Available online at www.sciencedirect.com

SCIENCE DIRECT®

European Journal of Pharmacology 500 (2004) 27–35

www.elsevier.com/locate/ejphar

Review

Sympathetic co-transmission: the coordinated action of ATP and noradrenaline and their modulation by neuropeptide Y in human vascular neuroeffector junctions

J. Pablo Huidobro-Toro*, M. Verónica Donoso

Centro de Regulación Celular y Patología J.V. Luco, Instituto Milenio para Biología Fundamental y Aplicada, MIFAB, Departamento de Fisiología, Unidad de Regulación Neurohumoral, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Casilla 114-D, Santiago, Chile

Accepted 1 July 2004
Available online 14 August 2004

Abstract

The historical role of noradrenaline as the predominant sympathetic neurotransmitter in vascular neuroeffector junctions has matured to include ATP and the modulator action of neuropeptide Y (NPY). Numerous studies with isolated blood vessels rings demonstrate the presence of key enzymes responsible for the synthesis of ATP, noradrenaline and NPY, their co-storage, and their electrically evoked release from sympathetic perivascular nerve terminals. Functional assays coincide to demonstrate the integral role of these neurochemicals in sympathetic reflexes. In addition, the detection of the diverse receptor populations for ATP, noradrenaline and NPY in blood vessels, either in the smooth muscle, endothelial cells or nerve endings, further contribute to the notion that sympathetic vascular reflexes encompass the orchestrated action of the noradrenaline and ATP, and their modulation by NPY. The future clinical opportunities of sympathetic co-transmission in the control of human cardiovascular diseases will be highlighted.

Keywords: Sympathetic neurotransmission; Co-transmission; ATP and purinergic mechanism; Noradrenaline and vasoconstriction; Neuropeptide Y modulation; Human vascular neuroeffector junction

Contents

* Corresponding author. Tel.: +56 2 686 2867; fax: +56 2 222 5515.
E-mail address: jphuid@bio.puc.cl (J. Pablo Huidobro-Toro).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.07.008

1. Introduction and historical perspective

The prevailing view that dominated the physiology, and oriented the pharmacology of sympathetic neurotransmission up to the mid-1970s, was based on the notion of noradrenaline as the sole sympathetic transmitter operating autonomic reflexes. Based on this understanding, the principles of adrenergic pharmacology were determinant in the development of cardiovascular disease therapies, including the management of some cardiac arrhythmias and immediately thereafter in the treatment of hypertension and congestive heart failure. Although there was some delay between theory and practice, the launching of drugs with adrenoceptor antagonist properties had a profound impact in internal medicine, particularly in the treatment of hypertension. These clinical opportunities boosted the field of autonomic pharmacology, which carried on the impetus raised in the late fifties by the clinical research with patients using drugs such as reserpine or guanethidine. During the decades of the 1960s and 1970s, the neurochemistry and pharmacology of adrenergic mechanisms experienced great growth. The relevant and influential monograms published by the Annual Reviews of Pharmacology and the Pharmacological Reviews witnessed the outburst of the field, together with the many new drugs and novel mechanisms discovered which were introduced to the clinic.

Newly developed chemical assays to detect catecholamines in living tissues and the emerging principles of histochemistry, used to visualize the enzymes responsible for the synthesis of noradrenaline at autonomic nerve terminals, further propelled the field. During the decade of the 1960s, the pharmaceutical industry busily assessed and tested novel structures to compare with propranolol, the golden standard of the β-adrenoceptor antagonists. The development of the relatively selective β_1-adrenoceptors antagonists, which as a by-product clarified the potential of selective β_2-adrenoceptor agonists, emerged shortly thereafter. The successful clinical applications of these drugs firmly established their role in general medicine, which together with the vigorous impetus of cellular physiology achieved within few years the purification and cloning of the human β-adrenoceptor (Fraser et al., 1987; Frielle et al., 1987) and immediately thereafter the α-adrenoceptor and the muscarinic receptors. Thus, within 20 years following the first clinical trials of the β-adrenoceptor antagonists, the road was paved for the blooming molecular pharmacology and the studies of the molecular physiology and pharmacology of the receptor itself.

The decade of the 1980s revealed the splendour of peptides in peripheral and central synapses and allowed the elucidation of the role of neuropeptide Y (NPY) as a sympathetic modulator at peripheral autonomic synapses. Basic and clinical Swedish researchers, both at Stockholm and Lund, extensively studied the pharmacology of NPY in the cardiovascular system and shortly after, postulated its modulator role in sympathetic reflexes. The decade of the 1990s raised firm grounds for the most original proposal by G. Burnstock in the early 1970s on the role of adenosine 5′-triphosphate (ATP), known to be synthesized and released from sympathetic nerve terminals, although its physiological relevance was highlighted only upon the cloning of the P2Y and P2X family of nucleotide receptors. These receptors are activated by purine and even pyrimidine di- or trinucleotides and limited synthetic derivatives. Multiple P2Y and P2X receptors are expressed in blood vessels either at their smooth muscles, endothelial cells or the sympathetic nerve endings. Facing the turn of the century, the field has received new momentum; the notion that sympathetic neurotransmission is governed by at least three neurochemicals: ATP, noradrenaline and neuropeptide Y (NPY) is firmly rooted, and beginning to be discussed in texts of physiology and autonomic pharmacology. The physiological and pathophysiological implications of the sympathetic triad in human vascular sympathetic reflexes await further clinical research. Likewise, research on the role of NPY as a modulator of peripheral autonomic vasoconstriction is paving its road for future clinical opportunities.

This review will discuss the main evidences supporting the notion of sympathetic co-transmission and the modulator role of NPY in human vascular neuroeffector junctions. In addition, it will discuss the published literature regarding the co-transmitter role of ATP and noradrenaline in human vascular sympathetic reflexes, which constitute a complex and integral signalling strategy of cell communication.

2. The co-storage of NPY, ATP and noradrenaline at presynaptic nerve terminals

In the early 1980s, Tatemoto's work at The Dept of Biochemistry at the Karolinska Institute in Stockholm focused on the discovery of novel peptides. He described a family of carboxyamidated peptides, one of which was present in the brain and named neuropeptide tyrosine (NPY) because of its tyrosine residues at the amino and carboxy ends; the carboxyl-terminus tyrosine is amidated and, in addition, there are four internal tyrosines in its 36 residue sequence (Tatemoto, 1982). Immunocytochemistry soon demonstrated the abundant immunoreactivity for NPY in the brain, which was often co-localized with catecholamines. Immediately thereafter, it was discovered that NPY was synthesized and co-stored together with catecholamines in central and peripheral neurons, particularly in the nerve endings surrounding blood vessels.

The notion that NPY is integral to vascular sympathetic responses became immediately accepted after the demon-

stration that the e.v. administration of exogenous NPY caused a prolonged rise in systemic blood pressure. Immunocytochemical staining of NPY in blood vessels, likely attributed to the abundance of NPY in perivascular sympathetic neurons of almost every species examined including humans, further supported this proposal (Lundberg et al., 1983; Ekblad et al., 1984). Presynaptic NPY, staining in perivascular sympathetic nerve endings, was further demonstrated by its co-localization with catecholamines and the catecholamines synthesizing enzymes, including tyrosine hydroxylase and dopamine β-hydroxylase (Lundberg et al., 1983; Ekblad et al., 1984). NPY tissue content plus the catecholamine titration in vascular biopsies soon was available and established that the amount of catecholamine in human vascular biopsies, as well as animals biopsies, showed a 40- to 100-fold predominance of noradrenaline over NPY in blood vessel biopsies from different vascular beds, including humans (Donoso et al., submitted for publication). This finding further supports a modulator role for NPY.

Regarding the presynaptic vesicle co-storage of the sympathetic triad, there seems to be consensus that while NPY is primarily co-stored in the large, dense-core vesicle, together with ATP and noradrenaline, the smaller vesicle contains essentially only ATP plus noradrenaline (Fried et al., 1985; Lundberg et al., 1989).

3. The co-release of the sympathetic triad

Much in the same way as NPY storage differs between the large and the small vesicle population, the release process seems also to be regulated differentially at vascular sympathetic neuroeffector junctions. Although this issue has not been extensively assessed, even less in human vascular biopsies. Donoso et al. (2002, 2004; and submitted manuscript) have provided good evidence in support of the notion that the α_2-adrenoceptor exclusively regulates the release of noradrenaline but not NPY.

The release of noradrenaline from human vascular sympathetic nerve endings appears to be highly regulated, and the mechanisms involved constitute no exception to those observed in central and peripheral autonomic neurons. Early studies assessed the electrically evoked release of tritium-labelled noradrenaline, while later studies quantified the release of endogenous noradrenaline by electrovoltametric techniques. However, few studies have been performed using human vascular biopsies. In human saphenous veins. Donoso et al. (2004) failed to detect a significant outflow of noradrenaline after electrical depolarization of perivascular nerve endings, even though measurable NPY overflow was detected. Notwithstanding, upon blockade of noradrenaline reuptake with phenoxybenzamine, a significant noradrenaline output was measured and identified chromatographically. We concluded that the high affinity and capacity of the nerve terminal and postjunctional catecholamine transporters obscures the quantification of the release process and obstructs further analysis of the release mechanism.

Due to the current experimental limitations, little has been accomplished with the release process of noradrenaline from human vascular biopsies; even less has been currently investigated with ATP, an issue that demands prompt assessment. In view of the recent claim that sympathetic nerve terminal depolarization co-releases ATP plus ectonucleotidases, enzymes that hydrolyse ATP into nucleosides and eventually to adenosine (Todorov et al., 1997), the further characterization of ATP release from human vascular neuroeffector junctions is challenging.

Regarding NPY release per se, there is not conclusive evidence that describes how the process is regulated. However, several reports indicate very significant increases in plasma levels of ir-NPY in human neonates, and in adults following demanding exercise (Lundberg et al., 1985), accounting for the corresponding rise in peripheral resistance attributed to the peptide. NPY and related peptide fragments have been detected in the plasma of hemodialyzed patients, indicating that it is released from sympathetic nerve terminals or the adrenals and reaches the blood stream. It is possible that along its routing to the plasma, it encounters degradation by several peptidases localized either at the synapse or surroundings or directly at the plasma, a relevant issue since some peptide fragments are biologically active and may even antagonize the vasomotor effect elicited by the native peptide (Hegbrant et al., 1995).

However, in spite that several clinical studies have measured plasma ir-NPY levels, which altogether are a manifestation of the release process, this data is rather limited to derive mechanistic conclusions on the dynamics of the release process. Elevated plasma ir-NPY levels were found in humans after myocardial infaction (Omland et al., 1994) or in patients suffering from brain or adrenal tumors (O'Hare and Schwartz, 1989). In addition, Erlinge et al. (1992) demonstrated a significant rise in plasma ir-NPY in patients with severe hypertension, but the administration of the selective NPY Y_1 receptor antagonist failed to lower systemic blood pressure, which has complicated the determining the role of NPY in primary hypertension (Zhao et al., 1997). In the case of human pheochromocytoma, peptide heterogeneity was observed in plasma samples (Tabarin et al., 1993), hinting at peptide fragmentation. A word of caution must be exercised in the proper interpretation of the plasma ir-NPY determinations since these release studies do not clarify whether NPY is secreted from the adrenals or from sympathetic nerve endings, nor is the chromatographic identity of the immunoreactive material established. Therefore, it is not possible to firmly identify, based on immunoreactivity measurements alone, whether NPY is fragmented, and therefore, which putative metabolite predominates or is more active; neither has it been determined whether disease modifies peptide metabolism.

This is not a trivial issue, since some NPY fragments have differential affinity for the two receptors present in the vascular tree (Wahlestedt et al., 1990), and therefore may modify the activity of NPY, obscuring the elucidation of its participation in cardiovascular disease. Even after the caveats aforementioned, the relevance of NPY to the pathophysiology of cardiovascular disease is slowly emerging. Clinical research in patients suffering from cardiovascular pathologies suggests a role for NPY in the clinical manifestations of an altered sympathetic tone.

A research model that has been useful to examine more directly the release of neurotransmitters from human vascular sympathetic nerve endings is blood vessel biopsies obtained from humans programmed for elective coronary surgery. Cardiac revascularization surgery offers a unique opportunity to examine relatively intact segments of human saphenous veins and mammary vessels as well as radial arteries for research purposes. This tissue was instrumental to identify the role of ATP as a human sympathetic transmitter (Rump and von Kugelgen, 1994) and its modulation by NPY (Racchi et al. (1999), as well as the role of calcium channels in autonomic neurotransmission (Fabi et al., 1993). Furthermore, in a recent study, Donoso et al. (2004) used this human tissue to establish the release and chromatographic identity of NPY following transmural electrical stimulation of human sympathetic perivascular nerve endings, and identified the release of an oxidized peptide metabolite together with the native NPY. In an extension of this work, Donoso et al. (2004) described the release of NPY and its oxidized counterpart from biopsies of human mammary arteries and veins as well as segments of human radial artery (Donoso et al., submitted for publication). Unfortunately, however, we have not been able to measure all three sympathetic chemicals simultaneously and to ascertain details of the amounts released and their sequential time course, a challenge that most likely will be met shortly by our research group (Donoso et al., unpublished observations). The use of specific receptor blockers acting selectively at the presynaptic nerve terminal will prove of great use to address this use in human vascular biopsies. The functionality of the co-release process in neuroeffector junctions is inferred by the vasomotor activity elicited by the exogenous application of ATP, noradrenaline or NPY, in an attempt to mimic the physiology of these chemicals at the neuroeffector junction (Racchi et al., 1999; Donoso et al., 2004), rather than by the measurements of their coordinated release process.

4. Postjunctional receptors and the concerted action of ATP, noradrenaline and NPY in human neuroeffector junctions

Receptor classification has been largely performed in the past by use of drugs with demonstrated specificity for the several receptor subtypes analysed. While this criterion allowed the successful classification of adrenoceptors into the α_1 and α_2-adrenoceptor subtypes, and the NPY receptors into the Y_1 and Y_2 subtypes, the lack of receptor-specific nucleotide agonists and antagonists has not permitted, at the time being, an entirely reliable classification of nucleotide receptors based solely on pharmacological testing. With the exception of α,β-methyleneATP, which is a relatively selective $P2X_1$ receptor agonist, and 2′,3′-*O*-(4-benzoyl-benzoyl) ATP, a selective $P2X_7$ receptor agonist, the rest of the nucleotide analogs do not have sufficient receptor specificity to guarantee unambiguous classification. Regarding antagonists, MRS 2179 and structurally related drugs are the only reliable $P2Y_1$ selective receptor tools that allow the identification of this nucleotide receptor as mediator of a vascular ATP response Buvinic et al., 2002). In the search of a more complete classification, recent studies have combined the use of classical pharmacological tools, such as preferential drug receptor agonists and antagonists plus receptor desensitization protocols, together with more modern protocols derived from cellular and molecular biology approaches, such as the identification of the mRNAs coding for the various nucleotide receptors by reverse transcription-polymerase chain reaction (RT-PCR) and in situ RNA hybridization (Nori et al., 1998; Valdecantos et al., 2003).

4.1. ATP

In most blood vessels, nucleotides have a dual action ensuing either a contractile or a dilator response. Perhaps the first unambiguous report on the release of ATP upon electrical stimulation of perivascular sympathetic nerves and its vasomotor effect came from studies on rabbit intestinal blood vessels (Ramme et al., 1987). Thereafter, numerous authors have reported similar observation in autonomically innervated vascular beds. The vasocontractile action of ATP and structural analogs is predominantly due to a direct action of the nucleotide on vascular smooth muscles, while the dilatation is mainly indirect in nature and due to endothelial cell signalling triggered by nucleotide receptor activation. The P2X receptors are ionic channels gated by extracellular ATP and structurally related analogs, their activation leads to rapid responses due to fast smooth muscle depolarization (Burnstock and Williams, 2000). In contrast, the P2Y receptors belong to the G-protein-coupled family of receptors and mediate responses that are slower than those gated by the P2X receptor; in most territories, these particular set of receptors are coupled to the synthesis of endothelial vasodilators from nitric oxide to arachidonate metabolites (Gao et al., 1999; Giaroni et al., 2002). The vasocontractile action of exogenous ATP in human mammary vessels is potentiated in a concentration-dependent manner by 1–10 nM NPY, much in the same way as that of exogenous noradrenaline (Fig. 1). The relatively low potency of ATP in these studies, compared to noradrenaline, likely indicates the extensive extracellular ATP metabolism

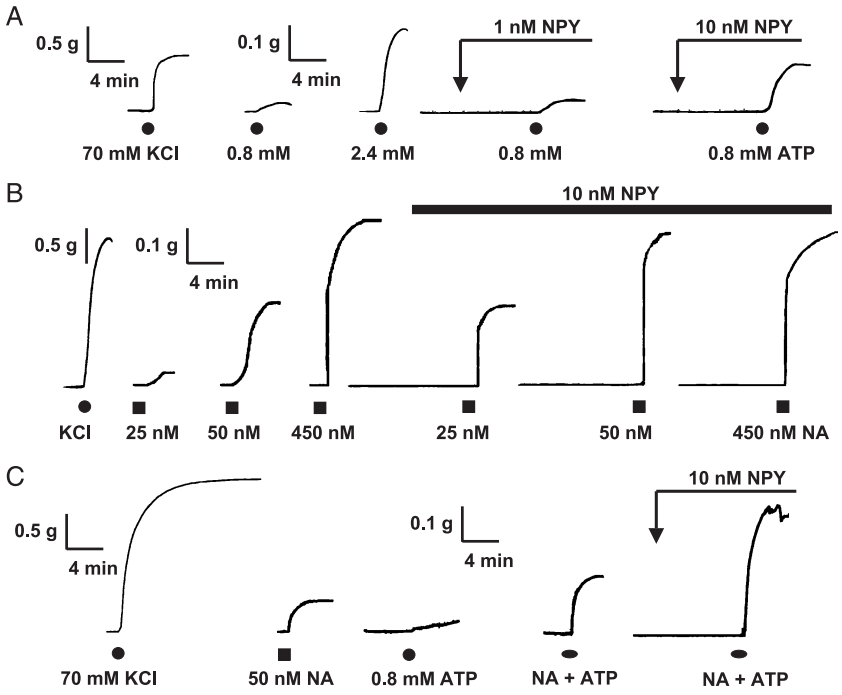

Fig. 1. NPY modulates the vasomotor action of ATP and noradrenaline; the joint application of exogenous ATP and noradrenaline causes a synergistic vasomotor response mimicking the coordinated action of ATP and noradrenaline as sympathetic co-transmitters. Representative protocols demonstrate the modulator role of NPY in isolated segments of human mammary veins from three separate patients: (A) 1.5–15 nM NPY potentiates concentration-dependently the ATP-evoked contractions; (B) 10 nM NPY potentiates the magnitude of the noradrenaline-evoked contractions, evidencing a leftward displacement of the noradrenaline concentration–response curve; (C) the joint application of ATP plus noradrenaline causes a synergic vasomotor response that is further potentiated by 10 nM NPY. Note that in these vessels, the sole application of NPY did not elicit a vasomotor response.

occurring at vascular neuroeffector junctions, an issue that at present has not been examined in sufficient detail, but in consent with its co-transmitters role.

We have focussed on the identification of the nucleotide receptors expressed in blood vessels; a starting point for this purpose has been the use of the human placenta as a source of meaningful biological samples. As in most animal tissues, the superficial chorionic arteries and veins of term placentas express the $P2X_1$, $P2X_4$, $P2X_5$, $P2X_6$ and $P2X_7$ receptors. In this study, we combined the use of rather receptor-selective agonists, desensitization protocols, and PCR identification of receptor mRNA (Valdecantos et al., 2003). We extended these observations in a second study, by perfusing human placental cotyledons with 2-(Methylthio)adenosine 5′-diphosphate (2-MeSADP) or ADP, two preferential $P2Y_1$ receptor agonists and observed that these agonists relax this territory by enhancing the production of endothelial nitric oxide (NO) and eliciting a consequent rise in tissue cGMP. Both of these effects are sensitive to 2′-Deoxy-N^6-methyladenosine 3′,5′-bisphosphate (MRS2179) blockade, supporting $P2Y_1$ receptor activation (S. Buvinic, doctoral dissertation, studies in progress). In contrast, the UTP-mediated vasodilatation, which is also indirectly mediated by NO production and increased cGMP synthesis, is not blocked by MRS2179, a likely indication that $P2Y_2$ receptors or the $P2Y_6$ receptors may mediate this UTP-gated response (Buvinic et al., 2004, in press).

4.2. Noradrenaline

While noradrenaline is a potent and universal vasoconstrictor, acting through α_1- and α_2-adrenoceptors located in vascular smooth muscles, several reports indicate that the nature of the receptors vary with blood vessels, suggesting that gene expression is differentially regulated within vascular territories. Although only a few studies have identified the adrenoceptor subtypes in human vascular tissues by means of pharmacological tools, there appears to be a consensus that among the α_1-adrenoceptors, all three subtypes are expressed but in different vascular beds and with great variance among territories (Bevan, 1979; He and Yang, 1998; Guimaraes and Moura, 2001; Giessler et al., 2002). A prototype protocol used to identify the functional subtypes of α_1-adrenoceptors present in human vascular neuroeffector junctions is presented in Fig. 2, which examines the vascular reactivity of noradrenaline in isolated segments of human mammary vessels. Human mammary artery and vein biopsies obtained from programmed for coronary graft surgery, coincide in concluding that while 5-methylurapidil is a competitive noradrenaline antagonist with selectivity for the α_{1A}-adreneceptor, chloroethyl clonidine (CEC) is a non-equilibrium and irreversible noradrenaline receptor antagonist (Fig. 2) that covalently labels the α_{1B}-adrenoceptor subtype preferentially. The present results seem to indicate also that the human α_{1B}-adrenoceptor is abundantly expressed in conductance

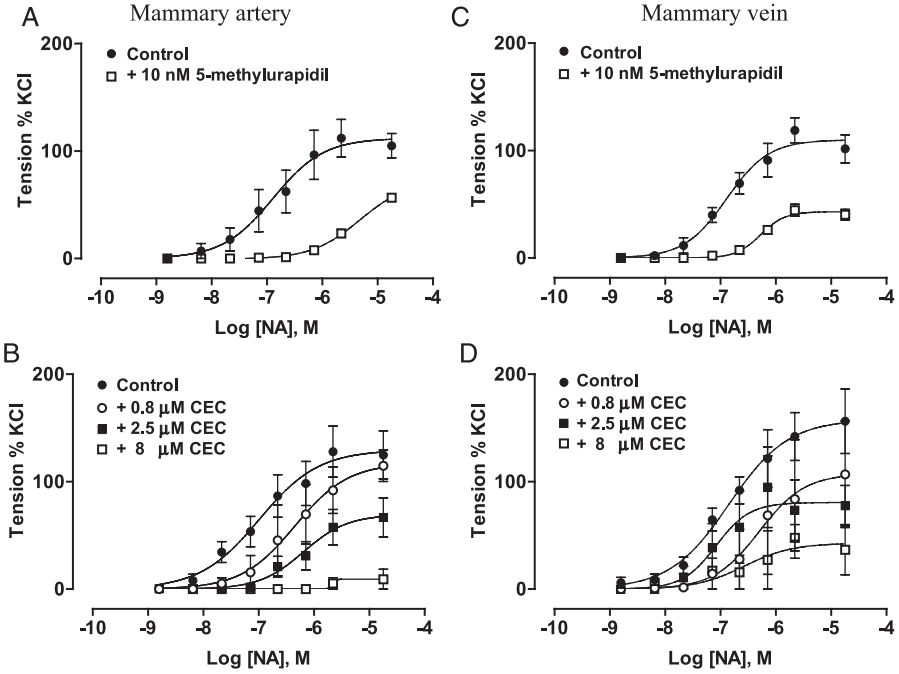

Fig. 2. Multiple α_1-adrenoceptors are expressed in human mammary arteries and veins and mediate the noradrenaline-induced vasoconstriction. Noradrenaline concentration–response curves were performed in isolated rings from biopsies of mammary arteries and veins; the isometric tension of the circular smooth muscle layer was recorded. The noradrenaline EC_{50} in artery (A and B) and vein (C and D) was 269±80 and 112±18 nM, respectively (n=15 and 11, respectively, $P<0.056$). A 5-min tissue pre-incubation with 10 nM 5-methyurapidil, a preferential α_{1A}-adrenoceptor antagonist, displaced downwards and to the right the noradrenaline concentration–response curve in artery and vein samples (A and C), while a 45-min preincubation with chloroethylclonidine (CEC), followed by extensive washout of this selective α_{1B}-adrenoceptor antagonist, caused a concentration-dependent downward displacement of the noradrenaline curves, evidencing its non-competitive mechanism of blockade in either vessel (B and D). Symbols indicate mean values; bars, S.E.M.

vessels such as the mammary veins. Even fewer studies have addressed the identification of the human α_2-adrenoceptor, although based on the extensive ophthalmologic use of selective α_2-adrenoceptor agonists this proposal warrants special studies.

As extensively reported in several blood vessels from animals and humans, the noradrenaline-evoked vasoconstriction is potentiated by nanomolar concentrations of NPY (Fig. 2), an effect that is ameliorated by using several NPY Y_1 receptor antagonists (Donoso et al., 2004). Furthermore, a remarkable synergism of the vasomotor response is observed upon the co-application of ATP plus noradrenaline, and effect that is further potentiated by the co-administration of 10 nM NPY (Fig. 2C). This result mimics the action of sympathetic co-transmitters plus the NPY-induced modulation occurring at vascular neuroeffector junctions, highlighting the relevance of co-transmission and how these properties are involved in human vascular contractility.

4.3. NPY

In vascular sympathetic neuroeffector junctions, NPY may act on two NPY receptor subtypes, the Y_1 and Y_2 receptors. The NPY Y_1 receptor is preferentially located postjunctionally, at the surface of the vascular smooth muscle. There is consensus that this receptor only exceptionally causes a direct vasomotor response and generally participates in sympathetic vascular reflexes either by potentiation of the transmural electrically evoked vasoconstriction of isolated human vascular, or the facilitation, of the ATP or the noradrenaline-evoked vasomotor responses in a vast number of vessels, including human segments of the inferior mesenteric vessels or saphenous vein (Racchi et al., 1997, 1999; Donoso et al., 2004), mammary vessels (Donoso et al., 2004, submitted for publication), as well as omental arteries and vessels from other territories (Bergdahl et al., 1996). Critical to clarify the role of NPY Y_1 receptors in the vasomotor action elicited by transmural nerve stimulation is the use of selective non-peptide Y_1 receptor antagonists, amongst which (*R*),-N^2-(diphenylacetyl)-*N*-(4-hydroxyphenyl)-methyl-D-argininea-mide (BIBP 3226) proved the best and most used tool. The exact molecular signalling mechanism triggered by NPY Y_1 receptor activation remains unknown; the importance of protein kinase C in the potentiation is being further evaluated.

In contrast, to the postjunctional location of the Y_1 receptor, the Y_2 receptor is presynaptic in origin, and its activation reduces the release of neurotransmitters both in the central nervous system as well as sympathetic perivascular nerve terminals. In addition, only in some restricted vascular territories, such as the brain and coronary circulation, NPY can induce a direct vasoconstriction

(Edvinsson, 1985; Macho et al., 1989; You et al., 2001). In this regard, Racchi et al. (1999) reported that only in few human saphenous vein rings (11/92), NPY per se constricted these biopsies, with an EC_{50} close to 100 nM, a finding that manifests the intricacies of NPY's vascular actions. This effect of NPY was blocked by 1 μM BIBP 3226, the best characterized NPY Y_1 receptor antagonist.

Instrumental to the identification of the Y_1 receptors in vascular beds by means of RT-PCR amplification has been the development and availability of selective antagonists for the NPY Y_1 receptor, such as BIBP 3226 and analogs. These drugs have allowed to functionally link the Y_1 receptor to the co-modulator role of NPY. Several studies demonstrate the competitive nature of BIBP antagonism in humans (Sautel et al., 1996), and a few of these studies focus on the blockade of the potentiation of vasocontractile mechanisms by endogenously released NPY. One of these studies showed that BIBP 3226 blocked the modulator role of NPY upon the electrically evoked human saphenous vein contractions (Racchi et al., 1999) while Donoso et al. (2004, submitted for publication) demonstrated that BIBP 3226 annulled the NPY-induced potentiation of the ATP or noradrenaline-evoked vasoconstrictions in human mammary vessels. All in all, the blockade of the modulator role of NPY via Y_1 receptor antagonists has given ample support to the functional role of NPY as an integral component of human sympathetic vasomotor reflexes.

The physiology of the Y_1 receptor-induced potentiation of the vasocontractile action of ATP and noradrenaline in human vascular biopsies (Fig. 2), including its identification by RT-PCR studies, has been documented in several studies using both human resistance vessels such as the omental arteries (Bergdahl et al., 1996; Uddman et al., 2002) and conductance blood vessels such as the saphenous vein (Donoso et al., 2004) or mammary arteries and veins as well as segments of the radial artery (Donoso et al., submitted for publication).

5. The modulator role of NPY in cardiac and human vascular disease

Consistent with the significance of NPY to vascular sympathetic reflexes in humans, several studies have measured ir-NPY in human plasma following a variety of stimuli of physiological and pathophysiological relevance such as exercise, stress and hypoxia (Ahlborg et al., 1992; Kaijser et al., 1994; Takiyyuddin et al., 1994; Zukowska-Grojec, 1995). In addition, the administration of NPY to humans significantly increases vascular resistance (Feng et al., 2000), which together with the recent report of Donoso et al. (2004), demonstrating the release of NPY from segments of the human saphenous vein, emphasizes the co-modulator role of NPY in the control of human vasomotor tone. Although we deem premature to provide an unequivocal link between the activation of the sympathetic vascular tone and a consequent rise in ir-NPY levels, several clinical evidences point in this direction. It is possible that the co-modulator role of NPY, which may play a subtle role in the physiology of normal tone regulation, may be exacerbated under pathophysiological conditions, being determinant in severe cardiac and/or vascular disease as suggested by some recent reports in patients with cardiac infarction, eclampsia and pheochromocytoma (Omland et al., 1994; Eurin et al., 2000; Khatun et al., 2000).

6. The integrated role of purinergic mechanisms in vascular neuroeffector junctions

In our view, co-transmission implies not only the concerted postjunctional action of the sympathetic co-transmitters plus NPY, but also some presynaptic conditions among which the synthesis, co-storage and co-release from synaptic vesicles is decisive. The issue of separate co-transmitter storage in the small and large synaptic vesicle has not been addressed in this review since details of the human sympathetic varicosities and the dynamics of their synaptic vesicles is non-existent. Upon physiological nerve terminal depolarization, or experimentally following electrically induced depolarization, the sympathetic triad is released in proportion to co-transmitter storage. As an initial step, ATP and noradrenaline act postjunctionally as co-transmitters; the postjunctional potentiation of the vasomotor effect evoked by ATP and noradrenaline ensues in the presence of NPY, which is co-released from the large vesicle. Upon access to presynaptic receptors, the full set of presynaptic auto regulatory mechanisms, including α_2-adrenoceptors, the NPY Y_2, and a variety of presynaptic receptors for ATP belonging to the P2Y subtype and adenosine receptors, operate to finely tune the sympathetic transmitter to be released by a next electrical impulse. The complexity of the regulatory mechanisms that operate in a human neuroeffector junction is schematised in Fig. 3.

Regarding the mechanisms accounting for the coordinated action of ATP and noradrenaline, it is possible to recognize that while ATP mainly activates P2X receptors in vascular smooth muscles, which are ionic channels that lead to cell depolarization, noradrenaline activates essentially G-protein-coupled receptors linked to the intracellular mobilization of calcium stores via the activation of selective inositol 1,4,5-triphosphate (IP_3) receptors at the endoplasmic reticulum. Therefore, while ATP ensues depolarization of the vascular smooth muscle within milliseconds, noradrenaline ensues the mobilization of intracellular calcium plus protein kinase C events within seconds, which are necessary to boost the contractile machinery. These dual and complementary mechanisms, orchestrated within a distinctive time frame and a defined temporary sequence of metabolic events, establish the foundations for co-transmission. Consonant with this proposal, we have systemati-

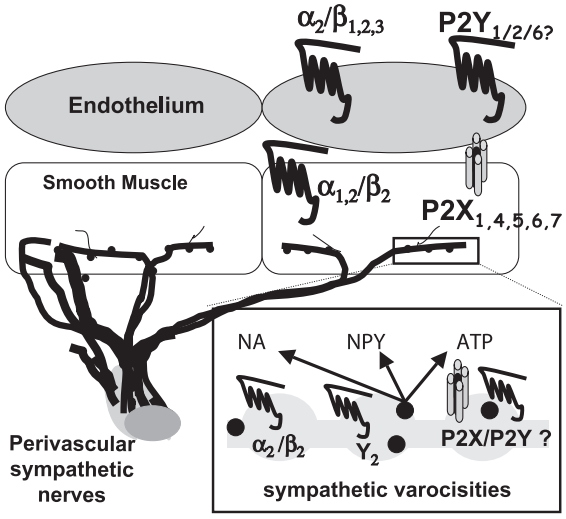

Fig. 3. Heuristic model shows the multiplicity of receptors and mechanisms that operate in human sympathetic vascular neuroeffector junctions, including possible endothelial receptors. Sympathetic nerve endings are in close proximity and spread stochastically on the vascular smooth muscle fibres. Upon nerve depolarization, the nerve endings secrete to the synaptic space the vesicle-stored transmitters, which, as in the case of the large, dense-cored vesicles, includes NPY in addition to ATP plus noradrenaline. ATP interacts selectively with a specific set of muscular P2X receptors in the smooth muscle, triggering smooth muscle depolarization, which coupled to the noradrenaline-mediated intracellular mobilization of calcium, ensues the vasoconstriction commanded by the sympathetic reflex. The scheme also depicts presynaptic autoreceptors for ATP, noradrenaline and NPY (insert), which tightly control the integrated sympathetic transmitter release. In addition, endothelial receptors for sympathetic co-transmitters that acts in a compounded manner, explaining the full complexity of the sympathetic reflex and the participation of three vascular wall cell types in the integrated sympathetic response.

cally observed that the joint application of the two sympathetic co-transmitters results in a synergic muscular response, illustrating the physiological implications of co-transmission. An example of the ATP–noradrenaline synergism, and the further potentiation of the vasomotor response by the modulator action of NPY, is illustrated in the protocol depicted in Fig. 2C.

7. Perspectives and concluding remarks

Altogether, this review summarized the progress over the last 20 years in favor of the notion that sympathetic reflexes, and in particular human vascular sympathetic reflexes are governed by the orchestrated action of ATP and noradrenaline, which in turn are modulated by the action of NPY. The findings discussed raise firm grounds to establish the role of ATP and related nucleotides in sympathetic neurotransmission, as was brilliantly and with great physiological insight put forward by Burnstock 18 years ago (Burnstock, 1986a,b). As to whether these findings open new avenues of clinical interventions and foster opportunities for therapeutic applications remains the challenge of the next two decades in exciting autonomic cardiovascular research. It has not escaped our attention that the hydrolysis of ATP by several ectoATPases apparently present in vascular neuroeffector junctions leads to adenosine, an active neuroeffector junctions metabolite. Adenosine is known to decrease the release of sympathetic co-transmitters and NPY, and to act either at the vascular smooth muscle as well as on the endothelium regulating blood flow. The view of the orchestrated action of sympathetic co-transmission together with the modulator action of NPY compounded to the regulatory role of adenosine in vascular neuroeffector junctions, promises new and excitement developments that may impact the clinic within the next decade.

Acknowledgements

This review was supported by FONDAP-BioMedicine grant 13980001; the Millennium Institute for Fundamental an Applied Biology, MIFAB, also contributed to the funding of the Center. To Prof. Dr. José Manuel Irarrázaval for his continual support in basic research and the many opportunities of collaborative studies using human vascular biopsies. We appreciate the collaboration of thoracic surgeons and their aid in the prompt delivery of biopsies to the pharmacology laboratory. We thank Ms. B. Watt for her editorial assistance.

References

Ahlborg, G., Weitzberg, E., Sollevi, A., Lundberg, J.M., 1992. Splanic and renal vasoconstrictor and metabolic responses to neuropeptide Y in resting and exercising man. Acta Physiol. Scand. 145, 139–149.

Bergdahl, A., Nilsson, T., Cantera, L., Nilsson, L., Sun, X.Y., Hedner, T., Erlinge, D., Valdemarson, S., Edvinsson, L., 1996. Neuropeptide Y potentiates noradrenaline-induced contraction through the neuropeptide Y Y1 receptor. Eur. J. Pharmacol. 316, 59–64.

Bevan, J.A., 1979. Some bases of differences in vascular response to sympathetic activity. Circ. Res. 45, 161–171.

Burnstock, G., 1986a. Autonomic neuromuscular junctions: current developments and future directions. J. Anat. 146, 1–30.

Burnstock, G., 1986b. The non-adrenergic non-cholinergic nervous system. Arch. Int. Pharmacodyn. Ther. 280, 1–15.

Burnstock, G., Williams, M., 2000. P2 purinergic receptors: modulation of cell function and therapeutic potential. J. Pharmacol. Exp. Ther. 295, 862–869.

Buvinic, S., Briones, R., Huidobro-Toro, J.P., 2002. $P2Y_1$ and $P2Y_2$ receptors are coupled to the NO/cGMP pathway to vasodilate the rat arterial mesenteric bed. Br. J. Pharmacol. 136, 847–856.

Donoso, M.V., Carvajal, A., Paredes, A., Tomic, A., Koenig, C., Huidobro-Toro, J.P., 2002. α_2-Adrenoceptors control the release of noradrenaline but not neuropeptide Y from perivascular nerve terminals. Peptides 23, 1663–1671.

Donoso, M.V., Miranda, R., Briones, R., Irarrazaval, M.J., Huidobro-Toro, J.P., 2004. Release and functional role of neuropeptide Y as a sympathetic modulator in human saphenous vein biopsies. Peptides 25, 53–64.

Edvinsson, L., 1985. Characterization of the contractile effect of neuropeptide Y in feline cerebral arteries. Acta Physiol. Scand. 125, 33–41.

Ekblad, E., Edvinsson, L., Wahlestedt, C., Uddman, R., Hakanson, R., Sundler, F., 1984. Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibers. Regul. Pept. 8, 225–235.

Erlinge, D., Ekman, R., Thulin, T., Edvinsson, L., 1992. Neuropeptide Y-like immunoreactivity and hypertension. J. Hypertens. 10, 1221–1225.

Eurin, J., Barthélemy, C., Masson, F., Maistre, G., Soualmia, H., Noé, E., Sarfati, F., Eurin, B., Carayon, A., 2000. Release of neuropeptide Y and hemodynamic changes during surgical removal of human pheochromocytomas. Regul. Pept. 86, 95–102.

Fabi, F., Chiavarelli, M., Argiolas, L., Chiavarelli, R., del Basso, P., 1993. Evidence for sympathetic neurotransmission through presynaptic N-type calcium channels in human saphenous vein. Br. J. Pharmacol. 110, 338–342.

Feng, Q., Lambert, M.L., Callow, I.D., Arnold, J.M., 2000. Venous neuropeptide Y receptor responsiveness in patients with chronic heart failure. Clin. Pharmacol. Ther. 67, 292–298.

Fraser, C.M., Chung, F.Z., Venter, J.C., 1987. Continuous high density expression of human beta 2-adrenergic receptors in a mouse cell line previously lacking beta-receptors. J. Biol. Chem. 262, 14843–14846.

Fried, G., Lundberg, J.M., Theodorsson-Norheim, E., 1985. Subcellular storage and axonal transport of neuropeptide Y (NPY) in relation to catecholamines in the cat. Acta Physiol. Scand. 125, 145–154.

Frielle, T., Collins, S., Daniel, K.W., Caron, M.G., Lefkowitz, R.J., Kobilka, B.K., 1987. Cloning of the cDNA for the human beta 1-adrenergic receptor. Proc. Natl. Acad. Sci. U. S. A. 84, 7920–7924.

Gao, J., Zhao, J., Rayner, S.E., Van Helden, D.F., 1999. Evidence that the ATP-induced increase in vasomotion of guinea-pig mesenteric lymphatics involves an endothelium-dependent release of thromboxane A2. Br. J. Pharmacol. 127, 1597–1602.

Giaroni, C., Knight, G.E., Ruan, H.Z., Glass, R., Bardini, M., Lecchini, S., Frigo, G., Burnstock, G., 2002. P2 receptors in the murine gastrointestinal tract. Neuropharmacology 43, 1313–1323.

Giessler, C., Wangemann, T., Silber, R.E., Dhein, S., Brodde, O.E., 2002. Noradrenaline-induced contraction of human saphenous vein and human internal mammary artery: involvement of different alpha-adrenoceptor subtypes. Naunyn-Schmiedeberg's Arch. Pharmacol. 366, 104–109.

Guimaraes, S., Moura, D., 2001. Vascular adrenoceptors: an update. Pharmacol. Rev. 53, 319–356.

He, G.W., Yang, C.Q., 1998. Characteristics of adrenoceptors in the human radial artery: clinical implications. J. Thorac. Cardiovasc. Surg. 115, 1136–1141.

Hegbrant, J., Thysell, H., Ekman, R., 1995. Circulating neuropeptide Y in plasma from uremic patients consists of multiple peptide fragments. Peptides 16, 395–397.

Kaijser, L., Pernow, J., Berglund, B., Grunnström, J., Lundberg, J.M., 1994. Neuropeptide Y release form human heart is enhanced during prolonged exercise in hipoxia. J. Appl. Physiol. 76, 1346–1349.

Khatun, S., Kanayama, N., Belayet, H.M., Bhuiyan, A.B., Jahan, S., Begum, A., Kobayashi, T., Terao, T., 2000. Increased concentrations of plasma neuropeptide Y in patients with eclampsia and preeclampsia. Am. J. Obstet. Gynecol. 182, 896–900.

Lundberg, J.M., Terenius, L., Hokfelt, T., Goldstein, M., 1983. High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. Neurosci. Lett. 42, 167–172.

Lundberg, J.M., Martinsson, A., Hemsen, A., Theodorsson-Norheim, E., Svedenhag, J., Ekblom, B., Hjemdahl, P., 1985. Co-release of neuropeptide Y and catecholamines during physical exercise in man. Biochem. Biophys. Res. Commun. 133, 30–36.

Lundberg, J.M., Rudehill, A., Sollevi, A., Fried, G., Wallin, G., 1989. Co-release of neuropeptide Y and noradrenaline from pig spleen in vivo: importance of subcellular storage, nerve impulse frequency and pattern feedback regulation and resupply by axonal transport. Neuroscience 28, 475–486.

Macho, P., Pérez, R., Huidobro-Toro, J.P., Domenech, R., 1989. Neuropeptide Y: a coronary vasoconstrictor and potentiator of catecholamine-induced coronary constriction. Eur. J. Pharmacol. 167, 67–74.

Nori, S., Fumagalli, L., Bo, X., Bogdanov, Y., Burnstock, G., 1998. Coexpression of mRNAs for P2X1, P2X2 and P2X4 receptors in rat vascular smooth muscle: an in situ hybridization and RT-PCR study. J. Vasc. Res. 35, 179–185.

O'Hare, M.M.T., Schwartz, T., 1989. Expression and precursor processing of neuropeptide Y in human pheochromocytoma and neuroblastoma tumors. Cancer Res. 49, 7010–7014.

Omland, T., Opstad, P.K., Dickenstein, K., 1994. Plasma neuropeptide Y levels in the acute and early convalescent phase after myocardial infarction. Am. Heart J. 127, 774–779.

Racchi, H., Schliem, A.J., Donoso, M.V., Rahmer, A., Zuñiga, A., Guzman, S., Rudolf, K., Huidobro-Toro, J.P., 1997. Neuropeptide Y Y_1 receptors are involved in the vasoconstriction caused by human sympathetic nerve stimulation. Eur. J. Pharmacol. 329, 79–83.

Racchi, H., Irarrázabal, M.J., Howard, M., Morán, S., Zalaquett, R., Huidobro-Toro, J.P., 1999. Adenosine 5′-triphosphate and neuropeptide Y are co-transmitters in conjunction with noradrenaline in the human saphenous vein. Br. J. Pharmacol. 126, 1175–1185.

Ramme, D., Regenold, J.T., Starke, K., Busse, R., Illes, P., 1987. Identification of the neuroeffector transmitter in jejunal branches of the rabbit mesenteric artery. Naunyn-Schmiedeberg's Arch. Pharmacol. 336, 267–273.

Rump, L.C., von Kugelgen, I., 1994. A study of ATP as a sympathetic cotransmitter in human saphenous vein. Br. J. Pharmacol. 111, 65–72.

Sautel, M., Rudolf, K., Wittneben, H., Herzog, H., Martinez, R., Munoz, M., Eberlein, W., Engel, W., Walker, P., Beck-Sickinger, A.G., 1996. Neuropeptide Y and the non-peptide antagonist BIBP 3226 share an overlapping binding site at the human Y_1 receptor. Mol. Pharmacol. 50, 285–292.

Tabarin, A., Cassiede, P., Minot, A.P., Cuber, J.C., Roger, P., Ducassou, D., 1993. Heterogeneity of neuropeptide Y immunoreactivity in patients with pheochromocytoma: influence on the diagnostic power of measuring plasma NPY using antisera with different specificities. Acta Endocrinol. (Copenh) 128, 243–250.

Takiyyuddin, M.A., Brown, M.R., Dinh, T.Q., Cervenka, J.H., Braun, S.D., Parmer, R.J., Kennedy, B., O'Connor, D.T., 1994. Sympatho-adrenal secretion in humans: factors governing catecholamine and storage vesicle peptide co-release. J. Auton. Pharmacol. 14, 187–200.

Tatemoto, K., 1982. Neuropeptide Y: complete amino acid sequence of the brain peptide. Proc. Natl. Acad. Sci. U. S. A. 79, 5485–5489.

Todorov, L.D., Mihaylova-Todorova, S., Westfall, T.D., Sneddon, P., Kennedy, C., Bjur, R.A., Westfall, D.P., 1997. Neuronal release of soluble nucleotidases and their role in neurotransmitter inactivation. Nature 387, 76–79.

Uddman, R., Moller, S., Nilsson, T., Nystrom, S., Ekstrand, J., Edvinsson, L., 2002. Neuropeptide Y Y1 and neuropeptide Y Y2 receptors in human cardiovascular tissues. Peptides 23, 927–934.

Valdecantos, P., Briones, R., Moya, P., Germain, A., Huidobro-Toro, J.P., 2003. Pharmacological identification of P2X1, P2X4 and P2X7 nucleotide receptors in the smooth muscles of human umbilical cord and chorionic blood vessels. Placenta 24, 17–26.

Wahlestedt, C., Grundemar, L., Hakanson, R., Heilig, M., Shen, G.H., Zukowska-Grojec, Z., Reis, D.J., 1990. Neuropeptide Y receptor subtypes, Y1 and Y2. Ann. N.Y. Acad. Sci. 611, 7–26.

You, J., Edvinsson, L., Bryan Jr., R.M., 2001. Neuropeptide Y-mediated constriction and dilation in rat middle cerebral arteries. J. Cereb. Blood Flow Metab. 21, 77–84.

Zhao, X.H., Sun, X.Y., Edvinsson, L., Hedner, T., 1997. Does the neuropeptide Y Y1 receptor contribute to blood pressure control in the spontaneously hypertensive rat? J. Hypertens. 15, 19–27.

Zukowska-Grojec, Z., 1995. Neuropeptide Y. A novel sympathetic stress hormone and more. Ann. N.Y. Acad. Sci. 29 (771), 219–233.