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特別講演 I

Autonomic Neuroeffector Mechanisms in Smooth Muscle

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This article reviews some of the new discoveries of the past decade about autonomic neuroeffector mechanisms, with particular emphasis on the increase in putative neurotransmitter substances and the co-existence of transmitters in single nerve terminals; some suggestions will be made about how such systems might operate physiologically.

Structural Relationships of Autonomic Nerves and Smooth Muscle

Transmission was initially studied most extensively at the skeletal neuromuscular junction (see Katz, 1966) and at ganglionic synapses (see Skok, 1973). Electron microscopy revealed that both these junctions were elaborate with separation of specialised pre- and postjunctional membranes of about 50 nm or less. Transmitters released from presynaptic sites diffused across the narrow cleft to occupy receptors on postjunctional membranes.

One of the most important observations of more recent years from studies of the autonomic nervous system (Hillarp, 1946; Bennett and Burnstock, 1968; Burnstock, 1970) is that many nerves have extensive terminal varicose regions free of Schwann cell envelopments, where vesicle-filled varicosities (1-2 μm in diameter) releasing transmitter *en passage* are separated by narrow (0.1-0.3 μm diameter) intervaricose regions. While prejunctional varicosity membranes sometimes show thickenings, there are rarely postjunctional specialisations and the minimum junctional cleft may vary from as little as 20 nm in some densely-innervated tissues (like vas deferens or iris) to as much as 2,000 nm in some large elastic arteries (see Burnstock, 1975 a; Burnstock *et al.*, 1980). It is still not known what proportion of varicosities release transmitter during a single nerve pulse, but it seems likely that junctions with such a wide cleft and open geometry are amenable to both pre- and postjunctional modulatory influences from locally-released or circulating substances, as well as being the sites of neurotransmission (see Fig. 1).

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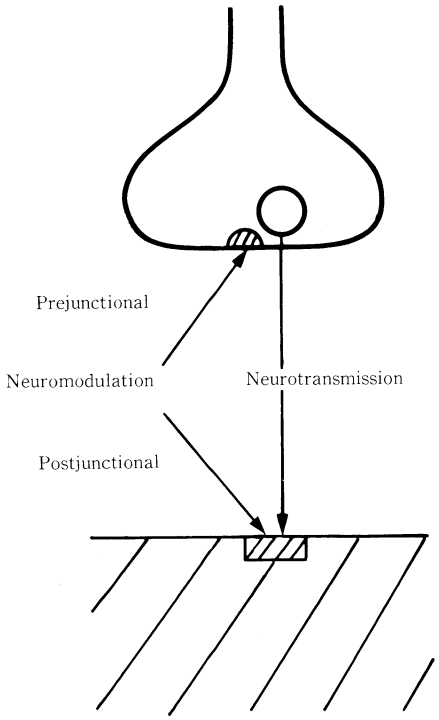

Fig. 1. Diagrammatic representation of neuromodulation. Prejunctional neuromodulators increase or decrease release of transmitter; postjunctional neuromodulators alter the extent and/or time course of transmitter action. (From Burnstock, 1982d).

Neurotransmitters

For many years the only autonomic neurotransmitters recognised were acetylcholine (ACh) and noradrenaline (NA). However, in the early 1960s, inhibitory junction potentials were recorded in intestinal smooth muscle during stimulation of enteric nerves in the presence of cholinergic and adrenergic blocking agents (Burnstock *et al.*, 1963), and the existence of non-adrenergic, non-cholinergic nerves was clearly established in the following years, not only in the gut, but in many other visceral and vascular organs (see Burnstock, 1969, 1981 a).

Many substances were examined as putative transmitters in non-adrenergic, non-cholinergic nerves, supplying the smooth muscle of the gut and urinary bladder. The following criteria were used: synthesis and storage in the nerve terminals; Ca^{2+}-dependent release on nerve stimulation; occupation of specific postjunctional receptors resulting in actions that mimic those produced by nerve stimulation; inactivation by enzymes and/or uptake synthesis; and agents that produce parallel block (or potentiation) of the responses to nerve stimulation and exogenous application of the substance. The substance that best satisfied these criteria was a purine nucleotide, probably adenosine-5′-triphosphate (ATP), and the purinergic nerve hypothesis was proposed (Burnstock, 1972). Since that time considerable evidence has accumulated in support of this hypothesis, although there have also been several reports that oppose it (see Burnstock, 1975 b, 1979 ; Gillespie, 1982).

In the mid-1970s, several important new findings suggested that further transmitters might be present in other components of the autonomic nervous system. For example, at least nine ultrastructurally distinguishable types of axon profile were described in the enteric nervous

Table 1. Transmitters proposed in the autonomic nervous system

ACETYLCHOLINE	ACh
NORADRENALINE	NA
ADENOSINE TRIPHOSPHATE	ATP
5-HYDROXYTRYPTAMINE	5-HT
γ-AMINOBUTYRIC ACID	GABA
DOPAMINE	DA
PEPTIDES	
ENKEPHALIN	Enk
VASOACTIVE INTESTINAL POLYPEPTIDE/ PEPTIDE HI	VIP/PHI
SUBSTANCE P	Sub P
GASTRIN RELEASING PEPTIDE/BOMBESIN	GRP/BN
SOMATOSTATIN	ST
NEUROTENSIN	NT
LUTEINIZING HORMONE RELEASING HORMONE	LHRH
CHOLECYSTOKININ/GASTRIN	CCK/G
NEUROPEPTIDE Y/PANCREATIC POLYPEPTIDE	NPY/PP
(BRADYKININ)	
ANGIOTENSIN	AII
ADRENOCORTICOTROPHIC HORMONE	ACTH
CALCITONIN GENE RELATED PEPTIDE	CGRP

system (Cook and Burnstock, 1976); a number of biologically active polypeptides, including enkephalin, somatostatin, vasoactive intestinal polypeptide (VIP), substance P and neurotensin, were localised with immunocytochemical methods in autonomic nerves (see Hökfelt *et al.*, 1980; Furness and Costa, 1980); and both 5-hydroxytryptamine (5-HT) (see Gershon, 1981) and γ-aminobutyric acid (GABA) (Jessen *et al.*, 1979) were localised in enteric nerves with autoradiographic methods. As a result of these and later studies about 16 substances, in addition to ACh, NA and ATP are now regarded as putative transmitters in the autonomic nervous system (see Burnstock *et al.*, 1979 a; Burnstock, 1981 a, 1983 a and Table 1).

Classical Neurotransmission

In classical neurotransmission, a single neurotransmitter is released by exocytosis from its vesicular storage site in the nerve terminal to diffuse across the junctional cleft to occupy specific receptors on the postjunctional membrane, leading to changes in membrane conductance and effector cell activity. Fine control is exercised by higher centres by variations in the frequency pattern of impulses in the nerves and also by the more recently recognised autoregulatory system, where negative feedback of transmitter release is mediated by presynaptic receptors of a different type from those present in postjunctional membranes (see Vizi, 1979). In adrenergic and purinergic junctions, these have been identified, i.e. α_1 and α_2 adrenoceptors (Docherty and McGrath, 1980) and P_1 and P_2 purinoceptors (Burnstock, 1981 b), but the existence of different pre- and postjunctional muscarinic receptors at cholinergic junctions is still being debated (Brown *et al.*, 1980; Fuder *et al.*, 1982).

It is also known that ACh has an inhibitory effect on responses to sympathetic nerve

stimulation via prejunctional muscarinic receptors (see Story *et al.*, 1975), and that NA released from sympathetic nerve terminals reduces the release of ACh from cholinergic nerves in the gut, thereby inhibiting gastrointestinal motility (Paton and Vizi, 1969). There is an anatomical basis for interactions or 'cross talk' between adrenergic and cholinergic nerves which have opposite actions on effector cells, since examples of close apposition of adrenergic and cholinergic nerve varicosities, often enclosed within the same Schwann cell sheath, have been described (Burnstock and Costa, 1975). Pharmacological findings add further support to this concept. For example, ACh released by stimulation of intrinsic cholinergic nerves in the rabbit atria (Story *et al.*, 1975) and in a variety of blood vessels (Vanhoutte, 1974 ; Su, 1978) can lead to a decrease in release of NA during adrenergic transmission.

Co-existence of Neurotransmitters and the Physiology of Co-transmission

The suggestion that some nerve cells store and release more than one transmitter was made in 1976 (Burnstock, 1976) largely on the basis of comparative studies of the evolution of the autonomic nervous system (Burnstock, 1969) and evidence for the co-existence of biologically active substances in certain invertebrate nerves (e.g. Brownstein *et al.*, 1974 ; Cottrell, 1976). Since then considerable evidence has accumulated in support of this possibility (see Cuello, 1982 ; Osborne, 1983). Nearly all nerve profiles examined under the electron microscope contain more than one type of vesicle, which is consistent with the multiple-transmitter concept, although the whole question of the identification of vesicle types to particular transmitter substances is unresolved and must await high electron microscopic resolution of preparations treated with highly specific cytochemical methods for transmitters and related enzymes (see Burnstock, 1982 a).

Acetylcholine and noradrenaline

There is compelling evidence that under certain conditions *in vitro* single sympathetic neurones may release NA, ACh, or a mixture of these two transmitter substances (Hill *et al.*, 1976 ; Furshpan *et al.*, 1976 ; Patterson *et al.*, 1976 ; Bunge *et al.*, 1978). It seems likely that this represents a true reflection of events that occur *in vivo* during perinatal development (Hill and Hendry, 1977). It appears that a population of sympathetic nerve cells are present at birth that have the potential to synthesize both NA and ACh. These multipotential cells require Nerve Growth Factor (NGF) to survive and they respond to NGF with an increased production of both choline acetyltransferase and tyrosine hydroxylase, enzymes that are involved in the synthesis of ACh and NA respectively. Under the influence of conditioning factors, most of the cells appear to differentiate into either cholinergic or adrenergic neurones soon after birth. However, it is possible that some sympathetic neurones, supplying some organs in some animals, retain the ability to produce and release both ACh and NA (see Burn and Rand, 1965 ; Burnstock, 1978 a).

Acetylcholine and adenosine-5′-triphosphate

A detailed account of the evidence for co-existence of acetylcholine and noradrenaline with ATP is available (Burnstock, 1982 c). Further, in a recent paper by Potter, Furshpan and Landis (1983), it was shown that some cultured sympathetic neurones secrete, in addition to NA and ACh a third transmitter, probably adenosine or a phosphorylated derivative. Thus

purinergic function is expressed with adrenergic or cholinergic function or with both (triple function).

Cholinergic vesicles isolated from the electric organ of various elasmobranch fish contain ATP in addition to the principal neurotransmitter ACh (Dowdall *et al.*, 1974 ; Israel *et al.*, 1979 ; Zimmerman, 1979). The ACh : ATP molar ratio in the three species studied is 4-10 : 1. The major nucleotide in these vesicles is ATP (83 per cent of the total), with adenosine diphosphate (ADP) (15 per cent) and traces of adenosine monophosphate (AMP) also being present. Studies of the turnover of adenine nucleotides in cholinergic synaptic vesicles have shown that ATP and ACh are depleted to the same extent (about 50 per cent) during nerve stimulation, that adenosine is an effective precursor of vesicular adenine nucleotides and that the new population of vesicles that appears following nerve stimulation has a high turnover rate for both ATP and ACh (Zimmerman and Denston, 1977 ; Zimmerman, 1979). Furthermore, a saturable uptake system for adenosine into nerve terminals isolated from the *Torpedo* electric organ with a K_m value of 1 μM has been reported, which is comparable to that of the high affinity choline uptake system (Dowdall, 1978). Evidence for axonal flow of ATP, as for ACh, in organelles other than mitochondria has also been reported (Davies, 1978).

Considerable quantities of ATP have also been reported to be released from the endings of phrenic nerves in the rat diaphragm during stimulation (Silinsky and Hubbard, 1973 ; Silinsky, 1975). This compares well with the levels of ATP released on stimulation of some regions of the cortex (Pull and McIlwain, 1972 ; Wu and Phillis, 1978) or cortical synaptosomes (White *et al.*, 1980). Release of [^{3}H]-adenine derivates has been shown to occur in the cholinergic septal system ; these were considered as possible cotransmitters with ACh (Rose and Schubert, 1977).

Botulinum neurotoxin virtually abolished the atropine-resistant response of the guinea-pig bladder to field stimulation, suggesting that ATP, which is a strong contender for the non-cholinergic transmitter to this preparation (Burnstock *et al.*, 1978), is being released as a cotransmitter with ACh (MacKenzie *et al.*, 1982). Hoyes *et al.* (1975) have presented ultrastructural evidence which supports this view.

It seems likely that in these situations the two substances are contained in the same vesicles (Fuldner and Stadler, 1982) and there is convincing evidence that the release of ACh and ATP from electric organ synaptosomes is precisely in parallel, as well as the cycle of reuptake and resynthesis (Zimmerman *et al.*, 1979 ; Morel and Meunier, 1981). Therefore, differential release of the cotransmitters at different impulse frequencies seems unlikely. However, several examples of the functional significance of this type of co-existence are now examined.

(1) *Skeletal neuromuscular junction.* Modulation of the activity of the principal transmitter, ACh, can occur through pre- and/or postjunctional actions of the cotransmitter.

ATP and adenosine have been shown to act on prejunctional purinergic receptors to modulate the release of ACh from cholinergic motor nerves in skeletal muscle of the rat diaphragm (Ginsborg and Hirst, 1972 ; Ribeiro and Walker, 1975), frog sartorius (Ribeiro and Dominguez, 1978 ; Branisteanu *et al.*, 1979) and fish electric organ (Israel *et al.*, 1977). These responses are blocked by methylxanthines (Ginsborg and Hirst, 1971 ; Ribeiro and Dominguez, 1978) indicating that they are mediated by P_1 purinoceptors (Burnstock, 1978 b). It has been suggested that occupation of the presynaptic P_1 purinoceptors leads to decrease in the entry of

Ca^{2+} with consequent reduction in release of ACh (Ribeiro, 1979 ; Dowdle and Maske, 1980 ; Israel *et al.*, 1980 ; Hayashi *et al.*, 1981). The frequency, but not the mean amplitude, of miniature endplate potentials and the amplitude of the nerve-evoked endplate potentials are reduced, indicating that the actions of adenosine (and ATP via adenosine) are presynaptic (Ginsborg and Hirst, 1972 ; Ribeiro and Walker, 1975). Further, ATP in concentrations sufficient to produce modulatory effects had no direct postsynaptic action (Ribeiro, 1977).

ATP has also been shown to be a postjunctional modulator of the action of ACh at the skeletal neuromuscular junction. Increase in ACh receptor sensitivity by ATP has been demonstrated at the motor endplate (Saji *et al.*, 1975 ; Ewald, 1976 ; Akasu *et al.*, 1981). The amplitude of the current induced by ionophoretic application of ACh to the motor endplate in frog skeletal muscle is increased in the presence of ATP, and kinetic analysis has suggested that ATP increases ACh sensitivity by acting on the allosteric site of the receptor-ionic channel complex without changing the affinity of ACh for its recognition site (see Stone, 1981).

(2) *Brain and gut.* ATP and adenosine have been shown to act on presynaptic purinergic receptors leading to modulation of the release of ACh from cholinergic motor nerves in other preparations, including brain (Kluge *et al.*, 1977) and intestine (Kosterlitz and Lees, 1972 ; Takagi and Takayanagi, 1972 ; Mori *et al.*, 1973 ; Gintzler and Musacchio, 1975 ; Hayashi *et al.*, 1978 ; Moritoki *et al.*, 1978 ; Okwusaba and Cook, 1980). These responses are blocked by methylxanthines (Sawynok and Jhamandas, 1976 ; Vizi and Knoll, 1976) indicating that they are mediated by P_1 purinoceptors. ATP does not act by way of P_2 purinoceptors, but is rapidly broken down to AMP and adenosine which occupy the P_1 purinoceptors on the cholinergic nerve terminals in the intestine (Moody and Burnstock, 1982). Low concentrations of adenosine or ATP also reduce evoked excitatory postsynaptic potentials in brain to half control values by way of presynaptic receptors (Kuroda *et al.*, 1976 ; Scholfield, 1978).

Noradrenaline and adenosine-5′-triphosphate

It has been known for a number of years that ATP is stored and released together with catecholamines from adrenal chromaffin cells (Douglas and Poisner, 1966 ; Stevens *et al.*, 1972). It has also been suggested that medullary granule-associated nucleotides may act locally as 'co- agonists' with biogenic amines and may additionally provide a circulatory pool of purines for use by heart and lungs (Van Dyke *et al.*, 1977). Storage of ATP together with NA in adrenergic nerves was recognised in the early literature (see Stjärne and Lishaijko, 1966 ; Geffen and Livett, 1971). The first indication that ATP might be released from adrenergic nerves was the demonstration that stimulation of periarterial adrenergic nerves led to release of tritium from taenia coli preincubated in [^{3}H]-adenosine (which is taken up and converted to [^{3}H]-ATP) ; both the release of tritium and NA were blocked by guanethidine (Su *et al.*, 1971). Later Langer and Pinto (1976) suggested that the substantial residual non-adrenergic, non-cholinergic response of the cat nictitating membrane following depletion of NA by reserpine, may be due to release of the ATP remaining in adrenergic nerves.

(1) *Vas deferens.* The physiology of co-transmission has been most fully explored in this tissue. The co-existing substances, NA and ATP act as synergistic neurotransmitters via postjunctional receptors, as well as exerting modulatory effects on each other via both pre- and postjunctional mechanisms (Fig. 2).

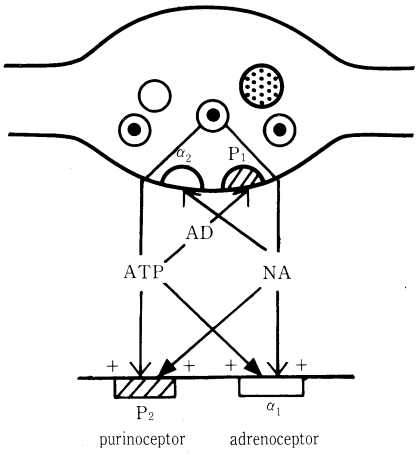

Fig. 2. Schematic representation showing that ATP and NA are released as co-transmitters from the sympathetic nerves supplying the vas deferens and some blood vessels. ATP acts on P_2-purinoceptors on the smooth muscles to initiate e.j.p.s, action potentials and the phasic contraction. NA acts on α_1-adrenoceptors to produce the second phase of the contraction by a different mechanism. Prejunctional α_2-adrenoceptors and P_1 purinoceptors can reduce transmitter release when activated by noradrenaline and adenosine, respectively. Note that in this model ADP and NA are stored in the same vesicle (From Burnstock, 1983b).

Evidence has been presented that ATP is stored and released as a co-transmitter together with NA from adrenergic nerves supplying the guinea-pig vas deferens (Westfall *et al.*, 1978 ; Fedan *et al.*, 1981 ; Stone, 1981). The initial phasic component of the excitatory response to sympathetic nerve stimulation is selectively antagonised by arylazido amino propionyl ATP ($ANAPP_3$), which is claimed to be a specific P_2 purinoceptor antagonist (Hogaboom *et al.*, 1980) or by selective desensitisation of the P_2-purinoceptor by α, β-methylene ATP (Meldrum and Burnstock, 1983) ; while the secondary more tonic component of the response is antagonised by prazosin or reserpine (Fedan *et al.*, 1981). The calcium channel blocker nifedipine has also been shown to block the initial, but not the secondary responses, of this preparation to nerve stimulation and contractions to ATP, but not to NA (Stone, 1981). More recently the excitatory junction potentials (EJP's) recorded in smooth muscle cells of the vas deferens in response to sympathetic nerve stimulation have been blocked by both $ANAPP_3$ (Sneddon and Westfall, 1984) and α, β-methylene ATP (Sneddon and Burnstock, 1984). Furthermore, local application of ATP by pressure ejection from a micropipette produced a transient depolarisation comparable to the EJP, which was also blocked by α, β-methylene ATP ; NA applied in a similar manner produced no such responses (Sneddon and Burnstock, 1984).

ATP and adenosine have been shown to inhibit NA release from adrenergic nerves supplying the vas deferens (Clanachan *et al.*, 1977 ; Wakade and Wakade, 1978, and see Paton, 1981). The prejunctional receptor that mediates these actions is the P_1-purinoceptor, since the inhibitory actions of ATP as well as adenosine are blocked by methylxanthines and because the slowly degradable methylene analogs of ATP are ineffective (De Mey *et al.*, 1979). It has been suggested that occupation of prejunctional P_1 purinoceptors leads to decrease in Ca^{2+} influx with subsequent reduction in NA release (Wakade and Wakade, 1978).

Purine nucleotides or nucleosides also act as postjunctional neuromodulators in the vas deferens and iris enhancing the actions of NA, while NA can potentiate the responses of the vas deferens and seminal vesicle to ATP (Hedqvist and Fredholm, 1976 ; Gustafsson, 1982 ; Holck and Marks, 1978).

(2) *Blood vessels*. Su (1975 ; 1978) used tritium-labelled adenosine and NA to show that

ATP is released together with NA from sympathetic nerves supplying the rabbit aorta and portal vein. Co-existence of NA and ATP has also been demonstrated in rabbit ear artery (Head *et al.*, 1977) and in dog basilar artery (Muramatsu *et al.*, 1981). ATP as well as NA release from guinea-pig portal vein has been shown to be abolished following sympathectomy (Burnstock *et al.*, 1979 b). Fluorescence in nerves of the rat portal vein following incubation in quinacrine, which binds to ATP (Irvin and Irvin, 1954 ; Olson *et al.*, 1976), is also abolished by sympathectomy (Burnstock *et al.*, 1984 a). In the rat tail artery, electrical responses to stimulation of the sympathetic nerves consist of two components, namely a fast depolarisation to each stimulating pulse and a slow maintained depolarisation as the train of stimuli progresses ; the slow component is blocked by phentolamine, suggesting it is mediated by α-adrenoceptors, while the fast depolarisations are blocked by α, β-methylene ATP, suggesting mediation by P_2 purinoceptors (Burnstock *et al.*, 1984 b).

Pre- and postjunctional modulation of responses to sympathetic nerve stimulation have been demonstrated in mesenteric, basilar and pulmonary arteries (Su, 1975, 1978 ; Verhaeghe *et al.*, 1977 ; Muramatsu *et al.*, 1981 ; Katsuragi and Su, 1982).

Established transmitters with polypeptides

Certain peripheral endocrine cells, particularly those located in the gastro-intestinal tract, contain both a biogenic amine, such as 5-hydroxytryptamine (5-HT) or histamine, and a peptide hormone, such as substance P, somatostatin or neurotensin. These cell systems are part of the so-called APUD ('Amine content of Precursor Uptake Decarboxylation') system (Pearse, 1969). Pearse postulated that this situation may also exist in neurones.

In the peripheral nervous system, somatostatin-like immunoreactivity has been observed in about 60-70 per cent of all principal adrenergic ganglion cells of the inferior mesenteric ganglion and of the coeliac superior mesenteric ganglion complex (Hökfelt *et al.*, 1977), suggesting the co-existence of NA and somatostatin or a structurally-related peptide in the same peripheral sympathetic neurone. In the superior cervical ganglion of the rat, enkephalin-like immunoreactivity has been observed in a rather small proportion of ganglion cells, at least some of which contain NA (Schultzberg *et al.*, 1979). Lundberg and co-workers (Lundberg *et al.*, 1979) have discovered a further example of co-existence by combining immunocytochemistry and acetylcholinesterase (AChE) staining : it was shown that the AChE-rich cells of the cat sympathetic L7-S1 ganglia contain a vasoactive intestinal polypeptide (VIP)-like peptide. Examples of co-existence have also been observed in the central nervous system, for example, in the lower brain stem, substance P-like immunoreactivity has been observed in 5-HT-containing neurones (Chan-Palay *et al.*, 1978 ; Hökfelt *et al.*, 1978).

Evidence is accumulating that many peptides are stored in large granular vesicles separately from the established transmitters ACh or NA, which are stored predominantly in small vesicles (Johansson and Lundberg, 1981 ; Sundler *et al.*, 1982). This separate storage system for co-existing transmitters, in contrast to the storage of ATP and established transmitters in the same vesicles described earlier, would appear to allow differential release of the cotransmitters at different impulse frequencies.

(1) *Cat exocrine gland.* There are many examples now of the co-existence of established neurotrnasmitters with various peptides. However, the only preparation where an analysis

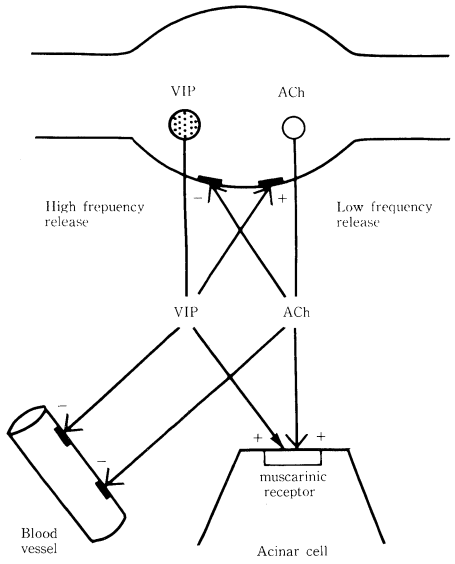

Fig. 3. Schematic representation of transmission where vasoactive intestinal polypeptide (VIP) is a co-transmitter with acetylcholine (ACh) in parasympathetic nerves supplying the cat salivary gland. Note that ACh and VIP are stored in separate vesicles; they can be released differentially at different stimuation frequencies to act on acinar cell and glandular blood vessels. Co-operation is achieved by selective release of ACh at low impulse frequencies. Pre- and postjunctional modulation is indicated (From Burnstock, 1983b).

has been carried out on the conditions for release and the sites and types of actions of the cotransmitters is the cat exocrine gland (Lundberg, 1981 ; Edwards and Bloom, 1982). In the salivary gland, ACh released from parasympathetic nerves at low frequencies causes salivary secretion from acinar cells and some dilation of blood vessels in the gland. VIP released by nerve stimulation at higher frequencies (>15 Hz) produces marked vasodilatation, and although it has no direct effect on acinar cells, it does substantially enhance the effect of ACh on acinar cell secretion and the release of ACh from the nerve endings via prejunctional responses (see Fig. 3).

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

Evidence has been presented that in some instances peptides may be stored within the same vesicles as the established transmitter. For example, substance P and 5-HT in dense-cored vesicles (60-90 nm) in nerve terminals in brain and spinal cord (Pelletier *et al.*, 1981), and opiate-like peptides and NA in large dense-cored vesicles in bovine splenic nerve (Wilson *et al.*, 1980). Co-operation between cotransmitters in these situations is therefore likely to be different from that employed by VIP and ACh in the cat salivary gland.

(2) *Sensory fibres.* The 'axon reflex' concept (see Langley, 1923 ; Lewis, 1927 ; Dale, 1935) involved rlease of transmitter following antidromic impulses down collateral branches of primary afferent sensory fibres to account for vasodilatation particularly of skin vessels, although the possibility that 'axon reflexes' from sensory collaterals also occur in stomach

(Delbro and Fändriks, 1982), carotid body (McDonald and Mitchell, 1981) and blood vessels in the lung (Lundberg and Saria, 1982) has also been raised. Thus, this type of physiological control mechanism may be more widespread than originally visualised. The transmitter released during the 'axon reflex' is not yet clearly established. Substance P is a strong contender (Hagermark *et al.*, 1978 ; Gamse *et al.*, 1980 ; Bernstein *et al.*, 1981), ATP is another (Holton, 1959 ; Jahr and Jessel, 1983), and it has been suggested that substance P and ATP may co-exist in some primary afferent nerve fibres (Burnstock, 1977).

Local Regulatory Mechanisms

The local regulatory substances considered here will be limited to those that are produced secondarily as the result of neurotransmitter action and which themselves have potent biological actions.

Cyclic AMP is perhaps the best recognised 'second messenger'. In addition to being released when catecholamines occupy β-adrenoceptor sites, putative neurotransmitter substances such as peptides and purine nucleosides are also known to act on adenylate cyclase systems leading to production of cAMP (see Daly, 1975 ; Amiranoff and Roselin, 1982).

The discovery that ATP is a potent inducer of prostaglandin synthesis (Needleman *et al.*, 1974) led Burnstock *et al.* (1975) to suggest that ATP released from purinergic nerves may be linked with prostaglandins in peristalsis. These authors showed that the prostaglandin synthesis inhibitor indomethacin, blocked the 'rebound contraction' that follows the inhibitory responses of the guinea-pig taenia coli to purinergic nerve stimulation and ATP. This concept of a functional link between ATP and prostaglandins has been extended to other intestinal preparations (Kamikawa *et al.*, 1977) and to atropine-resistant excitation of the urinary bladder (Dean and Downie, 1978). Prostaglandin synthesis can also be induced by other substances such as angiotensin, bradykinin and NA (Collier *et al.*, 1976 ; Goldberg *et al.*, 1976).

Some putative transmitter substances, including ATP (Kiernan, 1974) and some vasoactive peptides, particularly substance P (Johnson and Erdös, 1973), release histamine from mast cells.

There is growing evidence that peptides and amines contained in some APUD endocrine cells in the gastrointestinal tract may be released secondarily as a result of the action of various neurotransmitters (see Bloom, 1978). Matsuo and Seki (1978) report nerve varicosities containing vesicles within 200-300 nm of gastrin-containing and parietal cells in rat stomach.

'Trophic' Factors

In addition to release of substances (neurotransmitters) that are involved in short-term communication between excitable cells, there is growing evidence for the release of 'trophic' factors from nerves and effector cells that are concerned with long-term communication during development (see Burnstock, 1981 a, c ; 1982 b).

Influence of nerve on muscle development

Undifferentiated smooth muscle cells divide and proliferate in culture until a confluent monolayer is formed ; in contrast, differentiated smooth muscle cells from more mature animals dedifferentiate before proliferation takes place (Chamley *et al.*, 1974). As soon as a confluent monolayer is formed redifferentiation occurs, as indicated by the appearance of

myosin immunofluroescence and of thick filaments (Gröschel-Stewart *et al.*, 1975). The cells then aggregate into either clumps or chains. Spontaneous contractions develop which become synchronous as gap junctions form low-resistance pathways between neighbouring cells. This sequence of changes is similar to that described during normal development *in vivo* (Yamauchi and Burnstock, 1969) and in anterior eye chamber transplants of smooth muscle (Campbell *et al.*, 1971). The presence of sympathetic nerves delays the process of dedifferentiation. This effect is not mimicked by NA, ACh or spinal cord extract, but is mimicked by either sympathetic chain extract or dibutyryl cyclic AMP (Chamley and Campbell, 1975). These results could be explained by the release of a trophic substance from sympathetic nerves which acts on adenylate cyclase resulting in the production of cyclic AMP, which in turn promotes differentiation, delaying dedifferentiation and proliferation.

Formation of muscle effector bundles and gap-junctions occurs in culture following confluence in the absence of sympathetic nerves, but this is accelerated by their presence (Chamley *et al.*, 1974). In small clumps of muscle supplied by nerves, foci of synchronous contraction appear much earlier than in similar clumps without nerves, and there is an approximately two-fold increase in gap-junctions in the muscle clamps. Muscle effector bundle formation in anterior eye chamber transplants occurs at about the same time that varicose adrenergic nerves penetrate into the muscle layer (Malmfors *et al.*, 1971 ; Burnstock *et al.*, 1971), suggesting that in this situation too, nerves might influence muscle differentiation and aggregation.

Influence of muscle and associated tissue on nerve development

Explants of autonomic effector organs influence the growth of nerves from sympathetic ganglia *in vitro* (Levi-Montalcini *et al.*, 1954 ; Chamley-Campbell *et al.*, 1979 ; Burnstock, 1981 d). 'Attraction' of sympathetic nerves is evident soon after the nerve fibres emerge from ganglion explants, and it has been suggested that a chemical substance released from the smooth muscle explants might be involved (Chamley *et al.*, 1973 a). There are a number of indications that this substance is NGF : (i) there is increased growth of sympathetic or sensory nerves in the presence of NGF-producing mouse sarcomas (Bueker, 1948 ; Levi-Montalcini *et al.*, 1954 ; (ii) sensory nerves grow towards the tip of capillary tubes containing a solution of NGF, but not to tubes containing saline (Charlwood *et al.*, 1972) ; (iii) there are higher levels of NGF in densely innervated tissues than in sparsely-innervated tissues (Levi-Montalcini and Angeletti, 1961 ; Johnson *et al.*, 1971) ; and densely innervated tissues stimulate greater nerve growth (Chamley *et al.*, 1973 a).

The development and growth of the sympathetic nervous system *in vivo* also appears to be under the influence of NGF (Levi-Montalcini and Angeletti, 1968 ; Zaimis and Knight, 1972 ; Hendry and Campbell, 1976 ; Coughlin *et al.*, 1977). NGF is present in sympathetically innervated tissues during early embryogenesis and is taken up by adrenergic nerve terminals and transported by retrograde axonal flow to the cell body, where it exerts its major actions (Hendry *et al.*, 1974). It increases the size of immature neurones, the survival of differentiated neurones, the rate of growth of adrenergic axons, and the content of NA (Thoenen *et al.*, 1971). NGF appears to affect cholinergic as well as adrenergic synthetic enzymes in immature sympathetic neurones, since high doses of NGF in 2-day-old rats result in increases in en-

dogenous activities of choline acetyltransferase as well as tyrosine hydroxylase (Hill and Hendry, 1977).

At an early stage of postnatal life, most sympathetic neurones receive functional preganglionic innervation. At about the same time there is a rapid rise in tyrosine hydroxylase and dopamine-β-hydroxylase activity. This rise is prevented by preganglionic section of ganglionic blockade, indicating that maturation of the adrenergic neurone depends in part on transynaptic influences resulting from preganglionic nerve activity (Black, 1973). In addition, it has been shown that retrograde regulation by target organs also plays an important role (Hendry and Iversen, 1973, Hendry and Campbell, 1976 ; Dibner *et al.*, 1977).

When the interactions between sympathetic nerve fibres and single, isolated smooth muscle cells were studied for the first time (Chamley *et al.*, 1973 b ; Mark *et al.*, 1973), nerve fibres, upon random contact with a cell, appeared able to distinguish between fibroblasts and muscle cells within about an hour. Nerves did not maintain contact with fibroblasts after this time (and nerves never came closer than 30-40 nm), whereas they formed long-lasting, intimate relationships with smooth muscle cells of the potentially densely-innervated vas deferens, which were often maintained for up to several weeks. This process is called 'recognition' (see Burnstock, 1981 d). The junctions that formed between sympathetic nerves and smooth muscle cells became functional 1-3 days after first contact (Purves *et al.*, 1974). ACh or NA receptors do not appear to be involved in the mechanisms of 'recognition' of cardiac muscle cells by sympathetic nerves, since long-lasting associations still occurred in the presence of propranolol (an adrenergic β-blocker) and hyoscine (a cholinergic muscarinic blocker) (Campbell *et al.*, 1978). This is consistent with the finding that cholinergic nicotinic receptors are not necessary for the formation of skeletal neuromuscular junctions *in vitro* (Obata, 1977). 'Recognition' sites do not appear to distinguish between adrenergic and cholinergic nerves. For example, adrenal medullary cells, which are normally supplied by cholinergic nerves, form longlasting contacts with either cholinergic fibres growing from explants of ciliary ganglion or with adrenergic nerves growing from explants of superior cervical ganglion (Unsicker *et al.*, 1977).

Identity of trophic factors

The identification of trophic factors released from nerves is at a very early stage, but it is interesting that both purine nucleotides and nucleosides and polypeptides, compounds known to be co-stored and released together with classical transmitters, have been claimed to have trophic actions in development and regeneration (see Henderson and Scott, 1980 ; Burnstock, 1982 b), as well as nerve growth factors and related neuromuscular agents (Thoenen and Barde, 1980 ; Varon and Skaper, 1981).

A detailed consideration of neuropeptides as trophic factors has been presented recently (Burnstock, 1982 b). A few examples follow : enkephalin and β-endorphin enhance sprouting of noradrenergic nerves in the medulla and cerebellum following damage to adrenergic nerves after treatment of newborn rats with 6-hydroxydopamine (Harston *et al.*, 1981) ; β-endorphin immunoreactivity is detectable in motoneurones in the immature rat spinal cord up to 28 days postpartum, and it has been suggested that β-endorphin released from developing motoneurones regulates the activities of the different molecular forms of acetylcholinesterase that take place during development of skeletal muscles (Haynes and Smith, 1982) ; substance P appears

to prevent degeneration of noradrenergic neurones in the cortex produced by treatment of newborn rats with 6-hydroxydopamine (Jonsson and Hallman, 1982) ; substance P stimulates the outgrowth of neuronal processes in explant cultures of the chick trigeminal ganglion, but not explant cultures of the hippocampus from foetal rats (Lindner and Grosse, 1981).

Adenosine is a potent modulator of growth-related proceses (see Fox and Kelley, 1978 ; Henderson and Scott, 1980 ; Stone, 1981). Chronic administration of adenosine produces a marked increase in the growth of the capillary vessels in vascular beds of the heart and skeletal muscle (Hudlicka *et al.*, 1983). An effect of purine nucleotides and nucleosides on tumour growth has been known for some time, and it has been proposed that endogenously released adenosine may act by regulating the vascular supply to neoplastic tissue (Phillis and Wu, 1981). Adenosine-uptake inhibitors such as dipyridamole or diazepam stimulate the growth of transplanted mammary tumours (Karmali *et al.*, 1978), while adenosine antagonists reduce the size of primary tumours and the numer of metastases (Janik *et al.*, 1980). 5′-N-ethylcarboxamide adenosine (NECA), a potent P_1 purinoceptor agonist, is synergistic with NGF in producing neurite outgrowth in PC12 cells, clones derived from a pheochromocytoma tumour (Guroff *et al.*, 1981). Some of the adenosine involved in these trophic effects seems likely to arise from the rapid breakdown by ectoenzymes of ATP released from nerves.

Summary

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

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特別講演 II

脳腸ペプチド産生細胞
——ハムスターの副腎髄質におけるカテコールアミンとオピオイドに関する免疫組織化学——

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I. はじめに

藤田は，古典的には神経細胞（ニューロン）とは考えられないが，構造的にも機能的にも分泌物の観点からも，ニューロンに似ている一群の細胞をパラニューロン（paraneurons：para-には空間的な近傍であり，性質が似ているという意味がある）と呼んだ（藤田，小林，1975；Fujita, 1976）．パラニューロンには消化管粘膜の基底顆粒細胞，副腎クロム親和細胞，頸動脈小体の主細胞，甲状腺の傍濾胞細胞，下垂体前葉の内分泌細胞，膵臓の島細胞，皮膚のMerkel細胞，松果体細胞などが含まれる．

脳腸ペプチド産生細胞とは，脳腸ペプチドの存在が証明できるニューロンとパラニューロンである．神経分泌ニューロンはもとより，自律神経系のニューロンの多くは免疫組織化学的に検出できる量の脳腸ペプチドを含んでいる．また，脳腸ペプチドの存在が証明されていないニューロンであっても潜在的には脳腸ペプチド産生能をもつものは多い．一方，甲状腺の濾胞細胞や副腎皮質のステロイド分泌細胞は，内分泌機能を営むにもかかわらず，細胞生物学的には脳腸ペプチド産生細胞から明瞭に区別される．

私どもは，副腎クロム親和細胞をモデルにして，脳腸ペプチド産生細胞に関する形態学的な研究を行なってきた（Kobayashi, 1977）．哺乳類の副腎髄質は，クロム親和細胞が大きな塊をなし，またこれを支配する交感神経節前線維の終末が大きいので，私どもの研究目的には材料として適当である．本稿では，ハムスターの副腎クロム親和細胞におけるカテコールアミンとオピオイドペプチドの共存問題について報告する．さらに，副腎クロム親和細胞とシナプスを形成する交感神経節前線維に含まれるオピオイドペプチドの局在についても論ずる．私どもは，副腎髄質で観察される細胞生物学的現象の基本は，すべての脳腸ペプチド産生細胞に通用すると想像している．

II. 材料と方法

成熟したハムスターの副腎を使用した．飼育条件には特に注意をはらわなかった．

ネンブタール麻酔下に左心室から固定液を全身に灌流し，ただちに副腎髄質を取り出した．固定液は0.1 Mリン酸緩衝液でpH 7.4に調節した4%パラホルムアルデヒド液または4%パラホルムアルデヒド−1%グルタールアルデヒド混合液である．固定が終了した組織片はアラルダイトまたはLowicryl K4Mに包埋，光顕用にはガラスナイフで0.5 μm厚の切片とし，スライドガラスに貼付した．一方，電顕用にはダイヤモンドナイフで超薄切片（70 nm厚）を作成し，ニッケル製のグリッドに貼付して免疫組織化学反応を行なった．

光顕レベルでの免疫組織化学にはPAP法を使用した．一方，電顕レベルでの実験はプロテインA・金コロイド法を用いた。詳細な手技について綜説をまとめて発表した（内田，小林，

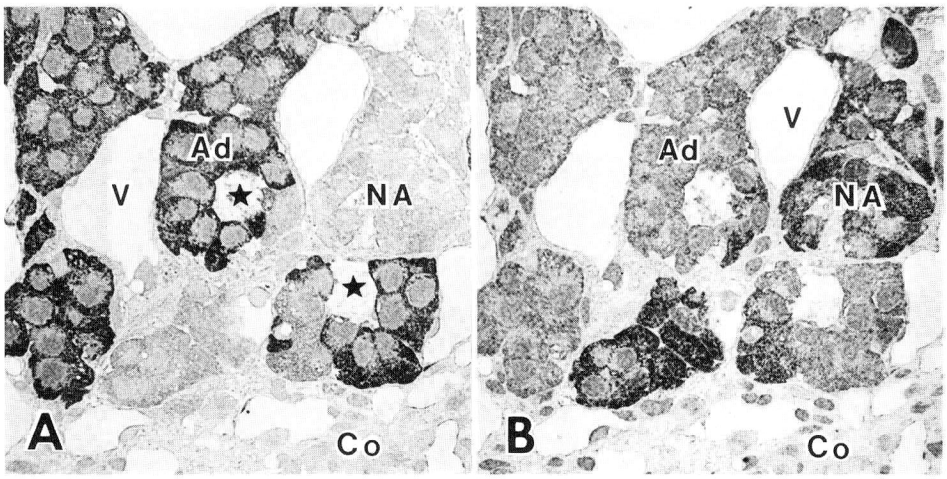

図1. 厚さ0.5 μmの連続切片を，A：抗アドレナリン血清(AP16-2, Verhofstad, Nijmegen, The Netherlands)と，B：抗ノルアドレナリン血清（NAP1-18, Verhofstad）で免疫染色した．アドレナリン様免疫活性はA細胞（Ad）に限局して認められる（A）．ノルアドレナリン様免疫活性はNA細胞（NA）に強い（B）．しかしA細胞（Ad）も弱いノルアドレナリン様免疫活性を示す（B）．星印は濾胞腔を示す．
V：血管，　Co：副腎皮質．　×460

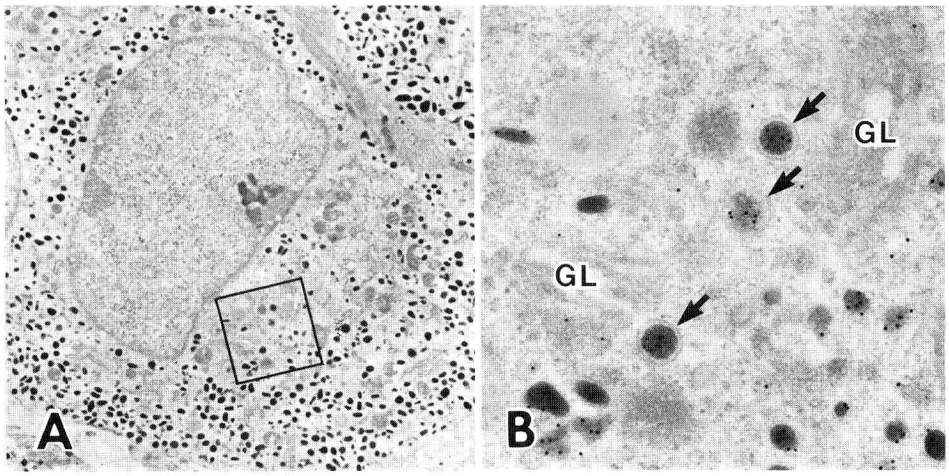

図2. 抗Met-エンケファリン-Arg-Phe血清（AP3-311，中尾，井村，京都大学医学部）で免疫染色したクロム親和細胞．BはAの枠内の拡大．免疫活性の局在を示す金粒子は成熟した分泌顆粒の他に，未熟な分泌顆粒（矢印）にも認められる．　GL：Golgi層板．　A：×5,000，　B：×25,000.

1984)．なお本研究に用いた抗血清はKobayashi, *et al.* (1983 a) が用いたものと同じである．

III. 結　果　と　考　察

1. クロム親和細胞でのカテコールアミンとオピオイドペプチドの共存

副腎髄質ではアドレナリンはA細胞から，一方，ノルアドレナリンはNA細胞から分泌される(図1A, B)．A細胞に含まれるノルアドレナリンはアドレナリンの前駆体とみなされる(Kobayashi, *et al.*, 1983 a)．

近年，副腎クロム親和細胞が分泌するオピオイドペプチドとその遺伝子が研究者の関心を集めている（菅野，1983；Pearse, 1983)．クロム親和細胞では核からの遺伝情報に基づいてプレプロエンケファリンが合成され，粗面小胞体で

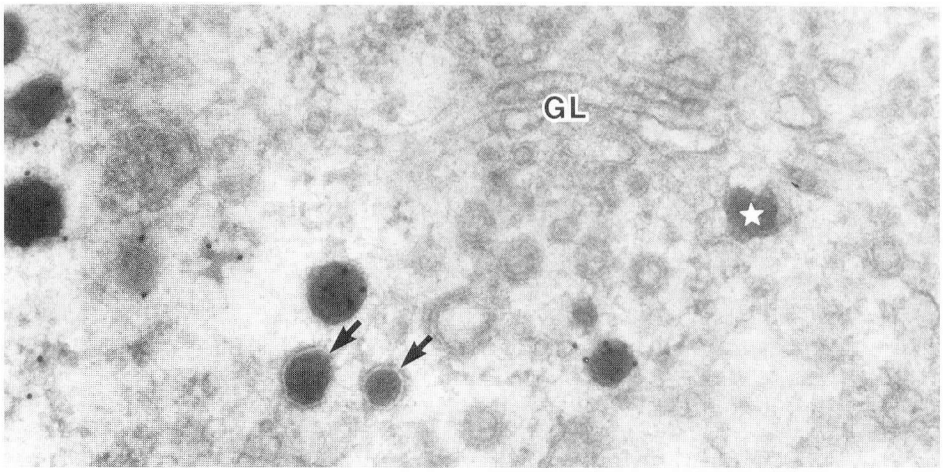

図3.　A細胞のアドレナリン様免疫活性．成熟した分泌顆粒のみが免疫活性を示し，未熟な分泌顆粒（矢印），Golgi層板（GL）や層板内の暗調物質（星印）には免疫活性は認められない．　×72,000

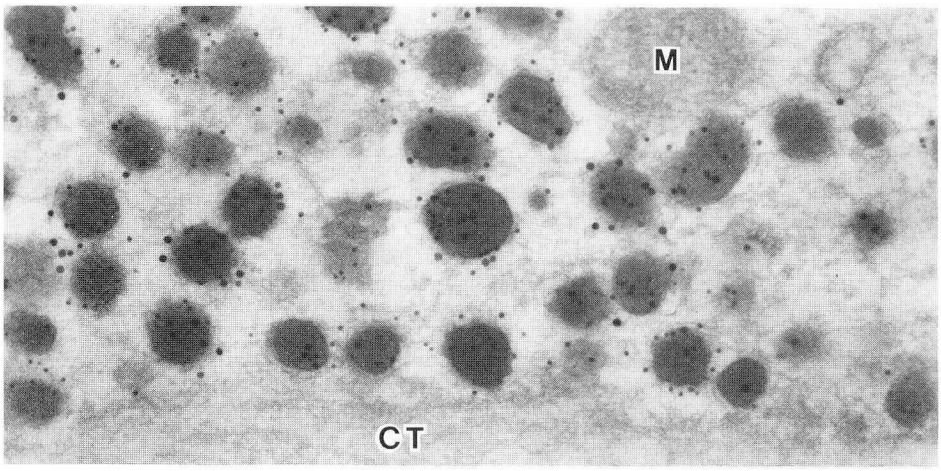

図4.　A細胞におけるカテコールアミンとエンケファリンの共存を示す電顕写真．アドレナリン様免疫活性（小粒子，直径8 nm）とMet-エンケファリン-Arg-Phe様免疫活性（大粒子，直径12 nm）は同一の分泌顆粒に認められる．　M：糸粒体，　CT：結合組織．　×77,000

プロエンケファリンが生じ，細胞内輸送の過程でプロセシングを受けて短鎖のオピオイドペプチドが生産される。カテコールアミンが添加されるのはGolgi装置で分泌顆粒が形成されて以後のことである（Kobayashi, 1977）．

図2にはプロテインA・金コロイド法でハムスターの副腎クロム親和細胞に，プロエンケファリンA（Noda, *et al*., 1982）のC末端にあるMet-エンケファリン-Arg-Pheを証明した電顕写真を示す．この写真によってMet-エンケファリン-Arg-Pheが主として分泌顆粒に含まれていることがわかる．Golgi層板の中の顆粒や，Golgi装置の周辺にある幼若な分泌顆粒にもこのオピオイドペプチドの免疫活性を証明できる（図2B）．

図3にはプロテインA・金コロイド法でハムスターの副腎クロム親和細胞にアドレナリンを検出した電顕写真を示す．クロム親和細胞ではアドレナリンは主として成熟した分泌顆粒に含まれ，Golgi装置の中およびその周辺にある幼若な分泌顆粒には含まれていない．

さて，プロテインA・金コロイド法では，，直

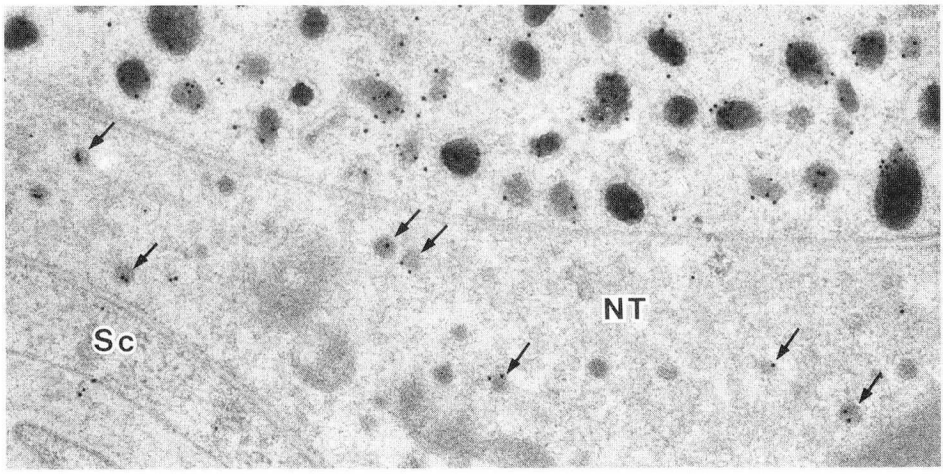

図5. クロム親和細胞と，それに接する交感神経節前線維の神経終末で，Met-エンケファリン-Agr-Phe様免疫活性を検出した．クロム親和細胞の分泌顆粒と神経終末（NT）に含まれる芯あり小胞（矢印）の両者が免疫活性を示す． Sc：Schwann細胞． ×28,000

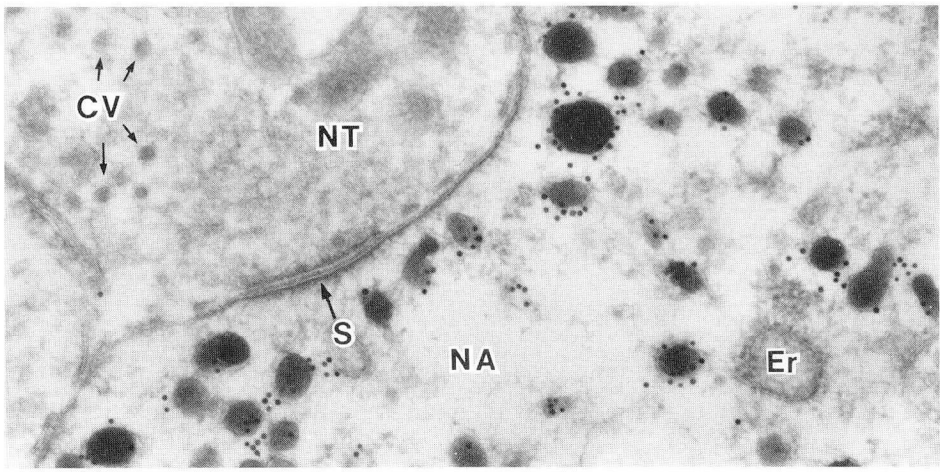

図6. NA細胞（NA）とシナプス結合（S）している交感神経節前線維の神経終末（NT）．抗ノルアドレナリン血清で免疫染色した．免疫活性はNA細胞（NA）の分泌顆粒に認められ，神経終末（NT）の芯あり小胞（CV）は免疫活性を示さない． Er：粗面小胞体． ×60,000

径の異なる2種類の金コロイドを使用して，二重染色をおこなうことが可能である（内田，小林，1984）．私どもは電顕用切片の表と裏とを染め分ける方法を用いて，ハムスターの副腎クロム親和細胞の分泌顆粒におけるカテコールアミンとオピオイドペプチドの共存問題を研究した．図4に示す電顕写真では，アドレナリンとMet-エンケファリン-Arg-Pheが同一の分泌顆粒内に共存していることが証明されている．

2. 交感神経節前線維の終末に含まれるオピオイドペプチド

副腎髄質のクロム親和細胞を支配する交感神経節前線維の終末については，生理学的・薬理学的研究が数多くなされているにもかかわらず，実際にその姿を示した形態学的研究はほとんど発表されていない．

従来からのGolgi法，Cajal法，Bielschowsky法をはじめとする鍍銀法やメチレンブルー法やコリンエステラーゼ法では，交

感神経の副腎枝の終末を明瞭にみることができない（Coupland, 1965）．電子顕微鏡的にはシナプスが同定できる．しかし電顕的方法には，視野が狭いことおよび平面的な像しか得られないという弱点があった（Coupland, 1965）．

私どもは，副腎クロム親和細胞に含まれるエンケファリン関連ペプチドについて，免疫組織化学的に研究している最中に，ラットの副腎で交感神経節前線維の終末が，非常に強いオピオイドペプチドの免疫活性を示すことを見いだした（Kobayashi, *et al.*, 1983 b）．ハムスターの副腎髄質では交感神経節前線維だけでなく，クロム親和細胞もオピオイドペプチドの強い免疫活性を呈するために，光顕的には交感神経節前線維を同定することは困難である．しかし，プロテインA・金コロイド法による電顕免疫組織化学では神経終末内におけるオピオイドペプチドの局在を明瞭に証明できる．

図5にはハムスターの副腎クロム親和細胞を支配する交感神経節前線維に含まれるMet-エンケファリン-Arg-Pheの局在を，プロテインA・金コロイド法による電顕免疫組織化学で示した．この写真にはクロム親和細胞の一部も含まれている．Met-エンケファリン-Arg-Phe様免疫活性はクロム親和細胞の分泌顆粒に含まれているほかに，交感神経節前線維の終末に含まれる大型芯あり小胞にも検出できる．しかし，小型で芯のないシナプス小胞や殻あり小胞（coated vesicles）にはこれを検出できない．またミトコンドリアや細胞膜にも陽性反応を認めない．

図6はハムスターの副腎クロム親和細胞に終る交感神経節前線維とそれをとりまくクロム親和細胞の細胞質にノルアドレナリンを検出したプロテインA・金コロイド法による電顕写真である．クロム親和細胞の分泌顆粒にはノルアドレナリンが存在するが，交感神経節線維の大型芯あり小胞には認められない．

3．副腎クロム親和細胞の神経支配に関する新しい図式

副腎クロム親和細胞に終る交感神経節前線維にオピオイドペプチドを検出したのはSchultzberg, *et al.*（1978）が最初である．しかし，Schultzberg, *et al.*（1978）の結論には不完全な点がいくつか認められる．まずアセチルコリンを含む神経終末と，オピオイドペプチドを含む神経終末が異なるとみなしたように思われる．さらに光顕的な免疫組織化学による観察をおこなったのであって，交感神経節前線維の終末におけるアセチルコリンとオピオイドペプチドの関係が不明瞭である．また，交感神経節前線維に含まれる大型の芯あり小胞と小型で芯のないシナプス小胞が区別されていない．

私どものプロテインA・金コロイド法による電顕免疫組織化学の結果によれば，ハムスターの副腎髄質のA細胞の分泌顆粒にはプロエンケファリンAに関連するオピオイドペプチドとア

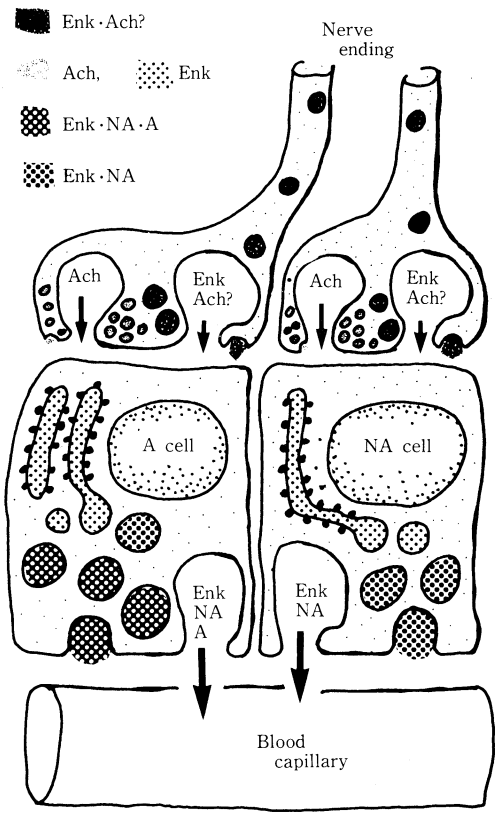

図7．副腎髄質クロム親和細胞とそれを支配する交感神経節前線維に関する私どもの仮説を示す図式．詳細は本文参照．
Enk：エンケファリン，Ach：アセチルコリン，NA：ノルアドレナリン，A：アドレナリン．

ドレナリンおよびノルアドレナリンが共存する．NA細胞の分泌顆粒にはアドレナリンは認められない．ここではオピオイドペプチドとノルアドレナリンが共存する．

副腎クロム親和細胞を支配する交感神経節前線維については小型の芯のない小胞にはオピオイドペプチドは含まれない．ここには主としてアセチルコリンが存在するであろう（Coupland, 1965；Kobayashi, 1977）．大型芯あり小胞にはオピオイドペプチドが含まれている．ここにアセチルコリンが存在することを示す証拠はない（図7）．

IV. おわりに

Pearse (1969) は，ペプチドホルモンを産生するいくつかの内分泌細胞が同時にアミンを含むか，またはその前駆体を特異的に取り込んでアミンに転換する能力をもつことを指摘し，APUD (amine precursor uptake and decarboxylation)という細胞系を提唱した．一方，藤田のパラニューロン学説では，神経と内分泌（ペプチド／アミン産生系に限る）はあらゆる性質について連続的である．パラニューロン学説では，アミンとペプチドの共存は，多岐にわたる神経と内分泌の共通現象のひとつにすぎない．Pearse (1983) は最近“APUD cells as neurons or paraneurons”という副題のついた論説を発表した．これによってAPUD系の概念の創始者にもパラニューロン学説が正しく評価されたことがわかる．

菅野（1983）によれば，現在脳腸ペプチドには20種類以上のものが知られている．「ひとつのニューロンがひとつの伝達物質を放出する．」という原則を普通「Daleの原理」と呼ぶ．ただし，Dale自身はニューロンの単一性を強調して，「知覚神経の中枢枝と末梢枝から同じ物質が放出される．」としただけであって，ひとつのニューロンにふたつの生理活性物が共存することは，厳密にはDaleの述べたところと矛盾しない．ともあれ，脳腸ペプチドの局在が免疫組織化学的に電顕レベルで証明できるようになってみると，ひとつのニューロンがひとつの伝達物質を放出するという従来の考えはむしろ例外であって，いくつかの脳腸ペプチドとアミンが同一の分泌顆粒またはシナプス小胞に共存し，同時に開口放出されるほうが一般的であることが判明した．

最近の遺伝子工学の進歩により，20種類以上ある脳腸ペプチドは，すべて「それぞれの大分子プレプロホルモンとしてまず合成され，プロホルモンを経てプロセシングが進行し，短鎖のペプチドとなる」．との原則が確立してきた(菅野, 1983)．プロエンケファリンAに関連するオピオイドペプチドについては，私どもは副腎髄質のほか，頸動脈小体，小腸粘膜，交感神経節，Auerbach神経叢，膵臓内の神経節に免疫活性をもつ細胞を検出している．これらの細胞群はおそらくプロエンケファリンAの遺伝子をもつという点で同質であり，また一方では多様な形態分化をとげてそれぞれの器官で異なった機能をいとなむようになったと思われる．

プロエンケファリンA以外の脳腸ペプチドに関しても，これを産生する細胞は中枢神経系，末梢神経系および内分泌系に広く分布している (Pearse, 1983)．特定の脳腸ペプチドとアミンの共存という同質性をふまえて，一連のニューロンとパラニューロンの多様な形態と機能の分化を総合的に考える時期になったと感じられる．

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