6 The Autonomic Neuroeffector Junction

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The autonomic neuromuscular junction differs in several important respects from the better known skeletal neuromuscular junction; it is not a synapse with the well defined prejunctional and postjunctional specializations established for the skeletal neuromuscular synapse or ganglionic synapses. A model of the autonomic neuroeffector junction has been proposed on the basis of combined electrophysiologic, histochemical, and electron-microscopical studies. The essential features of this model are that the terminal portions of autonomic nerve fibers are varicose, transmitter being released *en passage* from varicosities during conduction of an impulse, although excitatory and inhibitory junction potentials are probably elicited only at close junctions. Furthermore, the effectors are muscle bundles rather than single smooth muscle cells, which are connected by low-resistance pathways (gap junctions) that allow electrotonic spread of activity within the effector bundle. In blood vessels, the nerves are confined to the adventitial side of the media muscle coat, and this geometry appears to facilitate dual control of vascular smooth muscle by perivascular nerves and by endothelial relaxing and contracting factors. Neuroeffector junctions do not have a permanent geometry with postjunctional specializations, but rather the varicosities are continuously moving and their special relation with muscle cell membranes changes with time, including dispersal and reformation of receptor clusters. For example, varicosity movement is likely to occur in cerebral blood arteries, where there is a continuously increasing density of sympathetic innervation during development and aging and in hypertensive vessels or those that have been stimulated chronically *in vivo*, where there can be an increase in innervation density of up to threefold.

STRUCTURE OF THE AUTONOMIC NEUROMUSCULAR JUNCTION

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Varicose Terminal Axons

In the vicinity of the effector tissue, axons become varicose, varicosities occurring at 5–10 μm intervals (Fig. 6.1A), and branches intermingle with other axons to form the autonomic ground plexus, first described by Hillarp in 1946. The extent of the branching and the area of effector tissue affected by individual neurons varies with the tissue. Autonomic axons combined in bundles are enveloped by Schwann cells. Within the effector tissue they partially lose their Schwann cell envelope, usually leaving the last few varicosities naked.

The density of innervation, in terms of the number of axon profiles per 100 muscle cells in cross-section, also varies considerably in different organs. For example, it is very high in the vas deferens (Fig. 6.2A), iris, nictitating membrane, and sphincteric parts of the gastrointestinal tract, but low in the ureter, uterus, and longitudinal muscle coat of the gastrointestinal tract. In most blood vessels, the varicose nerve plexus is placed at the adventitial border and fibers rarely penetrate into the medial muscle coat (Fig. 6.2B).

Junctional Cleft

The width of the junctional cleft varies considerably in different organs. In the vas deferens, nictitating membrane, sphincter pupillae, rat parotid gland, and atrioventricular and sinoatrial nodes in the heart, the smallest neuromuscular distances range from 10 to 30 nm. The minimum neuromuscular distance varies considerably in different blood vessels. Generally, the greater the vessel diameter, the greater the separation of nerve and muscle. Thus, minimal neuromuscular distances in arterioles and in small arteries and veins are about 50 to 100 nm, in medium to large arteries the separation is 200 to 500 nm, whereas in large elastic arteries where the innervation is sparser, the minimum neuromuscular distances are as wide as 1000 to 2000 nm. Serial sectioning has shown that at close junctions in both visceral and vascular organs there is fusion of prejunctional and postjunctional basal lamina (see Fig. 6.1B). In the longitudinal muscle coat of the gastrointestinal tract, autonomic nerves and smooth muscle are rarely separated by less than

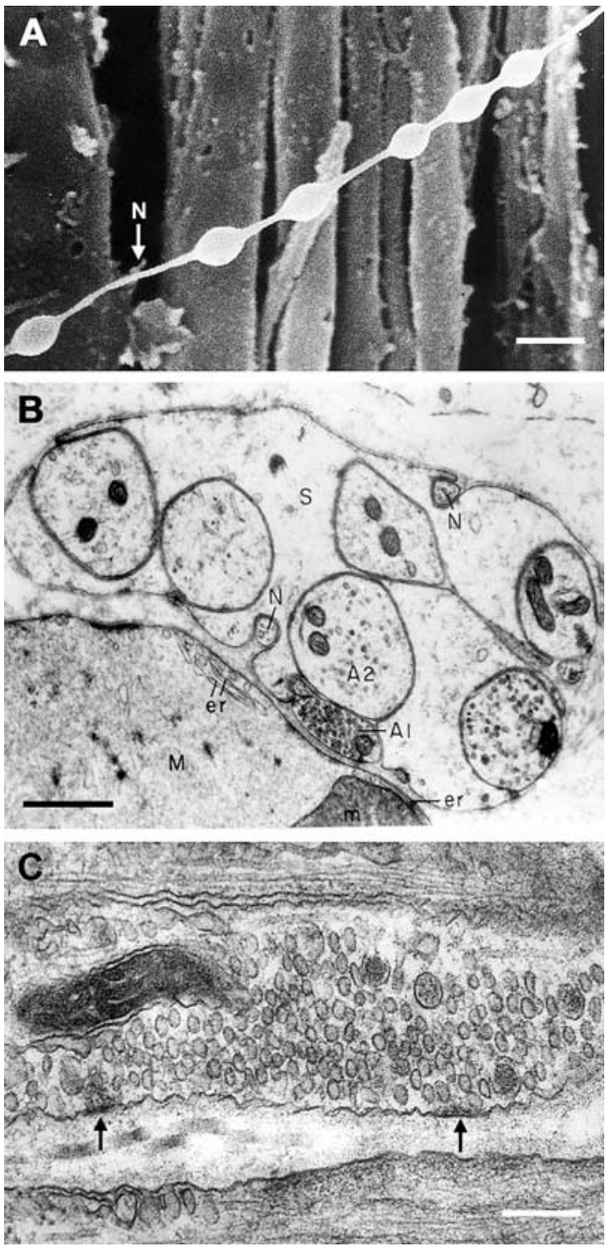

FIGURE 6.1 **A**, Scanning electron micrograph of a single terminal varicose nerve fibre lying over smooth muscle of small intestine of rat. Intestine was pretreated to remove connective tissue components by digestion with trypsin and hydrolysis with HCl. Scale bar = 3 µm. (Reproduced with permission from Burnstock, G. 1988. Autonomic neural control mechanisms. With special reference to the airways. In *The airways. Neural control in health and disease.* M. A. Kaliner, and P. J. Barnes, Eds, 1–22. New York: Marcel Dekker.) **B**, A medium-sized intramuscular bundle of axons within a single Schwann cell (S). There is no perineurial sheath. Some axons, free of Schwann cell processes, contain "synaptic" vesicles (e.g., *A1* and *A*). For nerve profile A1, there is close proximity (about 80 nm) to smooth muscle (*M*; *m*, mitochondria; *er*, endoplasmic reticulum) with fusion of nerve and muscle basement membranes. Most of the axons in bundles of this size have few vesicles in the plane of section, but they resemble the vesicle-containing axons of the larger trunks in that they have few large neurofilaments. The small profiles (*N*), less than 0.25 µm in diameter, are probably intervaricosity regions of terminal axons. Scale bar = 1 µm. (Reproduced with permission from Merrillees, N. C. R., G. Burnstock, and M. E. Holman. 1963. Correlation of fine structure and physiology of the innervation of smooth muscle in the guinea pig vas deferens. *J. Cell Biol.* 19:529–550.) **C**, Autonomic varicosities with dense prejunctional thickenings and bunching of vesicles, probably representing transmitter release sites (*arrows*), but there is no postjunctional specialization. Scale bar = 0.25 µm. (Courtesy of Phillip R. Gordon-Weeks.)

100 nm. However, in the circular muscle coat, close (20 nm) junctions are common, sometimes several axon profiles being closely apposed with single muscle cells.

Prejunctional and Postjunctional Specialization

Although there are many examples of prejunctional thickenings of nerve membranes in varicosities associated with accumulations of small synaptic vesicles, representing sites of transmitter release (see Fig. 6.1C), there are no convincing demonstrations of postjunctional specializations, such as membrane thickening or folding or indeed absence of micropinocytic vesicles; this is in keeping with the view that even close junctions might be temporary liaisons.

Muscle Effector Bundles and Gap Junctions

The smooth muscle effector is a muscle bundle rather than a single muscle cell—that is, individual muscle cells being connected by low-resistance pathways that allow electrotonic spread of activity within the effector bundle. Sites of electrotonic coupling are represented morphologically by areas of close apposition between the plasma membranes of adjacent muscle cells. High-resolution electron micrographs have shown that the membranes at these sites consist of "gap junctions" (see Fig. 6.2C). Gap junctions (or nexuses) vary in size between punctate junctions, which are not easily recognized except in freeze-fracture preparations, and junctional areas more than 1 µm in diameter. The number and arrangement of gap junctions in muscle effector bundles of different sizes in different organs and their relation to density of autonomic innervation have not been fully analyzed. It is interesting that partial denervation has been shown to result in an increase in gap junctions.

AUTONOMIC NEUROTRANSMISSION

Electrophysiology

Excitatory and inhibitory junction potentials can be recorded in smooth muscle cells in response to stimulation of the autonomic nerves in both visceral (Fig. 6.3A and B) and vascular organs, which represent the responses to

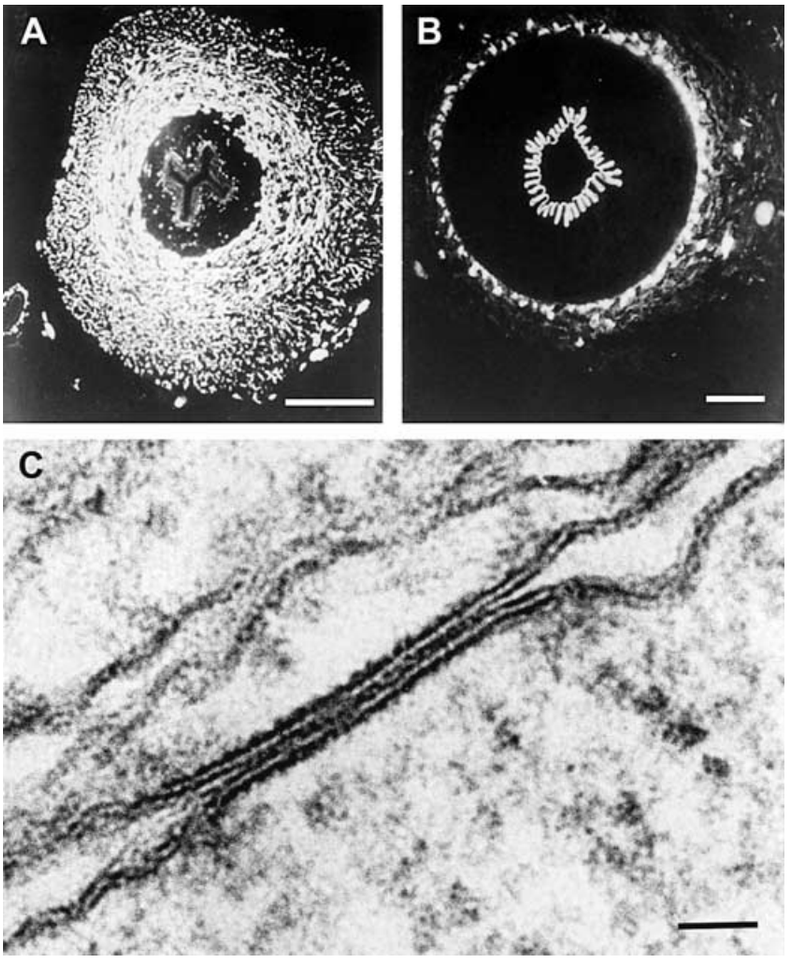

FIGURE 6.2 Comparison between the adrenergic innervation of the densely innervated vas deferens of the guinea pig (**A**) and the rabbit ear artery (**B**) in which the adrenergic fibers are confined to the adventitial–medial border. The inner elastic membrane shows a nonspecific fluorescence (autofluorescence). (**A** and **B**, Reproduced with permission from Burnstock, G., and M. Costa. 1975. *Adrenergic neurones: Their organisation, function and development in the peripheral nervous system*. London: Chapman and Hall.) **C**, A gap junction between two smooth muscle cells grown in tissue culture. (Reproduced with permission from Campbell, G. R., Y. Uehara, G. Mark, and G. Burnstock. 1971. Fine structure of smooth muscle cells grown in tissue culture. *J. Cell Biol.* 49:21–34.) Scale bar = 500 µm (**A**), 50 µm (**B**), and 50 nm (**C**).

adenosine triphosphate, released as a cotransmitter with noradrenaline from sympathetic nerves, as a cotransmitter with acetylcholine from parasympathetic nerves in the bladder, and as a cotransmitter with nitric oxide from nonadrenergic, noncholinergic inhibitory enteric nerves. Detailed analysis of these responses revealed that transmitter released from varicosities close (10–100 nm) to smooth muscle cells would produce junction potentials, although transmitter released from varicosities up to 500 to 1000 nm away (especially in large blood vessels) was likely to produce some muscle response; and that only about 1–3% of the varicosities release transmitter with a single impulse, although the probability for release increased to about 25% with repetitive nerve stimulation.

Receptor Localization on Smooth Muscle Cells

The distribution of P2X purinoceptors on smooth muscle cells in relation to autonomic nerve varicosities in urinary bladder, vas deferens, and blood vessels has been examined recently by using immunofluorescence and confocal microscopy. Antibodies against the $P2X_1$ receptor, the dominant receptor subtypes found in smooth muscle, and an antibody against the synaptic vesicle proteoglycan SV2

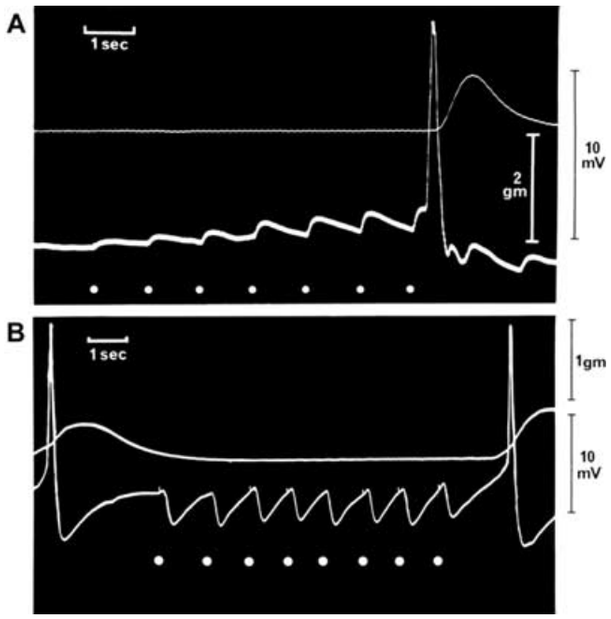

FIGURE 6.3 Electrophysiology of transmission at autonomic neuromuscular junctions. **Top trace**, Mechanical record. **Bottom trace**, Changes in membrane potential recorded with a sucrose-gap method (Burnstock & Straub, 1958). The junction potentials recorded with this method are qualitatively similar to those recorded with intracellular microelectrodes. **A**, Excitatory junction potentials (EJPs) recorded in smooth muscle of the guinea pig vas deferens in response to repetitive stimulation of postganglionic sympathetic nerves (*white dots*). Note both summation and facilitation of successive EJPs. At a critical depolarization threshold, an action potential is initiated that results in contraction. **B**, Inhibitory junction potentials (IJPs) recorded in smooth muscle of the atropinized guinea pig taenia-coli in response to transmural stimulation (*white dots*) of the intramural nerves remaining after degeneration of the adrenergic nerves by treatment of the animal with 6-hydroxydopamine (250 mg/kg intraperitoneally for 2 successive days) 7 days previously. Note that the IJPs in response to repetitive stimulation results in inhibition of spontaneous spike activity and relaxation. (Reproduced with permission from Burnstock, G. 1973. The autonomic neuroeffector system. *Proc. Aust. Physiol. Pharmacol. Soc.* 4:6–22.)

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showed clusters of receptors (about $0.9 \times 0.2\,\mu m$ in size) located beneath varicosities. Many more small clusters (about $0.4 \times 0.04\,\mu m$) were present on the whole surface of smooth muscle cells unrelated to varicosities; they may represent pools of receptors that can migrate toward varicosities to form large clusters. In blood vessels, small clusters of P2X receptors are present on cells throughout the medial muscle coat, whereas large clusters are restricted to the muscle cells at the adventitial surface. Alpha-adrenoceptors appear to be located only in extrajunctional regions, so that the possibility that noradrenaline is released from more distant varicosities has been raised. There are hints from studies of receptor-coupled green fluorescent protein chimeras that the receptor clusters are labile, dispersing when a varicosity moves to a new site where clusters reform, perhaps within a 20- to 30-minute time scale.

MODEL OF AUTONOMIC NEUROEFFECTOR JUNCTION

A model of the autonomic neuromuscular junction has been proposed on the basis of combined electrophysiologic, histochemical, and electron-microscopical studies described earlier (Fig. 6.4A and B). The essential features of this model are that the terminal portions of autonomic nerve fibers are varicose, transmitter being released *en passage* from varicosities during conduction of an impulse, although excitatory junction potentials and inhibitory junction potentials are probably elicited only at close junctions. Furthermore, the effectors are muscle bundles rather than single smooth muscle cells, which are connected by low-resistance pathways (gap junctions) that allow electrotonic spread of activity within the effector bundle. In blood vessels, the nerves are confined to the adventitial side of the media muscle coat, and this geometry appears to facilitate dual control of vascular smooth muscle by endothelial relaxing and contracting factors and perivascular nerves.

Neuroeffector junctions do not have a permanent geometry with postjunctional specializations, but rather the varicosities are continuously moving and their special relation with muscle cell membranes changes with time. For example, it is likely to occur in cerebral blood arteries, where there is a continuously increasing density of sympathetic innervation during development until old age, and in vessels that have been stimulated chronically *in vivo*, where there can be an increase in innervation density of up to threefold, including an increase in the number of varicosities per unit length of nerve from 10 to 20 per 100 µm to 30 per 100 µm.

Autonomic effector junctions appear to be suitable not only for neurotransmission, but also for neuromodulation. A neuromodulator is defined as any substance that modifies the process of neurotransmission. It may achieve this either by prejunctional action that increases or decreases transmitter release or by postjunctional action that alters the time course or extent of action of the transmitter, or both (Fig. 6.4B).

Finally, it should be emphasized that if this model of the autonomic effector junction is true, then the earlier emphasis on looking for images of specialized nerve–cell close apposition may not be appropriate; even if a varicosity has a passing close relation with a cell, releasing transmitter for which receptors are expressed on that cell (e.g., mast cells, epithelial cells, or even immune cells) expressing receptors for those transmitters, then, in effect, that cell is innervated.

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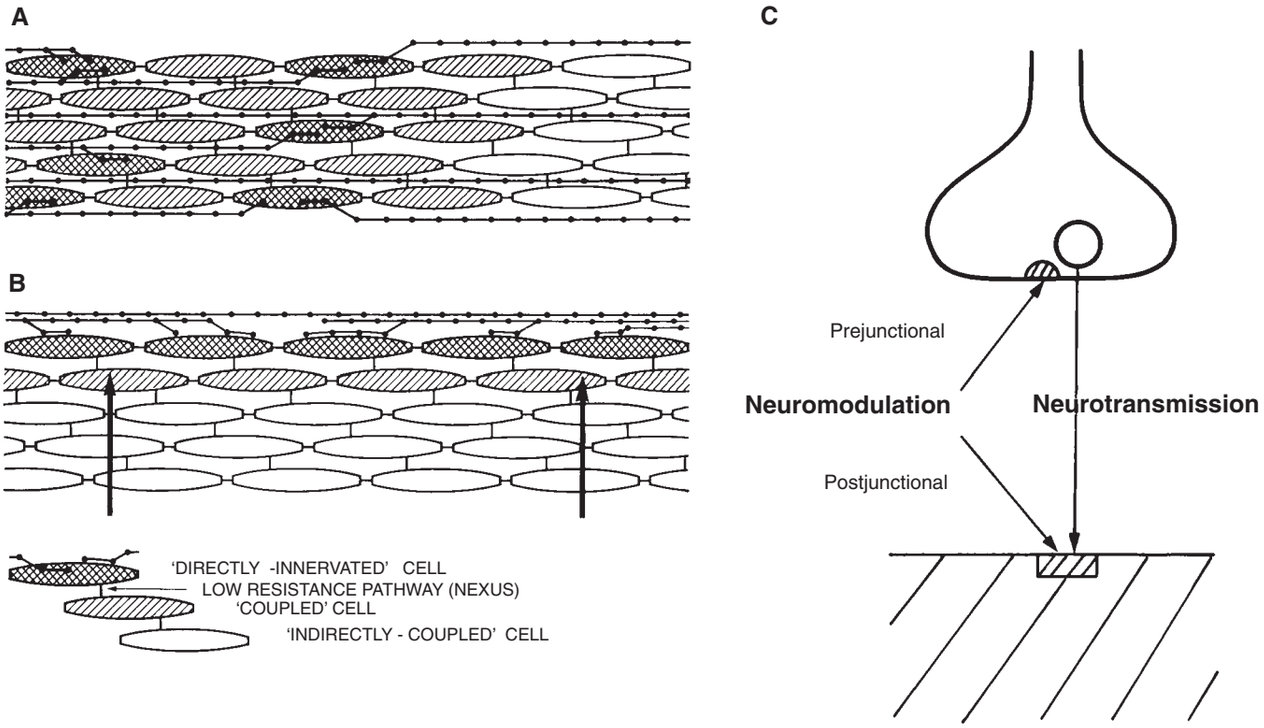

FIGURE 6.4 **A**, Schematic representation of control of visceral smooth muscle. "Directly innervated" cells (*cross-hatched*) are those that are directly activated by neurotransmitter; "coupled cells" (*hatched*) are those where junction potentials spread from "directly innervated" cells, when a sufficient area of the muscle effector bundle is depolarized, a propagated action potential will activate the "indirectly coupled" cells (*white*). **B**, Schematic representation of control of vascular smooth muscle by nerves (-●●-) and endothelial factors (*arrows*). (**A** and **B**, Reproduced with permission from Burnstock, G. 1975. Control of smooth muscle activity in vessels by adrenergic nerves and circulating catecholamines. In *Smooth muscle pharmacology and physiology. Les colloques de l'INSERM, Vol. 50*, 251–264. Paris: INSERM.) **C**, Schematic representation of prejunctional and postjunctional neuromodulation. (Reproduced with permission from Burnstock, G. 1982. Neuromuscular transmitters and trophic factors. In *Advanced medicine 18*, M. Sarner, Ed, 143–148. London: Pitman Medical.)

References

1. Bennett, M. R., and W. G. Gibson. 1995. On the contribution of quantal secretion from close-contact and loose-contact varicosities to the synaptic potentials in the vas deferens. *Philos. Trans. R. Soc. Lond B Biol. Sci* 347:187–204.
2. Brain, K. L., V. M. Jackson, S. J. Trout, and T. C. Cunnane. 2002. Intermittent ATP release from nerve terminals elicits focal smooth muscle Ca2+ transients in mouse vas deferens. *J. Physiol.* 541:849–862.
3. Burnstock, G. 1979. Autonomic innervation and transmission. *Br. Med. Bull.* 35:255–262.
4. Burnstock, G. 1986. Autonomic neuromuscular junctions: Current developments and future directions. The Third Anatomical Society Review Lecture. *J. Anat.* 146:1–30.
5. Burnstock, G., and T. Iwayama. 1971. Fine structural identification of autonomic nerves and their relation to smooth muscle. In *Progress in Brain Research, 34, Histochemistry of Nervous Transmission*. O. Eränkö, Ed, 389–404. Amsterdam: Elsevier.
6. Dutton, J. L., P. Poronnik, G. H. Li, C. A. Holding, R. A. Worthington, R. J. Vandenberg, D. I. Cook, J. A. Barden, and M. R. Bennett. 2000. $P2X_1$ receptor membrane redistribution and down-regulation visualized by using receptor-coupled green fluorescent protein chimeras. *Neuropharmacology* 39:2054–2066.
7. Hansen, M. A., V. J. Balcar, J. A. Barden, and M. R. Bennett. 1998. The distribution of single P_{2X1}-receptor clusters on smooth muscle cells in relation to nerve varicosities in the rat urinary bladder. *J. Neurocytol.* 27:529–539.
8. Luff, S. E. 1996. Ultrastructure of sympathetic axons and their structural relationship with vascular smooth muscle. *Anat. Embryol. (Berl)* 193:515–531.
9. Sandow, S. L., D. Whitehouse, and C. E. Hill. 1998. Specialised sympathetic neuroeffector associations in rat iris arterioles. *J. Anat.* 192(Pt 1):45–57.
10. Stjärne, L. 1989. Basic mechanisms and local modulation of nerve impulse-induced secretion of neurotransmitters from individual sympathetic nerve varicosities. *Rev. Physiol Biochem. Pharmacol.* 112:1–137.