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COTRANSMISSION

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INTRODUCTION

Research in the last ten years has shown that individual neurons can contain two or more biologically active compounds, each a potential or proven transmitter substance. The discovery of such coexistence suggests that single neurons may simultaneously release more than one transmitter and that cotransmission may occur. This review describes evidence that cotransmission is a physiological process in vertebrates. It focuses on selected examples from the peripheral nervous system because proof of a transmitter role for one substance, let alone two, still requires that the transmission be both experimentally accessible and neurologically simple. The broader implications of the findings are then discussed.

It is appropriate to begin with two definitions: (*a*) *Transmitter substance:* Potter et al (1) use the simple definition that a transmitter is any substance, excluding trophic substances[1], released by a neuron to control its target cells. The definition may be the only one broad enough to include the clever theory (2) that K^+ ions, released from noradrenergic axons in the recovery phase of the axonal action potential, increase extracellular K^+ concentration and directly depolarize muscle membranes. In this way they produce the apparently nonadrenergic component of excitatory transmission to the vas deferens. As a transmitter, K^+ ions would have no synthesis, no storage vesicles, no breakdown mechanism, no postsynaptic receptors, nor any of the other accretions found in more complex definitions. But K^+ is indeed a transmitter, at least at one invertebrate synapse (3). (*b*) *Cotransmission:* This will be taken as the action of two transmitters, simultaneously released from the same neuron, on

[1]Trophic substances may be taken to affect the expression of the genome of a target cell, whereas transmitters act on the already expressed genome. There is every chance that some substances are both transmitters and trophic substances.

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a single target cell. The definition is narrow but, as described in "Discussion," broader definitions become meaningless.

BACKGROUND

Elliott (4) put forward the concept of chemical neurotransmission in 1905; in 1921 Loewi (5) provided

Dale (6) was able to propose that two basic chemical transmission processes, cholinergic and adrenergic, mediate nearly all peripheral transmissions in vertebrates. The evidence seemed clear that a substance, whether acetylcholine (ACh), norepinephrine (NE), or something else, was responsible for each transmission. For lack of evidence to the contrary, the concept that a nerve releases "a substance" came to mean "a single substance." The generally unstated belief that one neuron acts via one transmitter substance dominated ideas of transmission into the 1970s. The single-transmitter concept is not "Dale's principle" (7), which was, in essence, that a single neuron probably releases similar substances at all of its synapses (1).

The first serious attack on the single-transmitter concept came from Burn & Rand (8, 9) in the late 1950s. They noted that many drugs that act on cholinergic systems also affect adrenergic transmission. They proposed that when a noradrenergic axon is activated it releases ACh, which re-acts on that axon to trigger the further release of NE. Although the Burn–Rand theory did not survive rigorous inspection (10), it started cracks in the belief in solitary transmitters.

Almost as soon as histochemical techniques for demonstrating transmitter substances became available, evidence was produced that two chemically unrelated, biologically active substances could be colocalized in one neuronal type. In the sympathetic noradrenergic innervation of the pineal gland, NE and serotonin could be colocalized, not just to the same group of nerve fibers (11) but also to the same intraneuronal storage vesicle (12). However, this example may be dismissed as a local phenomenon: the serotonin is not synthesized in the neurons but is taken up into them from an extracellular pool of serotonin leaking out of pinealocytes (13). Once inside the neuron, serotonin parasitizes NE storage vesicles, from which both amines can be released by nerve stimulation (14). In this case, serotonin can be regarded as a false transmitter, and the neuron must still be regarded as adrenergic.

The next steps towards acceptance of cotransmission came from studies of identifiable giant neurons in molluscs. In brief, it was shown that one neuron can synthesize and store two potential transmitters (15, 16) and that a single cell can produce synaptic depolarizations in a target cell, mediated jointly by two transmitters, in this case ACh and serotonin (17). The validity of the

experiments is still disputed (18, 19), but these studies paved the way for the first, but still the clearest, example of cotransmission by a vertebrate neuron.

EXAMPLES

Sympathetic Neurons in Culture: NE-ACh

The clearest possible evidence of cotransmission was obtained from studies of sympathetic neurons grown in culture. The studies have been reviewed (e.g. 20–23), and primary references are not used in this summary.

When sympathetic postganglionic neurons are grown by themselves in culture, without non-neural cells or cell factors, they have the biochemical and structural characteristics of noradrenergic neurons. But when cultured with certain non-neural cells or in medium in which such cells have grown, the neurons develop cholinergic characteristics. During the change from noradrenergic to cholinergic function, the neurons show dual function and sustain a NE-ACh cotransmission. This cotransmission has been shown unequivocally in experiments in which a single neuron is allowed to grow on and to innervate a small cluster of heart myocytes. In these microcultures, the muscle acts not only as a bioassay of the transmitter (or transmitters) released from the neuron but also as the initiator of the conversion from noradrenergic to cholinergic function.

Stimulation of a neuron in an adrenergic form, as seen after brief culture, causes depolarization of the myocytes and acceleration of spontaneous firing, effects blocked by β-adrenoceptor antagonists. Electron microscopic study of such neurons loaded with 5-hydroxydopamine shows that 70–80% of the small synaptic vesicles in nerve varicosities have an electron-dense core, i.e. they are small granular vesicles, typical of noradrenergic axons. Neurons of cholinergic form are found in older cultures. When stimulated, they cause hyperpolarization and inhibit spontaneous firing of myocytes, effects blocked by atropine; the small synaptic vesicles contain no granular core (small, clear vesicles).

Most of the cultured neurons have mixed noradrenergic and cholinergic properties: stimulation commonly causes inhibition followed by excitation of myocytes, blocked by atropine and β-adrenoceptor antagonists respectively; the neurons contain a mixture of small clear and small granular vesicles, but there are fewer granular vesicles than in purely noradrenergic forms. In one single case, serial physiological studies were made on one neuron over a 45-day period, during which time the neuron changed from noradrenergic to dual-function to cholinergic.

The results leave no doubt that a single neuron can show NE-ACh cotransmission, at least as an interim state during development in vitro.

NE-ACh cotransmission might indeed occur as a temporary state during in vivo development of the sympathetic neurons innervating rat eccrine sweat glands (24), but there is little evidence that it occurs in any adult mammalian neuron (cf. 20). However, the definitive sympathetic postganglionic innervation of splenic muscle in the codfish *Gadus* may show such a cotransmission: the response to nerve stimulation is jointly mediated by NE and ACh (25), and both components of the response are eliminated by adrenergic neuron blockade or by treatment with 6-hydroxydopamine (25–27).

Some of the cultured neurons show another dual transmission involving either NE or ACh together with a different cardioinhibitory substance. The third substance appears to be adenosine or a related purine-based compound (23). To date, no neuron transmitting via all three compounds has been found, but there is no obvious reason why that should not happen.

Frog Sympathetic Ganglia: ACh-LHRH

The sympathetic chains of the bullfrog *(Rana catesbeiana)* contain a peptide that has luteinizing hormone–releasing hormone (LHRH)-like immunoreactivity (IR) (28). The LHRH-like material resembles authentic LHRH chromatographically (28), but is not identical to it (29). The peptide is released from the sympathetic chains in a Ca^{2+}-dependent manner by both high K^+ concentrations (28) and electrical stimulation of preganglionic nerves (30). The LHRH-IR has been localized to synaptic boutons on ganglion cells (30, 31). Both the immunoreactive boutons and the assayable LHRH-IR are largely lost after preganglionic denervation (30, 31).

The most posterior (ninth and tenth) ganglia of the sympathetic chains contain postganglionic neurons innervated in two different ways (see 32): B cells are innervated by rapidly conducting axons arising from the anterior sympathetic outflow; smaller C cells are innervated by slowly conducting axons arising from the spinal nerves 7 and 8. Stimulation of preganglionic fibers in the anterior chain produces, in B cells only, both fast and slow cholinergic excitatory postsynaptic potentials (epsp) mediated by nicotinic and muscarinic receptors, respectively. Stimulation of preganglionic fibers in spinal nerves 7 and 8 elicits a fast nicotinic epsp and a slow muscarinic inhibitory potential (ipsp) in C cells only. But, in addition, repeated stimulation of spinal nerves 7 and 8 produces, in both B and C cells, a noncholinergic depolarization lasting for several minutes, the late slow epsp (LSepsp) (33, 34).

Electrophysiological evidence shows that the LSepsp is mediated by the LHRH-like substance (30, 35). Ejection of authentic LHRH from a micropipette near a ganglion cell causes a prolonged depolarization and changes in membrane conductance, similar to the events of the LSepsp in both extent and time course. The LHRH-induced depolarization and the LSepsp are similarly

affected by applied shifts of neuronal membrane potential (see also 36). Certain LHRH analogs, for example [D-*p*Glu[1],D-Phe[2],D-Trp[3,6]]-LHRH, antagonize both the LSepsp and the LHRH-induced depolarization without affecting ACh-mediated transmission.

It appears that the LHRH-like material is in fact released from cholinergic preganglionic nerves. First, LHRH-like immunoreactivity is found in more than 90% of identified preganglionic terminals on C cells (30, 37) so it seems that there must be at least some overlap between the cholinergic and the LHRH-positive fiber populations. Second, as the strength of preganglionic stimulation is gradually increased, the cholinergic epsp shows up to five abrupt increments in height. Each increment represents the recruitment of another cholinergic fiber. With each recruitment, the LSepsp also increases in amplitude (37). Either each cholinergic fiber is matched by a peptidergic fiber with exactly the same threshold, or, more likely, each fiber is both cholinergic and peptidergic.

Curiously, although both B and C cells show the LSepsp, the B cells are remote from the LHRH-positive boutons, found predominantly on C cells (30). It seems that both the ACh and the peptide released from the terminals can reach C cells, but that only the peptide can survive diffusion over many micrometers to the B cells.

The peptidergic transmission may be physiological or an artifact of stimulation. The LSepsp can cause a discharge of postganglionic action potentials but it is normally subliminal (30, 33). But even a sub-threshold depolarization should make the nicotinic cholinergic transmission more effective (38). On the other hand, the LSepsp is not produced by a single stimulus and is best seen after periods of stimulation at 5–10 Hz (30), which may be greater than the (unknown) frequency of preganglionic action potentials in vivo.

Toad Cardiac Innervation: ACh-SOM

The postganglionic parasympathetic neurons innervating the heart of amphibians lie in the interatrial septum (see 39). The neurons innervate all cardiac chambers, including the single ventricle (40), and are the archetype of cholinergic neurons (5). In the toad *Bufo marinus,* the neurons appear to show cotransmission to heart muscle via ACh and somatostatin (SOM) (41).

All of the neurons on the toad interatrial septum contain SOM-like-IR. SOM-IR varicose nerve fibers lie on cardiac muscle throughout the heart. SOM applied to in vitro preparations acts slowly to inhibit the pacemaker and reduce the force of beat of driven atria, but is without effect on the ventricle. The negative chronotropic and inotropic effects of applied SOM are strongly tachyphylactic.

Vagal stimulation slows the spontaneously beating toad heart; it also inhibits the force of beat of driven atria and ventricles. Stimulation at 1 Hz can

stop the spontaneously beating heart within 2 beats and can rapidly suppress detectable beating of driven atria, effects that are abolished by muscarinic antagonists. But when stimuli are applied at 3 Hz or more, the effects on the pacemaker and on atrial force of beat are not abolished even by high concentrations of hyoscine. (Effects on the ventricle are abolished by hyoscine.) The responses surviving muscarinic blockade are slow to develop, and resemble the response to SOM. Similar responses, in this case to postganglionic nerve stimulation, are seen when the cholinergic transmission is inhibited by treatment with hemicholinium-3 (42).

Induction of tachyphylaxis to SOM specifically inhibits the nonmuscarinic vagal effects. Conversely, when vagal transmission is "fatigued" by repeated bursts of stimulation the response to SOM is suppressed, as if by a tachyphylaxis induced by stimulation. The simplest explanation of the results is that the vagal postganglionic neurons inhibit the pacemaker and atrial muscle by releasing SOM.

Since the cholinergic postganglionic neurons lie in the heart, and since all of the intracardiac neurons contain SOM-IR, it follows that all of the cholinergic postganglionic neurons contain SOM. The evidence does not prove that any one neuron can release both ACh and SOM, but that mechanism seems most likely.

It is not clear whether the SOM mechanism is used in the normal physiology of the animal. The frequencies of stimulation needed to produce SOM-mediated transmission, although quite low, may exceed vagal firing rates occurring in the intact toad.

Cholinergic Postganglionic Neurons: ACh-VIP

Vasoactive intestinal peptide (VIP) is a 28–amino acid peptide that is widely distributed in peripheral and central neurons (see 43). It relaxes many smooth muscles and may be a transmitter substance in its own right in, for example, the gut. Lundberg and his colleagues (44–46) have concluded that certain postganglionic neurons in both parasympathetic and sympathetic pathways, mediating vasodilation in exocrine glands (e.g. nasal, salivary, pancreatic, and sweat glands), act by the corelease of ACh and VIP.

The case for ACh-VIP cotransmission to blood vesels in exocrine glands is best documented for the parasympathetic vasodilator innervation of cat salivary glands. There is good evidence that the dilator response is mediated in part by ACh. Nerve stimulation causes ACh release (47). Atropine, which prevents the vasodilation caused by ACh (48), reduces the response to low-frequency nerve stimulation (49) and indeed abolishes the dilation elicited by a single stimulus (50). But the innervation also appears to act in part via VIP. The vasculature is provided with VIP-IR axons (see 51), and nerve stimulation, especially with higher frequencies, causes VIP release (44, 47,

51–54). Atropine does not antagonize the response to VIP (44, 48, 52) or to high-frequency stimulation (49, 55). Finally, infusion of VIP antiserum reduces the dilator response to stimulation (44, 56).

It might be argued that the VIP-mediated response seen after atropine is artifactual, since atropine markedly increases the release of VIP on nerve stimulation (53, 57), presumably by preventing a muscarinic presynaptic inhibition. However, the reduction of the normal response by VIP immunoblockade shows that a VIP transmission occurs even while cholinergic transmission is functional.

Until recently when antibodies for choline acetyltransferase became available, there was no reliable histochemical marker for cholinergic neurons. Lundberg and his colleagues (58) used acetylcholinesterase as a marker, while aware that the enzyme is not restricted to cholinergic neurons. They found that many peripheral neurons in the cat contain VIP-IR, but are not obviously reactive for acetylcholinesterase. But the neurons innervating exocrine glands are both VIP-IR and acetylcholinesterase-positive (44, 45, 58). For example, the cat sphenopalatine ganglion contains the cholinergic neurons innervating the nasal mucosa (59); since 98.5% of the neurons contain VIP-IR (60), ACh and VIP neurons almost certainly overlap. However, another study (61) showed that sphenopalatine neurons are normally either VIP-IR or rich in acetylcholinesterase; only after treatment with colchicine do all neurons contain both markers. The results seem to show that the neurons innervating exocrine glands are able to express both ACh and VIP, but the possibility remains that some specialize in VIP, whereas others produce mainly ACh. Apart from that evidence, the possibility that the neurons show ACh-VIP cotransmission remains a good working hypothesis.

The vasodilator cotransmission may in fact involve three transmitters. Immunoreactivity to another vasodilator polypeptide, PHI-27 (62), is also released from the submandibular gland by parasympathetic stimulation (57). The PHI-like material detected may have been identical to or homologous with a very similar peptide, PHM-27, which is part of the VIP precursor molecule synthesized in human neuroblastoma cells (63). Thus, both of the released peptides may arise from the one precursor molecule.

ACh-VIP-PHI vasodilator cotransmission probably occurs in vivo. Natural firing rates of the parasympathetic vasomotor nerves are unknown, but may resemble the firing rate in the secretomotor fibers. The fastest reflex salivation seen in dogs can be matched by parasympathetic stimulation at 4–8 Hz (64). The average firing rate of secretory nerve fibers in rabbit, recorded directly during a reflex salivation, ranged as high as 20 s^{-1}, and bursts of much higher frequency can be seen in the records (65). These firing rates exceed the rates of stimulation needed to produce both ACh- and VIP-mediated responses (49, 56).

Adrenergic Postganglionic Neurons: NE-NPY

Several biologically active peptides have been localized to noradrenergic sympathetic neurons, using double or sequential labeling with antibodies to the peptide and to a synthetic enzyme for NE (see 66, 67). For example, substance P-IR is found in many neurons of the rat superior cervical ganglion (68). Enkephalin-IR is also found in some neurons of this ganglion in the rat (69), but not the cat (70). SOM-IR occurs in some neurons in paravertebral ganglia of guinea pigs (71), but there are fewer in the rat (69) and apparently none in the cat (67, 70). Finally, a large subpopulation of noradrenergic neurons contains pancreatic polypeptide-like-IR in all mammals studied. Although there are several 36-amino acid peptides in the pancreatic polypeptide family, the material in noradrenergic neurons is probably neuropeptide Y (NPY) (see 72), and is referred to as such here. In this last case, there is some evidence for a NE-peptide cotransmission.

NPY-IR is found in a large proportion of sympathetic noradrenergic neurons (73–77). In the cat, for example, between 25% and 75% of the neurons in various para- and prevertebral ganglia contain NPY-IR (75). Some NE-NPY neurons in cattle also contain enkephalin-IR (78).

NPY-IR is found in apparently all noradrenergic neurons innervating mammalian blood vessels (73, 76, 77, 79–82) and in the adrenergic neurons innervating amphibian blood vessels (83). Certain nonvascular muscles also receive NE-NPY fibers, e.g. the heart (73, 75, 84, 85), the vas deferens (75, 86), and tracheobronchial muscle (75). However, the noradrenergic innervation of other nonvascular effectors, at least in some species, lacks NPY: iris (73); nictitating membrane (75); exocrine parenchyma of salivary glands (73, 76); myenteric and submucous ganglia of the intestine (80). The adrenergic innervation of the amphibian heart also lacks NPY (83).

The NPY-IR in noradrenergic nerve fibers can be depleted by treatment with the adrenergic neurotoxin 6-hydroxydopamine (73, 74, 76, 80, 82). Stimulation of vascular sympathetic nerves releases NPY, and the release is prevented by adrenergic neuron blockade (87, 88). Thus NPY appears to be both stored in and released from noradrenergic nerves, raising the possibility of NE-NPY cotransmission. In fact, NE and NPY have been argued to be vasoconstrictor cotransmitters to salivary gland vasculature (89), pial blood vessels (90, 91), and the spleen (78, 87, 88, 92). The latter case is particularly strong.

In the cat spleen (88), stimulation of the splenic nerve normally releases both NE and NPY. When the sympathetic innervation is intact, reserpinization depletes the splenic content of both NE and NPY: the neurogenic release of both NE and NPY is reduced, as are the splenic contractile and vasoconstrictor responses to nerve stimulation. But much of the action of reserpine on NPY seems to involve sympathetic nerve activity (93). Thus, reserpine has

little effect on NPY levels after preganglionic nerve section, although it depletes NE as usual. In the reserpinized, decentralized spleen, nerve stimulation does not release NE, but the release of NPY is greater than normal, and the nerve-mediated contraction and constriction are strong. These responses are mimicked by applied NPY: they are barely affected by adrenoceptor blockade, but are still abolished by adrenergic neuron blockade. Reserpinization of the pig spleen (94), even with the sympathetic innervation intact, reduces neurogenic NE release. However, NPY release is greater than normal: nerve stimulation now causes a marked vasoconstriction that is resistant to adrenoceptor blockade and is mimicked by NPY. Therefore, in both cat and pig spleen, NPY released from adrenergic nerves seems to be a significant vasoconstrictor transmitter, at least when the release of NE has been reduced. However there is little evidence that NPY is a transmitter when NE release is normal. In fact, the above findings suggest that released NE normally suppresses NPY release. Experiments with, for example, specific antagonists of NPY are needed to establish that there is a simultaneous release of both NPY and NE in physiologically significant amounts, i.e. that cotransmission occurs.

DISCUSSION

Coexistence, Corelease, and Cotransmission

In the excitement of novel research, imprecise terminology is often used, sometimes at the expense of clarity in experimental design. It is worth examining the three terms that head this section.

The term *coexistence* presents little semantic problem. Substances are found to coexist by means of anatomically based studies of tissues or cells, so that the colocalization is always referred to an anatomical unit, e.g. a neuron or a synaptic vesicle. It seems obvious that coexistence is a necessary, but not sufficient, condition for cotransmission. For example, the cultured sympathetic neurons described above form functional synaptic connections to themselves ("autapses"). Transmission at autapses seems to be wholly cholinergic, even in neurons that have NE-ACh cotransmission to heart cells (23). While NE and ACh probably coexist at the autapse, NE has no apparent effect on the neurons, and there is no cotransmission. Incidentally, virtually every active amine or peptide localized to a neuron is released by nerve stimulation in one preparation or another: therefore, when these substances are colocalized they are probably also coreleased.

Corelease has an apparently simple meaning: the release of two or more transmitter substances together from one neuron. However, the word lacks precision: it is arbitrary how closely related in space and time the releases must be before corelease occurs. For example, the release of two transmitters

by exocytosis from one synaptic vesicle is clearly corelease. But if a neuron, in defiance of the authentic "Dale's principle," released one transmitter from its dendrites and another from remote axon terminals, it would not constitute a meaningful corelease. The anatomical "unit of corelease" must lie between these extremes, but it can be defined only pragmatically. For instance, the NE-ACh cultured sympathetic neurons contain both small, granular vesicles, and small, clear synaptic vesicles, which may represent discrete stores of NE and ACh, respectively. If so, the smallest possible anatomical unit that could sustain NE-ACh corelease is a single varicosity, in which the two types of vesicle are mixed (23). But the minimal unit of corelease might prove to be much larger, e.g. several varicosities. In short, since the maximal anatomical unit for corelease cannot be defined absolutely, the word is only vaguely helpful. The critical limit in time for corelease is easier to define because that corelease clearly implies that substances emerge from a neuron in response to the same event, e.g. an action potential. From what is known of the transmitter-releasing effects of action potentials, the time window delimiting corelease might be as short as a few milliseconds, but it might be found to be much longer.

Corelease is a necessary, but not a sufficient, condition for cotransmission. In the ventricle of the toad heart, for example, SOM is probably released with ACh from vagal postganglionic neurons, but it has no known action on the muscle (41). In spite of the corelease, there is an apparently pure cholinergic transmission, not cotransmission.

Cotransmission could be defined from the point of view of either the releasing neuron or the target cell. It is used, confusingly, in both senses in the literature. The definition used here is expressed in terms of the target, for the following reasons. A chemically transmitting neuron is a device for converting an input into a release of substances. What is released is, in a sense, irrelevant to the neuron; the process of release probably reflects a single action, e.g. an entry of Ca^{2+}, regardless of how simple or complex the mixture of substances released and whether all of the substances released are transmitters. It is only when a target is defined, even if the target is the neuron itself, that the source and the nature of what is released become important. For example, it has been proposed that noradrenergic axons in the vas deferens corelease NE and NPY, the NE to act on the muscle and the NPY to act back on the axon, inhibiting further release (74, 95, 96). Such a transmission would clearly show a duality, but, in its effect, it would not differ from two completely separate transmission processes. It seems that, if cotransmission has any unique physiological significance, it would be that two or more coreleased transmitters act on the same target cell, so that the net result of transmission incorporates interactive effects of the transmitters. Again using the vas deferens as an example, researchers have proposed that neurogenic

excitation is mediated in part by adenosine triphosphate and in part by NE (see 97–99). Since the whole response is antagonized by, for example, destruction of the noradrenergic innervation with 6-hydroxydopamine (100), it seems that the NE and adenosine triphosphate must be coreleased from noradrenergic nerves. In this case, where both substances act on the muscle, the conditions for cotransmission would be met.

The definition of cotransmission from the point of view of the target cell leads to conclusions that are, at first sight, strange. For example, the amphibian preganglionic ACh-LHRH neurons cotransmit to sympathetic C cells, but at the same time these terminals are not cotransmitting to B cells, which are affected only by the peptide (30).

Cotransmission as a Physiological Process

Cotransmission, as defined above, clearly does occur in experimental situations. The first two examples considered, NE-ACh neurons in culture and amphibian ACh-LHRH neurons, are the only vertebrate cases that show convincingly that one neuron can transmit to a single target cell via at least two transmitters. With regard to mammalian ACh-VIP neurons and amphibian ACh-SOM neurons, there is no direct evidence that any one neuron acts via both substances, but cotransmission is a reasonable hypothesis. The final example, that of NE-NPY neurons, was chosen to represent the many other instances of coexistence of active substances in neurons (101, 102), for which the rudiments of a case for cotransmission are available.

There is virtually no evidence that cotransmission occurs during normal behavior. In most of the postulated instances of cotransmission, the transmitters each produce obvious effects such as contraction, secretion, or altered membrane potential. In these straightforward systems, pharmacological dissection in vivo should be able to eliminate the possibility that only one of the cotransmitters is normally used. However, no reports of appropriate experiments have been found. For example, it seems that nobody has determined whether atropine does or does not abolish the vasodilation that presumably occurs during salivation in response to feeding; without such evidence it remains possible that the parasympathetic ACh-VIP cotransmission to the vasculature of salivary glands is an experimental artifact.

With that proviso in mind, the scope for cotransmission as a physiological process seems to be enormous. On one hand, many substances are now recognized as potential transmitters. Added to the classical amine and amino acid transmitters are the active peptides, more than a dozen of which have been identified in neurons (e.g. 66). There are also unexpected additions to the list of potential transmitters. For example, the corelease of dopamine-β-hydroxylase and NE (103) seems to be an accidental consequence of the sequestration of the enzyme within NE storage vesicles. But extracellularly

applied dopamine-β-hydroxylase can affect cells, e.g. it can restore NE effects on desensitized pinealocyte β-adrenoceptors (104), and it might conceivably be a transmitter.

On the other hand, conceptions of what is meant by an "effect" of a transmitter substance are expanding. Koketsu (105) distinguished four types of synaptic actions of transmitters on target cells: (*a*) changing membrane potential or conductance, i.e. typical synaptic transmission; (*b*) changing the amplitude or time course of action potentials; (*c*) changing the amount of transmitter released, i.e. presynaptic modulation; and (*d*) changing the sensitivity of receptors for some other transmitter. This last type of effect, which might be called receptor modulation, is quite novel. In some cases, a potential transmitter has been shown to affect the availability or affinity of receptors for another compound, e.g. opiates and nicotinic receptors on adrenal medullary cells (106) and NPY and α_2-adrenoceptors in rat brain (107). In others, the efficacy of receptor occupation is changed, e.g. adenosine triphosphate and nicotinic receptors on frog skeletal muscle (108).

In each of the chosen examples of receptor modulation there is some evidence that both transmitter substances involved coexist in neurons in the organ. If cotransmission occurs, the "modulatory" transmitter might have the sole function of regulating the effect of the "major" transmitter, and have no effect at all if released alone. Indeed, evidence suggesting cotransmission by a major and a modulatory substance is already available. The salivation caused by parasympathetic stimulation in cats is fully blocked by atropine (55), and transmission has been regarded as simply cholinergic. However, treatment with VIP antiserum reduces the secretory response, suggesting partial mediation by VIP (56), but VIP by itself does not cause secretion (44, 52). It emerges that VIP increases the secretory response to ACh (48), perhaps by increasing the affinity of muscarinic receptors for ACh (109). Thus, it seems that the role of VIP in the salivatory ACh-VIP cotransmission is to promote transmission by ACh.

Until recently the complexity of nervous systems has seemed to derive from extensive, precisely located synaptic connections between neurons. Synaptic transmission has seemed relatively simple and susceptible to only a few integrative processes, e.g. spatial or temporal summation, facilitation, and fatigue. The existence of many possible transmitters and of many possible modes of action would necessitate a complex nervous system, even if each neuron released only one transmitter. But the occurrence of corelease means that one neuron may affect several different targets or, in the special case of cotransmission, a single target via a number of substances. This occurrence suggests a new order of complexity in the function of the nervous system, with interactions occurring between transmitters at both pre- and postsynaptic sites. One should not, however, be overwhelmed by the potential for

complexity. Dale (6) knew of only a few peripheral transmissions that he could not readily class as adrenergic or cholinergic. Even now, many transmissions still seem to be explicable in terms of one transmitter.

The rather simple appearance of many peripheral transmissions in spite of the potential for complexity raises several possibilities. First, substances coreleased with a major transmitter might often be receptor modulators, acting in ways not easily detectable unless reliable antagonists for the individual cotransmitters are available. Second, routine experimental protocols may be inadequate to detect cotransmission. For example, we tend to look for short-term effects of transmitters. But peptides can have actions lasting for an hour or more (e.g. 110). Yet, stimulus intervals in most experiments are 10 min or less, during which time the effects of a long-acting substance would be essentially constant. In hindsight, the only overt sign left by long-acting cotransmitters in many experiments might be the changes in response that usually occur over the first few stimulus repetitions at the beginning of an experiment, i.e. those that we tend to dismiss as "equilibration" of the preparation. Finally, transmissions seeming simple may indeed involve only one transmitter: active substances coreleased with a major transmitter might not play any role in transmission, either because the concentrations achieved are too low or because responsive targets are not present.

Can Neuronal Types Still be Recognized?

When it seemed that neurons acted via single transmitters, only three types of peripheral motor neuron were distinguished on functional grounds. Dale (6) recognized adrenergic and cholinergic nerves fifty years ago. In the 1960s, nonadrenergic, noncholinergic inhibitory neurons in the gut, lung, and some other tissues were recognized as a third type, acting perhaps via adenosine triphosphate or VIP (see 111, 112). Evidence for further distinct types is less secure. For example, the gut contains excitatory motor nerves that seem to act via substance P, but it remains possible that the substance P is in fact released as a cotransmitter from cholinergic nerves (113).

The many examples of colocalization show that, at the least, the previous neuronal types can be subdivided. A subdivision of noradrenergic neurons into four types, based on the peptides colocalized in them, has been discussed (66, 67). Similarly, the cholinergic (i.e. choline acetyltransferase-IR) neurons of the submucous plexus in guinea pig ileum can be divided into three subtypes: ACh-substance P; ACh-NPY-SOM-cholecystokinin; and ACh without known peptides (114). But, in principle, there is no reason why, for example, SOM neurons should not be subdivided, e.g. SOM-ACh (toad vagus, guinea-pig ileum) and SOM-NE (guinea-pig sympathetic). The associations of active substances in neurons might even be chaotic, so that neurons would be more realistically classified in a multidimensional array that ac-

knowledges no major transmitters. It even appears that neurons can express incomplete transmitter mechanisms. For example, the definitive adult ACh-VIP sympathetic neurons innervating rat eccrine sweat glands, having appeared in the neonate as noradrenergic, no longer synthesize detectable NE, yet they still express a presumably functionless catecholamine uptake (24). Half of the neurons in the guinea pig paracervical ganglion show neither tyrosine hydroxylase-IR nor detectable NE, but they express dopamine-β-hydroxylase-IR (J. L. Morris & I. L. Gibbins, personal communication), which, if biochemically active, would similarly have no obvious function.

However, the disposition of active substances is probably not merely chaotic. For example, some evidence points toward preferred groupings of "transmitters." Cultured rat sympathetic neurons can express SOM, substance P, choline acetyltransferase, and tyrosine hydroxylase. The conditions of culture can affect these expressions independently, but most circumstances favoring SOM expression also promote tyrosine hydroxylase, whereas substance P and choline acetyltransferase also are often regulated together (115). Furthermore, some of the neuronal subtypes that can be distinguished by colocalization of transmitters seem to be "natural" groups, related to a particular function. The noradrenergic innervation of the guinea pig ileum shows a detailed allocation of different neuronal subtypes to specific targets: NE-NPY fibers innervate blood vessels; NE-SOM fibers innervate muscle layers, submucous ganglia, and mucosa; NE fibers without known peptide complement innervate myenteric ganglia and share the innervation of mucosa (116). So a chemically defined neuronal type can in some circumstances be related to a specific target for innervation. The most likely reason for this specificity is that the innervated target determines at least part of the transmitter complement (21, 117, 118).

The existence of natural groupings of transmitters is supported by comparisons of homologous neurons between species. For example, the parasympathetic vasodilator innervation of salivary glands operates by ACh-VIP cotransmission in cat (45), dog (119), and rat (120, 121), but in rabbit it seems to be cholinergic with no other cotransmitter (122, 123). When cardiac vagal postganglionic neurons are compared between amphibians, ACh-SOM cotransmission is found in *Bufo* (41) and the unrelated clawed toad *Xenopus* (G. Campbell, unpublished observation). But in several other genera *(Neobatrachus, Limnodynastes, Litoria)* the neurons lack SOM-IR, the heart is unresponsive to SOM, and vagal transmission is fully blocked by muscarinic antagonists (G. Campbell, unpublished observation), as it is in *Rana* (124). If it is the influence of the target organ that dominates the range of substances expressed in the innervation, these two examples might be read as follows: (*a*) in each case, the target organ always permits, or perhaps causes, the expression of ACh in this part of its innervation; (*b*) in each organ, the neurons can express only certain other substances, e.g. VIP but not SOM in the salivary

gland, SOM but not VIP in the amphibian heart; and (*c*) such coexpression with ACh is not obligatory. In terms of evolution, it may be that each target has repeatedly, in the course of history, fostered the expression of the particular colocalized substance, but that the substance has been cemented into place in the cellular machinery only when a function for it has been found, in this case as a cotransmitter.

In closing, it is interesting that the "stable" transmitter in these last examples, ACh, provided support for the earlier classification of neurons into three types, adrenergic, cholinergic, and something else inhibitory. There may yet be truth in the older classification, but it would have to be a subtler truth than was thought even ten years ago.

SUMMARY

Cotransmission, defined here as the control of a single target cell by two or more substances released from one neuron in response to the same neuronal event, does occur in experimental situations. It has not been shown to occur in the normal operation of an animal, but the likelihood that it does is great.

There are many examples of potential transmitters coexisting in one neuron, suggesting that cotransmission might be widespread in the peripheral nervous system. But many transmissions still seem to be mediated by a single transmitter. In such cases, coreleased substances might act on other targets or modulate the receptors for the main transmitter. But the possibility also exists that some colocalized "transmitters" have no function in transmission.

It is increasingly difficult to retain a simple classification of neuronal types based on transmitter substances. However, there are indications that some combinations of colocalized substances are "preferred" and that certain combinations typify the innervation of a particular target tissue.

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Literature Cited

1. Potter, D. D., Furshpan, E. J., Landis, S. C. 1981. Multiple-transmitter status and "Dale's principle". *Neurosci. Comment.* 1:1–9
2. von Euler, U. S., Hedqvist, P. 1975. A tentative transmission mechanism for the twitch elicited in the guinea-pig vas deferens by field stimulation. *Med. Hypotheses* 1:214–16
3. Yarom, Y., Spira, M. E. 1982. Extracellular potassium ions mediate specific neuronal interaction. *Science* 216:80–82
4. Elliott, T. R. 1905. The action of adrenalin. *J. Physiol.* 32:401–67
5. Loewi, O. 1921. Über humorale Übertragbarkeit der Herznervenwirkung. I. *Pfluegers Arch.* 189:239–42

6. Dale, H. H. 1933. Nomenclature of nerve fibres in the autonomic system and their effects. *J. Physiol.* 80:10–11P
7. Dale, H. H. 1935. Pharmacology and nerve endings. *Proc. R. Soc. Med.* 28:319–32
8. Burn, J. H., Rand, M. J. 1959. Sympathetic postganglionic mechanisms. *Nature* 184:163–65
9. Burn, J. H., Rand, M. J. 1965. Acetylcholine in adrenergic transmission. *Ann. Rev. Pharmacol.* 5:163–82
10. Ferry, C. B. 1966. Cholinergic link hypothesis in adrenergic neuroeffector transmission. *Physiol. Rev.* 46:420–56
11. Owman, C. 1964. Sympathetic nerves probably storing two types of monoamines in the rat pineal gland. *Int. J. Neuropharmacol.* 2:105–12
12. Jaim-Etcheverry, G., Zieher, L. M. 1968. Cytochemistry of 5-hydroxytryptamine at the electron microscope level. II. Localization in the autonomic nerves of rat pineal gland. *Z. Zellforsch. Mikr. Anat.* 86:393–400
13. Jaim-Etcheverry, G., Zieher, L. M. 1982. Co-existence of monoamines in peripheral adrenergic neurones. See Ref. 101, pp. 189–206
14. Jaim-Etcheverry, G., Zieher, L. M. 1980. Stimulation-depletion of serotonin and noradrenaline from vesicles of sympathetic nerves in the pineal gland of the rat. *Cell Tissue Res.* 207:13–20
15. Kerkut, G. A., Sedden, C. B., Walker, R. J. 1967. Uptake of dopa and 5-hydroxytryptophan by monoamine-forming neurones in the brain of *Helix aspersa. Comp. Biochem. Physiol.* 23:159–62
16. Brownstein, M. J., Saavedra, J. M., Axelrod, J., Zeman, G. H., Carpenter, D. O. 1974. Coexistence of several putative neurotransmitters in single identified neurons of *Aplysia. Proc. Natl. Acad. Sci. USA* 71:4662–65
17. Cottrell, G. A. 1977. Identified amine-containing neurones and their synaptic connexions. *Neuroscience* 2:1–18
18. Osborne, N. N. 1979. Is Dale's principle valid? *Trends Neurosci.* 2:73–75
19. Osborne, N. N. 1982. Co-existence of neurotransmitter substances in a specifically identified invertebrate neurone. See Ref. 101, pp. 207–22
20. Burnstock, G. 1978. Do some sympathetic neurons synthesize and release both noradrenaline and acetylcholine? *Prog. Neurobiol.* 11:205–22
21. Patterson, P. H. 1978. Environmental determination of autonomic neurotransmitter function. *Ann. Rev. Neurosci.* 1:1–17
22. Potter, D. D., Landis, S. C., Furshpan, E. J. 1981. Adrenergic-cholinergic dual function in cultured sympathetic neurons of the rat. In *Development of the Autonomic Nervous System, Ciba Found. Symp.*, 83:123–38. London: Pitman
23. Furshpan, E. J., Potter, D. D., Landis, S. C. 1982. On the transmitter repertoire of sympathetic neurons in culture. *Harvey Lect.* 76:149–91
24. Landis, S. C., Keefe, D. 1983. Evidence for neurotransmitter plasticity in vivo: developmental changes in properties of cholinergic sympathetic neurons. *Dev. Biol.* 98:349–72
25. Nilsson, S., Grove, D. J. 1974. Adrenergic and cholinergic innervation of the spleen of the cod *Gadus morhua. Eur. J. Pharmacol.* 28:135–43
26. Holmgren, S., Nilsson, S. 1976. Effects of denervation, 6-hydroxydopamine and reserpine on the cholinergic and adrenergic responses of the spleen of the cod, *Gadus morhua. Eur. J. Pharmacol.* 39:53–59
27. Winberg, M., Holmgren, S., Nilsson, S. 1981. Effects of denervation and 6-hydroxydopamine on the activity of choline acetyltransferase in the spleen of the cod, *Gadus morhua. Comp. Biochem. Physiol.* 69C:141–43
28. Jan, Y. N., Jan, L. Y., Kuffler, S. W. 1979. A peptide as a possible transmitter in sympathetic ganglia of the frog. *Proc. Natl. Acad. Sci. USA* 76:1501–5
29. Eiden, L. E., Eskay, R. L. 1980. Characterization of LRF-like immunoreactivity in the frog sympathetic ganglia: non-identity with LRF decapeptide. *Neuropeptides* 1:29–37
30. Jan, L. Y., Jan, Y. N. 1982. Peptidergic transmission in sympathetic ganglia of the frog. *J. Physiol.* 327:219–46
31. Jan, L. Y., Jan, Y. N., Brownfield, M. S. 1980. Peptidergic transmitters in synaptic boutons of sympathetic ganglia. *Nature* 288:380–82
32. Dodd, J., Horn, J. P. 1983. A reclassification of B and C neurons in the ninth and tenth paravertebral sympathetic ganglia of the bullfrog. *J. Physiol.* 334:255–69
33. Nishi, S., Koketsu, K. 1968. Early and late after discharges of amphibian sympathetic ganglion cells. *J. Neurophysiol.* 31:109–21
34. Libet, B., Chichibu, S., Tosaka, T. 1968. Slow synaptic responses and excitability in sympathetic ganglia of the bullfrog. *J. Neurophysiol.* 31:383–95
35. Kuffler, S. W. 1980. Slow synaptic responses in autonomic ganglia and the

pursuit of a peptidergic transmitter. *J. Exp. Biol.* 89:257–86
36. Kuffler, S. W., Sejnowski, T. J. 1983. Peptidergic and muscarinic excitation at amphibian sympathetic synapses. *J. Physiol.* 341:257–78
37. Jan, Y. N., Jan, L. Y. 1981. Coexistence and co-release of acetylcholine and the LHRH-like peptide from the same preganglionic fibers in frog sympathetic ganglia. *Neurosci. Abstr.* 7: 603
38. Schulman, J. A., Weight, F. F. 1976. Synaptic transmission: long-lasting potentiation by a postsynaptic mechanism. *Science* 194:1437–39
39. McMahan, U. J., Kuffler, S. W. 1971. Visual identification of synaptic boutons on living ganglion cells and of varicosities in postganglionic axons in the heart of the frog. *Proc. R. Soc. London Ser. B* 177:485–508
40. Gaskell, W. H. 1882. On the rhythm of the heart of the frog, and on the nature of the action of the vagus nerve. *Philos. Trans. R. Soc. London* 173:893–1033
41. Campbell, G., Gibbins, I. L., Morris, J. L., Furness, J. B., Costa, M., et al. 1982. Somatostatin is contained in and released from cholinergic nerves in the heart of the toad *Bufo marinus*. *Neuroscience* 7:2013–23
42. Campbell, G., Jackson, F. 1985. Independent co-release of acetylcholine and somatostatin from cardiac vagal neurones in toad. *Neurosci. Lett.* 60:47–50
43. Said, S. I., ed. 1982. *Vasoactive Intestinal Peptide*. New York: Raven
44. Lundberg, J. M., Änggård, A., Fahrenkrug, J., Hökfelt, T., Mutt, V. 1980. Vasoactive intestinal polypeptide in cholinergic neurons of exocrine glands: functional significance of co-existing transmitters for vasodilatation and secretion. *Proc. Natl. Acad. Sci. USA* 77:1651–55
45. Lundberg, J. M. 1981. Evidence for coexistence of vasoactive intestinal polypeptide (VIP) and acetylcholine in neurons of cat exocrine glands. Morphological, biochemical and functional studies. *Acta Physiol. Scand. Suppl.* 496:1–57
46. Lundberg, J. M., Änggård, A., Fahrenkrug, J., Johansson, O., Hökfelt, T. 1982. Vasoactive intestinal polypeptide in cholinergic neurons of exocrine glands. See Ref. 43, pp. 373–89
47. Lundberg, J. M., Änggård, A., Fahrenkrug, J., Lundgren, G., Holmstedt, B. 1982. Corelease of VIP and acetylcholine in relation to blood flow and salivary secretion in cat submandibular salivary gland. *Acta Physiol. Scand.* 115:525–28
48. Lundberg, J. M., Änggård, A., Fahrenkrug, J. 1982. Complementary role of vasoactive intestinal polypeptide (VIP) and acetylcholine for cat submandibular gland blood flow and secretion. III. Effects of local infusions. *Acta Physiol. Scand.* 114:329–37
49. Darke, A. C., Smaje, L. H. 1972. Dependence of functional vasodilatation in the cat submaxillary gland upon stimulation frequency. *J. Physiol.* 226:191–203
50. Emmelin, N., Garrett, J. R., Ohlin, P. 1968. Neural control of salivary myoepithelial cells. *J. Physiol.* 196:381–96
51. Uddman, R., Fahrenkrug, J., Malm, L., Alumets, J., Håkanson, R., et al. 1980. Neuronal VIP in salivary glands: distribution and release. *Acta Physiol. Scand.* 110:31–38
52. Bloom, S. R., Edwards, A. V. 1980. Vasoactive intestinal polypeptide in relation to atropine resistant vasodilation in the submaxillary gland of the cat. *J. Physiol.* 300:41–53
53. Lundberg, J. M., Änggård, A., Fahrenkrug, J. 1981. Complementary role of vasoactive intestinal polypeptide (VIP) and acetylcholine for cat submandibular gland blood flow and secretion. I. VIP release. *Acta Physiol. Scand.* 113:317–27
54. Andersson, P. O., Bloom, S. R., Edwards, A. V., Järhult, J. 1982. Effects of stimulation of the chorda tympani in bursts on submaxillary responses in the cat. *J. Physiol.* 322:469–83
55. Heidenhain, R. 1872. Über die Wirkung einiger Gifte auf die Nerven der Glandula submaxillaris. *Pfluegers Arch.* 5:309–18
56. Lundberg, J. M., Änggård, A., Fahrenkrug, J. 1981. Complementary role of vasoactive intestinal polypeptide (VIP) and acetylcholine for cat submandibular gland blood flow and secretion. II. Effects of cholinergic antagonists and VIP antiserum. *Acta Physiol. Scand.* 113:329–36
57. Lundberg, J. M., Fahrenkrug, J., Larsson, O., Änggård, A. 1984. Corelease of vasoactive intestinal polypeptide and peptide histidine isoleucine in relation to atropine-resistant vasodilatation in cat submandibular salivary gland. *Neurosci. Lett.* 52:37–42
58. Lundberg, J. M., Hökfelt, T., Schultzberg, M., Uvnäs-Wallensten, K., Kohler, C., et al. 1979. Occurrence of vasoactive intestinal polypeptide (VIP)-

like immunoreactivity in certain cholinergic neurons of the cat: evidence from combined immunohistochemistry and acetylcholinesterase staining. *Neuroscience* 4:1539–59
59. Eccles, R., Wilson, H. 1973. The parasympathetic secretory nerves of the nose of the cat. *J. Physiol.* 230:213–23
60. Lundberg, J. M., Änggård, A., Emson, P., Fahrenkrug, J., Hökfelt, T. 1981. Vasoactive intestinal polypeptide and cholinergic mechanisms in cat nasal mucosa: studies on choline acetyltransferase and release of vasoactive intestinal polypeptide. *Proc. Natl. Acad. Sci. USA* 78:5255–59
61. Håkanson, R., Sundler, F., Uddman, R. 1982. Distribution and topography of peripheral VIP nerve fibers: functional implications. See Ref. 43, pp. 121–44
62. Tatemoto, K., Mutt, V. 1981. Isolation and characterization of the intestinal peptide porcine PHI (PHI-27), a new member of the glucagon-secretin family. *Proc. Natl. Acad. Sci. USA* 78:6603–7
63. Itoh, W., Obata, K., Yanaihara, N., Okamoto, H. 1983. Human preprovasoactive intestinal polypeptide (VIP) contains the coding sequence for a novel PHI-27-like peptide, PHM-27. *Nature* 304:547–49
64. Emmelin, N., Holmberg, J. 1967. Impulse frequency in secretory nerves of salivary glands. *J. Physiol.* 191:205–14
65. Kawamura, Y., Matsuo, R., Yamamoto, T. 1982. Analysis of reflex responses in preganglionic parasympathetic fibres innervating submandibular glands of rabbits. *J. Physiol.* 322:241–55
66. Hökfelt, T., Lundberg, J. M., Skirboll, L., Johansson, O., Schultzberg, M., et al. 1982. Coexistence of classical transmitters and peptides in neurones. See Ref. 101, pp. 77–125
67. Lundberg, J. M., Hökfelt, T., Änggård, A., Terenius, L., Elde, R., et al. 1982. Organizational principles in the peripheral sympathetic nervous system: subdivision of coexisting peptides (somatostatin-, avian pancreatic polypeptide- and vasoactive intestinal polypeptide-like immunoreactive materials). *Proc. Natl. Acad. Sci. USA* 79:1303–7
68. Kessler, J. A., Adler, J. E., Bohn, M. C., Black, I. B. 1981. Substance P in principal sympathetic neurons: regulation by impulse activity. *Science* 214: 335–36
69. Schultzberg, M., Hökfelt, T., Terenius, L., Elfvin, L. G., Lundberg, J. M., et al. 1979. Enkephalin-immunoreactive nerve fibres and cell bodies in sympathetic ganglia of the guinea-pig and rat. *Neuroscience* 4:249–70
70. Lundberg, J. M., Hökfelt, T., Änggård, A., Uvnäs-Wallensten, K., Brimijoin, S., et al. 1980. Peripheral peptide neurons: distribution, axonal transport and some aspects on possible function. In *Neural Peptides and Neuronal Communication*, ed. E. Costa, M. Trabucchi, pp. 25–36. New York: Raven
71. Hökfelt, T., Elfvin, L. G., Elde, R., Schultzberg, M., Goldstein, M., et al. 1977. Occurrence of somatostatin-like immunoreactivity in some peripheral sympathetic noradrenergic neurons. *Proc. Natl. Acad. Sci. USA* 74:3587–91
72. Emson, P. C., DeQuidt, M. E. 1984. NPY—a new member of the pancreatic polypeptide family. *Trends Neurosci.* 7:31–35
73. Lundberg, J. M., Hökfelt, T., Änggård, A., Kimmel, J., Goldstein, M., et al. 1980. Coexistence of an avian pancreatic polypeptide (APP) immunoreactive substance and catecholamines in some peripheral and central neurones. *Acta Physiol. Scand.* 110:107–9
74. Lundberg, J. M., Terenius, L., Hökfelt, T., Martling, C. R., Mutt, V., et al. 1982. Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. *Acta Physiol. Scand.* 116:477–80
75. Lundberg, J. M., Terenius, L., Hökfelt, T., Goldstein, M. 1983. High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. *Neurosci. Lett.* 42:167–72
76. Olschowka, J. A., Jacobowitz, D. M. 1983. The coexistence and release of bovine pancreatic polypeptide-like immunoreactivity from noradrenergic superior cervical ganglia neurons. *Peptides* 4:231–38
77. Ekblad, E., Edvinsson, L., Wahlestedt, C., Uddman, R., Håkanson, R., et al. 1984. Neuropeptide Y co-exists and cooperates with noradrenaline in perivascular nerve fibers. *Regulatory Peptides* 8:225–35
78. Fried, G., Terenius, L., Brodin, E., Efendic, S., Dockray, G., et al. 1986. Neuropeptide Y, enkephalin and noradrenaline coexist in sympathetic neurons innervating the bovine spleen. Biochemical and histochemical evidence. *Cell Tissue Res.* 243:495–508
79. Uddman, R., Edvinsson, L., Håkanson, R., Owman, C., Sundler, F. 1982. Immunohistochemical demonstration of

APP (Avian pancreatic polypeptide)-immunoreactive nerve fibers around cerebral blood vessels. *Brain Res. Bull.* 9:715–18

80. Furness, J. B., Costa, M., Emson, P. C., Håkanson, R., Moghimzadeh, E., et al. 1983. Distribution, pathways and reactions to drug treatment of nerves with neuropeptide Y- and pancreatic polypeptide-like immunoreactivity in the guinea-pig digestive tract. *Cell Tissue Res.* 234:71–92
81. Uddman, R., Ekblad, E., Edvinsson, L., Håkanson, R., Sundler, F. 1985. Neuropeptide Y-like immunoreactivity in perivascular nerve fibres of the guinea-pig. *Regulatory Peptides* 10: 243–57
82. Morris, J. L., Murphy, R., Furness, J. B., Costa, M. 1986. Partial depletion of neuropeptide Y from noradrenergic perivascular and cardiac axons by 6-hydroxydopamine and reserpine. *Regulatory Peptides* 13:147–62
83. Morris, J. L., Gibbins, I. L., Campbell, G., Murphy, R., Furness, J. B., et al. 1986. Innervation of the large arteries and heart of the toad *(Bufo marinus)* by adrenergic and peptide-containing neurons. *Cell Tissue Res.* 243:171–84
84. Gu, J., Adrian, T. E., Tatemoto, K., Polak, J. M., Allen, J. M., et al. 1983. Neuropeptide tyrosine (NPY)—a major cardiac neuropeptide. *Lancet* 1983: 1008–10
85. Sternini, C., Brecha, N. 1985. Distribution and colocalization of neuropeptide Y-like immunoreactivity in the guinea-pig heart. *Cell Tissue Res.* 241:93–102
86. Stjernquist, M., Emson, P., Owman, C., Sjöberg, N. O., Sundler, F., et al. 1983. Neuropeptide Y in the female reproductive tract of the rat. Distribution of nerve fibres and motor effects. *Neurosci. Lett.* 39:279–84
87. Lundberg, J. M., Änggård, A., Theodorsson-Norheim, E., Pernow, J. 1984. Guanethidine-sensitive release of neuropeptide Y-like immunoreactivity in the cat spleen by sympathetic nerve stimulation. *Neurosci. Lett.* 52:175–80
88. Lundberg, J. M., Fried, G., Pernow, J., Theodorsson-Norheim, E., Änggård, A. 1986. NPY—a mediator of reserpine-resistant, non-adrenergic vasoconstriction in cat spleen after preganglionic denervation. *Acta Physiol. Scand.* 126: 151–52
89. Lundberg, J. M., Tatemoto, K. 1982. Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to α-adrenocoptor blockade. *Acta Physiol. Scand.* 116:393–402
90. Auer, L. M., Edvinsson, L., Johansson, B. B. 1983. Effect of sympathetic nerve stimulation and adrenoceptor blockade on pial arterial and venous calibre and on intracranial pressure in the cat. *Acta Physiol. Scand.* 119:213–17
91. Edvinsson, L., Emson, P., McCulloch, J., Tatemoto, K., Uddman, R. 1984. Neuropeptide Y: immunocytochemical localization to and effect upon feline pial arteries and veins in vitro and in situ. *Acta Physiol. Scand.* 122:155–63
92. Lundberg, J. M., Änggård, A., Pernow, J., Hökfelt, T. 1985. Neuropeptide Y-, substance P- and VIP-like immunoreactive nerves in cat spleen in relation to autonomic volume and vascular control. *Cell Tissue Res.* 239:9–18
93. Lundberg, J. M., Saria, A., Franco-Cereceda, A., Theodorsson-Norheim, E. 1985. Mechanisms underlying changes in the contents of neuropeptide Y in cardiovascular nerves and adrenal gland induced by sympatholytic drugs. *Acta Physiol. Scand.* 124:603–11
94. Lundberg, J. M., Rudehill, A., Sollevi, A., Theodorsson-Norheim, E., Hamberger, B. 1986. Frequency- and reserpine-dependent chemical coding of sympathetic transmission: differential release of noradrenaline and neuropeptide Y from pig spleen. *Neurosci. Lett.* 63:96–100
95. Allen, J. M., Adrian, T. E., Tatemoto, K., Polak, J. M., Hughes, J., et al. 1982. Two novel related peptides, neuropeptide Y (NPY) and peptide YY (PYY) inhibit the contraction of the electrically stimulated mouse vas deferens. *Neuropeptides* 3:71–77
96. Lundberg, J. M., Stjärne, L. 1984. Neuropeptide Y (NPY) depresses the secretion of ^{3}H-noradrenaline and the contractile response evoked by field stimulation in rat vas deferens. *Acta Physiol. Scand.* 120:477–79
97. Sneddon, P., Westfall, D. P. 1984. Pharmacological evidence that adenosine triphosphate and noradrenaline are cotransmitters in guinea-pig vas deferens. *J. Physiol.* 347:561–80
98. Sneddon, P., Burnstock, G. 1984. Inhibition of excitatory junction potentials in guinea-pig vas deferens by α,β-methylene ATP: further evidence of ATP and noradrenaline as co-transmitters. *Eur. J. Pharmacol.* 100:85–90
99. Stjärne, L., Åstrand, P. 1984. Discrete events measure single quanta of adenosine 5'-triphosphate secreted from sym-

pathetic nerves of guinea-pig and mouse vas deferens. *Neuroscience* 13:21–28
100. Fedan, J. S., Hogaboom, G. K., O'Donnell, J. P., Colby, J., Westfall, D. P. 1981. Contribution by purines to the neurogenic response of the vas deferens of the guinea-pig. *Eur. J. Pharmacol.* 69:41–53
101. Cuello, A. C., ed. 1982. *Co-transmission*. London: Macmillan
102. Osborne, N. N., ed. 1983. *Dale's Principle and Communication Between Neurones*. Oxford: Pergamon
103. Weinshilboum, R. M., Thoa, N. B., Johnson, D. G., Kopin, I. J., Axelrod, J. 1971. Proportional release of norepinephrine and dopamine-β-hydroxylase from sympathetic nerves. *Science* 1349–51
104. Thomas, J. A., Sakai, K. K., Holck, M. I., Marks, B. H. 1980. Dopamine-β-hydroxylase: a modulator of beta adrenergic receptor activity. *Res. Commun. Chem. Pathol. Pharmacol.* 29:3–13
105. Koketsu, K. 1984. Modulation of receptor sensitivity and action potentials by transmitters in vertebrates neurons. *Jpn. J. Physiol.* 34:945–60
106. Kumakura, K., Karoum, F., Guidotti, A., Costa, E. 1980. Modulation of nicotinic receptors by opiate receptor agonists in cultured adrenal chromaffin cells. *Nature* 283:489–92
107. Agnati, D. F., Fuxe, K., Benfenati, F., Battistini, N., Härfstrand, A., et al. 1983. Neuropeptide Y in vitro selectively increases the number of α_2-adrenergic binding sites in membranes of the medulla oblongata of the rat. *Acta Physiol. Scand.* 118:293–95
108. Akasu, T., Hirai, K., Koketsu, K. 1981. Increases of acetylcholine-receptor sensitivity by adenosine triphosphate: a novel action of ATP on ACh-sensitivity. *Br. J. Pharmacol.* 74:505–7
109. Lundberg, J. M., Hedlund, B., Bartfai, T. 1982. Vasoactive intestinal polypeptide enhances muscarinic ligand binding in cat submandibular salivary gland. *Nature* 295:147–49
110. Potter, E. K. 1985. Prolonged non-adrenergic inhibition of cardiac vagal action following sympathetic stimulation. Neuromodulation by NPY? *Neurosci. Lett.* 54:117–21
111. Burnstock, G. 1972. Purinergic nerves. *Pharmacol. Rev.* 21:247–324
112. Campbell, G., Gibbins, I. L. 1979. Non-adrenergic, non-cholinergic transmission in the autonomic nervous system: purinergic nerves. In *Trends in Autonomic Pharmacology*, ed. S. Kalsner, 1:102–49. Baltimore: Urban & Schwarzenberg
113. Barthó, L., Holzer, P. 1985. Search for a physiological role of substance P in gastrointestinal motility. *Neuroscience* 16:1–32
114. Furness, J. B., Costa, M., Keast, J. R. 1984. Choline acetyltransferase– and peptide-immunoreactivity of submucous neurons in the small intestine of the guinea-pig. *Cell Tissue Res.* 237:329–36
115. Kessler, J. A. 1985. Differential regulation of peptide and catecholamine characters in cultured sympathetic neurons. *Neuroscience* 15:827–39
116. Costa, M., Furness, J. B. 1984. Somatostatin is present in a sub-population of noradrenergic nerve fibres supplying the intestine. *Neuroscience* 13:911–19
117. Kessler, J. A. 1984. Environmental coregulation of substance P, somatostatin and neurotransmitter synthesizing enzymes in cultured sympathetic neurons. *Brain. Res.* 321:155–59
118. Kessler, J. A., Adler, J., Jonakait, G. M., Black, I. B. 1984. Target organ regulation of substance P in sympathetic neurons in culture. *Dev. Biol.* 102:417–25
119. Shimizu, T., Taira, N. 1979. Assessment of the effects of vasoactive intestinal peptide (VIP) on blood flow through and salivation of the dog salivary gland in comparison with those of secretin, glucagon and acetylcholine. *Br. J. Pharmacol.* 65:683–88
120. Thulin, A. 1976. Blood flow changes in the submaxillary gland of the rat on parasympathetic and sympathetic nerve stimulation. *Acta Physiol. Scand.* 97: 104–9
121. Bloom, S. R., Bryant, M. G., Polak, J. M., van Noorden, S., Wharton, J. 1979. Vasoactive intestinal peptide-like immunoreactivity in salivary glands of the rat. *J. Physiol.* 289:23P
122. Morley, J., Schachter, M., Smaje, L. H. 1966. Vasodilatation in the submaxillary gland of the rabbit. *J. Physiol.* 187:595–602
123. Edvinsson, L., Fahrenkrug, J., Hanko, J., Owman, C., Sundler, F., et al. 1980. VIP (vasoactive intestinal peptide)-containing nerves of intracranial arteries in mammals. *Cell Tissue Res.* 208:135–42
124. Hartzell, H. C. 1979. Adenosine receptors in frog sinus venosus: slow inhibitory potentials produced by adenine compounds and acetylcholine. *J. Physiol.* 293:23–49