expected larvae to be more sensitive for all species. In *Melanoplus*, female instars are the least sensitive of all. The albino strain is less resistant than its wild type counterpart, especially at the adult stage. Whether these variations reflect, for instance, differential repair mechanisms is open to speculation. It is interesting to note, however, the existance of a variety of strains of *Drosophila* (treated as larvae) which display variable degrees of sensitivity to MMS; male-female differences are also noted but are found to be strain-dependent[12]. Comparing the acridids with 2 other organisms, it can be seen that their range of response overlaps that of the mouse, but is about 6–17 times more sensitive than *Drosophila*. These results are somewhat paralleled by MMS but the differences are not as great as for EMS. It would appear, therefore, that the radiosensitive acridid is also mutagen-sensitive, at least for EMS and possibly MMS.

Table 2. Median lethal doses of alkylating agents introduced by injection into a number of organisms including 3 species of acridids

	MLD (molarity) ± SE	mg/g
Locusta-L*		
EMS	0.2828 ± 0.0381 (15 µl)	1.1095
MMS	0.0251 ± 0.0029 (15 µl)	0.0837
DES	0.1518 ± 0.0441 (15 µl)	-
MNU	0.0501 ± 0.0096 (15 µl)	-
EMS		
Locusta-A*	0.5404 ± 0.0726 (15 µl)	1.1079
Albino-L	0.2549 ± 0.0490 (15 µl)	1.0001
Albino-A	0.3821 ± 0.0654 (15 µl)	0.7834
Aiolopus-L	0.0719 ± 0.0107 (5 µl)	0.3656
Melanoplus-L	0.1416 ± 0.0329 (5 µl)	0.4949
Melanoplus-L (♀)	0.2639 ± 0.0317 (5 µl)	0.8429
Melanoplus-A	0.3249 ± 0.0412 (5 µl)	0.6232
Drosophila-A[9]	-	6.9884
Mouse-A[10]	-	0.45–0.50
MMs		
Drosophila-A[9]	-	0.25
Mouse-A[13,14]	-	0.05–0.12

*L, 5th-instar larvae, A, adult.

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Cholinergic and adrenergic innervation of the penis artery of the bull: Transmitter concentrations and synaptic vesicles

E. Klinge, O. Eränkö and N.O. Sjöstrand[1]

Department of Pharmacology, University of Helsinki, SF-00170 Helsinki 17 (Finland), Department of Anatomy, University of Helsinki, SF-00170 Helsinki 17 (Finland), and Department of Physiology, Karolinska Institutet, S-10401 Stockholm 60 (Sweden), 16 May 1978

Summary. The penile artery of the bull contained significant amounts of acetylcholine, noradrenaline and dopamine, and its axon profiles contained either numerous small granular or agranular vesicles, as well as some large granular vesicles. In the dorsal metatarsal artery, only noradrenaline and dopamine were detectable, and the axon profiles observed contained numerous small granular vesicles. In the penile artery, the axons with small agranular vesicles, probably cholinergic axons, were in close contact with axons containing small granular vesicles. It is suggested that, in the penile artery of the bull, one of the functions of the cholinergic nerves is suppression of excitatory adrenergic neurotransmission.

In mammals, initiation of penile erection involves dilation of the penile artery as well as simultaneous relaxation of other smooth muscle effectors of erection, if present, i.e. of the retractor penis muscle and the muscles in the cavernous bodies[2]. It is likely that this functional entity of smooth muscles has an identical innervation consisting of an excitatory adrenergic component and 1 or more obscure inhibitory components. Thus in several mammals, neurogenic relaxation of these muscles is atropine-resistant and in vitro usual muscarinic concentrations of acetylcholine (ACh) do not relax but rather contract the muscles[2,3]. ACh is therefore hardly the inhibitory transmitter acting directly upon the smooth muscle cells. However, evidence has been presented indicating that cholinergic nerves are present in the smooth muscle effectors of erection, and that the function of these nerves might be suppression of excitatory adrenergic neurotransmission[3,4]. But the evidence for this concept has hitherto been essentially confined to studies on the retractor penis muscle.

In the present study we have made an attempt to clarify further the innervation of the penile artery of the bull by measuring its ACh, noradrenaline (NA) and dopamine (DA) contents, and by examining the ultrastructure of its axon profiles. For the sake of comparison, the dorsal metatarsal artery of the same bulls was studied in an identical way. In the bull, the penile and the dorsal metatarsal arteries have about the same outer diameter, but the former has a more elastic and soft consistence. Contrary to the penile artery, the dorsal metatarsal artery seems to be devoid of inhibitory nerves[3].

Materials and methods. Bulls weighing 250–500 kg were killed in the slaughter house and bled. Samples of the stem of the penile artery were obtained within 20–50 min after killing and were cut from the area lying between the branches serving the cavernous bodies[3]. ACh was determined biologically on the superfused frog rectus abdominis muscle[5]. The average amount of tissue used for extraction was 1.4 g. NA and DA were determined spectrophotofluo-

rometrically[6,7]. For electron microscopy, thin longitudinal strips of vessels were fixed in a stretched state at 0 °C for 2 h in 3% potassium permanganate in Krebs-Ringer-glucose solution at pH 7.1[8]. Sections of the epon-embedded material were cut at 50–80 nm and examined at 40 or 60 kV in an EM 300 (Philips) electron microscope.

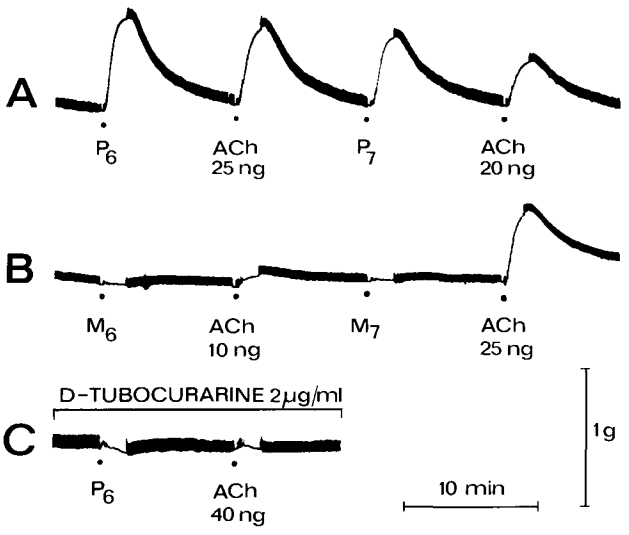

Fig. 1. Bioassay of acetylcholine (ACh) on the superfused frog rectus abdominis muscle. P, penile artery; M, dorsal metatarsal artery. P_6 and M_6 are samples from one and the same bull, and P_7 and M_7 from another bull. They all represent 0.2 ml extracts corresponding to about 70 mg of arterial tissue. *A* The samples of the penile artery induce clear ACh-like contractions. *B* The samples of the dorsal metatarsal artery are devoid of sufficient amounts of contracting substance. *C* d-Tubocurarine abolishes the contractions.

Results and comments. Extracts of the penile artery elicited strong ACh-like contractions that were abolished by d-tubocurarine, while identical extracts of the dorsal metatarsal artery elicited no contractions (figure 1). If there is any ACh in the dorsal metatarsal artery, its concentration is below the sensitivity of the present method and more than 10 times smaller than that in the penile artery (table). There was no significant difference between the NA concentrations in the 2 arteries, while the penile artery had a greater DA concentration ($p < 0.01$). It is not known whether this reflects a difference in neuronal DA or in non-neuronal DA-containing cells, or in both. In the penile artery, the molar ratio ACh:NA was about 1:3, i.e. the same as in the bull retractor penis[4]. The transmitter levels in the penile artery were, however, just one third of those in the bull retractor penis[4].

There were few nerve fibres as compared with the number of smooth muscle cells in both the penile and the dorsal metatarsal artery. Figure 2 shows a small nerve trunk in the penile artery. In both arteries axon profiles were observed which contained numerous small granular vesicles about 50 nm in diameter and some large granular vesicles. Ensheathed in the same Schwann cell sheath with such profiles, presumably adrenergic axons, there were in the

Acetylcholine, noradrenaline and dopamine concentrations in bovine penile and dorsal metatarsal arteries

	ACh	NA	DA
Penile artery	0.32±0.04 (7)	1.02±0.12 (5)	0.27±0.04* (5)
Dorsal metatarsal artery	<0.03 (7)	0.81±0.08 (5)	0.10±0.03 (5)

Values are means±SE (µg/g of wet tissue). Figures in brackets refer to number of bulls. *$p<0.01$ (difference from the corresponding value in the dorsal metatarsal artery).

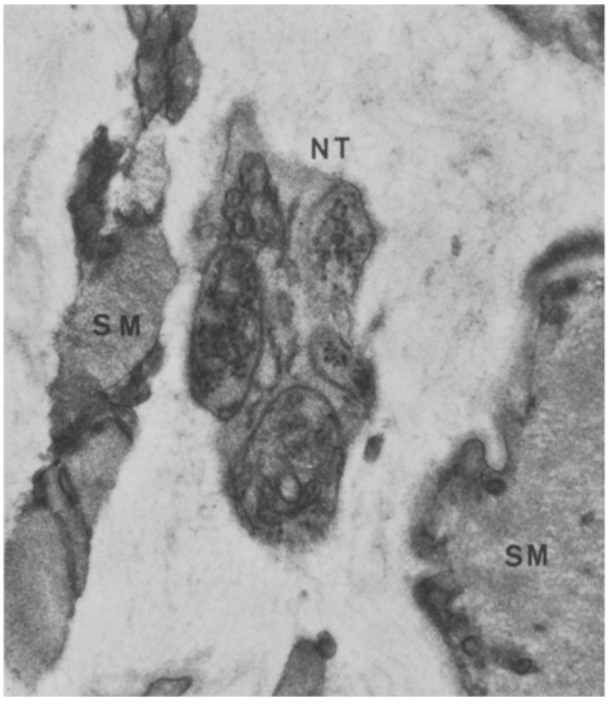

Fig. 2. Small nerve trunk (NT) with 4 axon profiles in the connective tissue space between smooth muscle (SM) cells of the penile artery of the bull. Fixation with potassium permanganate. × 16,500.

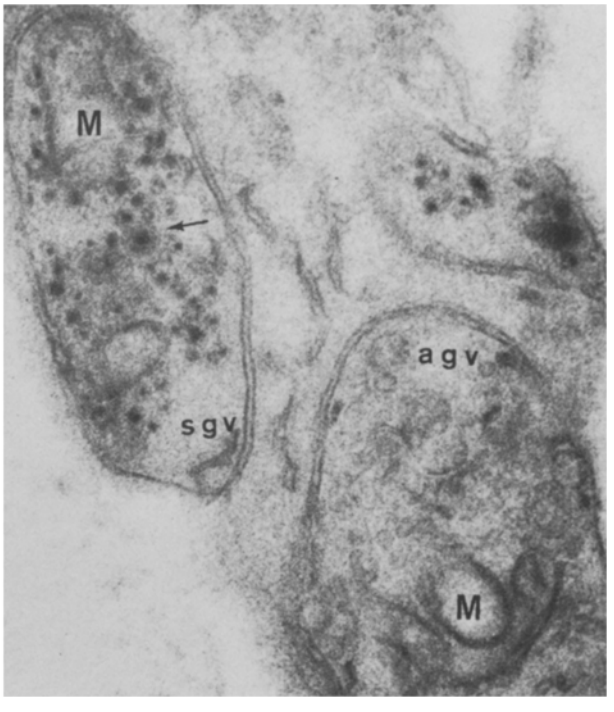

Fig. 3. Higher magnification of the nerve trunk shown in figure 2. Axon profiles can be seen containing mitochondria (M) and numerous small granular vesicles (sgv) or agranular vesicles (agv). A large granular vesicle is indicated by the arrow. × 45,000.

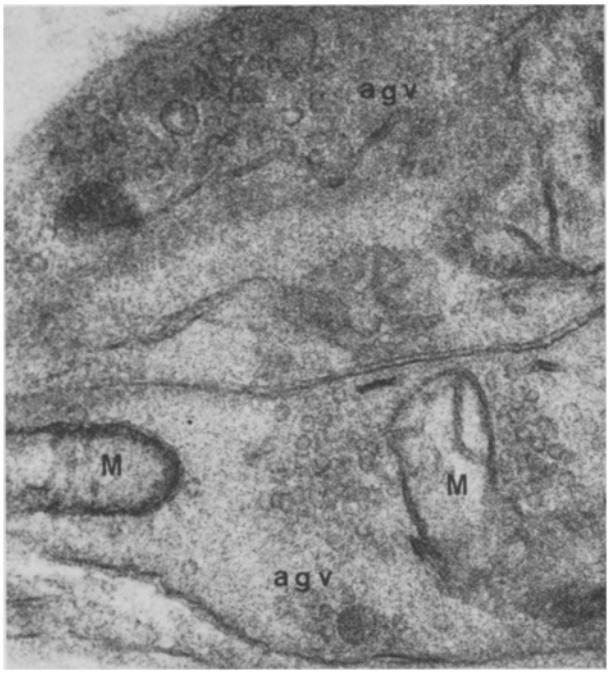

Fig. 4. Nerve trunk with 2 axon profiles containing agranular vesicles (agv) in the penile artery of the bull. M, mitochondrion. Fixation with potassium permanganate. × 45,000.

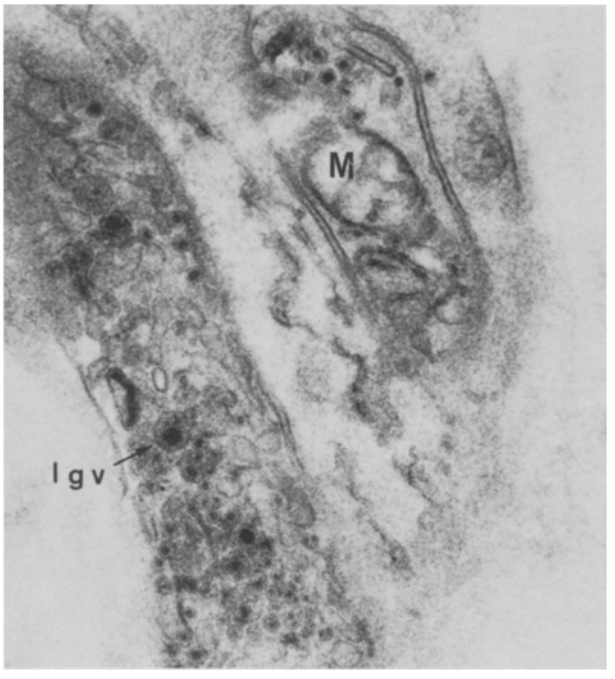

Fig. 5. 2 axon profiles with numerous small granular vesicles in the dorsal metatarsal artery of the bull. 1 large granular vesicle (lgv) is indicated by the arrow. M, mitochondrion. Fixation with potassium permanganate. × 45,000.

penile artery also axon profiles containing numerous small agranular vesicles about 50 nm in diameter (figure 3). In the penile artery, there were also nerve trunks with several axon profiles containing numerous small agranular vesicles (figure 4). These profiles were of the type generally observed in cholinergic synapses[8,9]. No profiles with small agranular vesicles were found in the dorsal metatarsal artery (figure 5). However, owing to the general paucity of nerve fibres in this artery, the presence of the cholinergic type cannot be entirely excluded.

In the penile artery of the bull, axons containing small granular or agranular vesicles also contained some large opaque vesicles, previously illustrated in the axons of the retractor penis muscle of the same species[10]. However, the number of such vesicles, which are possibly fragments of large granular vesicles, seemed to be smaller in the penile artery than in the retractor penis.

Discussion. The significant amount of ACh found in the bovine penile artery but not in the dorsal metatarsal artery, supports the concept that the axons containing small agranular vesicles are cholinergic. Recently obtained pharmacological evidence suggests the existence of muscarinic inhibitory receptors in the adrenergic axons innervating this artery[3]. The close juxtaposition of the cholinergic axons with the adrenergic axons is in agreement with the idea that they really can exert an inhibitory effect upon adrenergic neurotransmission. Furthermore, there is strong evidence pointing to an endogenous muscarinic suppression of adrenergic neurotransmission in the retractor penis[4,10]. In view of recent observations[11] and the present results, the existence of inhibitory dopaminergic receptors in the adrenergic axons of the bovine penile artery has also to be considered.

While ACh may contribute to the establishment of penile erection by prejunctional inhibition of the excitatory adrenergic neuromuscular transmission, evidence is lacking that ACh can relax the smooth muscles concerned by direct action on the muscle cells. Attempts to demonstrate muscarinic inhibitory receptors in the smooth muscle cells of the stem of the penile artery, or in those of the branches serving the cavernous bodies, have been unsuccessful[2,3]. Only in perfusion experiments has it been possible to demonstrate humoral muscarinic inhibitory receptors in an undefined but probably more peripheral part of the bovine penile vascular bed[12].

In conclusion, the present results support the concept that, in the penile artery of the bull, one of the inhibitory nervous mechanisms could indeed be cholinergic suppression of motor adrenergic neurotransmission. The evidence against an inhibitory purinergic neurotransmission in this vessel, and the possible significance of the large granular and the large opaque vesicles have been considered in 2 recent reports[3,10].

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