Studies on the contractile activity and ultrastructure of the boar testicular capsule*

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Summary. The ultrastructure and the spontaneous and drug-induced contractility of the testicular capsule of 18 boars were investigated. Isometric recordings were obtained *in vitro* using strips of the tunica albuginea isolated from various regions of the testis. Maximal contractile activity was found in the strips of the posterior border of the testis, in which the histological studies (light and electron microscopy) showed abundant typical smooth muscle cells distributed in layers parallel to the testicular long axis. These cells were largely aggregated in the inner layer of the testicular capsule, which displayed contractile activity similar to that of the entire tunica albuginea. The outer layer of the tunica albuginea was almost totally devoid of smooth muscle fibres and showed little or no contractility. The spontaneous contractions were rhythmic and exhibited an amplitude of 20–70 mg and a frequency of 5–30 contractions/10 min. Norepinephrine, acetylcholine and oxytocin all produced an increase of the contractility of the tunica albuginea, consisting mainly in a rise of the tone.

Introduction

It has been demonstrated that the testicular capsules of various mammals can contract spontaneously or in the presence of smooth muscle stimulating agents, both *in vivo* and *in vitro* (Davis & Langford, 1970, 1971; Hargrove, Seeley & Ellis, 1975; Firlit, King & Davis, 1975; Hargrove & Ellis, 1976), and also following electrical stimulation of the spermatic nerves (Rikimaru & Shirai, 1972; Rikimaru & Suzuki, 1972; Bell & MacLean, 1973). Light and electron microscope studies have demonstrated various amounts of smooth muscle cells within the connective tissue framework of the tunica albuginea (Holstein 1967a, b; Langford & Heller, 1973; Gorgas & Böck, 1974; Leeson & Cookson, 1974), and it is these cells which are considered responsible for the contractile movements. The contractions cause change in the intratesticular pressure (Holstein & Weiss, 1967; Rikimaru, Maruyama, Shirai & Dendo, 1972; Hargrove & Ellis, 1976) and in the pressure of the perfused testicular artery of the rabbit testis (Langford, Waites & Archer, 1976). These findings led to the suggestion that such influences aid sperm transport and circulation of blood and lymph within the testis (Holstein, 1967b; Holstein & Weiss, 1967; Davis & Langford, 1970; Firlit *et al.*, 1975; Hargrove, MacIndoe & Ellis, 1977). At present, the possible involvement of capsular contractions in such processes cannot be excluded nor can it yet be considered as clearly demonstrated. In this paper we describe the contractility and ultrastructure of the tunica albuginea of the boar.

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Materials and Methods

Testes were obtained from 18 healthy boars of the Duroc–Jersey breed. All animals were killed by bleeding following electrocution in a local abattoir. Immediately after death, the testes were removed and immersed in Tyrode's solution at 4°C for transport to the laboratory. Fresh specimens were also placed in appropriate fixatives for light and electron microscopy.

Within 45–60 min after removal, longitudinal pieces were cut from the mid-anterior and the mid-posterior regions of the testicular capsules and further reduced to 20 × 4 mm strips. As far as possible, all attached testicular parenchyma was thoroughly removed from the tunica albuginea.

The tunica albuginea of the boar consisted typically of a thick outer collagen-rich layer, a middle layer containing the testicular blood vessels (the so-called tunica vasculosa), and an inner connective layer directly apposed to the testicular parenchyma. In this latter layer, in all the specimens examined, the amount of smooth muscle cells was distinctly greater than in the outer layer. In some experiments the inner and outer layers were separated to ascertain whether the contractile activity of the tunica albuginea was due to the smooth muscle fibres of the inner layer. The contractility of both layers was recorded separately and then compared with that of whole strips.

All strips were placed in a 50 ml organ bath containing Tyrode's solution maintained at 34°C. To obtain isometric recordings, one end of each strip was attached by a hook to the bottom of the bath and the opposite end was tied by a cotton thread to a Statham strain gauge (0.15 oz). A four-channel Sanborn Poly-Viso was used as recording system. Solutions were changed through a polyethylene catheter attached to the inner wall of the bath. The apparatus was calibrated to give a positive deflection of 1 cm on the recording paper for every 50 mg tension applied.

In all experiments, the preparation was allowed to equilibrate for 90 min and then several agents were assayed. Norepinephrine (Levophed: Winthrop Laboratories, New York), acetylcholine (Sigma Chemical Co., St Louis, Missouri), and oxytocin (Syntocinon: Sandoz, Basel) were assayed at random in every experiment. Each drug was dissolved in Tyrode's solution and given at threshold and increasing doses. Each dose was left in the bath for 10 min, then the preparation was washed, and after 20 min another dose of the same preparation was given.

After the experiment was completed (about 6–8 h), the strips were fixed in Bouin's fluid overnight and then processed together with specimens fixed immediately after death of the animals (see above). For light microscopy, the material was embedded in paraffin wax in the usual way and 5 µm sections were stained with the PAS–haematoxylin technique, the Van Gieson method and the Cajal–Gallego's trichrome method to demonstrate smooth muscle fibres within the tunica albuginea and to assess the normal appearance of the testes included in this study. For electron microscopy, 3.5% glutaraldehyde, buffered to pH 7.3 with 0.1 M-phosphate buffer at 4°C, was injected slowly into the tunica albuginea *in situ*. The tissue was cut into 1 × 5 mm blocks, which were immersed in the above fixative for 10 h. These specimens were further reduced to 1 mm³ blocks, which were post-fixed in 1% aqueous osmium tetroxide for 2 h. After dehydration with ethanol and acetone, the material was embedded in Araldite and sectioned. Thick sections (1 µm) were stained with toluidine blue for examination with the light microscope. Thin sections (60–80 nm) were stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop I electron microscope at 80 kV.

Results

The mid-anterior strips were consistently inactive and did not respond to the addition of drugs (6 experiments); they also appeared almost completely devoid of smooth muscle cells. For this reason, they were not further studied.

Contractility studies

Spontaneous contractions. All whole strips obtained from the mid-posterior border of the testis (15 experiments) contracted spontaneously. Initial contractions were irregular, but after 60–90 min more than half of the capsular preparations showed rhythmic contractions. The amplitude of contractions varied between 20 and 70 mg, and the rate was 5–30 contractions/10 min (Text-fig. 1).

Text-fig. 1. Different patterns of spontaneous contractions of the testicular capsule of the boar (mid-posterior border of the testis).

The amplitude and frequency of contractions of the inner layer preparations isolated from whole strips of the mid posterior region (6 experiments) were similar to those of whole strips, while the outer layer preparations exhibited only poor activity or even remained quiescent (6 experiments).

Repeated rinsing of the preparations with fresh Tyrode's solution neither abolished nor modified the pattern of spontaneous contractions in any of the experiments.

Drug-induced contractions. All three agents induced an increased contractility of whole strips of tunica albuginea. The effect consisted of a rise in tone, sometimes accompanied by modifications of the amplitude and rate of the contractions (Text-fig. 2). The effect of the drugs was therefore evaluated by measuring tone modifications (mg tension developed) (Table 1).

The threshold dose for norepinephrine was 0.01 $\mu\text{g/ml}$ and doses of 0.1 and 1.0 $\mu\text{g/ml}$ were used to obtain a dose-response curve (Table 1). The correlation coefficient was significant ($r = 0.66$, $P < 0.005$). In some of the experiments in which the spontaneous contractility remained irregular, the administration of norepinephrine elicited a rhythmic activity. Only 1 preparation failed to respond to norepinephrine stimulation.

The correlation coefficient for the dose-response curve for acetylcholine (Table 1) was significant ($r = 0.42$, $P < 0.05$).

Whole strips of tunica albuginea were quite sensitive to oxytocin since the threshold dose was 0.001 $\mu\text{g/ml}$. The dose-response curve differed from that for norepinephrine and acetylcholine in that the highest dose (0.1 $\mu\text{g/ml}$) elicited a lower contractile response than did 0.01 $\mu\text{g/ml}$ (Table 1). The correlation coefficient was not significant ($r = 0.34$, $P > 0.05$).

The inner layer preparations of the tunica albuginea behaved like the whole strips (6 experiments), whereas the outer layer preparations (6 experiments) did not respond at all to the drugs.

Text-fig. 2. Some examples of the stimulatory effect of (a) norepinephrine, (b) acetylcholine and (c) oxytocin on the contractility of the testicular capsule of the boar (mid-posterior border of the testis). The main effect of the drugs consisted of a rise in tone.

Table 1. The effects (mean $\pm$ s.e.m., no. of expts in parentheses) of drugs on the contractility of isolated whole strips of the testicular capsule of the boar

Norepinephrine (14)		Acetylcholine (12)		Oxytocin (12)	
Dose ($\mu\text{g/ml}$)	Tension (mg)	Dose ($\mu\text{g/ml}$)	Tension (mg)	Dose ($\mu\text{g/ml}$)	Tension (mg)
0.01	19.87 $\pm$ 10.46	0.1	28.96 $\pm$ 16.34	0.001	21.12 $\pm$ 5.73
0.1	135.83 $\pm$ 32.31	1.0	128.22 $\pm$ 35.02	0.01	65.75 $\pm$ 15.06
1.0	311.81 $\pm$ 63.13	10.0	112.37 $\pm$ 17.99	0.1	55.26 $\pm$ 17.43

Histological studies

Light microscopy. The tunica albuginea of the boar consisted of (1) a thick layer of dense collagenous tissue immediately beneath the squamous mesothelium of the tunica vaginalis, (2) a well-developed middle layer of a more loose connective tissue containing the large and medium-sized blood vessels, and (3) a thin inner layer composed mainly of sparse collagen fibrils, fibroblasts, numerous Leydig cells and abundant capillaries, arterioles, venules, and lymph vessels (Pl. 1, Fig. 1).

PLATE 1

Fig. 1. General view of the inner layer of the tunica albuginea of the boar testis showing numerous smooth muscle nuclei (smn) Leydig cell nuclei (L) and blood vessels (Bv). $\times$ 280.
Fig. 2. Low power electron micrograph of the inner layer of the boar tunica albuginea. Several layers of smooth muscle cells are clearly seen interspersed among collagen fibres (co). These cells showed an irregular contour and typical cell processes (cp). At this magnification, cytoplasmic dense bodies (db) and micropinocytotic vesicles (arrows) are visible. $\times$ 24 800.

PLATE 1

(Facing p. 82)

Although the smooth muscle cells were observed in almost all areas of the tunica albuginea examined, they were predominantly located in the posterior border of the testis, and the following description refers exclusively to this region. In all cases, the smooth muscle cells were distributed singly or formed layers, 3–5 cells wide, predominantly disposed parallel to the long axis of the testis. They were very scarce in the outer collagenous layer of the tunica albuginea. Their number increased markedly towards the middle vascular layer and reached a maximum in the inner layer. The great majority of the smooth muscle cells of each section were found among the other cells and vessels of this layer. Incidentally, in sections stained with the Cajal–Gallego's method, numerous mast cells were found throughout the whole tunica albuginea and predominantly near blood vessels.

Electron microscopy. Numerous typical smooth muscle cells were found disseminated between the collagen fibres of the tunica albuginea. They had an extremely irregular shape and exhibited several slender, twisted cytoplasmic processes extensively interdigitated with those of neighbouring cells (Pl. 1, Fig. 2). There were numerous gap junctions usually in the form of short, blunt processes from one cell abutting against the plasma membrane of an adjacent cell (Pl. 2, Fig. 3). The smooth muscle cells were surrounded by a thin basal lamina interrupted at the level of the plasma membrane appositions. They contained abundant, irregularly distributed thin myofilaments which occupied the major portion of the cytoplasm. There were also abundant dense bodies closely apposed to the plasma membrane and located between the cytoplasmic filaments. Micropinocytotic vesicles were numerous in all of the cell surfaces except at the sites of plasma membrane-associated dense areas. The cytoplasm contained a few mitochondria, free ribosomes and rough endoplasmic reticulum, and numerous granules which seemed to be glycogen. The nucleus was quite large, rich in heterochromatin and had characteristic indentations (Pl. 2, Fig. 4). The tunica albuginea also contained numerous typical fibroblasts, collagen fibres, elastic material and mast cells.

Discussion

In the present study we have described the presence of smooth muscle cells and the spontaneous and drug-induced contractility of the boar testicular capsule.

The smooth muscle cells of the tunica albuginea showed all the fine structural features encountered in other organs. They appeared scattered singly or forming well defined layers, in agreement with the findings of Langford & Heller (1973) and Leeson & Cookson (1974) in human, rat, dog and cat testes. These cells were different from the so-called myofibroblasts recognized in a wide variety of tissues and organs, and also in the tunica albuginea of the rat testis (Gorgas & Böck, 1974).

Like that of the rabbit (Davis & Langford, 1970; Hargrove, Johnson & Ellis, 1971; Johnson, Hargrove & Ellis, 1971; Seeley, Hargrove, Sanders & Ellis, 1973), the testicular capsule of the boar showed rhythmic, spontaneous contractility. However, we could not reproduce the findings of Hargrove, Seeley, Johnson & Ellis (1973) who demonstrated that changing the Tyrode's solution in the organ bath resulted in a marked decrease or even complete abolition of the

PLATE 2

Fig. 3. This picture shows the extensive contact between two adjacent smooth muscle cells. Note the presence of numerous micropinocytotic vesicles (white arrow heads); dense bodies attached to the plasma membrane (white arrows) and in the cytoplasm (db); basal lamina (bl); some collagen fibrils transversely cut (co) and a typical gap junction (g). $\times 44\ 000$. *Inset:* higher magnification of the same gap junction. $\times 66\ 000$.

Fig. 4. Partial view of a smooth muscle cell which clearly shows: a nucleus with typical indentations (n); cytoplasmic bundles of myofilaments (mf); cytoplasmic and membrane-attached dense bodies (db); micropinocytotic vesicles (mv) and cytoplasmic granules (g). $\times 32\ 500$.

spontaneous contractions of the rabbit tunica albuginea. These authors suggested that the spontaneous contractility observed in the rabbit was dependent upon the release of endogenous substances, most probably prostaglandins, into the bath. The same effect was described by Buhrley & Ellis (1975) in a parallel study on the contractility of isolated rat seminiferous tubules following the addition of prostaglandin F-1 α . In another study, however, Seeley *et al.* (1974) showed that the contractions of the rabbit testis *in vivo* differed from those of the isolated capsules in not being abolished by successive changes of the bath medium. The significance of these differences cannot be explained and requires further study. The contractility of the human testicular capsule also differs greatly from that observed in the boar since it is periodic and extremely powerful (Firlit *et al.*, 1975).

The differences in capsular contractility between species might be due to differences in structure and function of the tunica albuginea (Ellis, Buhrley & Hargrove, 1978). The posterior area of the boar tunica albuginea exhibited the highest contractility, which correlated well with the finding of the maximal amounts of smooth muscle cells in this region. In all mammalian species examined so far, the bulk of the smooth muscle cells within the tunica albuginea has been found at the posterior border of the testis, irrespective of specific differences in the general architecture of the organ (Holstein & Weiss, 1967; Davis & Langford, 1970; Leeson & Cookson, 1974). In addition, our results indicate that the inner layer of the tunica albuginea essentially retained all the ability to contract of the entire capsule strips. This finding was not totally unexpected since we found the great majority of the smooth muscle cells within this inner layer, close to the testicular parenchyma. The almost complete inactivity of the outer layer of the testicular capsule further strengthens this finding and suggests that the contractility of the vascular smooth muscle does not contribute in a significant degree to the contractions of the boar tunica albuginea.

There is at present little information about the regulation of the contractile activity of the tunica albuginea. As in other species (Davis & Langford, 1970, 1971; Firlit *et al.*, 1975), norepinephrine and acetylcholine markedly increased the tone of the boar testicular capsule, indicating that they may regulate the intratesticular pressure. In addition, the presence of cholinergic and adrenergic nerves within the tunica albuginea has been demonstrated in human, dog, cat and rat testes (Bell & Maclean, 1973; Leeson & Cookson, 1974). All these findings suggest that the autonomous nervous system may regulate the contractility of the tunica albuginea. Our results indicate that oxytocin may also play a role in the regulation of the testicular pressure; this is in agreement with the work of Voglmayr (1975) showing that oxytocin influences the sperm and fluid output by the testis of the ram *in vivo*. However, the effect of oxytocin cannot be extrapolated to other species at present since the testicular capsule of the rabbit did not show any response to oxytocin *in vitro* (Hargrove, Seeley, Johnson & Ellis, 1973).

There is some work suggesting that other factors, e.g. prostaglandins and steroid hormones, may also be involved in modulating the contractility of the tunica albuginea (Seeley, Hargrove, Johnson & Ellis, 1972; Seeley *et al.*, 1974; Hargrove & Ellis, 1976; Hargrove *et al.*, 1977). The effects of these agents have been mainly studied in laboratory animals and we hope to perform similar studies with domestic animals.

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