The gubernaculum in testicular descent and cryptorchidism

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The gubernaculum plays an essential role in the complex mechanism of testicular descent and inguinal hernia closure. Understanding this complex developmental process is gradually allowing us insight into how to regulate normal descent and also treat maldescended testes.

Key words: gubernaculum, Mullerian Inhibiting Substance, genitofemoral nerve, CGRP.

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Despite much controversy, the gubernaculum or genitoinguinal ligament is emerging as the key anatomical structure in control of testicular descent. During the last twenty years, much research has focussed around a two-step model. The first or transabdominal step was proposed to be caused by enlargement of the gubernacular bulb, thereby anchoring the testis during embryonic enlargement. During the second step, the gubernaculum was proposed to migrate from the external inguinal ring to the scrotum. Simultaneously the gubernaculum becomes hollowed out by a diverticulum of the peritoneum, tunica vaginalis, to allow the intra-abdominal fetal testis to reach the scrotum. Following complete descent, the peritoneum proximal to the testis obliterates preventing development of an inguinal hernia. The evidence about hormonal control of descent suggested that the first step was controlled by a non-androgenic factor, while the second step did appear to be under control of testosterone. However, how the hormones actually caused changes in the gubernaculum was was not known at that time[1,2].

Since the development of the two-step model, a number of challenges have arisen to its validity. The first controversial issue was whether or not Mullerian Inhibiting Substance/ Anti-Mullerian Hormone was the primary

Table I. Anatomical Phases of Testicular Descent and Their Hormonal Control

	Transabdominal Phase	Inguinoscrotal Phase
Anatomy	1. Regression of cranial suspensory ligament 2. Enlargement of genitoinguinal ligament (gubernaculum) ♦ Anchors testis near groin as embryo grows	1. Gubernaculum migrates from external inguinal ring to scrotum 2. Processus vaginalis grows inside gubernaculum 3. Testis descends inside processus vaginalis 4. Processus vaginalis obliterates after descent
Hormonal Control	1. Testosterone triggers suspensory ligament involution 2. Insl3 (+MIS) stimulates swelling	1. Testosterone controls migration indirectly via GFN and release of CGRP (sensory nerve endings) ♦ Stimulates growth of gubernacular tip by trophism and chemotaxis

hormone controlling the first step with enlargement of the gubernacular bulb. There was a lot of indirect evidence that Mullerian Inhibiting Substance (MIS) may be involved, including intra-abdominal testes in patients with persistent mullerian duct syndrome as well as undescended testes and preservation of the mullerian duct in mice treated by estrogens during development. However, there was no direct evidence that MIS was able to cause gubernacular enlargement. In recent years this controversy has been mostly resolved by the identification of insulin-like hormone 3 (Insl3) and its role in gubernacular enlargement. A detailed study of the different hormones in gubernacular enlargement done in our own laboratory suggest that Insl3 is the primary factor, but that MIS has a adjunctive role, at least in the mouse gubernaculum[3]. We have tested both synthetic rat and human Insl3 in the culture system and shown a primary effect of Insl3 and an auxiliary effect of MIS[4].

Fifteen years ago evidence began to emerge that the effect of testosterone on stimulation of gubernacular migration in the second step may be occurring indirectly via the genitofemoral nerve[5]. The genitofemoral nerve was found to contain the neuropeptide, calcitonin gene-related peptide (CGRP), which in organ culture of rat gubernaculum was shown to cause rhythmic contractility of the cremaster muscle[6-8]. There were many challenges to this indirect model, since it seemed intuitive that testosterone was much more likely to directly affect the gubernaculum[9]. However, since the gubernaculum needs to migrate from the inguinal region to the scrotum, not only does it need to be stimulated to grow, but it needs to grow in a particular direction. The genitofemoral nerve provided an anatomical and functional explanation, which might allow the gubernaculum to migrate in a specific direction. Our laboratory found a very tight correlation between contraction of the rat gubernaculum in organ culture and animal models with interference with testosterone (flutamide-treated rat)[10-12]. In addition there was interference in function of the contraction when the genitofemoral nerve was either cut surgically or the nerve was treated with capsaicin, which is a sensory nerve toxin[13]. Initially there was the belief that CGRP was released from the motor branches of the

genitofemoral nerve, but recent evidence has confirmed that CGRP is actually in the sensory branches of the nerve[14,15].

Figure 1. The maximum mitotic index is in the tip (arrow) of the gubernacular bulb, as measured by BUdR uptake.

Another challenge to the genitofemoral nerve hypothesis as an explanation for the second stage of gubernacular development was the suggestion that the human gubernaculum may respond totally differently because the amount of cremaster muscle in the human gubernaculum is significantly less than in the rat[9]. It was therefore suggested that rhythmic contractility as seen in the rat gubernaculum might be irrelevant for human testicular descent. Our laboratory has addressed this challenge recently by studying the tip of the gubernacular bulb of the rat gubernaculum *in vivo* and in organ culture[16-18]. We have found that the gubernaculum grows by cell division right at the very tip of the gubernaculum, as measured by BUdR uptake (Figure 1). This suggested that the gubernaculum grew from the very tip, like a plant root. We proposed that the gubernaculum grew towards the scrotum because of the release of CGRP from the genitofemoral nerve along the migration pathway. Recently we have shown that BUdR uptake in the tip of the gubernaculum is decreased in the flutamide- and capsaicin - treated animals, and increased in the transcrotal rat, which is known to have excess amounts of CGRP in the genitofemoral nerve, despite maldescent[18]. Taken together, these studies suggest that CGRP release from the nerve might

trigger migration of the gubernaculum to the scrotum by elongation of the tip with active growth. This could occur similarly in the human as in the rat.

Our current work on this area includes a study of neonatal rat gubernacula *in vitro*, cultured with or without CGRP. Preliminary results suggest that BUdR labelling is significantly increased in the presence of exogenous CGRP. This suggests that CGRP directly stimulates the growth of the gubernaculum [Huynh et al., unpublished].

In a related study, we have tested whether the gubernaculum can respond to a chemotactic gradient of CGRP. Neonatal rat gubernacula were placed in culture adjacent to agarose beads that had been soaked in CGRP. Over 24-72 hours the gubernacular tip turned towards the beads if they contained CGRP, consistent with the notion that CGRP is providing a chemotactic gradient [Yong et al., unpublished].

A recent challenge to the genitofemoral nerve hypothesis has been the suggestion that gubernacular development may actually be controlled by sympathetic nerves rather than the sensory branches of the genitofemoral nerve[19-21]. We have tested this hypothesis directly in an organ culture of the rat gubernaculum, and shown that CGRP causes rhythmic contractility of the rat gubernaculum, which is neither augmented nor inhibited by sympathetic agonists or antagonists such as isoprenaline and guanethidine. These studies suggest that sympathetic innervation is unlikely to be a key factor in gubernacular migration. Because any proposed sympathetic nerve action may be mediated by calcium flux across the cell membrane, we are currently testing beta agonists and antagonists in the presence or absence of extra calcium ions in the medium [Sasaki et al., unpublished].

Our research into the role of CGRP release from the genitofemoral nerve during descent has been extended to study of obliteration of the processus vaginalis in the human gubernaculum[22,23]. We now have evidence that inguinal hernia closure can be regulated by exposure to CGRP, suggesting that the genitofemoral nerve causes obliteration of inguinal hernia in the human following descent of the testis. The mechanism whereby CGRP causes inguinal hernia closure is not fully understood, and is currently under study in our laboratory. We have looked for CGRP receptors in the processus vaginalis, and found them in the mesenchyme, and recently we have identified the receptor for hepatocyte growth factor (HGF) in the processus vaginalis peritoneum (Figure 2). However, the c-met receptor for HGF is also present in parietal peritoneum, so the regulatory control of obliteration must be elsewhere [Ting et al., unpublished].

Figure 2. Expression of c-met on the epithelial lining of the processus vaginalis.

A final area of research related to the role of the gubernaculum in testicular descent and cryptorchidism is the study of acquired undescended testis[24]. Our research has shown that a significant number of children present late with cryptorchidism, which was initially thought to be secondary to delayed diagnosis[25]. It is now being accepted in many centers that this is actually a delayed presentation of an acquired variant of undescended testis rather than delayed diagnosis of congenital undescended testis. Our studies on these patients suggest that the cause of this acquired maldescent is failure of the processus vaginalis to completely obliterate. Following inguinal hernia closure, the peritoneal membrane and adjacent fibrous tissue must completely disappear, so that the vas deferens and testicular vessels can elongate during growth of the child. Since the distance from the inguinal ring to the scrotum is approximately 5 cm in a baby and 10 cm in a 10-year-old, the spermatic cord must double in length during the first 10 years after

birth. If the inguinal hernia tissue does not disappear completely, this leaves a fibrous string in the spermatic cord, which prevents elongation. Histological study of this fibrous string at orchiopexy confirms that this is a remnant of the peritoneal tissue, since there are islands of peritoneal cells in the fibrous string[26]. It is our proposal that this fibrous remnant of the peritoneum is sensitive to testosterone treatment, which is why acquired undescended testes appear to be amenable to postnatal hormone therapy. In addition, it has been known for some time that many of these testes descend spontaneously at puberty, consistent with an active response to the hormonal environment.

In conclusion, the gubernaculum plays an essential role in the complex mechanism of testicular descent and inguinal hernia closure. Understanding this complex developmental process is gradually allowing us insight into how to regulate normal descent and also treat maldescended testes.

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