokes a slow, long lasting depolarization (up to 8 mV) of the smooth muscle that is directly correlated with constriction [13]. NPY is known to inhibit ATP-sensitive K^+ channels, causing depolarization of arterial smooth muscle, but this has yet to be demonstrated in cerebral arteries. Membrane depolarization produced by NPY likely activates voltage-operated calcium channels (VOCC). NPY-induced vasoconstriction of cerebral arteries is dependent on the influx of calcium since it is blocked by either removal of extracellular calcium or addition of VOCC blockers, e.g., nifedipine, nimodipine, verapamil and diltiazem [1, 11, 14].

NPY inhibits cyclic adenosine monophosphate (AMP) formation in cerebral arteries, as it does in other tissues [4]. This effect is expected for G_i/G_o-coupled receptors; and it likely contributes to the vasoconstrictor effects of NPY since cyclic AMP is considered a vasodilator. In cat and guinea pig cerebral vessels, NPY decreases basal cyclic AMP content as well as forskolin-induced cyclic AMP production [15].

Inhibition of vasodilatation is another way that NPY increases vascular tone in various vascular beds. Interestingly, NPY inhibits vasodilator responses to acetylcholine, adenosine, norepinephrine (in the presence of phentolamine), substance P, and VIP without affecting calcitonin gene-related peptide (CGRP)-mediated dilation. In pre-contracted basilar arteries from guinea pig, acetylcholine or substance P induces relaxation, and this response is significantly inhibited by NPY. The inhibitory effect of NPY is reversed by the inhibitor α-trinositol. NPY-induced inhibition of adenylate cyclase, decrease in Na^+-K^+ pump activity, or membrane depolarization have been postulated as mechanisms underlying inhibition of relaxation by NPY [16, 17].

NPY-induced dilatation

Although NPY is best known for its ability to increase vascular tone, this peptide can also cause direct vasodilatation *in vivo* and *in vitro* [17]. Injection of NPY into the carotid artery of the cat produces a transient, but significant, increase in cerebral blood volume and flow. Administration of N^G-monomethyl-L-arginine (L-NMMA), a nitric oxide synthase (NOS) inhibitor,

prevents the volume increase, thus implying that NPY elicits transient vasodilatation via production of NO [18]. In isolated guinea pig cerebral arteries, NPY also causes a transient vasodilatation that is correlated with increased cyclic GMP levels [19]. When applied selectively to the lumen of rat middle cerebral artery segments, NPY, [Leu^{31}, Pro^{34}]-NPY, and NPY$_{13–36}$ all produce a concentration-dependent vasodilatation [17]. Pretreatment of the artery with a NOS inhibitor or removal of the endothelium prevents the dilatory response to NPY. In fact when the endothelium is removed, intraluminal NPY agonists produce constriction instead, no doubt by direct action on the smooth muscle [17]. Together, these studies indicate that NPY acts on the endothelium to release NO that in turn stimulates smooth muscle guanylate cyclase leading to relaxation.

There is some evidence for the presence of NPY receptors on endothelial cells. In cerebral arteries, the functional NPY antagonist α-trinositol attenuates NPY-stimulated increases in cyclic GMP formation without affecting basal cyclic GMP levels [19]. BIBP 3226, however, does not affect vasodilatation in response to intraluminal NPY or [Leu^{31}, Pro^{34}]-NPY [17] indicating that Y_1 receptors are not involved. It is hypothesized that endothelial Y_2 receptors are responsible for NPY-induced dilatation; however, other NPY receptor subtypes cannot be ruled out until more discriminating agents, e.g., a selective Y_2 receptor antagonist, are tested. Within the brain, NPY is often co-localized in NOS-containing nerves that appose intracerebral blood vessels [20]. While the functional consequences have never been demonstrated, it is possible that NPY and NO act synergistically to produce local vasodilatation.

Neuropeptide Y and the cerebral circulation

The influence of *in vivo* NPY administration on the cerebral circulation was first examined in the rat. Allen and colleagues [21] reported that a bolus injection of NPY into the carotid artery produced profound reductions in cortical blood flow. Decreases in flow were large in magnitude (up to 98%) and long in duration (at least 2 h). Although an identical protocol was used in another study, such a dramatic or prolonged reduction in cerebral blood flow was not seen [22]. The administration of 1 nM and 5 nM of NPY as a bolus into the internal carotid artery of the rat resulted in a dose-dependent decrease in ipsilateral striatal local blood flow as measured with the hydrogen clearance method. The decrease developed slowly and persisted for at least 2 h without affecting blood pressure. A bolus injection of NPY into the vertebral artery of anesthetized dogs resulted in a concentration-related reduction in vertebral blood flow as measured by electromagnetic flowmetry [23]. The decrease in vertebral blood flow produced by NPY reached its maximum at 3 min and remained depressed for up to 30 min.

Effect of neuropeptide Y on regional blood flow and metabolism

The importance of NPY in the regulation of regional cerebral blood flow (CBF) has been investigated in the rat striatum using quantitative autoradiographic techniques [24, 25]. Since NPY may influence local CBF either by a direct vasomotor action or by directly altering cerebral metabolism with secondary changes in blood flow, the effect of NPY on both striatal blood flow and striatal glucose use was examined [25]. Intrastriatal administration of NPY produced significant reductions of CBF within a limited number of regions of the CNS. The majority (30 of 40) of the regions investigated, however, did not exhibit changes in CBF or in glucose use.

In the caudate nucleus into which NPY had been administered, tissue perfusion was markedly reduced [24, 25]. Reduction in striatal blood flow extended from the most rostral to the most caudal portion of the caudate nucleus. In contrast, the overall rate of glucose utilization in the striatum was only minimally altered by the administration of NPY. Thus, the increase in striatal cerebrovascular resistance occurred independently of local changes in metabolism, indicating that NPY directly alters striatal blood flow. This is a relatively unusual observation, since changes in CBF are generally correlated with alterations in cerebral oxidative metabolism. Arteries that supply blood to the striatum (the middle cerebral and lenticulostriate arteries) also are innervated by NPY-like immunoreactive fibers and respond to neuropeptide Y with a dose-dependent contraction [24]. Together these observations further support a role for NPY in regional cerebrovascular regulation.

It is surprising that NPY caused profound reductions in CBF with minimal changes in glucose utilization in several brain regions far removed from the striatal injection site, e.g., the entorhinal cortex, amygdala, and perirhinal cortex [23–25]. The cause of the marked alterations in blood flow in these extrastriatal regions is uncertain. Since their distance from the injection site (greater than 2 mm) makes simple diffusion of NPY into these regions unlikely, it is improbable that blood flow changes in these regions resulted from a direct vasoconstrictor effect of NPY. It may be that constriction originating in the striatal arteries and arterioles is propagated to regions remote from the injection site. Another intriguing possibility is that the injection of peptide produced alterations in neuronal activity within intracerebral pathways involved in cerebral circulatory control. Thus, the dissociation between local CBF and local glucose may be evidence of a functional role played by cerebrovascular fibers originating within the CNS itself.

Modulation of autoregulation

Direct proof for involvement of NPY in cerebrovascular physiology comes from two sets of results. In the cat, Goadsby and Edvinsson [26] examined cortical microcirculation in conjunction with activation of the sympathetic nerves.

The NPY blocker α-trinositol was found to shift the autoregulation curve to the left, thus showing that part of the protective effect of the sympathetic system was mediated by NPY.

The second study was carried out by Vraamark et al. [27] who studied whole cerebral blood flow in rat using the Kety-Schmidt method. A marked influence on the upper limit of the autoregulation was also observed with the NPY blocker α-trinositol. Evidence now exists that both NPY and norepinephrine participate in protecting the brain against breakthrough of the upper limit of autoregulation in conditions of high blood pressure.

Neuropeptide Y in stroke

The possibility that NPY contributes to the development of cerebral vasospasm has been examined in experimental subarachnoid hemorrhage (SAH) [28, 29]. In the first study Abel et al. [28] observed that NPY in cerebrospinal fluid was markedly increased after injection of autologous blood. In these studies NPY was observed to be a strong vasoconstrictor. Depending on experimental conditions the responses to NPY were modified following SAH. NPY-like immunoreactivity (NPY-LI) also has been measured in CSF from patients with aneurysmal SAH [30, 31]. Both studies found that NPY was not significantly higher in SAH than in controls. However, Juul et al. [30] observed that in some patients there was a correlation between the degree of severity of spasm as studied with transcranial Doppler ultrasound and the content of NPY-LI in the external jugular vein. The possibility that NPY plays a role in a particular subset of SAH patients needs to be explored further.

Further support for a deleterious role of NPY in stroke has appeared [32]. There was increased immunoreactivity for neuropeptide Y (NPY) within the perilesional cortex following experimental middle cerebral artery occlusion (MCAO) or focal excitotoxic damage. NPY administration increased the relative infarct volume and reduced rCBF as observed during reperfusion. These results indicate that peripheral or central administration of NPY impairs reperfusion following experimental MCAO and worsens the outcome of focal cerebral ischemia [32].

Concluding remarks

The presence of NPY receptors on both endothelial and vascular smooth muscle cells indicates that blood-borne NPY as well as NPY stemming from surrounding nerves can affect contractile responses of cerebral vessels. In addition, endothelial cells may themselves be a source of NPY as well as a site of NPY metabolism. Understanding the roles of NPY in cerebrovascular regulation is complicated by multiple receptor subtypes, signalling pathways and cellular responses. Vasodilatory effects and vascular remodeling actions under-

score the fact that NPY cannot just be classified as a potent vasoconstrictor. NPY is an important sympathetic transmitter, but it clearly acts via intracerebral nerves as well to regulate local CBF.

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