The testis is descended through the processus vaginalis via the propulsive force generated by the muscles derived from the gubernaculum. After propelling the testis, the smooth muscle should undergo programmed cell death for obliteration of the processus vaginalis. Achievement of programmed cell death mandates a transient decrease in sympathetic, but an increase in parasympathetic, tonuses. Since the sympathetic tonus is androgen-dependent, the decrease in androgen levels during the third trimester appears to be responsible for the process. Alterations in timing, intensity or duration of the decrease in sympathetic tonus under the control of the central nervous system give rise to hernia, hydrocele or abnormal testis localizations. The persistence of decrease causes undescended, retracted, or ascended testis. Absence or inadequacy of the decrease in sympathetic tonus results in rescue of more smooth muscle, thus inhibiting the obliteration. Inadequacy in the intensity or duration rescues less smooth muscle and gives rise to a hydrocele. Persistence of signals towards inducing programmed cell death contributes to decrease in fertility, and provides a basis for epididymo-vasal anomalies. The reduction in the central regulatory mechanism that involves catecholaminergic activity explains the blunting of luteinizing hormone (LH) response to gonadotropin-releasing hormone (GnRH).

The explained mechanism precisely defines the role of all factors, and links all of the associations related to the process of descent.

Key words: gubernaculum, descent, testis, autonomic nervous system, cryptorchidism, G-proteins, apoptosis.
Although the remaining smooth muscles in sacs from any diagnostic origin do not reveal evidence of ongoing programmed cell death, sacs with various smooth muscle and myofibroblast contents may reflect arrests at different steps in the pathway of programmed cell death. Therefore a comparative evaluation is required to enlighten the mechanism of disappearance of smooth muscle, and reasons for persistence.

Programmed cell death

The central component of the process of programmed cell death is a proteolytic system that involves a family of proteases called caspases. Caspases are activated through two main pathways. One of the pathways, termed the extrinsic pathway, involves ligand binding at the cell surface receptors of the tumor necrosis factor family such as Fas and Fas-ligand system. While activation of this system directly executes programmed cell death in some cells, involvement of mitochondria is essential in other cells. Involvement of the mitochondria, termed the intrinsic pathway, depends on the depletion of Ca\(^{2+}\) stores via G-protein linked signal transduction. Activation of phospholipase C, and thus generation of diacylglycerol and inositol 1,4,5-trisphosphate (IP\(_3\)), is one of the initial steps. IP\(_3\) promotes the release of Ca\(^{2+}\) from internal stores via IP\(_3\) receptors. Although depletion of Ca\(^{2+}\) from the endoplasmic reticulum may also have a role, sustained elevations of cytosolic Ca\(^{2+}\) can switch on programmed cell death. Early increase of cytoplasmic Ca\(^{2+}\) is followed by delayed increase of mitochondrial Ca\(^{2+}\). Increases in mitochondrial Ca\(^{2+}\) facilitates Ca\(^{2+}\)-induced transient opening of mitochondrial permeability transition pore, and release of cytochrome c. A set of proteins takes part in the regulation of programmed cell death. Among those proteins, Bcl-2 inhibits apoptosis, and Bax is proapoptotic when overexpressed. Bax is a protein that is located in the cytosol, but inserts into mitochondrial membranes after a death signal, and induces opening of the permeability transition pore, release of cytochrome c, and activation of downstream caspase pathways. Precipitation of apoptosis is associated with sarco-endoplasmic reticulum Ca\(^{2+}\) pump overexpression, which augments cellular and sarco-endoplasmic calcium loading.

Both sympathetic tonus and androgens influence smooth muscle survival

Sympathetic nerves are among factors that exert trophic influences upon smooth muscles through increasing intracellular cAMP via beta-adrenergic receptors. The dedifferentiation and programmed cell death are associated events. Smooth muscle cells dedifferentiate earlier in the absence of sympathetic nerve fibers. Sympathetic tonus appears to be important for smooth muscle cells to survive and maintain the differentiated state. On the other hand, androgens are known to be important for smooth muscle in the prostate. Castration diminishes the volume of smooth muscle.

Since sympathetic tonus is androgen-dependent, and since smooth muscle also responds to androgens, at least two pathways exist to exert androgen effects upon smooth muscles.

Undescended testis and inguinal hernia

Although the processus vaginalis is patent in boys with undescended testis, clinical inguinal hernia is encountered in 10-15%. The smooth muscle content has also been proposed to give rise to dartos muscle around the tunica vaginalis. When an undescended testis is located in a cranial position, the smooth muscle retained to establish the dartos is in direct connection with the peritoneal cavity. Therefore the samples associated with undescended testis have been obtained from boys with patent processus vaginalis, but without an associated inguinal hernia for evaluation.

The mechanism of obliteration of processus vaginalis

Despite the fact that total Ca\(^{2+}\) contents have suggested the persistence of depletion of stores and increase in cytosolic calcium levels via IP\(_3\) in cremaster muscles of boys with undescended testis, sacs in the same boys have revealed an overload of Ca\(^{2+}\). On the other hand, total Ca\(^{2+}\) contents have not revealed the depletion of stores in cremaster muscles of boys with hydrocele, but sacs in the same boys have also revealed the overload of Ca\(^{2+}\). Since overload succeeds the initial depletion of calcium stores
and increase in cytosolic calcium levels via IP3, the depletion of stores has preceded in sacs associated with hydrocele and undescended testis. The stimuli towards depleting the calcium stores has occurred transiently in boys with hydrocele, but has persisted in boys with undescended testis. On the other hand, no evidence of depletion of stores in cremaster muscles or overload of calcium in sacs associated with inguinal hernia has been encountered. Since phospholipase C is activated by acetylcholine, the parasympathetic system is involved in initial depletion of calcium stores and increase in cytosolic calcium levels.

Previous evidence gained from cremaster muscles associated with undescended testis has indicated a persistent decrease in sympathetic, but an increase in parasympathetic, tonuses. The androgen dependency of sympathetic tonus and the role of androgens in the process have suggested the increase in parasympathetic tonus to reflect a relative increase due to decrease in sympathetic tonus. It appears that a transient decrease in sympathetic, but an increase in parasympathetic tonus to reflect a relative increase due to decrease in sympathetic tonus. This physiologic step for initiation of programmed cell death has not taken place in boys with inguinal hernia, but has become persistent in boys with undescended testis. On the other hand, more smooth muscle is rescued in sacs associated with hydrocele despite the fact that this step has been succeeded.

While Bcl-2 and Fas-L co-expressions to α-smooth muscle actin do not reveal differences among sacs, sacs associated with undescended testis reveal more Bax and Fas expressions. Higher Bax and Fas levels in sacs associated with undescended testis explain the difference between the amount of smooth muscles in sacs associated with hydrocele and undescended testis.

The precise mechanism by which Bax is upregulated is not clearly understood. It appears that Bax permits the triggering of programmed cell death in response to specific sets of multiple weak signals rather than to sufficiently strong individual stimuli. Since long-standing denervation results in an increase of Bax immunoreactivity, and since the expression of Bax is correlated with defective innervation of a muscle, expression of Bax appears to be affected by the pattern of innervation. On the other hand, androgens are known to attenuate, while androgen withdrawal is known to induce Bax expression. Additionally, Ca2+ and Bax are known to move synergistically. The factors that increase the expression of Fas are not clearly understood, but the overexpression of Fas makes the cells more prone to programmed cell death.

The least smooth muscle content is associated with elevations in Bax and Fas levels in addition to depletion of stores. Persistent decrease in sympathetic tonus revealed higher Bax and Fas levels. Since the smooth muscle requires both the sympathetic tonus and androgens for survival, the alterations in autonomic innervation required to release Ca2+ from internal stores may also have a role in upregulation of Bax and Fas expressions.

According to these evidences, the programmed cell death of smooth muscle required for obliteration of the processus vaginalis appears to be guided by a decrease in sympathetic and a concomitant increase in parasympathetic tonuses. A given intensity in alteration and/or sustenance at a critical time period appears to be required for the obliteration of the processus vaginalis.

A transient decrease in sympathetic, but increase in parasympathetic tonus is indeed a physiologic requirement for the obliteration of the processus vaginalis.

Pathogeneses of hernia, hydrocele and abnormal testicular localizations

After propelling the testis, the smooth muscle undergoes programmed cell death through a transient increase in parasympathetic and a decrease in sympathetic tonuses. Aberrations in timing, intensity or sustenance of this physiologic requirement give rise to hernia, hydrocele or abnormal testicular localizations. The alterations that determine the clinical outcome represent a spectrum that varies according to variations in each of the variables. If the alteration in autonomic innervation does not take place and/or the required intensity is not achieved and/or it is not sustained enough, the pathway of programmed cell death does not initiate. Smooth muscle persists and gives rise to an inguinal hernia. Since the sympathetic
tonus is sexually dimorphic and it is less in females, easier suppression may contribute to the lower incidence of inguinal hernia among females. If the decrease in sympathetic tonus is enough to deplete the calcium stores and to increase the cytosolic calcium, but not profound enough or does not sustain enough to increase the Bax and Fas levels, it results in a hydrocele. If the sympathetic tonus decreases before descent, the diminution of smooth muscle required to propel the testis together with inhibition of propulsive activity hinder the descent. A more cranial location of a testis indicates more profound alteration in autonomic innervation, thus a more profoundly affected testis. If the initial descent is not hampered, but parasympathetic dominance subtly persists, the descended testis is retracted or even ascended by the contracted cremaster muscle due to increase in the levels of cytosolic calcium.

Pathway links epididymo-vasal anomalies to undescended testis

Ductus epididymis develops progressively from the fetal period to 2-4 months of age\(^34\). The postnatal period of epididymal development overlaps with the period of postnatal androgen surge\(^35\). A decrease is shown in the size of efferent and epididymal ducts in boys with undescended testis. The smaller size has been accepted to be due primarily to under-development of the muscular wall. This data has been suggested to reflect the undescended testis as representing a primary congenital illness of the testis and spermatic ducts that would probably not be completely reversed through surgical treatment\(^36\).

Orchidectomy induces apoptosis in the epididymis\(^37\). On the other hand, chemical sympathectomy causes smooth muscle abnormalities within the vas deferens\(^38\). It is clear that androgen-dependent sympathetic tonus is important for the development of vaso-epididymal structures. Persistence of signals towards inducing programmed cell death in boys with undescended testis may explain the high incidence of vaso-epididymal anomalies.

The August-Copenhagen rat provides a model of congenital epididymal anomaly. Testes descend in spite of the epididymal anomaly\(^39\). On the other hand, total disappearance of the epididymis in this animal model has been suggested to take place postnatally\(^40\). The possibility of subsequent disappearance of the epididymis further strengthens the possible role of persistence of signals towards inducing programmed cell death in boys with undescended testis and vaso-epididymal anomaly association.

Testis and persistence of parasympathetic tonus

An initial wave of apoptosis within the testes has been suggested to be necessary for normal spermatogenesis during adulthood\(^41\). The physiologic decrease in sympathetic tonus may also act to initiate the physiologic apoptosis within the testes.

On the other hand, apoptosis in the testis has been suggested to be under hormonal control, and undescended testes reveal a decrease in apoptosis resulting from a reduction in the number of germ cells capable of undergoing apoptosis\(^42\). Permanent decrease in sympathetic tonus among males with undescended testis may lead to apoptosis beyond physiologic requirements in the testes and contribute to decrease in fertility.

The mechanism to decrease the sympathetic tonus

The obliteration of the processus vaginalis through programmed cell death of smooth muscle requires a decrease in sympathetic tonus at a given timing, intensity and duration. For understanding the basis of pathologies resulting from aberrations, attempts should first be directed to outlining the physiologic mechanism.

Since the sympathetic system is sexually dimorphic and responds dramatically to androgens\(^43\), the decrease in sympathetic tonus suggests the requirement of a preceding decrease in androgen levels.

Where do the androgens act to decrease the sympathetic tonus?

Since boys with an undescended testis reveal a persistent decrease in sympathetic tonus\(^21-23\), an evaluation from this point of view may help to resolve the mechanism.

The type II fibers, which are influenced more from sympathetic tonus, are larger in boys with normal descended testes. However type II fibers
in boys with undescended testis are as small as those encountered in girls. Similar type II fiber diameters suggest that cremaster muscles in boys with undescended testis lacks the effect exerted by sympathetic tonus.

The electron microscopic evaluation revealed a decrease in the number of sympathetic fibers among boys with undescended testis. Autonomic ganglia that control the reproductive system are sexually dimorphic. In addition to the presence of more neurons, the proportion of nor-adrenergic neurons in the pelvic ganglia is higher in males. The small type II fiber diameters as encountered among girls together with a decrease in the number of sympathetic fibers suggest the persistent decrease in sympathetic tonus to associate with an aberration in the establishment of sexual dimorphism in the sympathetic neurons.

The neurons are initially overproduced. Approximately half of the neurons undergo programmed cell death. More neurons are spared in sexually dimorphic systems. However, the precise location of androgen action in establishing sexual dimorphism is controversial. The possibilities of androgen action include sexually dimorphic target structures, peripheral nerves and the central nervous system.

An electromyographic evaluation of cremasteric reflex in boys with undescended testis has revealed a shortened response latency and prolonged duration of activity. The same electromyographic findings that have also been recorded for the contralateral descended testis in boys with unilateral undescended testis indicated the undescended testis to indeed be a bilateral disease.

Shortened response latency and prolonged duration of activity suggest the loss of inhibitory control over the spinal cord, and point out the central nervous system as the localization of persistent alterations in boys with undescended testis.

The decrease in central catecholaminergic activity explains the decrease in peripheral sympathetic neurons

Central catecholaminergic neurons are involved in sympathetic nerve discharge. Autonomic postganglionic neurons can alter their levels of transmitters and co-transmitters as a function of the activity of their preganglionic inputs. Transection or pharmacological blockage of the preganglionic inputs decreases the levels of tyrosine hydroxylase and increases the level of substance P in postganglionic neurons. Therefore the decrease in central catecholaminergic activity satisfactorily explains the decrease in the level of tyrosine hydroxylase and increases in the levels of afferent neurotransmitters in postganglionic neurons encountered in boys with undescended testis.

The ratio of pre- to post-ganglionic sympathetic neurons differs for a given ganglion. A preganglionic neuron contacts many postganglionic neurons. Among factors that determine the number of contacted postganglionic neurons, neural unit size has been suggested. The pre-ganglionic inputs may also play a role in determining the number of postganglionic neurons. The evidence of regulation of the peripheral sympathetic system by high centers in the central nervous system supports this view.

The brain is sexually dimorphic, and gonadal steroids exert permanent (organizational) and transient (activational) effects on the brain. The development and differentiation of sexually dimorphic brain structures and functions proceed during critical pre- and postnatal periods. The organizational effects of testosterone on the central nervous and reproductive systems are exerted during the first half of gestation. On the other hand, the diminution in nerve fibers suggests an alteration in the organizational effect. If the diminution reflects an aberration in the organizational effect, establishment of sexual dimorphism in sympathetic post-ganglionic neurons should last for a period that extends to the time of descent.

Since the decrease in central catecholaminergic activity provides satisfactory explanations for the decrease in sympathetic tonus through the decrease in sympathetic fibers and for the increase in afferent neurotransmitters encountered in boys with undescended testis, central catecholaminergic activity appears to reside within the center of regulation of decrease in sympathetic tonus required to initiate the programmed cell death.
The decrease in androgen levels

Testicular testosterone production is stimulated by the high levels of placental human chorionic gonadotropin (hCG) between 10 and 20 weeks in a male fetus\(^50\). The hypothalamic-pituitary-gonadal axis gains the control in the third trimester\(^51\), and the levels of luteinizing hormone (LH) decreases with advancing gestational age\(^52\). Testosterone secretion decreases dependently during late gestation\(^53\).

It appears that the decrease in androgen levels during the third trimester plays a role in the obliteration of the processus vaginalis through decreasing the sympathetic tonus.

The mechanism that decreases the activity of the hypothalamic-pituitary-gonadal axis becomes important for both the physiologic effects in the obliteration of the processus vaginalis and in the pathogenesis of pathologies of the inguinal region.

Gonadotropin-releasing hormone (GnRH) neurons in the hypothalamic region of the brain provide the central neural drive that directs the production of androgens. GnRH drive to the reproductive axis is controlled primarily by regulation of GnRH release. The regulation of pulsatile release has not yet been fully elucidated. GnRH neurons receive synaptic inputs from other GnRH neurons and dopaminergic, noradrenergic, and serotonergic neurons, and neurons containing B-endorphin, corticotropin releasing hormone, GABA, vasopressin, and glutamate. Thus GnRH neurons appear to be regulated by direct input from a number of different neural systems. However, the mechanism that decreases the activity during the third trimester is obscure. Progressive maturation of inhibitory electrical activity in the neocortex has a role in decreasing the activity\(^50\).

Do the alterations in central catecholaminergic activity precede or succeed the decrease in androgen levels?

Tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis, responds to androgens. Perinatal gonadectomy decreases tyrosine hydroxylase immunoreactivity in the central nervous system\(^54\). The sexual dimorphism of sympathetic tonus appears to be exerted through pre-ganglionic inputs. From that point of view, the decrease in androgen levels appears to precede the decrease in central catecholaminergic activity for decreasing the sympathetic tonus.

On the other hand, catecholaminergic neurons innervate GnRH neurons\(^55\). They are suggested to provide stimulatory afferent input to the GnRH neurons\(^56\).

Furthermore, they are proposed to be involved in the regulation of synthesis of GnRH from the hypothalamus\(^57\). From that point of view, a decrease in catecholaminergic activity should precede the decrease in the activity of the hypothalamic-gonadal axis.

Birth tells the precedent

The sympathetic tonus is low in the fetus. Birth is associated with a noradrenaline surge in the human brain, and a high level of plasma catecholamines are encountered after birth\(^58\). This surge of sympathetic outflow during birth is controlled by central mechanisms\(^59\). Preterm infants reveal an augmented catecholamine response\(^50\).

The early postnatal period in males is associated with activation of the hypothalamic-pituitary-testicular axis\(^35\). Although the postnatal surge of gonadotropins has been suggested as responsible for priming the testes for subsequent development and fertility\(^60\), the suppression of the hypothalamic-pituitary-gonadal axis during the neonatal period does not affect sperm counts or fertility\(^35\).

The increase in central catecholaminergic activity at birth not only provides a satisfactory explanation for early postnatal activation of the hypothalamic-pituitary-testicular axis but, as similarly encountered for sympathetic tonus, links the hypothalamic-pituitary-gonadal axis to the central catecholaminergic activity. Furthermore, the augmented catecholamine response among preterm infants explains the more marked and prolonged postnatal surge of testosterone in preterm infants than in term infants\(^61,62\).

Although controversies exist\(^63\), a secondary increase in testosterone among males has been suggested to occur by the second week of life\(^64,65\). Tyrosine hydroxylase activity is influenced by gonadal hormones\(^66\). The first increase in the levels of circulating androgens...
explain the second increase through activation of catecholamine synthesis in the central nervous system.

Reports that indicate normal postnatal surge, and baseline or stimulated levels of LH and testosterone levels among boys with undescended testis exist in the literature. On the other hand, reduction in levels of LH and testosterone during postnatal surge and blunting of LH response to GnRH have also been reported. Evaluation of three-month-old boys with at least one suprascrotal testis has revealed non-measurable androgen bioactivity. Those boys have been suggested to be exposed to less biologically active androgens during the postnatal activation of the hypothalamus-pituitary-testicular axis.

Since the decrease in sympathetic tonus appears to associate with a decrease in central catecholaminergic activity, and since catecholaminergic activity also takes part in the stimulation of GnRH-secreting neurons, the blunting in LH response to GnRH in boys with undescended testis seems reasonable.

Regulatory mechanism of obliteration of processus vaginalis

Central catecholaminergic activity takes part in the regulation of both sympathetic tonus and GnRH release. It is the center that regulates the obliteration of the processus vaginalis through decreasing the sympathetic tonus while also decreasing the activity of the hypothalamic-pituitary-gonadal axis. Aberrations in regulation result in inguinal hernia, hydrocele and undescended testis.

Decrease in central catecholaminergic activity explains blunting of LH response

Pulsatile stimulation of the pituitary is essential. Exposure of the pituitary to continuous GnRH rapidly leads to a downregulation of GnRH receptors and an accompanying complete inhibition of pituitary gonadotropin secretion. GnRH upregulates its own receptors on pituitary gonadotropes at physiological frequencies of pulsatile GnRH release. During periods of little GnRH neuron activity, the number of GnRH receptors also decreases. The decrease in central catecholaminergic activity may decrease the number of GnRH receptors to blunt the LH response to GnRH.

On the other hand, catecholamines facilitate GnRH-induced discharge of LH through acting on beta-adrenergic receptors in the pituitary. Noradrenergic neurons are directly modulated by substance P. A chronic decrease in noradrenaline may increase the release of substance P. The increase in levels of substance P, together with interactions between substance P and the nor-adrenergic system can also augment the blunting.

Determination of side(s) of presentation

If the undescended testis results from the alterations in the central nervous system, both testes should be affected. However, there is a right side predilection for undescended testis.

On the other hand, the descended side reveals similar alterations in electromyographic evaluation. Additionally, 20-25% of malignancies associated with undescended testis involve the descended gonad, and contralateral testis is affected in boys with unilateral undescended testis. Enough evidence exists to indicate the undescended testis as a bilateral disease.

Cerebral hemispheres do not mature symmetrically. Both the cerebral hemispheres and sexually dimorphic nuclei are not symmetric. The distribution of androgen receptors and sizes of sexually dimorphic nuclei differ among hemispheres.

There is a predominance of the right half of the brain in controlling gonadal functions. The right side of the hypothalamus contains significantly more GnRH neurons and GnRH than the left. The asymmetry is not restricted to the central nervous system. Asymmetry also exists at the level of gonads including their innervation. The right testis exhibits a greater response to LH.
Concluding remarks
The gubernaculum gives rise to both smooth and striated muscles. The testis descends through the processus vaginalis via the physical force generated by the propulsive activity of those muscles under the control of the sexually dimorphic autonomic nervous system. The smooth muscle should undergo programmed cell death for the closure of the processus vaginalis after the descent. Programmed cell death requires a decrease in sympathetic tonus that depends on androgens. Central catecholaminergic activity takes part in the regulations of both peripheral sympathetic tonus and GnRH release. While decreasing the peripheral sympathetic tonus, the decrease in central catecholaminergic activity also decreases the stimulatory drive to the GnRH neuronal system.

The aberrations in the regulation of the decrease in central catecholaminergic activity result in inhibition of descent, inguinal hernia or hydrocele. Premature decrease in activity, permanently decreases the number of postganglionic sympathetic fibers, diminishes the amount of smooth muscle to propel the testis, and impairs the propulsion. Those alterations may inhibit the descent or ascend the initially descended testis. Absence of the initially descended testis. Absence of the testis, and impairs the propulsion. Those explanations not only precisely defines the variables, but also satisfactorily links the features associated with the process of descent.

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