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Fine Structure of Muscle Cells of the Human Testicular Capsule: Basis of Testicular Contractions

Abstract. *Electron micrographs of human testicular capsule reveal large numbers of branching smooth muscle cells coursing through collagenous tissue of the tunica albuginea. These cells have subcellular morphology characteristic of smooth muscle cells, and they associate with one another through areas of close contact. These are the contractile cells responsible for spontaneous contractions of the human testicular capsule—contractions that may be important in transporting nonmotile sperm out of the testis.*

Confining the seminiferous tubules and interstitial tissue of the human testis is a capsule commonly referred to as the tunica albuginea. It has been described as a dense connective tissue consisting mainly of fibroblasts and collagen; like the capsules of other organs, the testicular capsule is considered to act as a support for the underlying parenchymal tissue.

Recently Davis and Langford (*1*) reported that the human testicular capsule is capable of spontaneous contractions and that it can be stimulated to contract by several autonomic drugs. These observations suggest that the capsule may indeed be more than an outer support for the testis. In animals, they were able to demonstrate not only contractions of the capsule but also similar contractions of the intact whole testis both in vitro and in the living animal (*1*, *2*). The possibility that smooth muscle cells may be responsible for these contractions became evident when cells resembling smooth muscle cells were found with the light microscope in the capsules of animal and human testes (*1–3*). Electron microscopy studies were therefore undertaken to define the cells responsible for the contractions of the capsule. We report here the fine-structural identification and morphology of the contractile cells located in the capsule of the human testis.

Biopsy specimens of the testicular capsule from young, healthy, adult volunteers were obtained. Capsule tissue was immediately transferred to glutaraldehyde, postfixed in osmium tetroxide, dehydrated in ethanol, and embedded in Epon (*4*). To improve contrast of membranes, some specimens were stained en bloc prior to embedding in Epon (*5*). Thin sections were doubly stained with uranyl acetate and lead citrate (*6*).

The three tissue layers of the human testicular capsule can readily be identified by light microscopy (*1*, *2*). The exterior tunica vaginalis visceral consists of a thin layer of serous cells. Directly beneath is the tunica albuginea, a dense collagenous tissue forming the major portion of the capsule. The interior tunica vasculosa is less dense than the

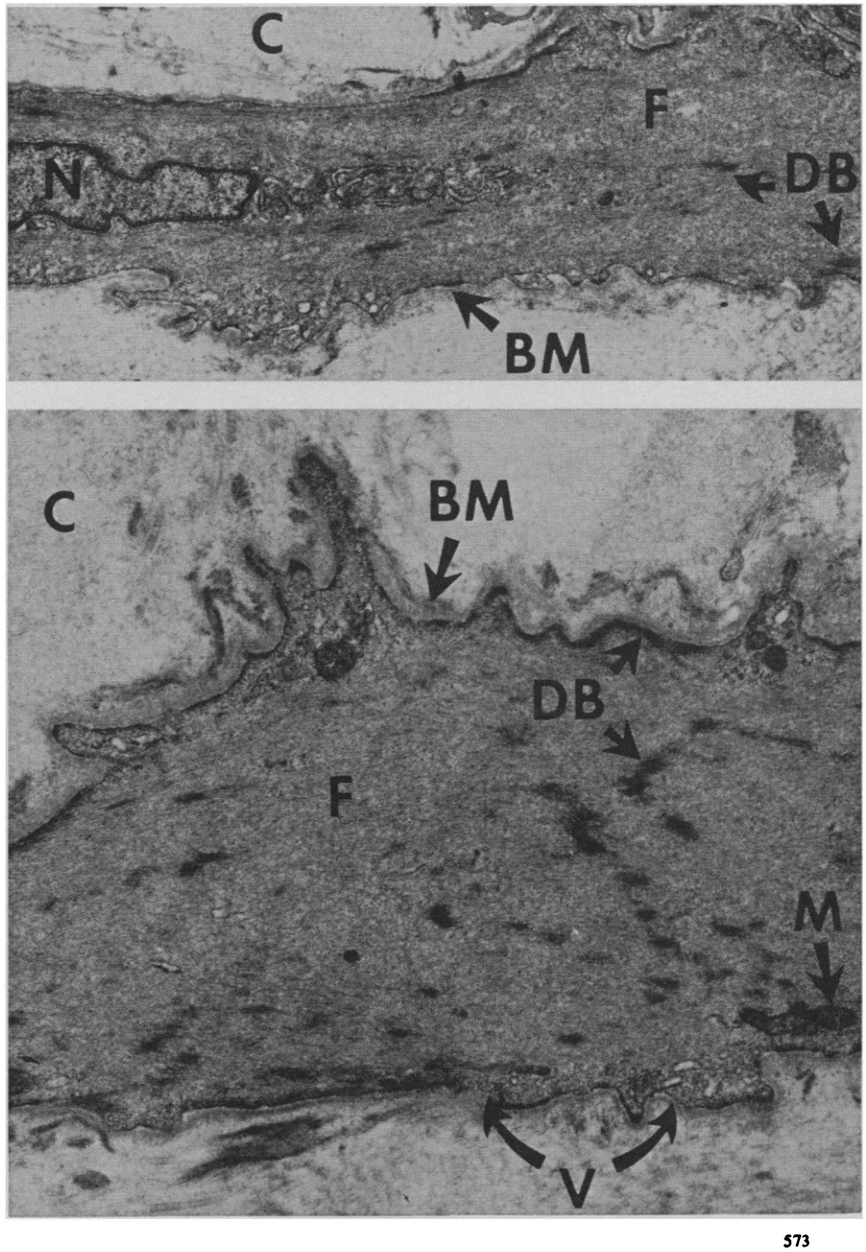

Fig. 1. Electron micrographs of smooth muscle cells (longitudinal section) located in the tunica albuginea of the human testicular capsule. (Top) A smooth muscle cell is surrounded by collagen (*C*). Myofilaments (*F*), dense bodies (*DB*), and a basement membrane (*BM*) can be seen. Most of the organelles extend from the poles of the nucleus (*N*) (× 9,900). (Bottom) Cytoplasm of the smooth muscle cells contains mainly myofilaments (*F*) and dense bodies (*DB*). Numerous micropinocytotic vesicles (*V*) are located at the cell periphery along with occasional mitochondria (*M*) (× 10,300).

tunica albuginea and contains many blood vessels. Our report is concerned with the tunica albuginea.

When observed with the electron microscope, the majority of cells of the tunica albuginea are branching smooth muscle cells with long, irregular profiles. The most predominant feature and that which occupies the major portion of these cells is tightly packed myofilaments oriented approximately parallel to the long axis of the cell (Fig. 1). Dense bodies, characteristic of other smooth muscle cells (*7*, *8*), are distributed among the myofilaments and also directly beneath the plasma membrane. The nucleus is elongate, with many invaginations of the membrane. Most of the cell organelles—such as mitochondria, Golgi apparatus, endoplasmic reticulum (which is often found dilated), and ribosomes—occur near the poles of the nucleus (Fig. 1). A smaller number of organelles, including the centrioles, are located in a narrow perinuclear zone that is surrounded by variable amounts of myofilaments. The plasma membrane forms numerous small cytoplasmic projections that are usually filled with micropinocytotic vesicles, identical with the plasmalemma vesicles found in other smooth muscle cells. These vesicle-filled areas of the plasma membrane usually alternate with the dense areas. Larger cytoplasmic projections containing endoplasmic reticulum, ribosomes, mitochondria, and micropinocytotic vesicles also occur along the cell periphery. Occasional mitochondria can be found among the myofilaments along the length of the cell, usually in close proximity to the plasma membrane (Fig. 1). Surrounding these smooth muscle cells is a basement membrane of variable thickness which separates each muscle cell from the enveloping collagenous connective tissue.

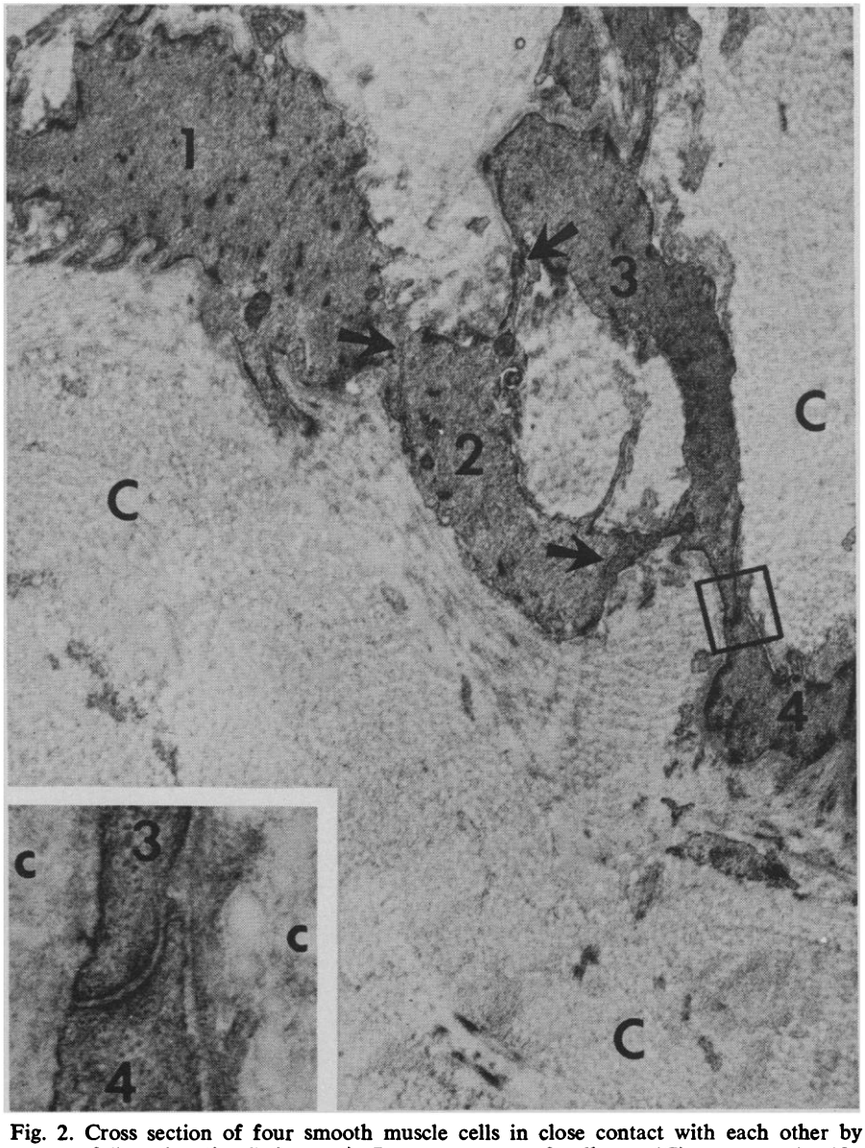

Fig. 2. Cross section of four smooth muscle cells in close contact with each other by means of "gap junctions" (arrows). Large expanses of collagen (*C*) are seen (× 10,050). The inset shows detail of close contact junction between muscle cells *3* and *4* (× 43,800).

Figure 2 shows the smooth muscle cells coursing through the large areas of collagen which usually separate the muscle cells from one another along most of the cell length. However, neighboring smooth muscle cells do make contact with each other, most often by means of cytoplasmic extensions. The size of the close contact areas of the plasma membranes of apposing cells is variable, tending to be quite short in cross section (inset in Fig. 2) and more extensive along the long axis of the cell. The contours of apposing cell membranes closely conform with one another and are separated by a space as small as 50 Å, considered a "gap junction," in which the basement membrane material is excluded. Such areas of close contact between smooth muscle cells of the capsule may represent specialized contact zones or "bridges" of relatively low resistance electrical pathways that permit myogenic conduction as reported for other smooth muscle tissues (*8*).

Although the functional significance of these smooth muscle contractions of the human testicular capsule is not certain, we speculate that capsule contractions may assist lymphatic and venous drainage from the testis and, perhaps most important, may be responsible for the transport of nonmotile sperm out of the testis into the epididymis where the sperm attain their motility.

When sperm are released from the germinal epithelium into the lumen of seminiferous tubules, they are essentially nonmotile cells suspended in fluid within the tubule lumen (*9*). The mechanism for transporting the nonmotile sperm from the seminiferous tubules into the epididymis is still not clear. Microscopic motions of isolated seminiferous tubule segments (*10*) have been attributed to peritubular myoid cells situated along the basement membrane of the seminiferous tubules (*11*); these motions were suggested as a possible influence in transporting nonmotile sperm out of the testis. However, what may be of greater significance to sperm transport, in view of the rhythmic interstitial pressure changes within the testis which are independent of blood pressure and respiration (*3*), are the powerful spon-

taneous contractions of smooth muscle cells in the testicular capsule—contractions that in man represent approximately a 40 percent shortening of the capsule (*1*).

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Dopaminergic and Noradrenergic Substrates of Positive Reinforcement: Differential Effects of d- and l-Amphetamine

Abstract. *Intracranial self-stimulation was elicited from electrodes located in either the lateral hypothalamus or substantia nigra of the rat. Facilitatory effects of* d- *and* l-*isomers of amphetamine on self-stimulation were assessed. The* d-*isomer was seven to ten times more effective than the* l-*isomer at the hypothalamic placement, whereas the two isomers were equipotent for substantia nigra electrodes. These data support the hypothesis that both dopaminergic and noradrenergic systems subserve positive reinforcement.*

Catecholaminergic involvement in the phenomenon of intracranial self-stimulation (ICS) is supported by neuroanatomical (*1–3*), neurochemical (*4, 5*), histochemical (*6*), and pharmacological (*7–9*) data. With respect to the pharmacological evidence, amphetamine increases the release and prevents the uptake of catecholamines in adrenergic neurons (*10–12*), while having pronounced facilitatory effects on ICS (*7–9*).

Although ICS may be obtained from a variety of subcortical sites (*13, 14*), the highest rates are obtained in the medial forebrain bundle (MFB) region of the lateral hypothalamus (LH), an area that coincides with a major rostral projection of noradrenergic fibers (*15*). Utilizing animal preparations with electrodes in the MFB, Stein and his co-workers have amassed evidence in support of their proposal that ICS is subserved by noradrenergic neurons (*5, 9*). However, the exclusive role of noradrenergic neurons has recently been challenged by neuroanatomical and histochemical evidence of a possible dopaminergic involvement in ICS. Stimulation in the region of the substantia nigra (SN) will maintain ICS (*2, 14*) and yet the fibers of passage and cell bodies in this region are almost exclusively dopaminergic rather than noradrenergic (*15*).

It has recently been demonstrated that *d*- and *l*-amphetamine have differential effects on the uptake of dopamine and noradrenaline in the brain, the levo isomer being approximately ten times less potent in blocking catecholamine uptake into noradrenergic neurons, but being equally efficient in blocking uptake into striatal dopaminergic neurons (*16, 17*). Because *d*-amphetamine was shown to be ten times more potent in enhancing locomotor activity but only twice as potent in producing compulsive gnawing behavior, it was suggested that the former behavior was mediated by brain noradrenaline while the latter had dopaminergic substrates.

In view of these findings, our experiments were undertaken to determine if the noradrenergic and the dopaminergic substrates of ICS are pharmacologically identifiable. Specifically it was hypothesized that electrodes placed in a noradrenergic pathway would be approximately ten times more responsive to the rate increasing effects of *d*-amphetamine than of *l*-amphetamine, whereas dopaminergic placements would be approximately equally responsive to both isomers.

Two groups of ten male Wistar rats had bipolar electrodes implanted in either the MFB as it courses through the LH, or in the SN, in accordance with standard stereotaxic procedures (*18*). Those animals that did not learn to deliver electrical stimulation (0.2-second train of 5 to 100 μa, 60 hz sine wave a-c) to their brains by pressing a lever in a Skinner box, were rejected after seven daily 30-minute training sessions. Subjects displaying high rates of lever pressing during this period were given additional testing to establish the lowest current intensities that would maintain low but consistent response rates, that is, response threshold (*19*). Five days of responding at the response threshold ensured stable response rates at the newly established current intensities.

Six rats with LH electrodes displayed stable response rates at low current intensities and were subsequently injected with different doses of *d*- and *l*-amphetamine to assess the effects of these two isomers on ICS. To facilitate direct comparison of the "drug" test with the "no-drug" control test, a daily schedule of two 15-minute test sessions separated by a 20- to 30-minute intertest period was incorporated. Two "no-drug" days preceded each "drug" day, and the previously established current intensities were used throughout this phase of the experiment. On a "drug" day, intraperitoneal injections of either *d*-amphetamine (0.10, 0.25, 0.50, or 1.0 mg/kg) or *l*-amphetamine (0.50, 1.0, or 2.5 mg/kg) were randomly administered to all animals immediately after the first 15-minute test session. The animals were then placed in their home cages, and 20 minutes later they were returned to the test chamber so that we could assess the effects of the drug on the response rate for reinforcing brain stimulation.

Five of the ten animals with electrodes aimed at the dopaminergic fibers of the SN displayed stable ICS behavior when tested exactly as described above. The testing procedure used to assess the effects of *d*- and *l*-amphetamine on self-stimulation at these placements was