SCANNING ELECTRON MICROSCOPE OBSERVATION OF PENILE VASCULAR CASTS IN THE DOG: AN INQUIRY INTO THE POSSIBLE MECHANISM OF ERECTION BASED ON THE FINDINGS

KEN-ICHI KANO¹, SHUGO HANYU¹, TOSHIHIKO IWANAGA² and SHOTARO SATO¹
Departments of ¹Urology and ²Anatomy, Niigata University School of Medicine, Niigata 951, Japan

ABSTRACT

The vascular construction of the dog penis was investigated mainly by scanning electron microscopic observation of corrosion casts produced by injection of methylmethacrylate into the penile artery and its branches. The corpus cavernosum penis was supplied mainly by the penile deep artery; only its distal end received a few small branches from the dorsal artery. The helicine arteries were provided with polsters which protruded into the lumen so prominently that they might conspicuously reduce the luminal space during the flaccid state of the penis. The polsters were restricted to the helicine arteries, and could not be found in the penile deep artery or in veins of the penis. Cavernous sinuses strikingly anastomosed each other forming a vascular network as a whole. No connection of sinuses was present between the corpus cavernosum penis of either side. Postcavernous venules in the corpus cavernosum penis occurred only on its dorso-medial surface at the point where the crus penis of either side converged; a well-developed capillary network was found in the same region. The postcavernous venules ran for a distance beneath the tunica albuginea, and it is apparent that they are effectively compressed between the tunica albuginea and the peripheral sinuses during erection. In contrast, the cavernous sinuses in the corpus spongiosum urethrae directly emptied into a vein penetrating the thin tunica albuginea. The drainage vein with large calibers was unlikely to receive significant mechanical compression during erection. No arterio-venous anastomoses could be found either within or out of the cavernous bodies.

It is generally accepted that penile erection is induced by increased arterial inflow into cavernous tissues. Several different processes, however, have been proposed to account for the essential mechanism of erection (for reviews, 19, 30). This is partly due to divergence of opinion over the anatomical basis for the erectile tissues, especially that arising from insufficient knowledge of the vascular system.

The blood vessels of the penis have been reported to possess conspicuous luminal protrusions of their intima called 'wurst-oder polsterartige Verdickungen' (29), 'polsters' (7) or 'Ebner's pad' (17); in the present paper, we adopt the term 'polster'. Conti (7) has regarded the polsters with their active contraction and relaxation as the crucial part in his theory on the mechanism of erection. Although the polsters have been reported to occur both in arteries and veins in the human penis (6, 7, 28), Goldstein et al. (10) described that the polsters with physiological significance were restricted to the veins. On the other hand,
some researchers have interpreted these intimal structures as arteriosclerotic changes (5, 21).

In regard to the possible contribution of venous drainage to penile erection, two views have been available: one says that venous return is not involved in erection (9, 20, 25, 26), while the other declares that erection is established by the stemmed venous outflow (10, 12, 16).

In order to correctly criticise these controversial views and to lead a more appropriate hypothesis on the mechanism of erection, investigations of the penile vascular system seem urgently important. Several major problems with the morphology of the penile vascular system remain to be elucidated, due to their three-dimensional complexity. Lierse (14) first induced scanning electron microscopy (SEM) into the morphological analysis of penile vessels in humans, but could not obtain sufficient findings.

Vasodilatation of the arteries in the penis has been demonstrated to be caused by non-adrenergic and non-cholinergic relaxation of vascular smooth muscles (27). Recently it has become clear that nerve fibers containing a potent vasodilator, vasoactive intestinal polypeptide (VIP), are numerous in the penis, being concentrated on arteries (8, 13). Intragenital injection of VIP has been shown to induce tumescence or erection in human volunteers (22). Thus, morphological and physiological evidence suggests that VIP is a neurotransmitter responsible for penile erection (22, 31).

The present study deals with the SEM observations of penile vascular casts in the dog, combined with conventional SEM observation of the penile tissue and immunohistochemistry for VIP. The data will be discussed with regard to the mechanism of penile erection.

MATERIALS AND METHODS

SEM of Penile Vascular Corrosion Casts
Young male adult, mongrel dogs weighing 7.5-14.5 kg were used in this study. Anesthesia was introduced by ketamine hydrochloride and maintained by pentobarbital sodium. In fifteen animals, the bilateral internal iliac arteries were exposed by lower abdominal midline incision, and polyethylene tubes were inserted into them. The abdominal aorta and inferior vena cava were ligated just proximal to the aortic bifurcation, and the unilateral common iliac veins were incised for the purpose of bloodletting. The penis thereafter was perfused through the internal iliac arteries with a warmed Ringer solution until the glans turned pale. The perfusion of Ringer solution was followed by that of methylmethacrylate (Mercox, Dainippon Ink Co.) according to Murakami et al. (18). The penis was removed and immersed in 70% ethanol overnight in order to remove the fat. It was then immersed in a concentrated NaOH solution to macerate the tissue.

In additional ten dogs, the pubic bone was removed, and the trifurcation of the penile artery was carefully exposed. Three branches of the penile artery were separately cannulated and perfused with Ringer solution followed by methylmethacrylate, in order that the vascular casts of their distribution could be visualized.

Conventional SEM of Penile Tissue
Five dogs were perfused with Ringer solution through canules inserted into the bilateral internal iliac arteries, followed by 10% formalin in 0.1 M phosphate buffer, pH 7.4. The penis was removed, cut into blocks and immersed in the same fixative for one or two days. After washing in a phosphate buffer, the specimens were put into 2% tannic acid for 4 h, and immersed in 2% OsO4 for 4 h. The blocks were dehydrated in a graded series of ethanol and then dried in a critical point dryer using liquid carbon dioxide.

The casts and specimens were coated with platinum in an ion coater and observed under a Hitachi S-450 LB scanning electron microscope.

Light Microscopy and Immunohistochemistry for VIP
The formalin-fixed tissue blocks were obtained from various regions of the penis in the same dog used for the conventional SEM study. The specimens were dehydrated through a graded series of ethanol and embedded in paraffin. Paraffin sections were serially cut at 4-6 μm in thickness and stained with hematoxylin-eosin or aldehyde fuchsin-Masson-Goldner's trichrome.

For immunostaining of VIP-containing neurons, the fixed tissue blocks were rinsed in a 30% sucrose solution at 4°C overnight. They were rapidly frozen in liquid nitrogen and sectioned at a 20 μm thickness in a cryostat. The sections were processed to the peroxidase-
Fig. 1  Vascular corrosion cast of a dog penis, showing the trifurcation of the penile artery. The penile deep artery (b) rises as a branch of the dorsal artery (a) and bifurcates before entering the CCP. The bulbourethral artery (c) is the thickest, supplying the CSU. Bar=5 mm

Fig. 2  Dorsal view of the crus penis. Blood vessels enter the CCP in the region where two crura converge (intercrural angle). Cavernous sinuses in this region are smaller in caliber and form a finer meshwork (arrows). The CSU (asterisk) is seen between the corpora cavernosa. Bar=5 mm

antiperoxidase (PAP) method for the detection of VIP-like immunoreactivity. The VIP antiserum was raised in rabbits by using synthetic porcine VIP as antigen (33). For checking the specificity of immunoreactions, the antiserum was preincubated with the antigen (10 μg/ml diluted antiserum) for 24 h at 4°C. The absorbed antiserum did not show any immunoreactivity in the sections examined.

RESULTS

Deep Arteries of the Penis

The penile artery derived from the internal
pudendal artery pierced the urogenital diaphragm, and then trifurcated on the dorsal side of the ischiourethral muscle. These branches are the bulbourethral artery, the dorsal artery and the deep artery of the penis, each of them being paired. The last one was the smallest in diameter among the three arteries and arose as a branch of either the bulbourethral artery or dorsal artery (Fig. 1). Selective injection of methacrylate resin into each artery clearly revealed the region of its distribution: the bulbourethral artery and deep artery supplied the corpus spongiosum urethrae (CSU) and corpus cavernosum penis (CCP), respectively. The dorsal artery which extended toward the glans on the dorsal surface of the penis gave off three or four small branches along its course. These branches ran around the CCP (circumflex arteries), partly piercing the tunica albuginea. Therefore, the dorsal artery was confirmed to supply mainly the glans, and partly the distal end of the CCP.

The deep artery soon divided into two to four branches, all of them entering the CCP in the dorso-medial direction at the portion where the two crura approached each other (intercruval angle) (Fig. 3). Within the cavernous tissue, one of the branches ran toward the proximal portion of the crus. A thick distal branch became the deep artery in a narrow sense and passed the axis of the CCP (Fig. 4), but tapered over the mid point of the shaft. Thus, the distal portion of the CCP did not contain the deep artery.

**Helicine Arteries in the Cavernous Bodies**

The deep artery gave off several branches nearly at right angles along its course (Fig. 4). Each branch then divided, in a tree-like fashion, into small arteries which took a tortuous pathway within the cavernous tissue, thus representing helicine arteries (Fig. 5). The surface of the corrosion casts from the penile artery to the helicine arteries showed oval imprints by the nuclei of endothelial cells (Fig. 6). As coming down the helicine arteries, gradually occurred several large scaphoid cavities covered with longitudinally striated imprints (Fig. 6). These cavities apparently coincided with the intravascular protrusions.
of the polsters.

On the cut surface of cavernous tissues the polsters appeared as longitudinal protrusions covered with longitudinal undulations due to the smooth muscle fibers oriented in that direction. Two or more polsters were frequently seen in the cross-sectioned plane of a helicine artery (Fig. 7). The lumen of the helicine arteries was irregular in shape and almost closed where a polster was well-developed. The characteristic figures of polsters were also demonstrated in the light microscopic observation of tissue sections (Fig. 8). The helicine arteries endowed with polsters were numerous in the proximal portion of the CCP, but much fewer in the distal portion.

As the helicine artery approached the cavernous sinuse, the cavities on the surface of
Fig. 8  Light microscopic observation of a helicine artery in the CSU. Aldehyde fuchsin-Masson Goldner's trichrome staining. It is equipped with two polsters.  Bar=50 μm
Fig. 9  VIP-immunostaining of the dog CCP. Immunoreactive nerve fibers densely innervate two helicine arteries (arrows), whose lumina are markedly narrowed by polsters.  Bar=100 μm
Fig. 10  VIP-immunoreactive nerves distributed to smooth muscles in the trabeculae of the CSU.  Bar=100 μm
Fig. 11 The terminals of helicine arteries continuing to smooth-surfaced sinuses via a short transitional zone. Arrows indicate the beginning of the transitional zone. Bar = 100 μm.

Fig. 12 A vascular cast of a CCP after cavernous sinuses has been partly removed. A huge sinus (asterisks) is exposed in the axis of the CCP. Bar = 500 μm.

the vascular casts produced by the polsters and the round imprints by the epithelial cells abruptly disappeared, resulting in a smooth-surfaced cast (Fig. 11). This was taken to be the transitional portion between the helicine arteries and cavernous sinuses. This smooth-surfaced vascular portion extended for 150-400 μm and continued into a sinus, frequently via a constricted portion.

The bulbourethral artery entered the urethral bulb on its dorsal surface, 4-5 mm distal to its origin, and soon diverged into two branches; the proximal branch arborized and continued to helicine arteries. The distal branch advanced along the urethra and gave off twigs at right angles along its course, and then arborized into helicine arteries in the same manner as the penile deep artery. Helicine arteries with polsters were numerous in the bulbular region of the CSU but gradually decreased in number toward the glans. They showed an architecture identical to that of the helicine arteries in the CCP.

Nerve fibers containing VIP were numerous observed in the crus penis and in the proximal portion of the shaft, but were rarer in the distal portion of the CCP. This tendency of VIP-immunoreactive fibers was seen also in the CSU. The smooth muscles of the trabeculae and the arteries in both cavernous bodies received a rich innervation of VIP neurons. A plexus of VIP-positive nerves accompanied the helicine arteries in which polsters were well-developed (Fig. 9). Another dense distribution of VIP-immunoreactive nerve fibers was present in the bundles of smooth muscles in the trabeculae (Fig. 10).

Cavernous Sinuses

The cavernous sinuses were observed as largely expanded elements in the corrosion casts (Fig. 11). They were generally smooth in surface, the indentations by endothelial nuclei being unclear. The sinuses comprised a frequently anastomosed network, being connected with adjacent sinuses via small channels or constricted parts. In fact, when resin was injected directly into a sinus by use of a needle, it easily spread throughout the cavernous tissues. There was no vascular connection between the corpora cavernosa of both sides, due to the complete septum separating them.

The three-dimensional structure of sinuses
Fig. 13 The dorsomedial surface in the intercruval angle of the CCP. Postcavernous venules creep for a distance before turning vertically and penetrating the tunica albuginea. The penetrating veins are constricted in the region which corresponds to the inner half of the tunica albuginea (arrowheads).  Bar=500 μm

Fig. 14 Cavernous sinuses in the CSU arranged in parallel to the long axis of the CSU. They remarkably anastomose each other.  Bar=500 μm

Fig. 15 Cavernous sinuses in the bulbus of the CSU which are large and irregular in shape. Periferal sinuses directly empty into a drainage vein (arrow) without the constriction by the tunica albuginea.  Bar=1 mm

Fig. 16 A developed capillary network between a postcavernous venule (asterisk) and peripheral sinuses. Some capillary vessels are apparently continuous with the cavernous sinuses (CS).  Bar=100 μm
was clearly shown by binocular and SEM observations of corrosion casts. The arrangement and shape of the sinuses differed between the CCP and CSU, and also according to the position within the same cavernous body; the cavernous sinuses in the CCP tended to be arranged perpendicularly to the long axis of the CCP. A huge sinus frequently occurred along the axis of the corpus (Fig. 12). In the region where the deep artery of the penis entered the dorso-medial part of the CCP, sinuses were smaller than elsewhere and formed a finer meshwork (Fig. 2). Cavernous sinuses were arranged more loosely in the distal part of the CCP; they were divided by thick collagenous bundles into disc-like compartments arranged across the long axis of the CCP. On the other hand, the cavernous sinuses of CSU generally ran parallel to the long axis of the cavernous body (Fig. 14). The sinuses in the bulbus of the CSU were large and irregularly arranged (Fig. 15).

**Veins Draining the Cavernous Sinuses**

The venules draining the sinuses of the CCP arose on the dorsomedial surface at the point where both crura penis converged (Figs. 3 and 13), no vessels of venous nature being present in other regions of the CCP. The boundary between the postcavernous venules and the sinuses was generally identifiable (Fig. 3). The postcavernous venules without valves crept for 0.2–1.0 mm closely along the peripheral sinuses, joined each other in drainage veins and abruptly changed their course to be perpendicular (Fig. 13). As soon as the veins altered direction, they were constricted for a distance of 500–800 μm (Fig. 13). The portion corresponded to the inner half of the tunica albuginea. The 'penetrating veins' dilated again in the outer half of the tunica albuginea and become equipped with valves. They emptied into the deep vein of the penis on each side. No polsters were found in any of the vessels of venous nature.

The drainage vein of the CSU arose on the dorsal surface of the bulbus. The peripheral cavernous sinuses directly emptied into a vein with large calibers; no distinct postcavernous venules were present in the CSU. The drainage vein obliquely penetrated the thin tunica albuginea and continued to the bulbourethral vein (Fig. 15). This vein was equipped with valves and joined the deep vein of the penis.

Paired dorsal veins drained the glans penis (bulbus glandis). The dorsal veins on both sides joined each other and soon penetrated the median tendon of the ischiourethral muscle. It then divided again and emptied into the internal pudendal vein on each side. The external pudendal vein also drained the glans penis (pars longa), but turned round and left the penis. This vein probably corresponded to the superficial dorsal vein in human.

**Existence of Capillaries and Arterio-Venous Anastomoses within the Cavernous Tissues**

Capillary vessels were observed scattered in the trabeculae throughout the cavernous tissues. However, well-developed capillary networks were restricted to the dorsal surface of the CCP around the draining veins. These vessels were continuous with the helicine arteries and partly peripheral cavernous sinuses at one end, and with postcavernous venules at the other end (Fig. 16).

No direct connections between arteries and veins were found external to the tunica albuginea of the CCP. Within the cavernous tissues, the penile deep artery smoothly continued...
into the sinuses via the helicine artery; these arteries never formed a glomerular structure along their course as does the digital glomus.

The vascular construction in the CCP of dog penis is schematically summarised in Fig. 17.

DISCUSSION

The CCP Is Supplied Mainly by the Penile Deep Artery

Penile erection is due to the distention and subsequent hardening of the CCP. The CSU and glans penis never become rigid even during full erection. The present SEM observation of vascular casts showed that the erectile tissue, the CCP, was supplied exclusively by the deep artery of the penis. Previous hemodynamic studies on erection in the dog have estimated penile blood flow by way of the dorsal artery (9) or the dorsal vein (1). We believe that these data are not valid as reference for elucidating the hemodynamics of the CCP, because the blood flow of these vessels reflects mainly that of the glans penis, and not that of the CCP. Neither did Lue et al. (15), who attempted to measure hemodynamic changes during erection by means of the internal pudendal artery in the dog, estimate the actual blood flow of the CCP. At least in the dog, hemodynamic studies of erection must be carried out by means of the penile deep artery.

Do 'Polsters' Have a Functional Significance or Not?

Erection is caused by a particular regulation of arterial and venous flow. Early anatomists have attributed this regulating mechanism to the intimal thickenings of penile vessels (7). However, the presence and functional significance of the polsters have been disputed. The present observation by means of SEM and light microscope clearly demonstrated the existence of polsters in the all young, healthy dogs examined. They occurred in the helicine arteries and were never found in the drainage veins which have been reported to possess them (7). The polsters in dogs showed a peculiar but uniformly designed structure, distinguishable from any pathological changes which have been claimed to characterize the polsters (5, 21). It is likely that, during the flaccid state, the polsters of the helicine arteries narrow or even occlude the lumen for a long distance to reduce the blood flow into the sinuses.

Immunohistochemical studies have demonstrated numerous VIP-immunoreactive nerve fibers in the penis of the rat (8), cat (13), rabbit (31), monkey and human (11, 23). The VIP nerves have been shown to gather on the arterial wall and to be dispersed also in the trabecular smooth muscles. Dalil et al. (8) reported that the helicine arteries received a denser plexus of VIP nerves than did the penile deep artery in the rat penis. It has been shown morphologically and physiologically that the vasodilation of the deep artery and, especially, its helicine branches are caused by VIP-containing neurons (32). The dog penis has further been demonstrated in this study to contain numerous VIP nerve fibers densely distributed in the helicine arteries with developed polsters. These findings suggest that in the dog, the arterial inflow to cavernous sinuses is actively controlled by the polsters, while the venous outflow is not regulated by any polsters.

Existence of Arterio-Venous Anastomoses in the Penis

The existence of arterio-venous anastomoses at all levels of arterial branchings in the human penis has been reported for more than a century. The anastomoses are an important structure in the theory of Conti (7) who described anastomoses as located outside of the tunica albuginea and functioning as a bypass for arterial blood during the flaccid state. When an erection is induced, these anastomoses close and an increased volume of blood rushes into the cavernous sinuses; at the same time, venous outflow must be restricted. According to Conti (7), the opening and closing of arteries, veins and their anastomoses should be all actively controlled by the polsters. However, the present study made it clear that the polsters are restricted to the helicine arteries at least in dogs. Neither was the presence of such arteriovenous anastomoses as described by Conti (7) and Wagner (30) confirmed in the dog penis.

A significant capillary network was found in the present observation, occurring between the helicine arteries and postcavernous venules in the dog CCP. However, this capillary network was not developed enough to serve as a bypass during the flaccid state. Moreover, corrosion casts of the CCP made during the flaccid state always displayed a vascular system continuing from the helicine arteries to the cavernous
sinuses, suggesting that a modest amount of arterial blood pours into the sinuses through helicine arteries, even during the flaccid state. The existence and functional significance of routes bypassing the cavernous sinuses during the flaccid state need further morphological and hemodynamic studies.

Closed System of Penile Vessels

The blood pressure in the CCP is known to increase to a level just below the carotid pressure during erection (3). During rigidity, intracavernous pressure increases ten or more times higher than the systemic systolic blood pressure both in human and animals (16, 24). To maintain such high pressure, a closed system separated from the systemic circulation should be present as Beckett et al. (2, 3) and Goldstein et al. (10) suggested. On the other hand, some hemodynamic studies have supported the importance of venous obstruction for inducing and maintaining the erect state (10, 12, 16).

It is accepted that, since the helicine arteries open into cavernous sinuses in the core of the CCP, the expansion of peripheral sinuses compresses the non-muscular veins underlying the tunica albuginea. The present SEM observation of the vascular casts of the dog penis confirmed the characteristic course of drainage veins in the CCP: the veins draining sinuses first ran along the dorsal surface of the CCP for a considerable distance. These post-cavernous venules, without polsters and valves, might be readily compressed between the CCP and the stiff tunica albuginea during erection.

A large difference in the intracorporal pressure during rigidity has been recognized between the CCP and CSU in dogs (CCP 1,952 + 140 mmHg vs. CSU 123 ± 37 mmHg) (24). This gap in pressure is presumably due to the varying thickness of the tunica albuginea between the CCP and CSU and the lack of postcavernous venules taking a course underlying the tunica albuginea in the CSU. In the CSU, the drainage vein apparently is not efficiently compressed against the tunica albuginea.

The closed system separated from the systemic circulation must be valid also for the arterial side. Serial angiographic studies by Beckett et al. (4) in the goat described how the obstruction of arteries was due to a mechanical compression of the deep arteries either inside or outside of the tunica albuginea and of the internal pudendal arteries against the ischium. Our preliminary study (S. Hanyu, T. Iwanaga, K. Kano and S. Sato, the mechanism of penile erection in the dog: a pressure-flow study combined with the morphological observation of vascular casts; submitted for publication) shows that when a great amount of fluid is injected into the cavernous sinuses during the flaccid state and tumescence, little backflow into the deep arteries occurs. We believe that an obstruction of arteries is established inside of the tunica albuginea, although we cannot localize its site precisely as yet.

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