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Nervous control of smooth muscle by transmitters, cotransmitters and modulators

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This article reviews some of the new discoveries of the past decade about autonomic nervous control of smooth muscle, with particular emphasis on nonadrenergic, noncholinergic neurotransmitters and neuromodulators. Coexistence of transmitters in single nerve terminals is described, and some suggestions are made about how such systems might operate physiologically.

Structural relationships of autonomic nerves and smooth muscle

The autonomic neuroeffector junction differs significantly from the classical synapses of the skeletal neuromuscular junction and of those present in ganglia. Both these synapses are elaborate with separation of specialized pre- and postsynaptic membranes by about 20–50 nm. Transmitters released from presynaptic sites diffuse across the narrow cleft to occupy receptors localized on postjunctional membranes.

Studies of the relationship of autonomic nerve fibers to smooth muscle[5,27] have shown that the nerves have extensive terminal varicose regions free of Schwann cell envelopments, where vesicle-filled varicosities (1–2 μm in diameter) releasing transmitter en passage, are separated by narrow (0.1–0.3 μm diameter) intervaricose regions. While prejunctional varicosity membranes sometimes show thickenings, there are rarely postjunctional specializations, and the minimum junctional cleft may vary from as little as 20 nm in some densely-innervated tissues (like vas deferens or iris) to as much as 2000 nm in some large elastic arteries. It seems likely that only a small proportion of varicosities release transmitter during a single nerve pulse. The autonomic neurotransmitter junction with a variable and often wide cleft is amenable to both pre- and postjunctional modulatory influences from locally-released or circulating substances, as well as being the site of neurotransmission (see fig. 1).

Neurotransmitters

For many years the only autonomic neurotransmitters recognized were acetylcholine (ACh) and noradrenaline (NA). However, in the early 1960s, inhibitory junction potentials were recorded in intestinal smooth muscle during stimulation of enteric nerves in the presence of cholinergic and adrenergic blocking agents, and the existence of nonadrenergic, noncholinergic nerves was clearly established in the following years, not only in the gut, but in many other visceral and vascular organs[4,11].

Many substances were examined as putative transmitters in nonadrenergic, noncholinergic nerves supplying the smooth of the gut and urinary bladder. The following criteria were used: synthesis and storage in the nerve terminals; Ca^{2+}-dependent release on nerve stimulation; occupation of specific postjunctional receptors resulting in actions that mimic those produced by nerve stimulation; inactivation by enzymes and/or uptake mechanisms; and agents that produce parallel block (or potentiation) of the responses to nerve stimulation and exogenous application of the substance. The substance that best satisfied these criteria was a purine nucleotide, probably adenosine-5′-triphosphate (ATP), and the purinergic nerve hypothesis was proposed[6]. Since that time considerable evidence has accumulated in support of this hypothesis, although there have also been several reports that oppose it[10,29].

In the mid-1970s, several important new findings suggested that further transmitters might be present in other components of the autonomic nervous system. For example, at least nine ultrastructurally distinguishable types of axon profile were described in the enteric nervous system[19]; a number of biologically active polypeptides, including enkephalin, somatostatin, vasoactive intestinal polypeptide (VIP), substance P and neurotensin, were localized with immunocytochemical methods in autonomic nerves[25,31,56]; and both 5-hydroxytryptamine (5-HT)[28] and γ-aminobutyric acid (GABA)[34] were localized in enteric nerves using autoradiographic methods. As a result of these and later studies about 16 substances, in addition to ACh, NA and ATP, are now regarded as putative transmitters in the autonomic nervous system[11,15,16] (table).

Classical neurotransmission

In classical neurotransmission, a single neurotransmitter is released by exocytosis from its vesicular storage sites in

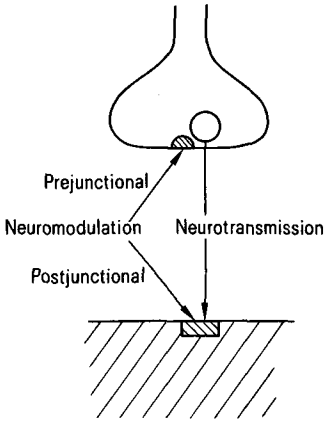

Figure 1. Diagrammatic representation of neuromodulation. Prejunctional neuromodulators increase or decrease release of transmitter; postjunctional neuromodulators alter the extent and/or time course of transmitter action. (From Burnstock[13a].)

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Transmitters proposed in the autonomic nervous system

Acetylcholine	ACh
Noradrenaline	NA
Adenosine triphosphate	ATP
5-Hydroxytryptamine	5-HT
γ-Aminobutyric acid	GABA
Dopamine	DA
Peptides	
Enkephalin endorphin	Enk/End
Vasoactive intestinal polypeptide/peptide HI	VIP/PHI
Substance P	Sub P
Gastrin releasing peptide/bombesin	GRP/BN
Somatostatin	ST
Neurotensin	NT
Luteinizing hormone releasing hormone	LHRH
Cholecystokinin/Gastrin	CCK/G
Neuropeptide Y/pancreatic polypeptide	NPY/PP
Galanin	GAL
Angiotensin	A II
Adrenocortico trophic hormone	ACTH
Calcitonin gene related peptide	CGRP

the nerve terminal to diffuse across the junctional cleft to occupy specific receptors on the postjunctional membrane, leading to changes in membrane conductance and effector cell activity. Fine control is exercised by higher centers by variations in the frequency pattern of impulses in the nerves and also by the more recently recognized autoregulatory system, where negative feedback of transmitter release is mediated by presynaptic receptors of a different type from those present in postjunctional membranes[57]. In adrenergic and purinergic junctions, these have been identified, i.e. α_1- and α_2-adrenoceptors[22] and P_1-and P_2-purinoceptors[12], but the existence of different pre- and postjunctional muscarinic receptors at cholinergic junctions is still being debated.

It is also known that ACh has an inhibitory effect on responses to sympathetic nerve stimulation via prejunctional muscarinic receptors, and that NA released from sympathetic nerve terminals reduces the release of ACh from cholinergic nerves in the gut, thereby inhibiting gastrointestinal motility. There is an anatomical basis for interactions or 'cross talk' between adrenergic and cholinergic nerves which have opposite actions on effector cells, since examples of close apposition of adrenergic and cholinergic nerve varicosities, often enclosed within the same Schwann cell sheath, have been described. Pharmacological findings add further support to this concept; for example, ACh released by stimulation of intrinsic cholinergic nerves in the rabbit atria and in a variety of blood vessels can lead to a decrease in release of NA during adrenergic transmission.

Coexistence of neurotransmitters and the physiology of cotransmission

The suggestion that some nerve cells store and release more than one transmitter was made in 1976[7] largely on the basis of comparative studies of the evolution of the autonomic nervous system[4] and evidence for the coexistence of biologically active substances in certain invertebrate nerves[2]. Since then considerable evidence has accumulated in support of this possibility[20, 44]. Nearly all nerve profiles examined under the electron microscope contain more than one type of vesicle, which is consistent with the multiple-transmitter concept, although the whole question of the identification of vesicle types to particular transmitter substances is unresolved and must await high electron microscopic resolution of preparations treated with highly specific cytochemical methods for transmitters and related enzymes[13].

Acetylcholine and noradrenaline

There is compelling evidence that under certain conditions in vitro single sympathetic neurones may release NA, ACh, or a mixture of these two transmitter substances[3, 26]. It seems likely that this represents a true reflection of events that occur in vivo during perinatal development. It appears that a population of sympathetic nerve cells that have the potential to synthesize both NA and ACh are present at birth. The multipotential cells require Nerve Growth Factor (NGF) to survive and they respond to NGF with an increased production of both choline acetyltransferase and tyrosine hydroxylase, enzymes that are involved in the synthesis of ACh and NA, respectively. Under the influence of conditioning factors, most of the cells appear to differentiate into either cholinergic or adrenergic neurones soon after birth. However, it is possible that some sympathetic neurones, supplying some organs in some animals, retain the ability to produce and release both ACh and NA[9].

In a recent paper by Potter, Furshpan and Landis[47], it was shown that some cultured sympathetic neurones secrete, in addition to NA and ACh, a third transmitter, probably adenosine or a phosphorylated derivative. Thus purinergic function is expressed with adrenergic or cholinergic function or with both (triple function).

Acetylcholine and adenosine-5′-triphosphate

A detailed account of the evidence for coexistence of ACh and NA with ATP is available[14]. Cholinergic vesicles isolated from the electric organ of various elasmobranch fish contain ATP in addition to the principal neurotransmitter ACh. The ACh:ATP molar ratio in the three species studied is 4–10:1. Studies of the turnover of adenine nucleotides in cholinergic synaptic vesicles have shown that ATP and ACh are depleted to the same extent (about 50%) during nerve stimulation, that adenosine is an effective precursor of vesicular adenine nucleotides and that the new population of vesicles that appears following nerve stimulation has a high turnover rate for both ATP and ACh[59]. ATP has also been reported to be released from the endings of phrenic nerves in the rat diaphragm during stimulation[50]. This compares well with the levels of ATP released on stimulation of slices of some regions of the cortex or of cortical synaptosome preparations[58].

The functional significance of this type of coexistence needs exploration. It seems likely that ACh and ATP are contained in the same vesicles, and there is convincing evidence that the release of ACh and ATP from electric organ synaptosomes is precisely in parallel, as well as the cycle of reuptake and resynthesis[43]. Therefore, differential release of the cotransmitters at different impulse frequencies seems unlikely.

At the skeletal neuromuscular junction, modulation of the activity of the principal transmitter, ACh, can occur through pre- and/or postsynaptic actions of the cotransmitter[14]. ATP and adenosine have been shown to act on prejunctional purinergic receptors to modulate the release of ACh from cholinergic motor nerves in skeletal muscle of the rat diaphragm, frog sartorius and fish electric organ. These responses are blocked by methylxanthines indicating that they are mediated by P_1-purinoceptors. It has been suggested that occupation of the presynaptic P_1-purinoceptors leads to decrease in the entry of Ca^{2+} with consequent reduction in release of ACh. The frequency, but not the mean amplitude, of miniature endplate potentials and the amplitude of the nerve-evoked endplate potentials are reduced, indicating that the actions of adenosine (and ATP largely via adenosine) are presynaptic. Further, ATP in concentrations sufficient to produce modulatory effects had no direct postsynaptic action on adult junctions. Receptors to ATP as well as ACh, however, appear to be present in the developing myotube[36].

In the gut, ATP and adenosine have also been shown to act on prejunctional purinergic receptors leading to modulation of the release of ACh from cholinergic motor nerves. These responses are blocked by methylxanthines indicating that they are mediated by P_1-purinoceptors. ATP does not act by way of P_2-purinoceptors, but is rapidly broken down to AMP and adenosine which occupy the P_1-purinoceptors on the cholinergic nerve terminals in the intestine[42].

ATP has been shown to be a postjunctional modulator of the action of ACh at the skeletal neuromuscular junction. Increase in ACh receptor sensitivity by ATP has been demonstrated at the motor endplate[1]. The amplitude of the current induced by ionophoretic application of ACh to the motor endplate in frog skeletal muscle is increased in the presence of ATP, and kinetic analysis has suggestd that ATP increases ACh sensitivity by acting on the allosteric site of the receptor-ionic channel complex without changing the affinity of ACh for its recognition site.

Noradrenaline and adenosine-5'-triphosphate

It has been known for a number of years that ATP is stored and released together with catecholamines from adrenal chromaffin cells. It has also been suggested that medullary granule-associated nucleotides may act locally as 'coagonists' with biogenic amines and may additionally provide a circulatory pool of purines for use by heart and lungs. Storage of ATP together with NA in adrenergic nerves was recognized in the early literature. The first indication that ATP might be released from adrenergic nerves was the demonstration that stimulation of periarterial adrenergic nerves led to release of tritium from taenia coli preincubated in [^{3}H]adenosine (which is taken up and converted to [^{3}H]ATP); both the release of tritium and NA were blocked by guanethidine[55]. Later, Langer and Pinto[37] suggested that the substantial residual nonadrenergic, noncholinergic response of the cat nictitating membrane following depletion of NA by reserpine, may be due to release of the ATP remaining in adrenergic nerves.

The physiology of cotransmission has been most fully explored in the vas deferens. The coexisting substances, NA and ATP, act as synergistic neurotransmitters via postjunctional receptors, as well as exerting modulatory effects on each other via both pre- and postjunctional mechanisms (fig. 2). Evidence has been presented that ATP is stored and released as a cotransmitter together with NA from adrenergic nerves supplying the guinea pig vas deferens[24,41,51,53]. The initial phasic component of the excitatory response to sympathetic nerve stimulation is selectively antagonized by arylazidoaminopropionyl ATP (ANAPP$_3$), which is claimed to be a specific P_2-purinoceptor antagonist, or by selective desensitization of the P_2-purinoceptor by α,β-methylene ATP; while the secondary more tonic component of the response is antagonized by prazosin or reserpine. The calcium channel blocker, nifedipine, has also been shown to block the initial, but not the secondary, responses of this preparation to nerve stimulation and contractions to ATP, but not to NA. More recently the excitatory junction potentials (EJP's) recorded in smooth muscle cells of the vas deferens in response to sympathetic nerve stimulation have been blocked by both ANAPP$_3$ and α,β-methylene ATP. Furthermore, local application of ATP by pressure ejection from a micropipette produced a transient depolarization comparable to the EJP, which was also blocked by α,β-methylene ATP; NA applied in a similar manner produced no such responses. ATP and adenosine have been shown to inhibit NA release from adrenergic nerves supplying the vas deferens[45,48]. The prejunctional receptor that mediates these actions is the P_1-purinoceptor[52]. It has been suggested that occupation of prejunctional P_1-purinoceptors leads to decrease in Ca^{2+} influx with subsequent reduction in NA release. Purine nucleotides or nucleosides also act as postjunctional neuromodulators in the vas deferens and iris enhancing the actions of

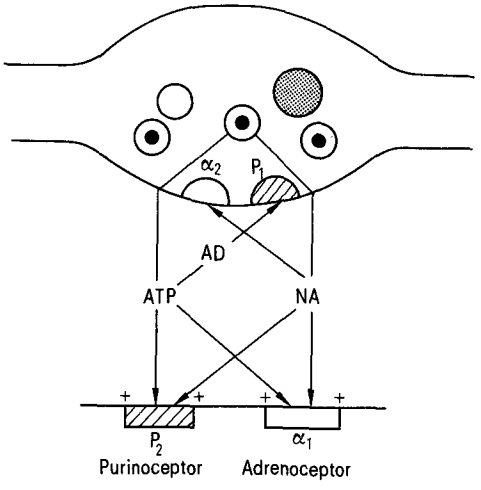

Figure 2. Schematic representation showing that ATP and NA are released as cotransmitters from the sympathetic nerves supplying the vas deferens and some blood vessels. ATP acts on P_2-purinoceptors on the smooth muscle to initiate EJP's, action potentials and the phasic contraction. NA acts on α_1-adrenoceptors to produce the second phase of the contraction by a different mechanism. Prejunctional α_2-adrenoceptors and P_1-purinoceptors can reduce transmitter release when activated by NA and adenosine, respectively. Note that in this model ATP and NA are stored in the same vesicle. (From Burnstock[15].)

NA[30,32], while NA can potentiate the responses of the vas deferens and seminal vesicle to ATP.

Cotransmission involving NA and ATP has also been studied in blood vessels. ATP is stored and released together with NA from sympathetic nerves supplying the rabbit aorta and portal vein[54]. Coexistence of NA and ATP has also been demonstrated in rabbit ear artery and dog basilar artery. ATP as well as NA release from guinea pig portal vein has been shown to be abolished following sympathectomy. Fluorescence in nerves of the rat portal vein following incubation in quinacrine, which binds to ATP, is also abolished by sympathectomy[17]. In the rat tail artery, electrical responses to stimulation of the sympathetic nerves consist of two components, namely a fast depolarization to each stimulating pulse and a slow maintained depolarization as the train of stimuli progresses; the slow component is blocked by phentolamine, suggesting it is mediated by α-adrenoceptors, while the fast depolarizations are blocked by α,β-methylene ATP, suggesting mediation by P_2-purinoceptors[18]. Pre- and postjunctional modulation of responses to sympathetic nerve stimulation have been demonstrated in mesenteric, basilar and pulmonary arteries[35].

Established transmitter with polypeptides

Certain peripheral endocrine cells, particularly those located in the gastrointestinal tract, contain both a biogenic amine, such as 5-HT or histamine, and a peptide hormone, such as substance P, somatostatin or neurotensin. These cell systems are part of the so-called APUD ('Amine content or Precursor Uptake and Decarboxylation') system[46]. Pearse[46] postulated that this situation may also exist in neurones.

There are many examples now of the coexistence of established neurotransmitters with various peptides[16,20,44]. For example, somatostatin-, enkephalin- and neuropeptide Y-like immunoreactivity have all been observed in sympathetic nerves, while coexistence of ACh with VIP-like peptide has been claimed in some parasympathetic nerves.

Evidence is accumulating that many peptides are stored in large granular vesicles separately from the established transmitters ACh or NA, which are stored predominantly in small vesicles. This separate storage system for coexisting transmitters, in contrast to the storage of ATP and established transmitters in the same vesicles described earlier, would appear to allow differential release of the cotransmitters at different impulse frequencies. The only preparation where an analysis has been carried out on the conditions for release and the sites and types of actions of the cotransmitters is the cat exocrine gland[23,38]. In the salivary gland, ACh released from parasympathetic nerves at low frequencies causes salivary secretion from acinar cells and some dilation of blood vessels in the gland. VIP released by nerve stimulation at higher frequencies (> 15 Hz) produces marked vasodilatation, and although it has no direct effect on acinar cells, it does substantially enhance the effect of ACh on acinar cell secretion and the release of ACh from the nerve endings via prejunctional receptors (see fig. 3).

The biological advantage of such a mechanism is that the cotransmitter can be released in more demanding situations to enhance the action of the principal transmitter. This enhancement may occur through several mechanisms: by postjunctional enhancement of transmitter action; by prejunctional enhancement of transmitter release; and by a separate synergistic action on blood vessels which provides for the increased metabolic needs of the tissue. When the emergency is over, reduction of stimulus frequency by central control centers would reduce cotransmitter release, which would be reinforced by prejunctional inhibition of its release by the principal transmitter.

Evidence has been presented that in some instances peptides may be stored within the same vesicles as the established transmitter. For example, substance P and 5-HT in dense-cored vesicles (60–90 nm) in nerve terminals in brain and spinal cord, and opiate-like peptides and NA in large dense-cored vesicles in bovine splenic nerve. Cooperation between cotransmitters in these situations is therefore likely to be different from that employed by VIP and ACh in the cat salivary gland.

The 'axon reflex' concept involved release of transmitter following antidromic impulses down collateral branches of primary afferent sensory fibers to account for vasodilatation particularly of skin vessels, although the possibility that 'axon reflexes' from sensory collaterals also occur in stomach[21], carotid body[40] and blood vessels in the lung[39] has also been raised recently. Thus, this type of physiological control mechanism may be more widespread than originally visualized. The transmitter released during the 'axon reflex' is not yet clearly established. Substance P is a strong contender, ATP is another[33], and it has been suggested that substance P and ATP may

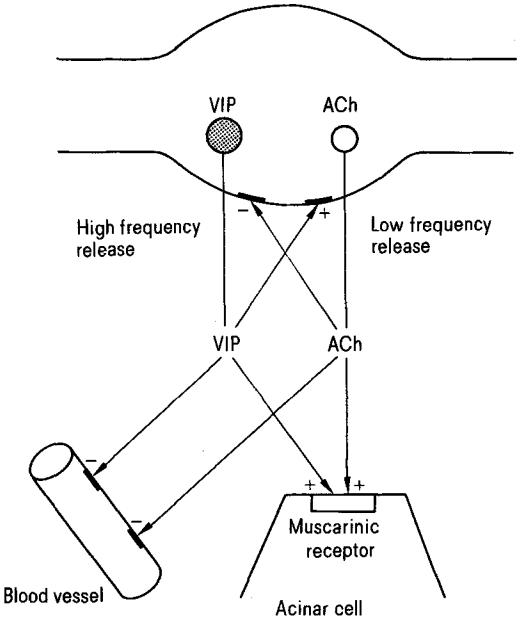

Figure 3. Schematic representation of transmission where vasoactive intestinal polypeptide (VIP) is a cotransmitter with acetylcholine (ACh) in parasympathetic nerves supplying the cat salivary gland. Note that ACh and VIP are stored in separate vesicles; they can be released differentially at different stimulation frequencies to act on acinar cells and glandular blood vessels. Cooperation is achieved by selective release of ACh at low impulse frequencies and of VIP at high frequencies. Pre- and postjunctional modulation is indicated. (From Burnstock[15].)

coexist in some primary afferent nerve fibers[8]. Calcitonin gene related peptide (CGRP) has also been claimed recently to be contained in sensory fibers[48a,49].

Conclusion

The part played by peripheral neuroeffector control mechanisms has been underestimated. These are additional to central and ganglionic control mechanisms and are much more elaborate than originally thought. While the classical view is that the autonomic nervous system consists largely of antagonistic cholinergic and adrenergic nerves, about sixteen putative neurotransmitters have been proposed in autonomic nerves in the past few years, including various monoamines, polypeptides, purines and amino acids. Modulatory transmitter mechanisms have also been recognized, including prejunctional inhibition or enhancement of transmitter release, postjunctional modulation of transmitter action, and the secondary involvement of locally synthesized hormones and prostaglandins. The existence of more than one transmitter substance in some nerves is now widely recognized, and suggestions have been made about the ways that this can lead to differential peripheral control mechanisms at nerve terminals themselves. The cotransmitters always have synergistic actions on postjunctional effector cells, but two different operating mechanisms are postulated. 1) If both substances are stored in the same vesicles (for example, ACh or NA with ATP), release is closely parallel at all impulse frequencies. Upon release, the cotransmitter, in addition to having a direct action on postjunctional cells, may facilitate the action of the other transmitter and/or act as an inhibitor of its release. Differential actions at different impulse frequencies are achieved post-junctionally by ATP and NA acting via EJP-spike and spike-independent mechanisms, respectively. 2) If the two substances are stored in separate vesicle types (for example ACh or NA with some peptides), then differential release is possible at different impulse frequencies; the peptides released at higher frequencies modulate the role of the classical transmitter, by both prejunctional enhancement of its release and postjunctional facilitation of its action.

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Neuromuscular transmission in arterioles

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Introduction

It is often assumed that superfusion of a smooth muscle organ with the putative transmitter, released by the nerves which innervate that organ, will produce a response identical to that produced by nerve stimulation. Many observations have been made which are in accord with this view. As simple examples, sympathetic nerve stimulation and superfusion with noradrenaline, the presumptive transmitter, each produce vasoconstriction in most vascular beds, frequently each of these mechanical responses are abolished by the same antagonist. Secondly, the sympathetic nerves which innervate many smooth muscle organs do not give rise to specialized