Responses of the isolated sympathetic nerve-ductus deferens preparation of the guinea-pig

S. Huković

Commentary by

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The vas deferens has featured in the pharmacological literature in the past 35 years to a greater extent than any other organ containing smooth muscle. Since its introduction by Seid Huković, this simple preparation has provided the vehicle for experimental evidence for some of the major concepts about autonomic neurotransmission that are widely accepted today. There had been earlier studies on the vas deferens *in situ* by Langley and Anderson in 1895 and as an isolated organ preparation for observations on the responsiveness of the smooth muscle to various agonists by Macht in 1917 and Martins and Volle in 1939, but these reports had largely been forgotten until they were resurrected by Huković.

Huković first set up his preparation when he was working in Oxford in 1959 with Professor J.H. Burn. At that time, Burn was vigorously pursuing evidence for a cholinergic link in adrenergic transmission — the so-called Burn-Rand hypothesis — and it occurred to him that it might be worthwhile to repeat and extend some earlier observations made in 1956 by a previous Jugoslavian visitor in his laboratory, Varagić, on the effects of physostigmine on responses of the rabbit isolated uterus to hypogastric nerve stimulation. In an article by Rand and Mitchelson in 1986, Mike Rand recalled how Huković was commissioned to set up the preparation; he went to the animal house and returned to the laboratory with a guinea-pig and slaughtered it. It was only when he came to open the abdomen that he noticed he had been given a male guinea-pig. Undaunted, he set about the preparation of an isolated vas deferens with its hypogastric nerve attached, rationalizing this course of action by reasoning that a vas deferens was roughly analogous to a uterine horn. It was immediately apparent that strong and regular contractions of the longitudinal smooth muscle in the vas deferens could be elicited by modest stimulation of the hypogastric nerve trunk, and these were undoubtedly due to propagated action potentials since the responses were abolished by crushing the nerve trunk distal to the electrodes. Huković's first experiments with the isolated vas deferens were carried out towards the end of his 12-month period in Oxford, and he had insufficient time to develop them until he returned to his own laboratory in Sarajevo, where he made the bulk of the observations obtained on this preparation then published with the help of Mike Rand in 1961.

Mike Rand, on his return to Australia, used the vas deferens for further studies and also introduced the preparation to Geoff Burnstock and Mollie Holman in 1960, who were working on the electrophysiology of smooth muscle. The Huković preparation was then used by them to demonstrate, for the first time, excitatory junction potentials (ejp's) in smooth muscle in response to sympathetic nerve stimulation. These ejp's were blocked by guanethidine, but surprisingly not by adrenoceptor antagonists, and more than 20 years elapsed before it was recognized that ejp's were due to ATP released as a cotransmitter with noradrenaline (NA), particularly as a result of the studies of Dave Westfall, Jeff Fedan and Peter Sneddon in West Virginia and later Nevada in the early 1980's.

Some of the earliest observations with Huković's preparation of the vas deferens were at variance with generally accepted views about the physiology and pharmacology of sympathetic neuroeffector junctions. For example, responses to repeated stimulation of the hypogastric nerve were gradually reduced and finally abolished by hemicholinium, a drug which was regarded to interact rather specifically with cholinergic nerves. This problem was resolved when it was shown that many of the axons in the hypogastric nerve were preganglionic.

The original Huković preparation of the vas deferens is now seldom used, since, in order to avoid complications arising from preganglionic stimulation in studies of neuromuscular transmission, the postganglionic nerves within the wall of the vas deferens are stimulated transmurally by short pulses (generally 0.5-2 ms) of electric current passed through the tissue, as described by Tony Birmingham and Alan Wilson in 1963. The responses so elicited are thought to be due entirely to stimulation of postganglionic axons, since they are unaffected by ganglion blocking drugs, but they are abolished by low concentrations of tetradotoxin and by surgical denervation. Another factor that must be taken into account in evaluating the literature on the vas deferens is that the reactivity of the smooth muscle to agonists and the nature of the response to field stimulation differ between the prostatic and epididymal ends of the vas deferens, so the reported observations depended to a considerable extent on exactly what portion of the organ was isolated.

Swedin first demonstrated in 1971 that field stimulation of the guinea-pig or rat vas deferens for periods of 30 s produced a biphasic contractile response. The second tonic phase was susceptible to blockade by α-adrenoceptor blocking drugs or reserpine pretreatment, but the initial rapid 'twitch' phase of the response was resistant to these treatments. As both phases of the response were abolished by postganglionic denervation or by adrenergic neurone blocking drugs, it was concluded that both phases of the response were produced by stimulation of sympathetic nerves.

As mentioned earlier, the vas deferens was the preparation used initially to support the idea of sympathetic cotransmission involving ATP and NA. ATP was shown to be more potent than NA in producing concentration-dependent contractions of the longitudinal muscle of the guinea-pig vas deferens and, typically for cotransmission, to have synergistic actions with NA. The initial twitch component was shown to be blocked by P_2-purinoceptor antagonists, the second tonic response was blocked by α-adrenoceptor antagonists or by reserpine depletion of NA. Local spritzed application of ATP mimicked the ejp, while NA did not. Ellis and Burnstock showed that neuropeptide Y, which Lundberg had shown was stored and released from sympathetic nerve varicosities, elicted prejunctional inhibition of release of both ATP and NA as well as, in low concentrations, postjunctional potentiation of their actions. Modulation of transmitter release also occurs via prejunctional α_2 adrenoceptors for NA and P_1-purinoceptors for adenosine that results from the breakdown of neurally released ATP and constitute an autoregulatory mechanism.

Recent studies of prejunctional modulation of cotransmitter release from sympathetic nerves in the vas deferens by Trachte and colleauges and Ellis and Burnstock surprisingly revealed different effects of various agents on the release of ATP and NA. This raises the possibility that ATP and NA may be contained in different vesicles in varicosities or that there may be subpopulations of sympathetic nerves some storing and releasing largely ATP, others releasing largely NA; other possible explanations are currently being explored. Another ongoing debate was prompted by the early serial electron microscopic study by Neil Merrillees of the nervous environment of single smooth muscle cells in the vas deferens. Varicosities from more than one branching sympathetic nerve fibre were found at variable distances from the muscle cells and while prejunctional thickenings representing sites of release of transmitter were evident, no postjunctional specialisations comparable to those found at synapses at the skeletal neuromuscular junction or between neurones, were seen. This prompted Burnstock to suggest that autonomic neuromuscular junctions are not fixed synapses, but that transmitter is released 'en passage' from varicosities at variable and perhaps changing distances from muscle cells. The alternative view that autonomic neuromuscular junctions are true synapses has been presented by David Hirst and his colleagues Luff and McLachlan.

The vas deferens continues to be a source of significant new developments in autonomic neuropharmacology. Lennart Stjärne and Tom Cunnane described a technique that allowed simultaneous recordings of pre- and postjunctional events and Max Bennett and colleagues in Sydney have recently developed a method for recording from single visualised axon varicosities, for studies of spontaneous and evoked transmitter release and its modulation. The first ligand-gated cation P_{2X}-purinoceptor was cloned and characterised from the vas deferens by Valera, Alan North and colleagues in 1994.

So, all in all, this has been one hellava useful preparation.

References

BIRMINGHAM, A.T. & WILSON, A.B. (1963). Preganglionic and postganglionic stimulation of the guinea-pig isolated vas deferens preparation. *Brit. J. Pharmacol. Chemother.* **21,** 569-580.

BROCK, J.A. & CUNNANE, T.C. (1987). Relationship between nerve action potential and transmitter release from sympathetic postganglionic nerve terminals. *Nature*, **226,** 605-607.

BURN, J.H. & RAND, M.J. (1965) Acetylcholine in adrenergic transmission. *Ann. Rev. Pharmac.* **5,** 163-212.

BURNSTOCK, G. & HOLMAN, M.E. (1961). The transmission of excitation from autonomic nerve to smooth muscle. *J. Physiol.* **155,** 115-133.

ELLIS, J.L. & BURNSTOCK, G. (1990). Modulation by prostaglandin E_2 of ATP and noradrenaline cotransmission in the guinea-pig vas deferens. *J. Auton. Pharmacol.* **10,** 363-372.

LAVIDIS, N.A. & BENNETT, M.R. (1992). Probablistic secretion of quanta from visualised sympathetic nerve varicosities in mouse vas deferens. *J. Physiol. (Lond.)* **454,** 9-26.

MERRILLEES, N.C.R. (1968). The nervous environment of individual smooth muscle cell of the guinea-pig vas deferens. *J. Cell. Biol*, **37,** 794-817.

SNEDDON, P., WESTFALL, D.P. & FEDAN, J.S. (1982). Cotransmitters in the motor nerves of the guinea-pig vas deferens: electrophysiological evidence. *Science*, **218,** 693-695.

STJÄRNE, L., LUNDBERG, J.M. & ASTRAND, P. (1986). Neuropeptide Y — a cotransmitter with noradrenaline and adenosine 5'-triphosphate in the sympathetic nerves of the mouse vas deferens? A biochemical, physiological and electropharmacological study. *Neuroscience*, **18,** 151-166.

SWEDIN, G. (1971). Studies on neurotransmission mechanisms in the rat and guinea-pig vas deferens. *Acta Physiol. Scand. Suppl.* **369,** 1-34.

VALERA, S., HUSSY, N, EVANS, R.J, ADAMI, N., NORTH, R.A., SURPRENANT, A. & BUELL, G.N. (1994). A new class of ligand-gated ion channel defined by P_{2x} receptor for extracellular ATP. *Nature*, **371,** 516-519.