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CHEMICAL CODING OF NEURONS AND PLURICHEMICAL TRANSMISSION

J. B. Furness[1], J. L. Morris[1], I. L. Gibbins[1], M. Costa[2]

Centre for Neuroscience, Departments of Anatomy & Histology[1] and of Physiology[2], Flinders University, Bedford Park, South Australia 5042, Australia.

INTRODUCTION

Two unexpected facts about neurons have been established in recent years: neurons contain and can release a large variety of substances that are capable of influencing target cells; and it is common for individual neurons to contain several such substances. Both of these findings have opened the way to the solution of old problems and have posed problems of their own. The discovery of a cornucopia of substances contained in neurons has led to a revitalization of microscopic neuroanatomy, spawning a new field termed "chemical neuroanatomy" (1). This burgeoning of knowledge of immunohistochemically identifiable neuronal markers occurred in the last decade and now encompasses thousands of publications, numerous reviews, several books, and even a Handbook series and a journal (1–8). On the other hand, it has been a considerably more difficult task for physiologists and pharmacologists to evaluate the roles of these substances and, in particular, to determine in which ways more than one substance can participate in the transmission process.

In the present review we look at two aspects of recent work in this field: the ways in which combinations of neuronal markers identify individual groups of neurons, a phenomenon referred to here as chemical coding; and the manner in which neurotransmission can involve the combined release and action of more than one substance, referred to as plurichemical transmission.

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There Are Many Neurotransmitters[1]

We have adopted the view that a substance can be called a transmitter when it is released from a nerve ending under conditions that are encountered physiologically or that mimic physiological conditions and, through its direct or indirect action, when it influences the excitability or metabolic state of another cell. This is a deliberately broad definition encompassing substances often referred to as classical neurotransmitters, as neuromodulators, or as trophic factors. It would include acetylcholine and noradrenaline, well-established as neurotransmitters, other amines such as adrenaline, dopamine, and serotonin, certain amino acids including γ-aminobutyric acid and glycine, and a large number of regulatory peptides, some of which clearly have transmitter roles and some of which may have such roles. The peptides are of particular interest, because they are so numerous, because they have been localized histochemically in a broad range of neurons, and because they have a wide range of biological actions.

CHEMICAL CODING

Colocalization of Potential Neurotransmitters

In the years between 1975 and about 1982 a large number of individual cases of neurons that contained several neuropeptides, or neuropeptides and amines, were documented. Given that 40 or more of these substances may contribute to the chemical coding of a neuron, it would perhaps simplify our analysis if certain substances were consistently found together. Unfortunately, this does not appear to be so, except where several peptides are derived from the same precursor molecule. We can take as an example the peptide VIP. Secretomotor neurons to the cat salivary gland contain substances that show immunoreactivity with anti-VIP antibodies (VIP–IR), and also contain ACh (9). In contrast, VIP–IR coexists with galanin and dynorphin in non-cholinergic secretomotor neurons (DYN/GAL/VIP neurons) in the guinea-pig small intestine (10). In the intestine of another species, the rat, VIP-IR is also in presumed intestinal secretomotor neurons, but in this case, VIP coexists with NPY (NPY/VIP neurons) (11). Vasodilator neurons to the uterine arteries in the guinea pig contain immunoreactivity to VIP which

[1]Abbreviations: ACh: acetylcholine; ATP: adenosine triphosphate; BPP: bovine pancreatic polypeptide; CCK: cholecystokinin; CGRP: calcitonin gene-related peptide; DA: dopamine; DBH: dopamine-β-hydroxylase; DYN: dynorphin; ENK: enkephalin; ESP: excitatory synaptic potential; GABA: γ-aminobutyric acid; GAL: galanin; GTP: guanosine triphosphate; 5HT: 5 hydroxytryptamine; IR: immunoreactivity; LHRH: luteinising hormone releasing hormone; NA: noradrenaline; NPY: neuropeptide Y; PHI: peptide histidine isoleucine; PP6: pancreatic polypeptide hexapeptide; PYY: peptide YY; SOM: somatostatin; SP: substance P; TRH: thyrotropin releasing hormone; VIP: vasoactive intestinal peptide.

coexists with DYN, NPY, and SOM (DYN/NPY/SOM/VIP neurons) (12, 13). VIP is contained in small sensory neurons of some species (14, 15), and in rats at least some of these neurons also contain CGRP (16). Instances of VIP coexistence with other potential transmitters also occur in the central nervous system; for example, VIP coexists with ACh in neocortical neurons (17) and with GABA in the hippocampus (18). PHI, which is cleaved from the same precursor as VIP, has been found in some of these neurons and may well occur in them all (19). Further examples of the variety of combinations in which individual substances participate are given elsewhere (20, 21, 22).

Not only can a particular potential neurotransmitter show different patterns of colocalization in a variety of neuronal types, but the type and numbers of substances located together also vary (22, 23). Thus neurons containing more than one non-peptide transmitter (often referred to as classical neurotransmitters) have been demonstrated. For example, some neurons in the lobster contain both 5-HT and DA (24), and certain neurons in the rat brain contain GABA and ACh (25). Other neurons contain several peptides but, as far as can be determined, contain no classical, non-peptide transmitter. Examples are the non-cholinergic secretomotor neurons in the guinea pig intestine which contain DYN/GAL/VIP (10), and unmyelinated sensory neurons containing CCK/CGRP/DYN/SP which project to the guinea pig skin (26). There are also numerous examples in which a non-peptide transmitter is in the same neuron as one or more neuropeptides (5, 6).

As some of the examples discussed above have demonstrated, individual neurons may contain four or more neurotransmitters. Further instances include raphe neurons that appear to contain GABA/5-HT/SP/TRH (27), and secretomotor neurons in the small intestine that contain CCK/CGRP/GAL/NPY/SOM and probably acetylcholine (10). The total number of potential transmitter substances found in these neurons may well be much greater when all the products of processing peptide precursor molecules have been identified. As more and more investigations are made, it appears that coexistence of potential transmitters is the rule, not the exception.

A further aspect of colocalization of potential neurotransmitters is their sites of intracellular storage. Most neurotransmitters are packaged and stored in intracellular vesicles in nerve cell bodies and axons. The few studies carried out to date examining the vesicular localization of multiple transmitters have indicated some heterogeneity in their storage. For example, NPY is restricted to the large vesicle fraction of sympathetic nerves in rat vas deferens and cat spleen, whereas noradrenaline occurs in both the small and large vesicle fractions (28, 29). It has not yet been determined directly whether NA and NPY are present in the same large vesicles. However, individual peptides may be contained in the same vesicles; both enkephalin and NPY are contained in the large vesicle fraction from bovine splenic nerve (30) and stellate ganglion (31), and SP and CGRP are stored in the same large vesicles in

guinea pig peripheral nerves (32). Electron microscopic immunocytochemical studies may help determine whether three or more neuropeptides are contained in the same, or different, neuronal storage vesicles.

Neurons Are Chemically Coded

Despite the variability in the combinations of substances coexisting in neurons, it has become apparent that there might be some system to the distribution and colocalization of potential neurotransmitter substances in specific groups of neurons in the central nervous system and the periphery (21, 22).

Lundberg et al (33) pointed out that noradrenergic neurons, which for a long time had been thought to be equivalent in their transmitter chemistry, could be subdivided into three populations. One population contained somatostatin along with noradrenaline, the second contained noradrenaline together with avian pancreatic polypeptide-like immunoreactivity (now known to be NPY), and the third contained noradrenaline only. These authors indicated that the separate populations appeared to show target specificity. Such specificity of target was clearly documented by Costa & Furness (34) who studied the projections of noradrenergic neurons from the coeliac ganglion to the small intestine of the guinea pig. The term "chemical coding" was introduced to describe the finding that noradrenergic neurons projecting to the intestinal vasculature contained the transmitter combination NA/NPY, those projecting to the submucous ganglia contained the combination NA/SOM, and those projecting to myenteric ganglia had none of the peptides investigated and were designated NA/-(noradrenaline alone) neurons. Thus it was established that fibres of functionally distinct noradrenergic neurons arising from the same ganglion were chemically different; the NA/NPY neurons are vasoconstrictor, the NA/SOM neurons secretomotor inhibitory neurons, and the NA/–neurons those inhibiting motility (35). Further analysis of the chemical coding within the coeliac ganglion (36, 37) showed that while there was some tendency toward preferential positioning of differently coded neurons, there was considerable mixing of the clumps of neurons. Thus from a mixed ganglion, nerve fibres with a specific chemistry and function run out in common nerve trunks, but segregate at the target organ to supply different elements in that organ.

Chemical coding can also be demonstrated for unmyelinated sensory neurons in guinea pigs. Many of the neurons contain combinations of CCK, CGRP, DYN, and SP–IR. A subpopulation with the peptide grouping CCK/CGRP/DYN/SP project almost exclusively to the skin, some forming a network under the epidermis and some surrounding cutaneous arterioles. Neurons from the same ganglia which contain CCK/CGRP/SP innervate arterioles of skeletal muscle; CGRP/DYN/SP nerve fibres mostly supply the

pelvic viscera, and CGRP/SP fibres run mainly to the heart, large arteries, and veins (26).

A similar precision of chemical coding has been revealed in the relationships between preganglionic and postganglionic neurons supplying specific target tissues. In the vagus nerve and its branches supplying the lung and esophagus in toads, postganglionic neurons that supply the lung contain ACh and SOM, while those supplying the esophagus are non-cholinergic and contain VIP (38). Other non-cholingergic VIP–IR nerve cell bodies lie close to both the lung and the esophagus. Preganglionic neurons connecting with VIP neurons supplying the esophagus contain IR to SP and a SOM-like peptide, while preganglionic neurons connecting with VIP–IR neurons close to the lung do not contain SP–, SOM– or VIP–IR. Moreover, SOM–IR neurons projecting to the lung receive preganglionic inputs from neurons that usually contain SP–IR but not SOM–IR.

Discrete connections between pre- and postsynaptic neurons containing specific combinations of peptides and non-peptides are also found in the guinea pig paracervical ganglia (39). In these ganglia, up to 11 populations of postganglionic neurons were identified immunohistochemically. Four of these populations of postganglionic neurons received histochemically defined inputs from neurons containing different combinations of peptides. For example, nerve terminals with SP–IR surrounded only those nerve cell bodies with IR to DBH/DYN/NPY/±SOM/VIP, while ENK–IR terminals were associated selectively with nerve cell bodies containing SOM–IR alone. Further examples of the specificity of ganglionic connections have been demonstrated in sympathetic ganglia of cats (40) and guinea pigs (36, 37).

Chemical coding of neuronal projections also occurs in the central nervous system, for example, among dopamine neurons of the *substantia nigra* and among noradrenergic neurons of the *locus coeruleus* (41, 42).

One consequence of the finding that multiple coexisting substances, and not a single substance, define the chemical code of a class of neurons, is that the same substance may be located in two functionally different groups of neurons supplying the same target tissue. For example, SP is contained both in cholinergic pupillo-constrictor neurons and in sensory nerve fibres supplying the guinea pig iris (43), while NPY is contained in sympathetic vasoconstrictor neurons, and in pelvic autonomic vasodilator neurons supplying the guinea pig uterine artery (12). In these cases SP and NPY coexist with different substances in the different neuronal classes. On the other hand, two groups of functionally similar neurons, the DYN/VIP neurons in the myenteric plexus of the guinea pig ileum which project different distances anally before supplying the circular muscle, contain different combinations of additional peptides (5). It remains to be determined whether the functions of the same peptide in different classes of nerve fibres supplying the same target are similar or different.

Chemical Coding is not Confined to Particular Classes of Animals or to Particular Neuronal Systems

The examples used above come from a variety of systems, but it is worth emphasizing that chemical coding of neurons has been demonstrated in animals from a broad range of vertebrate and invertebrate species. Moreover, colocalization of transmitters occurs in neurons of all systems: special and general sensory systems; somatic and autonomic motor neurons; associative neurons; and neurons in regions subserving higher integrative or cognitive functions. However, examples of chemical coding have not been encountered equally in all groups of neurons. With explorations made to date, chemical coding has been discovered in many neurons of the autonomic nervous system, in unmyelinated sensory neurons, and in certain brain regions, such as the hypothalamus, but has been less frequently noted in, for example, somatic motor neurons, large diameter primary afferent neurons, and the cerebellum. Whether there exist real differences in the extent of chemical coding between systems of neurons will only be determined as more investigations are undertaken, particularly to screen for novel substances in relatively poorly tested regions.

PLURICHEMICAL TRANSMISSION

Although there are many neurons in which coexistence of two or more substances has been demonstrated histochemically, there are very few for which the roles of the substances in neurotransmission have been investigated. We discuss first some examples where the release of more than one neurotransmitter from a single neuron has been demonstrated and then broaden the discussion to cover a range of possible roles of coreleased neurotransmitters. The concept that arises from this discussion is that neurotransmission commonly involves the release of several substances; the transmission process is the consequence of the combined actions and interactions of these substances—it is a plurichemical process.

Corelease

Release of two substances from the same neurons of the peripheral autonomic nervous system has been shown in recent years. Examples include release of ACh and LHRH from preganglionic nerve fibres to amphibian sympathetic ganglia (51), ACh and SOM from toad intracardiac neurons (49), ACh and VIP from parasympathetic neurons supplying cat salivary glands (54), ACh and SP from enteric excitatory neurons (5, 55, 56), and NA and NPY from sympathetic vasoconstrictor neurons in a number of organs (7, 57). The evidence for corelease was obtained from biochemical measurement of substances released in vitro after electrical stimulation of the appropriate neurons, as well as from selective pharmacological blockade of components of post-

synaptic events (58). In each of these examples there was some segregation of release of the non-peptide and peptide transmitters. Release of the non-peptide appears to be predominant at low frequencies of nerve stimulation (less than 10Hz), whereas peptide release is usually more effectively evoked at higher pulse frequencies (10–50Hz) (59, 60). The tendency for peptides to be released at higher frequencies of stimulation is not an indication that

Table 1 Examples of chemical coding in a range of species and neuronal systems. This table provides selected examples of colocalization of probable neurotransmitters in neurons of animals of different vertebrate and invertebrate classes. It is intended to illustrate the range of combinations that are found, the inconstancies of associations and the diverse species and neuronal systems in which coexistence has been demonstrated.

Combination	Location/Function	Reference
Mammals		
5-HT/SP/TRH/(GABA)	Medullary raphe neurons (rat)	Johansson et al (44) Belin et al (27)
CCK/CGRP/DYN/SP	Primary sensory neurons (guinea pig)	Gibbins et al (26)
DYN/NPY/SOM/VIP	Vasodilators to uterine arteries (guinea pig)	Morris et al (12, 13)
ATP/NA/NPY	Motor neurons to vas deferens (rodent)	Kasakov et al (45)
ACh/CCK/CGRP/GAL/NPY/SOM	Enteric secretomotor neurons (guinea pig)	Furness et al (10)
GAL/NA/NPY	Sympathetic neurons (cat)	Kummer (46)
Birds		
ACh/ENK/SP/VIP	Preganglionic fibres to ciliary ganglion	Reiner et al (47)
ACh/CGRP/SOM/VIP	Motor neurons to skeletal muscle during embryonic development	Villar et al (48)
Amphibians		
ACh/SOM	Postganglionic cardiac neurons in toad	Campbell et al (49)
5-HT/NA/SP	Cardiac neurons in mudpuppy	Neel & Parsons (50)
ACh/LHRH	Bullfrog sympathetic preganglionic neurons	Jan & Jan (51)
Insects		
CCK/ENK/PP	Blowfly central nervous system	Duve & Thorpe (52)
Glutamate/Proctolin	Cockroach muscle motor neurons	Adams & O'Shea (53)
Crustaceans		
DA/5-HT/Proctolin	Lobster commissural ganglion	Cournil et al (24)

the release is nonphysiological; there is good evidence for peptide release in response to reflex—rather than electrical or drug induced—stimulation of neurons (61–64).

The release of three substances from the same neuron occurs in the cat salivary gland; Lundberg and colleagues have demonstrated release of PHI in addition to VIP and ACh from parasympathetic neurons supplying the submandibular salivary gland (65). These two peptides, which are probably cleaved from the same precursor molecule in a 1:1 ratio (66, 67), were detected after release from the neurons in equimolar amounts. This was despite the 2:1 ratio of VIP:PHI found biochemically in salivary gland extracts (65). Deviation from a 1:1 storage ratio of VIP:PHI could occur by differential post-translational processing of the precursor molecule (68). Discrepancies between the ratio of peptides found in whole tissues and the ratio of peptides released on nerve stimulation may be due to different ratios of the peptides in vesicles, whose contents are released on nerve stimulation, compared with vesicles more distant from release sites. Different storage ratios could be achieved by differential cleavage of each peptide from the precursor molecule during passage of vesicles down the axons (69). On the other hand, the measured differences in peptide ratios may reflect differences in the effectiveness of recovery and detection of the peptides from tissue extracts or from perfusates.

Transmission involving three chemically unrelated substances released from the same neuron population has been demonstrated in the vas deferens of rodents, where sympathetic nerve terminals release NA, ATP, and NPY (45). The fast phase of the excitatory junction potential and smooth muscle contraction seems to be mediated primarily by neuronally released ATP. The slower electrical and mechanical events are at least partly attributable to the release and postsynaptic action of NA (70–72). NPY may contribute both to the slow and fast events (73). By combining information from biochemical and electrophysiological studies on sympathetic neurons in the vas deferens as well as in other tissues, Stjarne and colleagues have postulated that NA and ATP are packaged in small synaptic vesicles in a ratio of 50:1, and NA, ATP, and NPY are contained in large vesicles in a ratio of 100:2:1 (72, 73). They proposed that small vesicles, exclusively, are released at low rates of nerve activity, while large vesicles containing NPY, in addition to NA and ATP, are released in response to bursts of nerve impulses occurring at high frequency. Thus, differential vesicular packaging of transmitter substances can potentially lead to qualitative, as well as quantitative, differences in transmission at different levels of neuronal activation.

Diversity of Actions of Neurotransmitters

Any substance released from a nerve terminal may act on several target cells: on the nerve terminal itself to influence further neurotransmitter release; on

postsynaptic cells close to the site of release; or, after diffusion from the nerve terminal, on targets at a distance from the release site. For example, noradrenaline released from sympathetic neurons has both pre- and post-junctional actions (see below). Furthermore, a single substance released from a neuron may have several sites or modes of action on the same target. For example, acetylcholine can activate two different receptor types and three different ion channels in sympathetic neurons (74, 75), and GABA can activate two receptors linked to different cellular mechanisms in central neurons (76).

CORELEASED SUBSTANCES CAN ACT ON DIFFERENT TARGETS When multiple transmitters are released from the same nerve terminal, the role of each substance in the transmission process depends on the differential location and characteristics of receptors for the substances, as well as the relative quantities of substances released. Evidence now emerging indicates variation in the location and subtypes of receptors for coreleased substances, as well as variation in their potencies of action.

The sympathetic vasoconstrictor neurons provide a model for examining interactions between coreleased transmitters. Most of these neurons contain NPY in addition to NA, and in a number of cases NPY release, as well as NA release, has been demonstrated; moreover, some of the neurons appear to release ATP (7, 77). Each of these substances (or their metabolites) has a prejunctional and a postjunctional action.

NA constricts most blood vessels via α_1- or α_2-adrenoceptors, and can also constrict some vessels via junctional γ-receptors (78). In addition, NA produces presynaptic inhibition of NA and NPY release (79), an action mediated by α_2-receptors (80). NPY is a potent constrictor of many arteries (7). However, in other vessels, the predominant postjunctional effect of low concentrations of NPY is potentiation of constrictions produced by other agonists, including the cotransmitter NA (81). NPY can also produce inhibition of transmitter release from sympathetic nerve terminals. There is some evidence that prejunctional and postjunctional NPY receptors are different; the whole NPY sequence is more potent than NPY fragments on postjunctional receptors, while a C-terminal fragment of a closely related peptide, PYY 13–36, was almost as potent as NPY on prejunctional receptors in the vas deferens (82). Thus, products of NPY degradation may act preferentially on prejunctional NPY receptors.

ATP released from sympathetic nerve fibres initiates excitatory junction potentials and contraction in smooth muscle cells of a variety of blood vessels (79), but there is no strong evidence for a prejunctional action of ATP (83). Nevertheless, adenosine derived from breakdown of ATP has both pre- and postjunctional inhibitory actions (77).

It is apparent that smooth muscle receptors for one or two of these three substances predominate in any given vessel, and that the relative potencies of

cotransmitters vary greatly between vessels. Thus, sympathetic vasoconstriction in different vessels can be attributed to a range of postjunctional actions: NA acting on γ-receptors in mesenteric arterioles (78); the combined actions of ATP on purine receptors and NA on α_1-receptors in mesenteric arteries (84); or the actions of NA on α_1-receptors and NPY on specific receptors in the guinea pig uterine artery (85). It is as yet unclear whether substances not contributing directly to vasoconstriction are released from the sympathetic axons and, if they are, whether they act selectively on prejunctional, or on undetected postjunctional, receptors.

Two other examples can be mentioned where the targets for coreleased substances appear to be different. CGRP and SP are found in the same primary afferent fibres from which they are released together. When injected into the lumbar spinal cord of rats, SP causes a characteristic behavioral response that is not evoked by CGRP (86). Surprisingly, CGRP markedly accentuated the effect of SP. An explanation appears to be that the target for CGRP is an SP degrading endopeptidase, for which it is a potent inhibitor (87). At the peripheral ends of these primary afferents in the skin, CGRP potentiates the extravasation elicited by SP, probably by its action in causing dilatation of the cutaneous blood vessels (88). In bullfrog sympathetic ganglia ACh and LHRH are in the same nerve fibres that synapse on C cells, and on many of these cells they both have excitatory actions. However, LHRH has the additional effect, not shared by ACh, of diffusing to and exciting B cells that are not directly innervated by LHRH-containing preganglionic neurons (89).

INTERACTIONS BETWEEN CORELEASED TRANSMITTERS ON THE SAME TARGET CELL A variety of pharmacological and biochemical studies indicate that coreleased substances can interact on the same cell. Places at which actions can converge include the receptors themselves, the intracellular mechanisms, or the ion channels to which the receptors are coupled. Transmitters can also act through separate receptors and intracellular mechanisms to merge their actions in affecting a common ultimate cellular function, such as the contractile apparatus. In the following discussion we concentrate on the ways in which several different substances released from a neuron can interact.

Interactions at receptors Interactions at receptors can be of several types: the substances can be simply additive in their effects, they can interact to provide a diminished effect or the action of one substance can enhance another's effectiveness. There is evidence that VIP and PHI, which have about 50% sequence homology, act on the same receptors (90). In uterine smooth muscle of rabbits that is innervated by PHI/VIP fibers, these peptides are equipotent and their effects are simply additive (91). However, while VIP

and PHI each have hypotensive effects in rabbits, PHI appears to antagonize the hypotensive effect of VIP (91). In the salivary gland of cats, ACh, but not VIP, directly stimulates glandular secretion. However, VIP markedly enhances the action of ACh, apparently by increasing the affinity of ACh for its own receptors (92). In rat hippocampal slices SOM and muscarinic agonists both inhibit adenylate cyclase activity, but the combined effects of the agonists are nonadditive (93). Because a stable SOM analogue produced competitive displacement of muscarinic agonists and antagonists from their receptors, it was suggested that the two different agonists interact at a common receptor domain (93). Pancreatic polypeptide interacts with muscarinic receptors on rat pancreatic acini; this peptide inhibits carbachol-induced amylase secretion via displacement of carbachol from the muscarinic receptors (94).

Actions through separate receptors coupled to common intracellular mechanisms Somatostatin and noradrenaline are contained together in the endings of sympathetic neurons that supply submucous neurons in the guinea pig. Although NA and SOM act on different receptors on the neurons, they cause hyperpolarization through a common mechanism (95). Both substances activate an inwardly rectifying potassium channel via a mechanism involving a GTP binding protein. The actions of NA and SOM on secretomotor neurons appear to be simply additive (96). This type of coupling to common intracellular pathways occurs for pairs of agonists in many other systems (97, 98), but they have not been evaluated in relation to the corelease of neurotransmitters. There is, however, evidence that the potentiation of α_1 -adrenoceptor-mediated contraction of the rodent vas deferens by adenosine and NPY is due to enhancement of the adrenoceptor-induced accumulation of inositol triphosphate by these agonists (99).

Actions through different cellular mechanisms Some coreleased substances act on the same target cells via different receptors and different primary intracellular mechanisms. It is generally accepted that constriction of blood vessels mediated by α_1 -adrenoceptors involves the intracellular messengers inositol triphosphate and diacylglycerol, whereas prejunctional and postjunctional effects mediated by α_2 -adrenoceptors involve inhibition of adenylate cyclase (100). NPY also seems to produce constriction of some blood vessels by inhibition of adenylate cyclase (101, 102). Therefore vasoconstriction produced by activation of postjunctional α_1 -adrenoceptors seems to involve intracellular mechanisms different from those utilized during NPY-mediated constriction. Nevertheless, the prejunctional inhibition of transmitter release mediated by α_2 -adrenoceptors and NPY receptors may activate the same intracellular pathway (101).

Other potentially important interactions can occur when different types of

conductance changes are induced by different neurotransmitters. In neurons there are now many documented examples of slow synaptic events that last several minutes. The neurons are depolarized by transmitters that decrease membrane conductance to potassium (74). The presence of a slow ESP can thus enable subthreshold fast ESPs to reach threshold for action-potential firing and provide an increased effectiveness of fast ESPs that lasts many minutes. In bullfrog sympathetic ganglia, LHRH released together with ACh from preganglionic fibres produces slow ESPs that potentiate fast ESPs generated by ACh. Each of these transmitters acts on a separate receptor population linked to different membrane ion channels (89). Slow potentials in response to a natural stimulus, colonic distension, have recently been demonstrated in the inferior mesenteric ganglion (64).

More prolonged changes in membrane excitability are found in some neurons. For example, long-term potentiation in hippocampal neurons can persist for several hours after a second or less of activity in the presynaptic neuron (103). In these neurons, the transmission process not only involves rapid changes in membrane excitability, but changes that are dependent on the previous history of synaptic activation over several hours.

TROPHIC EFFECTS OF NEURONALLY RELEASED SUBSTANCES In addition to, or instead of, acute actions on presynaptic or postsynaptic effectors, some substances released from nerve terminals exert effects on growth or metabolism of neuronal and nonneuronal cells. NPY applied chronically to rats via the cerebral ventricles produced alterations in the characteristics of α_2 -adrenoceptors in the medulla (104, 105). Other peptides, most notably VIP and SP, promote survival or outgrowth of neurons in a number of systems (106–109). NPY, VIP, and SP all have been reported to have trophic effects on nonneuronal cells (110–112).

CONCLUSIONS

Throughout the nervous system there are neurons that contain more than one neurotransmitter. Histochemical studies indicate that complex patterns of coexistence of groups of transmitters provide a chemical coding that relates the chemistry of the neurons to their projections and functions. That neurotransmission involves the release and interaction of several transmitter substances appears to be a general phenomenon and may be true of the majority of neurons. The neurotransmitters can act at different locations, for example at postsynaptic and presynaptic sites, or on the same cells, where they can interact in a variety of ways. For example, one transmitter can enhance the action of the other, their effects can be just additive, or one can restrict the other's effectiveness. Some transmitters also have long-term

effects on cell excitability or metabolism. Neuropharmacology is changing in response to observations of plurichemical transmission, just as neuropharmacological research has helped in its discovery.

The neuropharmacological investigation of transmission tends to revolve around the use of agonists that can, to a greater or lesser extent, mimic the transmission process, and of antagonists that, by removing the effect of a transmitter, can be used to analyze its role. There has always been a problem in applying an agonist from an exogenous source in such a way that it reaches the appropriate receptors with the correct concentration profile and with the correct time-course to match the endogeneous transmitter. The greater neuropharmacological problem that now emerges is how to apply several exogeneous compounds in appropriate relative concentrations and appropriate, but perhaps different, time courses to adequately reconstruct the transmission process. The use of antagonists also presents problems, the most obvious being that pharmacologically useful antagonists have not yet been discovered for many of the novel neurotransmitter substances. Thus, a role of neuropharmacologists has necessarily become the time-consuming and difficult task of screening likely compounds for antagonist actions and of testing their specificity. Even when armed with an antagonist, the investigator's interpretation of its effects may not be at all simple, if its effect is to eliminate not just the action of a neurotransmitter on the excitability of a post-synaptic cell, but to withdraw that substance from the interactions it makes with other compounds that participate in the transmission process.

Neuropharmacology in the ensuing years can be seen as one of the most challenging and exciting areas of biological science, with far-reaching implications both for fundamental understanding of neurotransmission and for the development of therapeutic regimes.

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