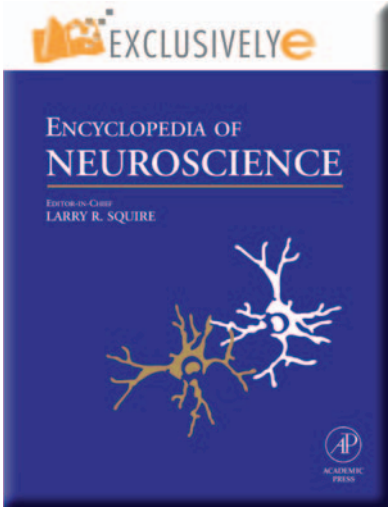




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# Cotransmission

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## Early Background

For many years, the understanding of neurotransmission has been dominated by the concept that one neuron releases only a single transmitter, known as 'Dale's Principle.' This idea arose from a widely adopted misinterpretation of Dale's suggestion in 1935 that the same neurotransmitter was stored in and released from all terminals of a single neuron, a suggestion which did not specifically preclude the possibility that more than one transmitter may be associated with the same neuron. Several lines of evidence emerged which were inconsistent with the single transmitter concept, and it is now known that individual neurons contain and can release a large number and variety of substances which are capable of influencing target cells. This phenomenon of 'cotransmission' is widespread, involving virtually all known transmitter systems.

Early hints of cotransmission came in the 1950s with evidence for the involvement of both noradrenaline (NA) and acetylcholine (ACh) in sympathetic transmission. Koelle identified acetylcholinesterase in some adrenergic neurons in 1955, while Burn and Rand introduced the concept of a 'cholinergic' link in adrenergic transmission in 1959. Another line of evidence provided by Hillarp concerned the coexistence of adenosine 5′-triphosphate (ATP) with catecholamines, first in adrenal chromaffin cells and later in sympathetic nerves. Inconsistencies in the single transmitter hypothesis provided by these and other studies from the early literature were rationalized in an article by Burnstock in 1976 with the provocative title,"Do some nerve cells release more than one transmitter?" Today it is widely accepted that cotransmission is an integral feature of neurotransmission.

A role for ATP as a cotransmitter in sympathetic, parasympathetic, sensory–motor, and enteric nonadrenergic, noncholinergic (NANC) inhibitory nerves was supported by research from Burnstock and colleagues, while Hökfelt and colleagues focused on the co-localization, vesicular storage, and release of peptides from both peripheral and central nerves.

Furness and Costa introduced the concept of 'chemical coding' to describe the combination of potential neurotransmitters found in enteric nerves, and this concept has since been applied to other nerve types, in both peripheral and central nervous systems CNSs (see Figure 1). Co-localized substances are not necessarily cotransmitters; they can (especially peptides) act as pre-and/or postjunctional neuromodulators of the release and actions of the principal cotransmitters. The proportions of cotransmitters vary considerably between species and organs and show plasticity of expression during development and in pathological conditions. In general, classical transmitters are contained in small synaptic vesicles, whereas peptides are stored in large granular (dense-cored) vesicles (LGVs), although small molecule transmitters are sometimes stored together with peptides in LGVs.

Pharmacological studies of pre- and postjunctional neuromodulation provide evidence which is complementary to the concept of cotransmission. For example, parallel presynaptic modulation of transmitter overflow supports the concept of closely associated co-release, while postjunctional synergism between co-localized transmitters provides justification of cotransmission in terms of transmitter economy.

## Sympathetic Nerve Cotransmission

There is substantial evidence to show that NA, ATP, and neuropeptide Y (NPY) are cotransmitters in sympathetic nerves, having differentially important roles as transmitters and neuromodulators, depending on the tissue, the species, and the parameters of stimulation. Most of the early studies establishing the model of cotransmission of NA and ATP were made on the vas deferens, a tissue with a high density of sympathetic nerves. Subsequently, numerous studies demonstrated that cotransmission of NA and ATP also occurs in other visceral organs and many different blood vessels in a variety of species.

The first indication that ATP might be released from sympathetic nerves was the demonstration that stimulation of sympathetic nerves by Su, Bevan, and Burnstock in 1971 led to release of tritium from taenia coli preincubated in [$^3$H]adenosine (which is taken up and converted to [$^3$H]ATP). Later Sol Langer and colleagues suggested that the substantial residual NANC response of the cat nictitating membrane observed after depletion of NA by reserpine was due to the release of ATP remaining in the sympathetic nerves. In retrospect, there was a good indication that the excitatory junction potentials (EJPs) recorded in the guinea pig vas deferens when the electrophysiology of sympathetic nerve smooth muscle transmission was first described by Burnstock and Holman in 1960 were due to ATP released as a cotransmitter from sympathetic nerves rather than to NA. It was puzzling at the time that adrenoceptor antagonists failed to block

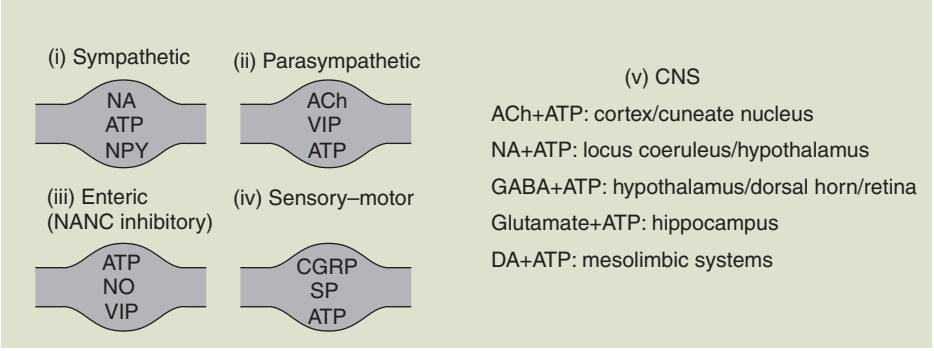


**Figure 1** Schematic diagram of the principal cotransmitters with ATP in the nervous system. Nerve terminal varicosities of (i) sympathetic, (ii) parasympathetic, (iii) enteric (NANC inhibitory), (iv) sensory–motor neurons, and (v) central nervous system (CNS). Reproduced from Burnstock G (2007) Physiology and pathophysiology of purinergic neurotransmission. *Physiological Reviews* 87: 659–797, with permission from The American Physiological Society.

the EJPs, although guanethidine, a drug that prevents the release of sympathetic transmitters, was effective. It was more than 20 years later that NANC EJPs were shown to be blocked by desensitization of the ATP (P2) receptors by $\alpha,\beta$-methylene ATP and mimicked by ATP. After destruction of sympathetic nerves with 6-hydroxydopamine, purinergic nerve-mediated responses were abolished. ATP is co-stored with NA in small and large vesicles. Differential prejunctional modulation of the release of NA and ATP by various agents has been shown in the vas deferens, perhaps indicating that NA and ATP are stored in different vesicles.

Cotransmission of NA and ATP in perivascular sympathetic nerves supplying the rabbit aorta, portal vein, and saphenous, pulmonary and mesenteric arteries and the dog basilar artery has been demonstrated. Electrophysiological studies have shown that in a number of vessels, the electrical response to stimulation of perivascular sympathetic nerves is biphasic: an initial fast, transient depolarization or EJP of the vascular smooth muscle is followed by a slow, prolonged depolarization. The EJP and slow depolarization are mimicked by the effects of ATP and NA, respectively. Considerable variation exists in the proportions of NA and ATP utilized by sympathetic nerves. For example, in guinea pig submucosal arterioles, both vasoconstriction and EJPs, evoked in response to electrical stimulation of sympathetic nerves, are mediated exclusively by ATP, with NA assuming the role of a neuromodulator, acting through prejunctional $\alpha_2$-adrenoceptors to depress transmitter release. At the other extreme, in rat mesenteric arteries, the purinergic component is relatively small. In addition, it has been noted that the purinergic component is optimal with short bursts of low-frequency stimulation, whereas longer durations of higher frequency favor adrenergic transmission.

NPY has been found to be present in LGV in most sympathetic nerves. The release of NPY, as well as

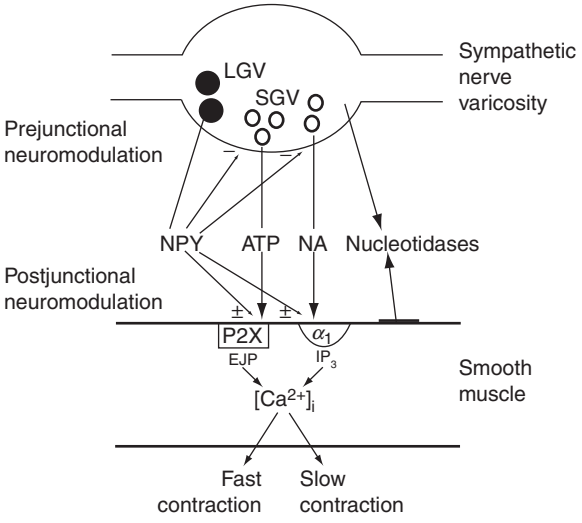


**Figure 2** Schematic of sympathetic cotransmission. Adenosine 5′-triphosphate (ATP) and noradrenaline (NA) released from small granular vesicles (SGV) act on P2X and $\alpha_1$ receptors on smooth muscle, respectively. ATP acting on inotropic P2X receptors evokes excitatory junction potentials (EJPs), increase in intracellular calcium ($[Ca^{2+}]_i$), and fast contraction while occupation of metabotropic $\alpha_1$ adrenoceptors leads to production of inositol triphosphate ($IP_3$), increase in $[Ca^{2+}]_i$, and slow contraction. Neuropeptide Y (NPY) stored in large granular vesicles (LGV) acts on release both as a prejunctional inhibitory modulator of release of ATP and NA and as a postjunctional modulatory potentiator of the actions of ATP and NA. Nucleotidases are released from nerve varicosities and are also present as ectonucleotidases.

NA and ATP, in response to electrical stimulation of sympathetic nerve terminals is prevented by guanethidine. The major role of NPY in the vasculature, and in the vas deferens, appears to be that of a pre- and/or postjunctional modulator of sympathetic transmission since it has little direct postjunctional action or causes contraction only at high concentrations (see Figure 2). Direct vasoconstrictor actions of NPY have, however, been demonstrated in some vessels. At the prejunctional level, NPY has potent inhibitory

effects, reducing the release of NA and ATP from sympathetic nerves. Postjunctionally, NPY generally acts to enhance the actions of sympathetic nerve stimulation, NA, and ATP.

Although 5-hydroxytryptamine (5-HT) immunofluorescent nerves have been localized in a number of vessels, for the most part 5-HT is not synthesized and stored in separate nerves but is taken up, stored in, and released as a 'false transmitter' from sympathetic nerves. Enkephalins have been shown to coexist with NA in cell bodies and fibers of some postganglionic sympathetic neurons. Both NA and met-enkephalin are co-released by transmural stimulation in a guanethidine-sensitive manner. The functional significance of sympathetic coexistence of opioids is likely to be related to their prejunctional inhibitory effects on sympathetic transmission.

## Parasympathetic Nerve Cotransmission

The classical evidence for cotransmission of ACh and vasoactive intestinal polypeptide (VIP) in certain postganglionic parasympathetic neurons comes from pharmacological studies performed by Lundberg in 1981 on cat salivary glands. ACh and VIP are released from the same parasympathetic nerve terminals in response to transmural nerve stimulation. During low-frequency stimulation, ACh is released to cause an increase in salivary secretion from acinar cells and also to elicit some minor dilatation of blood vessels in the gland. VIP is preferentially released at high frequencies to cause marked vasodilatation of blood vessels, and while it has no direct effect on acinar cells, it acts as a neuromodulator to enhance both the postjunctional effect of ACh on acinar cell secretion and the release of ACh from nerve varicosities via prejunctional receptors. Vasodilator nerves to the uterine arteries in the guinea pig contain immunoreactivity to VIP, which coexists with dynorphin, NPY, and somatostatin. NPY-like immunoreactivity has been reported in some of the choline acetyltransferase-/VIP-containing neurons of the parasympathetic ciliary, sphenopalatine, otic, and pterygopalatine ganglia with targets including the iris and cerebral vessels.

Autonomic control of penile erection, involving relaxation of the smooth muscle of the corpus cavernosum as well as dilatation of other penile vascular beds, has traditionally been attributed to the vasodilator effects of ACh and VIP released from parasympathetic nerves. More recently, nitric oxide (NO) released from nerves arising from nerves in the pelvic ganglia have been claimed to play a role in smooth muscle relaxation leading to penile erection. Fibers containing NO synthase, shown by lesion studies to arise from parasympathetic cell bodies in the sphenopalatine ganglia, have been localized in the adventitia of cerebral arteries, and many of these also contain VIP. A functional role for perivascular neuronal NO in cerebral arteries has been identified in studies showing that stimulation of adventitial nerve fibers causes vascular relaxation, which is attenuated by inhibitors of NO synthase.

Parasympathetic nerves supplying the urinary bladder utilize ACh and ATP as cotransmitters, in variable proportions in different species, and by analogy with sympathetic nerves, ATP again acts through P2X ionotropic receptors to produce EJPs and fast contraction, while the slow component of the response is mediated by a metabotropic receptor, in this case muscarinic. There is also evidence for parasympathetic, purinergic cotransmission to resistance vessels in the heart and airways.

## Sensory–Motor Nerve Cotransmission

The neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) are the principal transmitters of primary afferent nerves and have been shown to coexist in the same terminals. Furthermore, with the use of colloidal gold particles of different sizes, they have been shown to coexist in the same large granular vesicles. The motor (efferent) function of sensory nerves has been demonstrated in rat mesenteric arteries, where evidence exists for a role for CGRP as the mediator of vasodilatation following release from sensory–motor nerves. In contrast, SP is not co-released with CGRP by electrical stimulation, and SP has little or no vasodilator action on rat mesenteric arteries. While it is possible that SP released from nerves supplying the microvasculature could produce vasodilatation via SP receptors on endothelial cells, it is most unlikely to reach the endothelium without degradation in larger blood vessels. It may be that the role of the coexisting SP is either trophic or sensory (and not motor).

Other peptide and nonpeptide substances, including neurokinin A, somatostatin, VIP, and ATP, have been described in capsaicin-sensitive sensory neurons. Unmyelinated sensory neurons containing cholecystokinin (CCK)/CGRP/dynorphin/SP have been shown to project to cutaneous arterioles in guinea pig skin. Neurons from the same ganglia which contain CCK/CGRP/SP innovate arterioles of skeletal muscle, CGRP/dynorphin/SP nerve fibers mostly supply the pelvic viscera, and CGRP/SP fibers run mainly to the heart, large arteries, and veins. There is also evidence for a sensory role for ATP, and it has been proposed that ATP may coexist in sensory nerve terminals with SP and CGRP.

## Intramural Nerve Cotransmission

Intrinsic neurons exist in most of the major organs of the body. Many of these are part of the parasympathetic nervous system, but certainly in the gut and perhaps also in the heart and airways, some of these intrinsic neurons are derived from neural crest tissue, which differs from that which forms the sympathetic and parasympathetic systems and appears to participate in local reflex actions independent of the CNS.

The enteric nervous system contains several hundred million neurons located in the myenteric plexuses between muscle coats and the submucous plexus. The chemical coding of these nerves has been examined in detail. A subpopulation of these intramural enteric nerves provides NANC inhibitory innervation of the gastrointestinal smooth muscle. It seems likely that three major cotransmitters are released from these nerves. ATP produces fast inhibitory junction potentials, NO also produces inhibitory potentials but with a slower time course, while VIP produces slow tonic relaxations. The proportions of these three transmitters vary considerably in different regions of the gut and in different species; for example, in some sphincters, the NANC inhibitory nerves utilize largely VIP, in others largely NO, while in nonsphincteric regions of the intestine, ATP is more prominent. In recent papers, evidence has suggested that ACh and ATP are fast excitatory cotransmitters to myenteric neurons and that there may be co-localization of ACh, ATP, and serotonin in enteric S neurons. Detailed studies have allowed a very complete mapping of the complex neuronal markers and projections of enteric neurons. Several peptidergic substances, including NPY, VIP, peptide histidine isoleucine, SP, and CGRP, have been identified in enteric neurons, often coexisting (up to five peptides in the same neuron) with the neurotransmitters NA, ACh, 5-HT, NO, and ATP. The precise roles of the coexisting substances, however, have not for the most part been established, except for the proposed combinations of ACh and SP in the excitatory nerves and ATP, NO, and VIP in the NANC inhibitory neurons involved in peristaltic reflexes.

Studies of intrinsic cardiac neurons in culture have shown that subpopulations of intrinsic nerves in the atrial and intra-atrial septum contain and/or release cotransmitters, including ATP, NO, NPY, Ach, and 5-HT. Many of these nerves project to the coronary microvasculature and produce potent vasomotor actions. NO and ATP have been shown to be the mediators of NANC vasodilatation of the rabbit portal vein.

## CNS

Evidence for ATP's being a cotransmitter with established neurotransmitters in the CNS has been reported. In preparations of affinity-purified cholinergic nerve terminals from the rat cuneate nucleus, ATP and ACh are co-released. Co-release of ATP with catecholamines from neurons in the hypothalamus and locus ceruleus has been reported, and there is recent evidence for co-release of ATP with $\gamma$-aminobutyric acid (GABA) in dorsal horn and lateral hypothalamic neurons, and for ATP with glutamate in the hippocampus. Co-localization of functional nicotinic and ionotropic nucleotide receptors has been identified in isolated cholinergic synaptic terminals in the midbrain; ATP and dopamine (DA) are probably co-released from the terminals of ventral tegmental neurons in the nucleus accumbens.

Co-release and interaction of two fast inhibitory cotransmitters, GABA and glycine, in synaptic bouton preparations of the sacral dorsal commissural nucleus of the sacral spinal cord have been described. Earlier papers showed that glycine/GABA cotransmission occurred in brain stem motor neurons and spinal interneurons. Co-release of NA and DA from neurons in the cerebral cortex has also been reported. Neurons in the tuberomammillary nucleus in the posterior hypothalamus contain histamine, GABA, galanin, enkephalin, and SP as cotransmitters. GABA/somatostatin cotransmission has been reported at synapses in a subpopulation of amygdala projection neurons to the nucleus tractus solitarius, which might inhibit cardiovascular reflex responses to fear or emotion-related stimuli.

CCK is co-localized with DA in rat mesencephalic neurons, and with glutamate in corticostriatal neurons; released CCK appears to be involved in locomotor behavior. Synthesis and storage of glutamate, ACh, and GABA in basal forebrain neurons projecting to the entorhinal cortex have been reported. Galanin is co-stored with enkephalin, and often NPY, in some neurons of the substantia gelatinosa, whereas tachykinins and enkephalin, galanin, and SP are co-stored in neurons in deeper layers of the dorsal horn. Co-storage of galanin and neurotensin, as well as CGRP and SP, has been shown by postembedding immunocytochemistry to be present in LGVs. Reverse transcription polymerase chain reaction (RT-PCR) and *in situ* hybridization studies have shown co-storage of oxytocin and vasopressin mRNA in LGVs in hypothalamic neurosecretory neurons.

## Physiological Significance of Cotransmission

Several major themes have emerged about the physiology of cotransmission (see **Figure 3**).

### Fast and Slow Cotransmitters: Different Firing Patterns

Although single presynaptic action potentials release small molecule neurotransmitters, trains of impulses are needed to release neuropeptides. For sympathetic and parasympathetic cotransmission, release of ATP is favored at low-frequency stimulation, whereas NA and ACh are released at higher frequencies. There are instances where more than one fast cotransmitter is released (e.g., glutamate and ATP) together with one or more peptides. See Figures 3(a) and 3(b).

### Different Cotransmitters Act on Different Postjunctional Cells

Neurons using multiple transmitters may project to two or more targets (Figure 3(c)). For example, ACh released at low-frequency stimulation from parasympathetic nerves supplying salivary glands acts on acinus cells to produce secretion and a minor dilatation of vessels, whereas at higher frequency stimulation, its cotransmitter VIP causes powerful vasodilatation of vessels in the glands and postjunctional enhancement of ACh-induced saliva secretion.

### Presynaptic Neuromodulation of Cotransmitter Release

A cotransmitter can feed back on presynaptic receptors that increase or decrease its own release or that of its cotransmitter(s) (Figure 3(d)). For example, ATP released as a cotransmitter with glutamate from primary afferent fibers in lamina II of the spinal cord can act on prejunctional $P2X_3$ receptors to facilitate the release of its cotransmitter, glutamate, whereas adenosine resulting from ectoenzymatic breakdown of ATP acts on presynaptic P1 receptors to inhibit glutamate release. Both NA and ATP can prejunctionally modulate sympathetic transmission, NA via prejunctional $\alpha_2$-adrenoceptors and ATP via P1 receptors following breakdown to adenosine. Modulation of cotransmitter release and presynaptic action by other agents also occurs and might provide a new level of synaptic flexibility, in which individual neurons utilize more than one transmitter but retain independent control over their synaptic activity.

### Synergism

There are an increasing number of reports of the synergistic actions of cotransmitters (Figure 3(e)). ATP and NA released from sympathetic nerves have synergistic actions on smooth muscle of vas deferens and blood vessels, and ATP released with ACh from motor neurons facilitates the nicotinic actions of ACh at the skeletal neuromuscular junction. Cooperativity between receptors for ATP and *N*-methyl-D-aspartate (NMDA) in induction of long-term potentiation in hippocampal CA1 neurons has also been demonstrated. Thyrotropin-releasing hormone and serotonin have been reported to have synergistic actions in spinal cord neurons.

In view of the evidence for cotransmitter synergy, the reports that known nucleotide P2 receptor antagonists, such as suramin, have actions on nonpurinergic receptors need to be questioned. For example, the claims that suramin and reactive blue 2 have antagonistic actions on NMDA and GABA receptor channels in hippocampal neurons are probably explained by blockade of the P2 receptor-mediated responses of the cotransmitter ATP, thereby removing its synergistic potentiating effect.

The mechanisms underlying cotransmitter synergism are not well understood. However, it has been suggested that postjunctional synergism between the responses of vas deferens to NA and ATP is caused by the ability of NA to potentiate the contractile responses to ATP by sensitizing smooth muscle cells to $Ca^{2+}$ via an inhibitory action on myosin light chain phosphatase, an action mediated by protein kinase C.

### Negative Cross-Talk

Coapplication of nicotinic and P2X receptor agonists produces less than the additive responses predicted by independent receptor activation (Figure 3(f)). Inhibitory interactions between 5-$HT_3$ and P2X receptors have been described in submucosal and myenteric neurons. Cross-inhibition of $GABA_A$ and glycine receptors has been demonstrated in rat sacral dorsal commissural neurons.

### Cotransmitters and Trophic Factors

Some co-stored and co-released substances can act as long-term (trophic) factors, as well as neurotransmitters (Figure 3(g)). For example, ATP can act on P2 receptors, or P1 (adenosine) receptors after ectoenzymatic breakdown, to promote vascular cell proliferation, motility, differentiation, or death. NPY released from sympathetic nerves has cardiovascular trophic effects in end-stage renal disease. There is growing evidence that neurotrophic factors might be synthesized, stored, and released from nerve terminals together with fast neurotransmitters.

### Excitatory and Inhibitory Cotransmitters

Although cotransmitters generally have similar actions on postjunctional cells, there are some examples of cotransmitters having opposite actions. For example, in the mammalian uterus, one or other cotransmitter dominates depending on the hormonal and/or tonic status of the postjunctional muscle cells

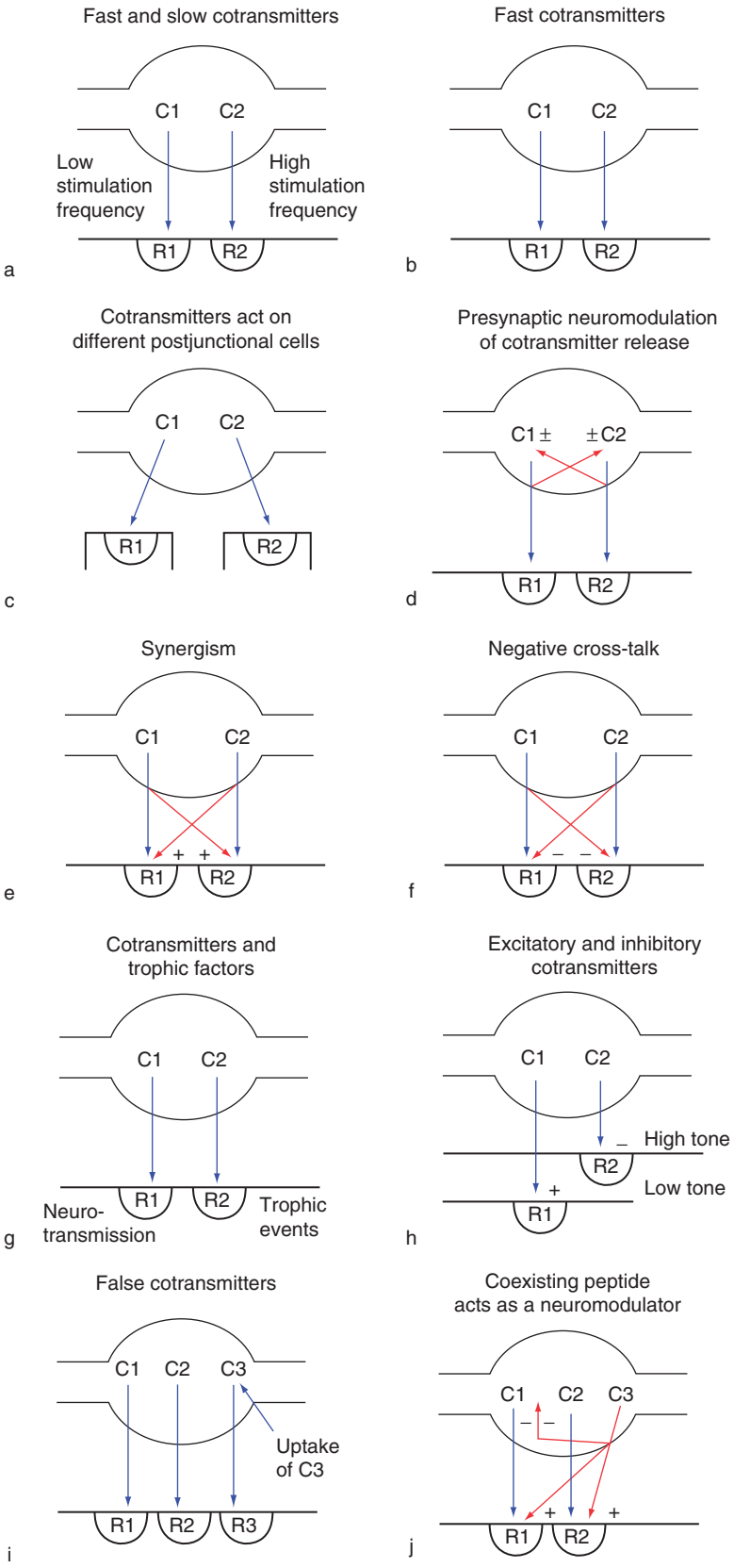


**Figure 3** Spectrum of signaling variations offered by cotransmission (blue arrows = neurotransmission; red arrows = pre- or postjunctional neuromodulation). (a) Fast transmission is usually produced by small molecules (C1) released at low-frequency nerve stimulation acting on ionotropic receptors (R1), whereas slow transmission is usually produced by release of peptides (C2) or other

(Figure 3(h)). Brain-derived neurotrophic factor (BDNF) increases the release of ACh and reduces NA release from sympathetic nerves to cause a rapid shift from excitatory to inhibitory transmission.

#### False Cotransmitters

For example, it has been known for some time that sympathetic nerves take up 5-HT, which can then be released as a 'false transmitter' rather than a genuine 'cotransmitter.' A false transmitter is a substance actively taken up and subsequently released by a neuron that does not synthesize it (Figure 3(i)).

#### Coexisting Peptide Acting as a Neuromodulator

For example, NPY released from sympathetic nerves acts as a pre- and postjunctional modulator of ATP and NA release and postjunctional actions (Figure 3(j)).

## Cotransmitter Plasticity: Control of Transmitter Expression

Cotransmitter plasticity occurs during development and aging, following trauma or surgery, and after chronic exposure to drugs, as well as in disease. There were some outstanding early studies of the factors influencing cotransmitter expression in sympathetic nerves, and a physiological role for neuropoietic cytokines in the control of VIP expression during the development of cholinergic sympathetic neurons was proposed. A more recent study presented evidence that cholinergic differentiation in sympathetic neurons is promoted by neurotrophic factors from three different protein families (glial cell line-derived neurotrophic factor, neurotrophin 3, and ciliary neurotrophic factor), whereas noradrenergic differentiation is promoted by nerve growth factor. In another study, BDNF was claimed to switch sympathetic neurotransmission to the heart from an adrenergic excitation to cholinergic inhibition; it was also shown that the action of BDNF was mediated by the P75 neurotrophic receptor. Histamine, galanin, and GABA acting as cotransmitters in neurons of the tuberomammillary nucleus (hypothalamus) have independent control mechanisms. Changes in chemical coding of myenteric neurons in ulcerative colitis have been reported, with a shift from cholinergic to more SP-positive cotransmission. In a study using primary cultures of neonatal rat spinal neurons, evidence was presented for the regulation of SP (NK1) receptor expression by CGRP.

CGRP-like immunoreactivity was found earlier than SP-like immunoreactivity in cerebrovascular nerves during development, and increased in old age, while the density of SP-like immunoreactive fibers did not change. NA and NPY also show different expression in cerebrovascular nerves during development. Direct evidence for changes in transmitter ratio in disease comes from a study of hypertension in which the purinergic component of sympathetic cotransmission has been claimed to be enhanced to the extent that it is the dominant component of the sympathetic response. In many isolated blood vessels, contractions produced by sympathetic nerve stimulation or due to vasoconstrictors including catecholamines and 5-HT are greater after the endothelium is removed or during antagonism of endothelium-derived relaxing factor. While part of this effect is likely to involve postjunctional mechanisms, evidence has been presented that substances released from the endothelium may act prejunctionally to influence neurotransmitter release from nerves. This may or may not involve endothelial-derived NO. ATP is also released from endothelial cells in response to physiological stimuli such as hypoxia or shear stress and may thus modulate the activity of perivascular nerves via prejunctional P1 receptors following breakdown to adenosine and diffusion through the vessel wall. Conversely, in the microvasculature, where neural-endothelial separation is small, cotransmitters released from nerves could act directly on endothelial cells to influence the release of endothelium-derived factors.

molecules at high frequency stimulation acting on G protein-coupled receptors (R2). (b) Cotransmitters C1 and C2 can both be fast messengers acting via ionotropic receptors (R1 and R2). (c) Cotransmitters C1 and C2 act on receptors (R1 and R2) localized on different postjunctional cells. (d) Cotransmitters C1 and C2 not only act postjunctionally via R1 and R2 receptors but can also act as prejunctional modulators to either inhibit (−) or enhance (+) the release of C1 and/or C2. (e) Cotransmitters C1 and C2 act synergistically to enhance the combined responses produced via R1 and R2 receptors. (f) Cotransmitters C1 and C2 act to inhibit the responses evoked via R1 and/or R2 receptors. (g) Cotransmitter C1 evokes neurotransmission via R1 receptors, while C2 evokes long-term (trophic) responses of postjunctional cells via R2 receptors. (h) Cotransmitter C1 produces excitation via R1 receptors when the postjunctional smooth muscle target has low tone, with C2 having little influence; however, when the smooth muscle tone is high, the dominant response might be relaxation produced by C2 via R2 receptors. (i) Substance C3 is taken up by nerve terminals rather than being synthesized and stored, as is true for the cotransmitters C1 and C2. C3 can then be released on nerve stimulation to act on postjunctional R3 receptors. In these circumstances, C3 would be known as a 'false transmitter.' (j) A coexisting substance C3 (often a peptide) can be synthesized and stored in a nerve but not act directly via a postjunctional receptor to produce changes in postjunctional cell activity. It could, however, act as a prejunctional inhibitor (−) of the release of the cotransmitters C1 and C2 or as a postjunctional enhancer (+) of the responses mediated by R1 and R2. Reproduced from Burnstock G (2004) Cotransmission. *Current Opinion in Pharmacology* 4: 47–52, with permission from Elsevier.

## Concluding Comments

To establish that compounds shown to be localized in nerves are actually cotransmitters, several criteria need to be satisfied:

1. The substance is synthesized and stored in the nerve.
2. The substance is released on nerve stimulation.
3. Specific receptors for the substance need to be identified on postjunctional sites that, when occupied, lead to changes in postjunctional activity.
4. A transport system needs to be present for the substance itself or its breakdown products, uptake of which leads to renewal of messenger storage in nerve terminals.

It has been particularly difficult to establish cotransmitter roles for the many peptides found in nerves, partly because specific receptors and physiological roles have not been established for some of these and partly because of the lack of selective antagonists. In some enteric neurons, up to five neuropeptides have been identified. It is important to distinguish between neuromodulator, neurotransmitter, and neurotrophic roles for released peptides.

It is becoming clear that ATP is a primitive signaling molecule that has been retained as a cotransmitter in every nerve type in both the peripheral and central nervous systems, although the relative role of ATP varies considerably in different species and pathophysiological conditions. ATP appears to become a more prominent cotransmitter in stress and inflammatory conditions. Most nerves contain and release ATP as a fast cotransmitter together with classical transmitters such as ACh, NA, glutamate, GABA, and one or more peptides. In view of this, it is recommended that the terms 'adrenergic,' 'cholinergic,' 'peptidergic,' 'purinergic,' 'aminergic,' and 'nitrergic' not be used when nerves are described, although adrenergic, cholinergic, peptidergic, purinergic, aminergic, or nitrergic transmission is still meaningful.

*See also:* Acetylcholine Neurotransmission in CNS; Adenosine Triphosphate (ATP); Adenosine Triphosphate (ATP) as a Neurotransmitter; Gamma-Aminobutyric Acid (GABA); Glutamate; Neutrotransmission and Neuromodulation: Acetylcholine; Noradrenaline; Serotonin (5-Hydroxtryptamine; 5-HT): Neurotransmission and Neuromodulation.

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