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Symposium on Neurobiology of incontinence, held at the Ciba Foundation, London, 11–13 October 1989

Editors: Greg Bock (Organizer) and Julie Whelan

The topic for this symposium was proposed by Dr Michael Swash

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Participants

K.-E. Andersson  Department of Clinical Pharmacology, University of Lund, S-221/85 Lund, Sweden

P. Arhan  Faculté Necker, Département de Physiologie, Hôpital des Enfants-Malades, 156 rue de Vaugirard, 75730 Paris cédex 15, France

D. C. C. Bartolo  Department of Surgery, Bristol Royal Infirmary, Marlborough Street, Bristol BS2 8HW, UK

J. G. Blaivas  Department of Urology, College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA

A. Bourcier  IFRUG, 2 Square la Fontaine, F-75016 Paris, France

A. F. Brading  University Department of Pharmacology, University of Oxford, South Parks Road, Oxford OX1 3QT, UK

G. S. Brindley  MRC Neurological Prostheses Unit, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, UK

G. Burnstock  Department of Anatomy & Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK

J. Christensen  Division of Gastroenterology, Department of Internal Medicine, University of Iowa, College of Medicine, Iowa City, IA 52242, USA

W. C. de Groat  Departments of Pharmacology & Behavioral Neuroscience, University of Pittsburgh, School of Medicine, 518 Scaife Hall, Pittsburgh, PA 15261, USA

J. O. L. DeLancey  Department of Obstetrics & Gynecology, University of Michigan Medical School, 1500 E Medical Center Drive, Ann Arbor, MI 48109-0718, USA
C. J. Fowler  Uro-neurology Unit, The National Hospital for Nervous Diseases, Queen Square, London WC1N 3BG, UK

R. S. Kirby  Department of Urology, St Bartholomew's Hospital, West Smithfield, London EC1A 7BE, UK

H. C. Kuijpers  Department of Surgery, Academic Hospital, POB 9101, 6500 Nijmegen, The Netherlands

C. A. Maggi  Department of Pharmacology, Division of Smooth Muscle, A Menarini Pharmaceutical Research Laboratories, Via Sette Santi 3, I-50131 Florence, Italy

P. H. G. Mahieu  Department of Radiology, Institut Chirurgical de Bruxelles, Université Catholique de Louvain, 59 Square Marie-Louise, B-1040 Brussels, Belgium

C. D. Marsden (Chairman)  Department of Clinical Neurology, Institute of Neurology, The National Hospital, Queen Square, London WC1N 3BG, UK

J. Nordling  Department of Urology, H111, Herlev Hospital, DK-2730 Herlev, Denmark

N. W. Read  Sub-Department of Gastrointestinal Physiology & Nutrition, Floor K, Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF, UK

M. Saito (Ciba Foundation Bursar)  Department of Urology, Nagoya University School of Medicine, 65 Turumae, Showa, Nagoya 466, Japan

S. L. Stanton  Urodynamic Unit, Department of Obstetrics & Gynaecology, St George's Hospital Medical School, Lanesborough Wing, Cranmer Terrace, London SW17 0RE, UK

D. Staskin  Division of Urology, Harvard University School of Medicine, Beth Israel Hospital, 330 Brookline Avenue, Boston, MA 02215, USA

M. Swash  Department of Neurology, The London Hospital, Whitechapel, London E1 1BB, UK

P. J. Tiseo  Department of Anesthesiology, T-018, University of California at San Diego, La Jolla, CA 92093, USA
Participants

G. Toson  Glaxo SpA, Research Department, Via Fleming 2, I-37100 Verona, Italy

W. D. Wong  Division of Colon & Rectal Surgery, Box 450, Mayo Building, University of Minnesota Medical School, 420 Delaware Street, SE, Minneapolis, MN 55455, USA
Introduction

C. D. Marsden

Department of Clinical Neurology, Institute of Neurology, The National Hospital, Queen Square, London WC1N 3BG, UK

Clinical neurologists are constantly referred a collection of patients whom I call the ‘wandering wounded’—people with various pain syndromes, such as backache, pain in the head or face, or fatigue syndromes, who are searching for an answer to their problem. They eventually end up in the hands of neurologists who are frequently quite incapable of providing an answer. Unexplained incontinence has been a similar problem, but this picture has totally changed as a result of developments that are discussed in great detail in this symposium.

I had one brief excursion into the field that joins the members of this symposium together. This stemmed from Merton and Morton’s demonstration (1980) that you can stimulate the human motor cortex electrically through the scalp. Pat Merton and I were wondering about the capacity of the human motor cortex to command muscles in different parts of the body; our particular interest was in the human hand. It came upon us late one evening that it would be interesting to see whether the external anal sphincter was commanded by the human motor cortex, as would be predicted by its capacity to protect one from emergencies. A small experiment with motor-cortical electrical stimulation demonstrated a very powerful cortico-motoneuron connection to the external anal sphincter.

I raise that simply because it illustrates one new technique that has revolutionized this subject, among a range of physiological methods now available for the assessment of both urinary and rectal continence mechanisms. With the advent of those sorts of techniques, many of the ‘wandering wounded’ who come to neurologists with complaints of incontinence can perhaps be put into categories that we begin to understand. I perceive this as the purpose of this particular symposium.

Against that background let me add that the first part of the symposium is devoted to discussion of the anatomy, physiology and pharmacology of the areas in question. We shall then move to the more clinically orientated aspects of incontinence in the second part of the symposium.

Reference

Innervation of bladder and bowel

G. Burnstock

Department of Anatomy and Developmental Biology and Centre for Neuroscience, University College London, Gower Street, London WC1E 6BT, UK

Abstract. The autonomic neuromuscular junction is described and neurotransmission, co-transmission and neuromodulation are defined, as well as the 'chemical coding' of sympathetic, parasympathetic, sensory-motor and intrinsic neurons in the wall of the bladder and bowel. A detailed description of the patterns of innervation of smooth muscle of the bowel, bladder and urethra and of the urethral and anal sphincters by intramural and extrinsic autonomic nerves is presented, and the functional and pharmacological features of this innervation are summarized. Finally, changes in the pattern of innervation and expression of co-transmitters and receptors in the bladder and bowel that occur during development and old age and following trauma, surgery and disease are discussed.

1990 Neurobiology of Incontinence. Wiley, Chichester (Ciba Foundation Symposium 151) p 2-26

The objective of this article is to provide an introductory overview of current opinions about mechanisms of autonomic neurotransmission, with particular attention to the innervation of the bladder and bowel.

General principles of autonomic neurotransmission

Definition of the autonomic neuromuscular junction

The autonomic neuromuscular junction differs significantly from the skeletal neuromuscular junction (Burnstock 1986a). The terminal regions of autonomic neurons are long and varicose with transmitter being released from only 1–3% of the varicosities during the passage of a single impulse. The varicosities do not form a fixed relationship with the muscle effectors, so that the geometry of a junction is variable and consequently there is no postjunctional specialization. The junctional cleft can vary between 20 nm and 2 μm, and receptors appear to be homogeneous on smooth muscle membranes. This type of junction means that neuromodulation is an important mechanism, as well as neurotransmission. A neuromodulator is any substance that alters the process of neurotransmission. Neuromodulation can occur in two different ways: prejunctional modulation is when occupation of receptors on nerve varicosities
leads to changes in the amount of neurotransmitter released, while postjunctional neuromodulation is when a substance acts at a postjunctional site to alter the time course or extent of neurotransmitter action.

Another important feature of the autonomic neuromuscular junction is that, unlike the striated muscle cell, a single smooth muscle cell is not the effector, but rather a bundle of cells in electrical continuity with each other via gap junctions. Thus, some smooth muscle cells in sparsely innervated tissues like the uterus or ureter may not be directly activated by neurotransmitter released from nearby nerve varicosities, but are activated indirectly by activity spreading through the muscle effector bundle from the directly innervated cells.

**Multiple transmitters**

For about 50 years it was generally assumed that only two neurotransmitters, namely acetylcholine (ACh) and noradrenaline (NA), were employed by the autonomic nervous system. In the early 1960s, however, it became apparent that there were nerves supplying the smooth muscle of the gut and the bladder that were neither adrenergic nor cholinergic, and, by the end of the 1960s, non-adrenergic, non-cholinergic (NANC) nerves were shown to supply many visceral organs and parts of the cardiovascular system (Burnstock 1969). In the early 1970s it was proposed that the purine nucleotide, adenosine 5'-triphosphate (ATP), was the principal transmitter in the NANC inhibitory nerves of the gut and the NANC excitatory nerves supplying the urinary bladder, and these nerves were termed 'purinergic' (Burnstock 1972). There has been much debate about the validity of this hypothesis but it is now widely accepted for those nerves supplying the intestine and bladder, and purinergic nerves have also been shown to supply the rabbit portal vein and the pulmonary resistance vessels (Burnstock 1986b, Inoue & Kannan 1988). 5-Hydroxytryptamine (5-HT) was also claimed as a neurotransmitter in some of the enteric neurons (Gershon 1979). In the mid-1970s it became clear, from detailed electron microscopic studies of nerve profiles in the gastrointestinal tract (Cook & Burnstock 1976) and from the introduction of immunohistochemistry, that other neurotransmitters, particularly neuropeptides, were present in autonomic nerves. Substances currently recorded as neurotransmitters in the autonomic nervous system are shown in Table 1.

**Co-transmission**

That some nerve cells release more than one transmitter was suggested in 1976 (Burnstock 1976) and it is now widely accepted that most nerves contain two or more neurotransmitters or neuromodulators in variable proportions. For example, the principal transmitters in most sympathetic nerves are NA, ATP and neuropeptide Y (NPY), although at many neuroeffector junctions the main
TABLE 1 Transmitters proposed in the autonomic nervous system

<table>
<thead>
<tr>
<th>Transmitter</th>
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<tbody>
<tr>
<td>Acetylcholine</td>
</tr>
<tr>
<td>Noradrenaline</td>
</tr>
<tr>
<td>Adenosine 5'-triphosphate</td>
</tr>
<tr>
<td>5-Hydroxytryptamine</td>
</tr>
<tr>
<td>γ-Aminobutyric acid</td>
</tr>
<tr>
<td>Dopamine</td>
</tr>
<tr>
<td>Peptides</td>
</tr>
<tr>
<td>Enkephalin/Endorphin</td>
</tr>
<tr>
<td>Vasoactive intestinal polypeptide/Peptide histidine isoleucine</td>
</tr>
<tr>
<td>Substance P</td>
</tr>
<tr>
<td>Gastrin-releasing peptide/Bombesin</td>
</tr>
<tr>
<td>Somatostatin</td>
</tr>
<tr>
<td>Neurotensin</td>
</tr>
<tr>
<td>Luteinizing hormone-releasing hormone</td>
</tr>
<tr>
<td>Cholecystokinin/Gastrin</td>
</tr>
<tr>
<td>Neuropeptide Y/Pancreatic polypeptide</td>
</tr>
<tr>
<td>Galanin</td>
</tr>
<tr>
<td>Angiotensin</td>
</tr>
<tr>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>Calcitonin gene-related peptide</td>
</tr>
</tbody>
</table>

role of NPY is as a postjunctional modulator potentiating the actions of both NA and ATP, while at higher concentrations NPY acts as a prejunctional modulator inhibiting NA and ATP release (see Fig. 1). The principal co-transmitters in parasympathetic nerves appear to be ACh and vasoactive intestinal polypeptide (VIP) (Lundberg 1981, Edwards & Bloom 1982), while in many sensory nerves (or ‘sensory-motor nerves’, as I believe they should now be termed), substance P, calcitonin gene-related peptide (CGRP) and ATP appear to coexist. Intrinsic nerves in the heart, bladder and airways also contain particular combinations of neurotransmitters.

**Chemical coding of autonomic nerves**

Currently many studies are being carried out to identify the precise combinations of substances contained in individual or groups of autonomic neurons, their projections and their central connections. This type of analysis has been termed ‘chemical coding’ and has been developed to the most sophisticated extent in the enteric nervous system (see Furness & Costa 1987). For example, neurons have been identified containing up to five neuropeptides that project to different effectors in the gut (Fig. 2).
Innervation of bladder and bowel

FIG. 1. Schematic representation of different interactions that occur between neuropeptide Y (NPY) and ATP and noradrenaline (NA) released from a single sympathetic nerve varicosity, in the vas deferens and many blood vessels. NA and ATP, probably released from small granular vesicles, act synergistically to contract (+) the smooth muscle via $\alpha_1$-adrenoceptors and $P_2$ purinoceptors, respectively. NPY, which is also released from the nerve, has little, if any, direct action on the muscle cell, but exerts potent neuromodulatory actions, both prejunctional inhibition (−) of the release of NA and postjunctional enhancement (+) of the action of NA. (From Burnstock 1987, by permission of the Raven Press.)

Dual control of blood flow

The pioneering work of Furchgott & Zawadzki (1980) has shown that blood vessels are controlled not only by perivascular nerves, but also by endothelial cell factors. Receptors for a variety of substances, including ACh, ATP, substance P, 5-HT and angiotensin II, are located on endothelial cells and when occupied lead to the release of endothelium-derived relaxing factor (EDRF) and consequent vasodilatation. Recent studies in our laboratory have shown that the origin of these substances, for all but the smallest vessels, is subpopulations of endothelial cells which release them during changes in flow (shear stress) or hypoxia, as a pathophysiological mechanism that protects tissues like the brain and heart from hypoxic damage (Burnstock 1988).
FIG. 2. 'Chemical coding' of neurons in the intestine, showing their projections and, in part, their central connections. DRG, dorsal root ganglion; SPLANC. N, splanchnic nerve; PREVERT. G, prevertebral ganglion; LM, longitudinal muscle; MP, myenteric plexus; CM, circular muscle; SM, submucosa; M, mucosa; ACh, acetylcholine; CCK, cholecystokinin; CGRP, calcitonin gene-related peptide; ChAT, choline acetyltransferase; DYN, dynorphin; ENK, enkephalin; GRP, gastrin-releasing peptide; NA, noradrenaline; NPY, neuropeptide Y; SOM, somatostatin; SP, substance P; VIP, vasoactive intestinal peptide. (From Costa et al 1986, by permission of Elsevier Science Publishers.)
Innervation of bladder and bowel

Innervation of the bladder

Bladder body

It has been known since the turn of the century that the responses of the detrusor to pelvic (parasympathetic) nerve stimulation were only partially blocked by atropine (Langley & Anderson 1895). Various explanations were put forward to account for atropine-resistant responses but, by the late 1960s, it was widely accepted that the atropine-resistant response was due to a NANC transmitter (Burnstock 1969, Ambache & Zar 1970, Moss & Burnstock 1985). Evidence was later presented that the identity of the NANC transmitter causing contraction of the detrusor was ATP (Burnstock et al. 1972, 1978a, Dean & Downie 1978, Theobald 1982, Kasakov & Burnstock 1983, Levin et al. 1986). This evidence included: close mimicry of the response to nerve stimulation and exogenously applied ATP; release of ATP during NANC nerve stimulation which was Ca\(^{2+}\) dependent; fluorescence localization of nerves in the bladder positively stained for quinacrine, a compound known to bind very strongly to ATP (Da Prada et al. 1978). More recently, the NANC responses have been shown to be blocked by arylazidoaminopropionyl ATP (ANAPP\(_3\)), an ATP receptor antagonist, and by \(\alpha,\beta\)-methylene-ATP, a stable analogue of ATP that specifically desensitizes \(P_2\) purinoceptors. Furthermore, excitatory junction potentials recorded in the smooth muscle of the bladder in response to NANC stimulation are also abolished by \(\alpha,\beta\)-methylene-ATP (Hoyle & Burnstock 1985, Fujii 1988). It has been suggested that ACh and ATP may be co-transmitters in intrinsic parasympathetic neurons in bladder (MacKenzie et al. 1982). In recent experiments in our own laboratory, we have demonstrated autoradiographic localization of the \(P_{2X}\) purinoceptor in the detrusor muscle of the rat and guinea pig and also in man (Bo & Burnstock 1989, and unpublished observations) (Fig. 3). Although it has been claimed that a NANC component is not present in human bladder (Sibley 1984), others have demonstrated atropine-resistant responses (Hindmarsh et al. 1977, Sjögren et al. 1982, Cowan & Daniel 1983, Negårdh & Kinn 1983). The current demonstration of ATP receptor binding (X. Bo & G. Burnstock, unpublished observations) and the recent work from Alison Brading’s laboratory (Speakman et al. 1989) and our own (Hoyle et al. 1989) confirm that this component is present and suggest that it is likely to be purinergic, although there seem to be marked regional variations in its distribution; for example, ATP responses and receptors are dense in the trigone region, but very low or perhaps even absent in the tip of the bladder dome. A NANC-mediated slow relaxation has also been described in human detrusor (Klarskov 1987).

In addition to the parasympathetic nerve pathway, there are sympathetic (hypogastric) nerves supplying the bladder which contain NPY as well as NA, and a number of neuropeptides have been localized in nerves in the bladder wall (Gu et al. 1984, Crowe & Burnstock 1989). It seems likely that SP and CGRP
FIG. 3. (A) shows a section of rat urinary bladder viewed with bright-field optics (stained with 0.5% Toluidine blue). Autoradiograph (B) shows the overall distribution of binding sites of $[{}^{3}H] \alpha,\beta$-methylene-ATP over (A) viewed with dark-field optics. Autoradiograph (C) shows the distribution of non-specific binding sites of $[{}^{3}H] \alpha,\beta$-methylene-ATP over the adjacent section to (A). bv, blood vessel; ep, epithelium; sm, smooth muscle. Calibration bar = 50 $\mu$m. (From Bo & Burnstock 1989, by permission of Elsevier Science Publishers.)
are contained in sensory-motor nerves which are abundant (Maggi et al 1988), especially in the trigone region, while VIP and [Leu]enkephalin immunofluorescence may be located in some parasympathetic nerves and/or in projections from intrinsic ganglia (Alm et al 1980, Kawatani et al 1983, Crowe et al 1986a). There are marked species variations in regional innervation and in the transmitters used (Moss & Burnstock 1985, Maggi et al 1987).

Both 5-HT (Holt et al 1985) and γ-aminobutyric acid (GABA) (Kusonoki et al 1984) have been implicated as neuromodulators in the bladder. Prostaglandins produced by ATP released from pelvic nerves also play a part in the contractile responses of the bladder (Burnstock et al 1978b, Andersson et al 1980).

**Urethra**

Not surprisingly, innervation of the urethra differs substantially from that of the bladder body (Klarskov et al 1983, Slack & Downie 1983, Ito & Kimoto 1985). There is a powerful innervation by adrenergic excitatory nerves, as well as a cholinergic innervation (Ekström & Malmberg 1984). In addition, there are NANC inhibitory nerves and there is some evidence to suggest that VIP and 5-HT, both of which relax the urethra, are NANC transmitter contenders (Hills et al 1984). ATP also relaxes the urethra, but only after it breaks down to adenosine, which then acts on P1 purinoceptors. Consistent with this finding is the absence of ATP binding in the urethra (Bo & Burnstock 1989).

**Intrinsic ganglia**

Whereas the presence of intrinsic ganglia in the wall of the bladder has been recognized for some time, only in recent years has it become apparent that these are not simple nicotinic parasympathetic relay stations, but consist of sophisticated local circuitry with the potential to support integrative activities in the bladder and urethra (Crowe et al 1986a, Burnstock et al 1987, Kumamoto & Shinnick-Gallagher 1987, Pittam et al 1987). NPY and somatostatin (SOM) are co-localized in many of the intrinsic neurons (James & Burnstock 1988, 1989). Receptors for ACh (muscarinic as well as nicotinic), VIP, substance P, enkephalin and ATP have been demonstrated on sub-populations of these neurons with electrophysiological and autoradiographic methods (Pittam et al 1987, James & Burnstock 1989). Intramural ganglia have been identified recently in the human urethra (Crowe et al 1988). It is not yet known whether the neuronal circuitry in the wall of the bladder and urethra includes sensory nerves that would allow local reflexes to occur independently of the central nervous system (CNS) (see also de Groat & Kawatani 1985).
Urethral sphincter-striated muscle

The intriguing possibility has been raised that there may be direct interactions between the autonomic nervous system and voluntary system in this sphincter, since both noradrenaline- and VIP-containing nerve fibres have been shown to run in close proximity to both individual and bundles of striated muscle fibres in this sphincter (Crowe et al 1986b, 1989, Lincoln et al 1986). These bundles are varicose and not simply related to blood vessels, but it is not known yet whether they have direct effects on the excitability of the striated muscle membrane or whether they act as modulators of neuromuscular transmission. There appears to be a substantial compensatory increase in the density of noradrenaline-containing fibres after sacral spinal injury in man (Crowe et al 1989).

Innervation of the bowel

Myenteric ganglia

There are two ganglionate plexuses in the wall of the gut, the myenteric plexus lying between the longitudinal and circular muscle coats, and the submucous plexus. Scanning and transmission electron microscopic studies have shown that the organization of these ganglia is closer to that of the CNS than that of sympathetic or parasympathetic ganglia (Paton 1957, Gershon 1979, Wood 1979, Jessen & Burnstock 1982). Glial cells and neurons are in close relationship and their processes form a dense neuropil; neither connective tissue nor blood vessels penetrate into the ganglia. Many neuropeptides, monoamines, purines and amino acids have been identified in subpopulations of neurons within these ganglia (see Fig. 2) (Burnstock 1982, 1986b, Furness & Costa 1987).

Innervation of smooth muscle

The main neural components innervating the bowel smooth muscle are: NANC excitatory nerves utilizing, as co-transmitters, ACh (which produces rapid excitatory junction potentials) and substance P (which produces slow responses); and NANC inhibitory nerves utilizing, probably as co-transmitters, ATP (which produces rapid inhibitory junction potentials) and VIP (which produces slow-onset, sustained relaxations) (see Hoyle & Burnstock 1989). The accumulation of evidence suggests that ATP is the dominant co-transmitter for the NANC inhibitory nerves in the small intestine and colon, while VIP is the dominant transmitter in the stomach. Substance P excitation of smooth muscle appears to be dominant in the ileum. A diagram proposed by Marcello Costa illustrating the involvement of these nerves in peristalsis is shown in Fig. 4.
Innervation of bladder and bowel

**FIG. 4.** This model has been put forward by Marcello Costa (Flinders University, Adelaide) to illustrate current views about the neural pathways involved in the peristalsis reflex. Sensory neurons (Dogiel Type 2 cells showing AH-type electrical activity and containing substance P and calbindin) carry impulses generated by stretching of the gut wall to activate, via interneurons (Dogiel Type 1 cells showing S-type electrical activity utilizing ACh), orally directed excitatory motor neurons utilizing ACh and substance P and anally directed NANC inhibitory neurons utilizing ATP and VIP. s.m., smooth muscle. (Figure first presented at the International Congress on Gastroenterology, Digestive Endoscopy and Colo-proctology organized by the Italian Society of Gastroenterology, Rome, 4–10 September 1988.)

It has become apparent (see Thuneberg 1982) that the interstitial cells of Cajal play a role as pacemakers in the activity of smooth muscle and, because these cells are innervated, it is important to take this system into consideration when investigating neuromuscular activity in the gastrointestinal tract.

**Sphincters**

In general, the sphincters are neurally controlled independently from the non-sphincteric smooth muscle. Stimulation of sympathetic (hypogastric) nerves leads to depolarization and contraction of the circular muscle of the internal anal sphincter, which is blocked by α-adrenoceptor antagonists, whereas stimulation of the parasympathetic nerves supplying this sphincter (second ventral sacral root, VS2) inhibits spontaneous activity resulting in relaxation mediated by NANC nerves in animals and man (Frenckner & Ihre 1976, Bouvier & Gonella 1981). ACh contracts the sphincter in most species. The identity of the NANC transmitter, however, is not clearly resolved (Burleigh 1983, Crema et al 1983, Biancani et al 1985, Goldberg et al 1986, Rattan & Shah 1988), although, since inhibitory junction potentials are evoked in the guinea pig internal sphincter, this would favour a purinergic mechanism in this species; VIP has never been shown to produce rapid hyperpolarizations of this kind, in contrast to ATP (Lim & Muir 1986).
Intrinsic enteric neurons containing a number of neuropeptides, although sparsely distributed in the anal canal, are also likely to project into the internal anal sphincter (Bouvier et al 1986, Krier 1989).

The external anal sphincter is innervated by the perineal inferior haemorrhoidal branches of the pudendal nerves (S2, S3, S4) as well as branching from the coccygeal plexus (S4, S5) (Schuster 1968, Krier 1985). Sensory and sensory-motor fibres containing neuropeptides and possibly ATP are also present in this sphincter as well as the internal anal sphincter (Krier 1989).

The relationship between the neural control of the smooth muscle of the internal anal sphincter and the striated muscle of the external anal sphincter is not resolved (see Krier 1989). Neuronally mediated interactions between motility of internal anal sphincter and bladder, however, have been examined (Bouvier & Grimaud 1984).

Plasticity of autonomic nerves

An important recent development has been to examine the changes in expression of co-transmitters and receptors in ageing and after surgery or trauma (see Burnstock 1990). Some examples follow.

Plasticity of nerves in the bladder

Long-term sympathectomy. Substantial increases in CGRP-immunofluorescent nerves were seen in rat bladder six or 20 weeks after sympathectomy by chronic exposure to guanethidine. The possibility that sympathetic nerve growth factor is involved in the mechanism of hyperinnervation by CGRP-containing sensory nerves has been raised (Aberdeen et al 1990).

Spinal injury. Relatively little attention has been paid to the autonomic innervation of the bladder and urethra after spinal cord injury, but some interesting examples of changes in expression of transmitters and receptors in the nerves that remain are beginning to be recognized. For example, adrenergic nerves appear to be increased in relation to the striated muscle of the external urethral sphincter of patients with sacral, but not cervical or thoracic, spinal lesions (Crowe et al 1989). In contrast, dense VIP-immunoreactive, but not noradrenaline-containing nerves, were found in the urethral smooth muscle in patients with thoracic lesions (Crowe et al 1988).

Diabetes. Taking into account the hypertrophy and distension of bladders from eight-week streptozotocin-treated diabetic rats, there was an increase in the activity of choline acetyltransferase and acetylcholinesterase and in dopamine levels, probably indicating an increase in activity of both sympathetic and parasympathetic nerves (Lincoln et al 1984).
Innervation of bladder and bowel

Plasticity of enteric nerves

*Hirschsprung's and Crohn's diseases.* It has been known for some years now that in the absence of enteric ganglia in the colon of man in Hirschsprung's disease, there is a striking hyperinnervation of the musculature by both adrenergic and cholinergic nerves (Gannon et al 1969). It is interesting that recent studies show that, in contrast to these extrinsic nerves, projections of the intrinsic enteric neurons containing peptides and purines do not appear to enter the aganglionic bowel (Hamada et al 1987). In contrast, in Crohn's disease there appears to be a compensatory increase in VIP in the diseased intestine (Bishop et al 1980).

*Diabetes.* Faecal incontinence associated with diabetes mellitus may be due to neuropathy (Schiller et al 1982). The streptozotocin-treated rat has been used as a model for diabetes mellitus; for example, there is good correlation between the loss of the VIPergic innervation of penile erectile tissue in rats and in impotent diabetic man (Crowe et al 1983). Increases in VIPergic innervation of the small and large intestine have been reported (Belai et al 1985), but there are concomitant decreases in CGRP-immunoreactive nerves (Belai et al 1987). Despite the increase in VIP levels in enteric nerves of diabetic rats there is no release of VIP from these nerves, although this can be reversed by acute application of insulin (Burnstock et al 1988).

*Laxatives.* Long-term use of purgative laxatives formulated from anthraquinone derivatives such as sennosides A and B, as in cascara and senna pods, causes neuropathy of the enteric plexuses of the large intestine. Smooth muscle atrophy accompanies loss of intrinsic innervation, myenteric nerve terminal damage and submucosal nerve terminal damage, leading to the condition known as cathartic colon. The typical lesions of cathartic colon implicate loss of absorptive and secretory function as well as loss of motor function. Although anthraquininone-derived laxatives can result in general neuropathy, the adrenergic neurons appear to be less susceptible than either cholinergic or peptidergic and purinergic neurons (Reimann et al 1980). Also, neurons that selectively take up quinacrine (an acridine dye related to anthraquinone, which binds to high concentrations of ATP) are damaged early (Smith 1968). The relationship between anthraquinone and NANC neurons is consistent with the observation that Reactive Blue 2, which is a sulphonic acid anthraquinone derivative, antagonizes purinergic inhibitory neuromuscular transmission and exogenous ATP in the large intestine of the rat and guinea pig and in the rat duodenum (Manzini et al 1986).

*Comment*

In view of the growing number of examples of autonomic nerve plasticity in adult animals and man it is suggested that neuropathologists need to pay
attention to compensatory increases in expression of transmitters, as well as seeking evidence for degrees of damage or loss of nerves; and that when new strategies for drug development are being considered, the age and pathological history of the patient should be taken into account. An exciting question to ask during the next phase of research is: 'what are the molecular mechanisms that control the expression of co-transmitters and receptors in ageing and disease?'

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DISCUSSION

Brindley: On the question of non-cholinergic, non-adrenergic nerves to the human bladder, there seems to be a straightforward, almost directly clinical