Different Types of Synaptic Vesicles in Axons of the Retractor Penis Muscle of the Bull

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Summary. Three types of axon profiles were observed in the smooth muscle of the retractor penis and the penile artery of the bull: 1. profiles containing small granular vesicles, presumably representing adrenergic axons; 2. profiles containing small agranular vesicles, presumably representing cholinergic axons; 3. profiles containing numerous large and small granular vesicles. The third type of profile was not found in the vas deferens or the metatarsal artery. It is therefore possible that this type of profile represents the non-adrenergic, non-cholinergic inhibitory nerves, the presence of which has previously been pharmacologically indicated in these tissues.

Innervation of the smooth muscle effectors of penile erection has long been a matter of dispute. While it seems clear that the excitatory innervation is adrenergic, pharmacological observations suggest that the inhibitory innervation is neither adrenergic nor cholinergic[2]. However, acetylcholinesterase has been histochemically demonstrated in the retractor penis[3,4] the penile artery[5], and the cavernous bodies[6,7] of different species, and acetylcholine has been found in some of these tissues[8,9]. These data suggest that there could be 3 types of efferent innervation of the smooth muscle effectors of penile erection: 1. adrenergic excitatory, 2. non-adrenergic, non-cholinergic inhibitory and 3. cholinergic innervation of unknown effect. Since previous ultrastructural studies dealing with the innervation are not available, we have studied the fine structure of the retractor penis muscle and also the penile artery in order to identify the types of synaptic vesicles in their axons.

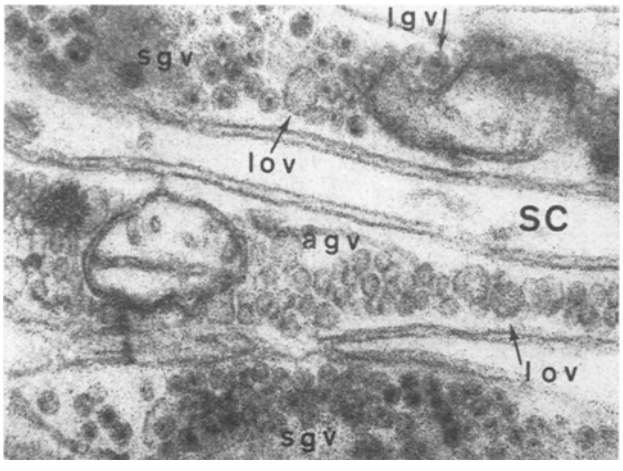

Fig. 1. Three axons in the retractor penis muscle of the bull. Note the close contact between the axon in the centre with agranular vesicles (agv) and large opaque vesicles (lov) and the axon below it, containing small granular vesicles (sgv). The uppermost axon contains large granular vesicles (lgv) besides sgv and lov. SC, Schwann cell. Fixation in potassium permanganate. × 54,000.

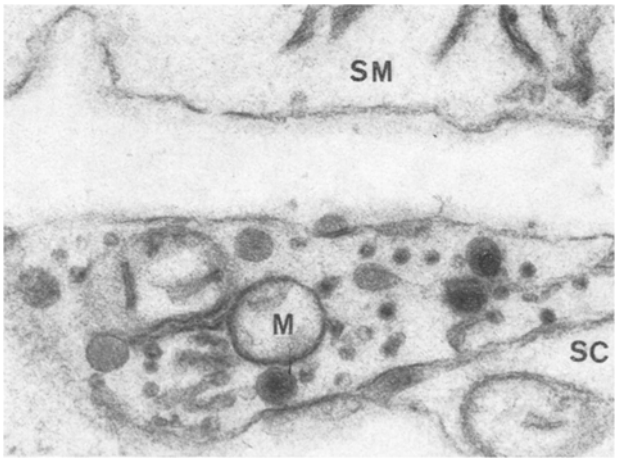

Fig. 2. Axon profile (below) and smooth muscle cell (SM) in the retractor penis muscle. Note the small granular vesicles and the large granular and opaque vesicles in the axon. M, mitochondrion. SC, Schwann cell. Permanganate fixation. × 44,000.

Material and methods. Young bulls weighing 250–400 kg were killed in the slaughter house and bled. Longitudinal strips 0.5–1 mm thick were cut within 15 min after killing from the middle third of the retractor penis muscle. Some specimens from the stem or the branch to the corpus cavernosum urethrae of the penile artery, as well as some specimens of the vas deferens and the dorsal metatarsal artery, were also examined.

Three types of fixations were used at 0 °C: 1. fixation for 1.5 h in 3% potassium permanganate in Krebs-Ringer-glucose solution at pH 7.0[10], 2. fixation for 3 h in 3.5% glutaraldehyde in 0.1 *M* phosphate buffer, pH 7.4[11], 3. fixation for 3 h in Karnovsky's[12] fixative containing 0.5% formaldehyde and 3% glutaraldehyde in 0.1 *M* phosphate buffer, pH 7.4. Tissues fixed in the glutaraldehyde-containing fixatives were rinsed in water and re-fixed for 1 h in 1% osmium tetroxide[11]. Longitudinal or cross sections of the epon-embedded material were cut at 50–80 nm and examined, unstained or after staining with uranyl acetate and lead citrate, at 40 or 60 kV in an EM 300 (Philips) electron microscope.

Results and comments. The smooth muscle cells of the retractor penis muscle formed bundles surrounded by a wide connective tissue space. Within each bundle, the muscle cells were separated by a narrow space containing collagen fibrils. Neighbouring muscle cells were in close contact with each other through processes bridging the

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[2] E. Klinge and N. O. Sjöstrand, Acta physiol. scand. *93*, Suppl. 420, 1 (1974).
[3] C. Bell and J. R. McLean, Z. Zellforsch. *106*, 516 (1970).
[4] E. Klinge, P. Pohto and E. Solatunturi, Acta physiol. scand. *78*, 110 (1970).
[5] E. Klinge and P. Pohto, Brain Res. *34*, 415 (1971).
[6] J. Grieten and M.-A. Gerebtzoff, Ann. Histochim. *2*, 127 (1957).
[7] E. Klinge and O. Penttilä, Ann. Med. exp. Fenn. *47*, 17 (1969).
[8] O. Penttilä, Ann. Med. exp. Fenn. *44*, Suppl. 9, 1 (1966).
[9] E. Klinge, Acta physiol. scand. *78*, 159 (1970).
[10] K. C. Richardson, Am. J. Anat. *114*, 173 (1964).
[11] R. E. Coupland and D. Hopwood, J. Anat. *100*, 227 (1966).
[12] M. J. Karnovsky, J. Cell Biol. *27*, 137 A (1965).

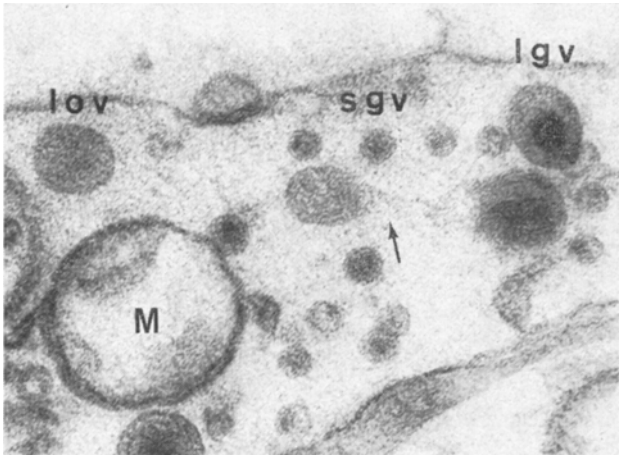

Fig. 3. The axon shown in Figure 2 at higher magnification. The arrow indicates the site of continuation between a large opaque vesicle and a tubule. M, mitochondrion; lgv, large granular vesicles; lov, large opaque vesicle; sgv, small granular vesicles. Permanganate fixation. × 86,000.

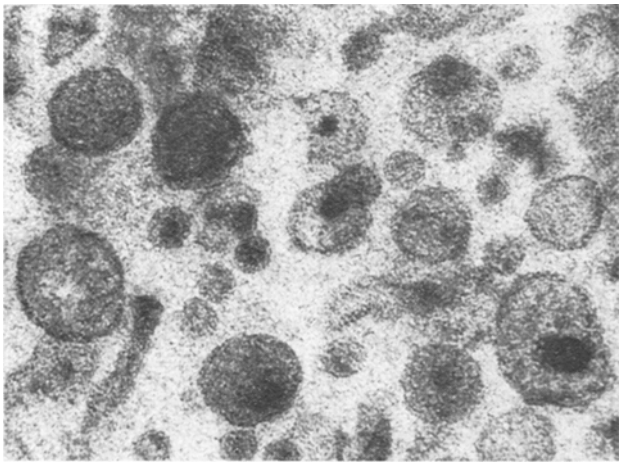

Fig. 4. Large and small granular vesicles in an axon profile in the retractor penis muscle. Note the eccentric location of the dense core in many vesicles. Permanganate fixation. × 86,000.

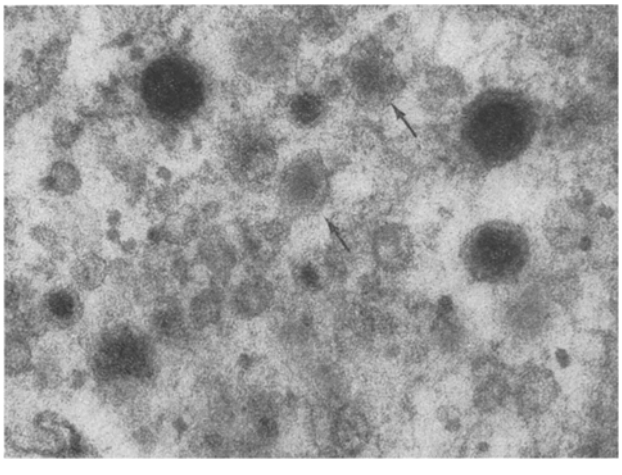

Fig. 5. Vesicles in an axon profile in the retractor penis muscle fixed in glutaraldehyde and osmium tetroxide. Note the 3 extremely dense large vesicles and the 2 large granular vesicles with a less dense core pointed out by arrows. × 86,000.

narrow space. Axons embedded in Schwann cell cytoplasm were seen both in the wide spaces between bundles and the narrow spaces between muscle cells. No striated muscle was seen.

In specimens fixed in potassium permanganate, the main part of the axon profiles of the retractor penis muscle contained numerous small granular vesicles about 50 nm in diameter, similar to those in typical adrenergic synapses[10,13]. Most of them also contained a varying number of large granular or agranular opaque vesicles of about 100 nm in diameter. The large vesicles were numerous in several profiles. There were also axons containing many small agranular vesicles about 50 nm in diameter of the type found in cholinergic synapses[10,13] and some large vesicles about 100 nm in diameter (Figure 1). Sometimes the axons with small agranular vesicles were in close, possibly synaptic, contact with those containing small granular vesicles (Figure 1). The axon profiles were usually only partly embedded in Schwann cell cytoplasm, presenting a free surface towards the smooth muscle (Figure 2). Similar profiles were also found in the penile artery.

Few large vesicles were seen in the axon profiles of the vas deferens and the dorsal metatarsal artery, in which small granular or agranular vesicles were the dominant types. While such profiles were also seen in the retractor penis muscle and the penile artery, the coexistence of numerous large granular or opaque vesicles characterized most axon profiles containing small granular vesicles in these tissues (Figures 3 and 4). The large vesicles tended to be more frequent in the narrow parts of the axon, but they were often numerous also in wider axon profiles of the retractor penis (Figure 4) or the penile artery muscle.

The diameter of the large vesicle profiles considerably varied around 100 nm. Since the section thickness was 50–80 nm, some of the large vesicles, whose diameter was about twice the section thickness, must have been cut off centre. Furthermore, since the dense core was often eccentrically located in the large vesicle (Figures 3 and 4), it is quite possible that the agranular large vesicle profiles were in fact agranular parts of large granular vesicles. Sometimes a large vesicle was continuous with a tubule, seemingly sprouting from its end (Figure 3, arrow).

After fixation in glutaraldehyde and osmium tetroxide, the electron density of the core greatly varied in individual large granular vesicles, some of which were dark almost throughout, while others had smaller cores of lower density (Figure 5). In glutaraldehyde-fixed specimens, only some small granular vesicles were seen in the axon terminals, a finding typical also of adrenergic axons in immersion-fixed material[14]. In specimens fixed in Karnovsky's formaldehyde-glutaraldehyde mixture and osmium tetroxide, all small vesicles were agranular, although dense cores were seen in some of the large vesicles. These differences in specimens fixed in different fixatives are probably due to differences in the amine-localizing power of the fixatives[10,13]. That dense cores almost filled the large vesicles in glutaraldehyde-fixed material (Figure 5) may be due to diffusion of the amine within the vesicle before formation of the insoluble reaction product during fixation.

Discussion. Some large granular vesicles are normally present together with numerous small granular vesicles in adrenergic terminal axons[10,13]. However, in the present study unusually numerous large granular vesicles were observed in most axon profiles of the retractor penis

[13] T. HÖKFELT, Progr. Brain Res. *34*, 213 (1971).
[14] A. PELLEGRINO DE IRALDI, R. GUEUDET and A. M. SUBURO, Progr. Brain Res. *34*, 161 (1971).

muscle. It may even be that profiles with numerous large granular vesicles, together with small granular vesicles (Figures 2–5), actually represent axons separate from axons containing almost exclusively small granular vesicles (Figure 1). If it is so, the retractor penis muscle of the bull would be innervated by 3 morphologically different types of nerve fibres: 1. axons containing small agranular vesicles, presumably cholinergic; 2. axons containing small granular vesicles, presumably adrenergic; 3. axons containing large and small granular vesicles, whose nature may differ from that of adrenergic axons. It must, however, be pointed out that the relative number of large granular vesicles may vary in different parts of the same axon.

In any case, numerous large granular vesicles characterize most of the axon profiles of the smooth muscle of the retractor penis and the penile artery of the bull, in contrast with those of the vas deferens and the metatarsal artery of the same animal, in which typical adrenergic axon profiles were seen with numerous small, but few large granular vesicles. In view of the pharmacological evidence for the presence of non-adrenergic, non-cholinergic inhibitory nerves in the retractor penis muscle[2], it is of special interest that large opaque vesicles have been considered as a characteristic of the inhibitory non-adrenergic, non-cholinergic, possibly purinergic axons innervating various tissues[15,16]. In those axons, small vesicles are present besides the large ones[16,17]. It is, however, unlikely that adenosine triphosphate serves as an inhibitory transmitter in the retractor penis muscle or the penile artery of the bull[2]. On the other hand, studies with different fixatives are necessary in order to characterize the fine structure of the so-called purinergic nerves.

[15] G. Burnstock, Proc. 6th Int. Congr. Pharmac. Helsinki (Ed. L. Ahtee; 1975), vol. 2, p. 49.
[16] G. Burnstock and T. Iwayama, Progr. Brain Res. *34*, 402 (1971).
[17] P. M. Robinson, J. McLean and G. Burnstock, J. Pharmac. exp. Ther. *179*, 149 (1971).

Origin of Ovarian Follicle Cells in Birds

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Summary. In the embryonic Japanese quail ovary, transplanted on chicken chorioallantoic membrane (CAM), follicle cells are derived from somatic cells of the ovarian surface epithelium. No evidence was found for a contribution of other cell groups of the quail ovary in the formation of follicle cells. This may be demonstrated on PAS stained sections, by following the transfer of carbon particles, initially applied on the surface epithelium.

It was classically accepted that follicle cells originate from the somatic cells of the 'germinal epithelium'. However, in several groups of vertebrates, the origin of follicle cells is still a matter of dispute[2]. For instance, in mammalia there is much doubt as to the cortical origin of follicle cells[3–6]. In birds, another higher vertebrate class, the formation of secondary sex cords (Pflüger or ovigerous cords) from the surface epithelium can usually be seen distinctly. However later, when the cortex has fully developed, a possible ingrowth of ovarian stroma cells into the ovigerous cords cannot be entirely ruled out after routine histological investigations.

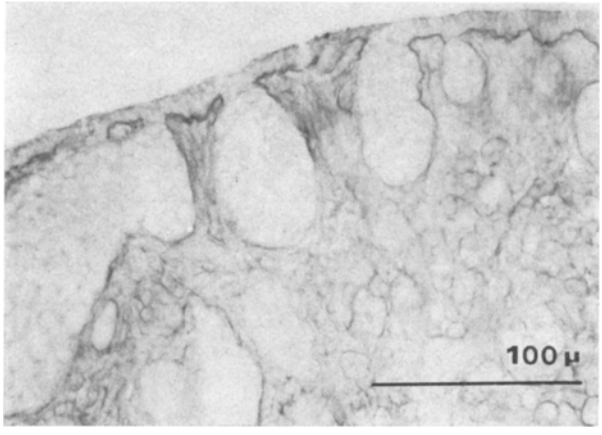

Fig. 1. PAS-stained section of the left ovary from a 15-day-old chicken embryo.

Material and Methods. Ovaries of 8- to 11-day-old chicken or Japanese quail embryos were used in this study. After opening the embryo's abdomen and removing the intraperitoneal organs, a small quantity of ultrasonicated animal charcoal is sprinkled on the surface of the left ovary, while it is still in situ. After excision the left ovary is transplanted on the CAM of a 7- to 9-day-old chicken embryo, according to the technique of Harris[7]. 1 to 9 days later, the graft is excised and fixed in acetic-alcohol (1:3 v) for 1 h. The sex of the host embryo is noted for each transplant. After embedding in paraffin, the transplanted ovaries are serially sectioned at 5 to 7 µm thickness. After deparaffination, the sections are stained with the PAS technique[8]. PAS-stained sections of ovaries from older embryos are used as controls. Some of the sections are counterstained with methyl green or Unna.

Results and discussion. During the formation of the cortex, PAS stained sections of embryonic quail or chicken ovaries clearly show the development of vase-shaped buds connected with the surface epithelium via a

[1] The author is very grateful to Prof. L. Vakaet, R.U.C.A. Antwerp, for his valuables suggestions, and to Miss C. van Hoecke for her technical assistance.
[2] S. Zuckerman, *The Ovary* (Academic Press, New York 1962), vol. 1.
[3] A. Fischel, Z. Anat. Entw. Gesch. *92*, 34 (1930).
[4] A. Gropp and S. Ohno, Z. Zellforsch. *74*, 505 (1966).
[5] H. Peters and T. Pedersen, Fertil. Steril. *18*, 309 (1967).
[6] A. Byskov and S. Lintern-Moore, J. Anat. *116*, 207 (1973).
[7] J. Harris, Ann. Acad. Sci. New York *76*, 764 (1958).
[8] J. MacManus, Nature, Lond. *158*, 202 (1946).