THE MORPHOGENESIS OF THE RENAL PLEXUS: RENAL ARTERY AND SYMPATHETIC FIBERS

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ABSTRACT

Objective: To examine the origin and development of the renal plexus and its relationship to the renal vessels in embryos and early human fetuses.

Method: Serial sections of 33 human embryos (stages 16 to 23 of Carnegie, four or 5-8 weeks) and 38 fetuses (9-19 weeks) were analyzed.

Results: Throughout the embryonic period, the kidney was not innervated by the renal plexus. Those nerves appeared at the beginning of the early fetal period (nine weeks) as branches given off by the immature autonomic abdominal plexus. The renal nerves started to approach to the kidney during the early fetal period at 9-10 weeks of development. They were distributed in close proximity to the renal arteries and their branches. They were observed first with the settlement of the renal veins.

Conclusions: The renal artery is present as a branch of the abdominal aorta at stage 19 (between six and seven weeks) prior to development of the renal plexus. The renal veins were not present during the embryonic period but appeared at the start of the fetal period, along with the renal nerves that emerged from segmented sympathetic para-aortic bodies (SPBs).

INTRODUCTION

The renal plexus and its relationship to the renal arteries in adults have been subjects of important recent investigations because this plexus is considered a target for treating refractory hypertension by catheter-based sympathetic denervation (Atherton et al., 2011; Tellez et al., 2013; Sievert et al., 2013; Tzafriri et al., 2014; Sakakura et al., 2014; Both et al., 2015; Okada et al., 2015; Mompeo et al., 2016).

The morphology of the renal plexus in adult humans has been addressed from anatomical, histological and immunohistochemical points of view (Petit-Dutaillis and Flandrin, 1923; Legueu and Flandrin, 1923; Mitchel, 1950 a, b; Atherton et al., 2011; Sakakura et al., 2014; Mompeo et al., 2016). However, few studies have been dedicated to the development of the renal nerve supply (Sariola et al., 1988; Liu and Barajas, 1993; Lumbers, 1995; Tiniakos et al., 2004).

The nerves of the kidney have been considered fundamental to renal morphogenesis and function in several nonhuman mammalian species such as mouse and sheep (Sariola et al., 1988; Lumbers, 1995). During human fetal development, intrinsic innervation of the kidney has been described from 20 weeks of gestational age in close apposition to the intrarenal arteries and their branches, which approach the afferent and efferent arterioles during the third trimester (Tiniakos et al., 2004). This type of intrinsic innervation has also been described during the fetal and postnatal periods in rats (Liu and Barajas, 1993). However, to the best of our knowledge, there have been no studies of the prenatal development of extrinsic nerves to the kidney arising from the renal

plexus in either humans or other mammalian species. Some studies suggest that extrinsic renal innervations could start during early fetal life. However, such innervations have not been described morphologically.

Therefore, we have undertaken this study with the aim of establishing the early development of the renal plexus and its relationship to the definitive renal vascular pattern using a reliable sample of human embryos and fetuses.

MATERIALS AND METHODS

We examined 34 human embryos corresponding to stages 15 to 23 of Carnegie and 38 fetuses with estimated gestational ages ranging between nine and eighteen weeks, all belonging to the collection of the Department of Human Anatomy and Embryology. The embryos and fetuses were donated to the Anatomy Department with parental consent and according to Law. They were fixed in 10% neutral formalin, processed for paraffin wax histology and serially sectioned at a thickness of 7µm or 10µm. The sections were stained with either hematoxylin-eosin, verde luz-orange g-acid fuchsin trichromic stain (VOF), Bielchowsky silver stain, or Azan stain. The embryos were classified using the criteria proposed by O'Rahilly and Müller (1986), and the fetuses were classified according to the criteria described by Patten (1973).

RESULTS

The embryos and fetuses were grouped on the basis of the similar morphologies of the autonomic nervous system and renal vascular supply.

Stages 15-16 (33-37 days)

During these stages, the mesonephroi are located in the abdominal zone and some mesonephric and segmental arterial branches emerge from the aorta (fig. 1A). During development, the initial metanephroi are located in the pelvis, and no metanephric arteries or blood vessels related to the metanephros could be seen. Some small vessels and some blood cells are distributed in the mesenchyme that surrounds the metanephros during development (figs. 1B and C). During these stages, two sympathetic-chromaffin cellular clusters are observed posterolateral to the aorta in front of the vertebral body (sympathetic cord). These clusters are connected by fibers to the spinal nerves. Two other similar cell clusters, the sympathetic para-aortic bodies (SPBs), are located anterolaterally beside the aorta (fig. 1A).

Stages 17-18 (41-44 days)

The metanephros is developing and is mainly located in the pelvis, though part of it is already located in the lower lumbar region. It is located posterior to the mesonephros at cranial levels. No arterial branches from the aorta towards the metanephros could be seen, but some venous vessels from the supracardinal and subcardinal veins were observed. Although nerve fibers run laterally to the aorta in an anterior direction, no nerve fiber running towards the metanephros was observed (fig. 1D)

Stages 19-20 (48-51 days)

At stage 19, only the caudal edge of the metanephros can be observed in the pelvis; the rest of it is located in the lower abdominal region (fig. 1E). The metanephros is posterior to the mesonephros. It is vascularized, and no arteries from the aorta approaching it or contribution from the mesonephric arteries are observed. There are two big SPBs in front of the aortic artery (fig. 1F), with nerve fibers from the sympathetic cellular clusters of the lumbar cord running towards them (fig. 1F). Fibers running to the body wall are also detected. However, no nerve fibers directed towards the metanephros were noted. At stage 20 (seven weeks), two branches from the aorta ran laterally to the kidneys . These arterial branches were crossed by nerve fibers going from the lumbar cord to the SPBs. There were no nerve fibers directed to the kidneys, and no renal veins were seen (fig. 1G).

Stages 21-23 (52-57 days)

At these stages, the definitive renal arteries are easily seen. They run towards the renal sinus, but there are no nerve fibers around them. Some nerve fibers run towards the suprarenal gland. The SPBs are better defined than in the previous stages. They consist of rounded masses of more lightly stained cells surrounded by layers of darker cells. There is a close relationship between these bodies and the renal arteries (fig. 1H).

Fetuses of 9-10 weeks

The renal veins are first seen in the 9-10 week fetus, approaching the renal sinus from the renal venous collar (fig. 2A). The more darkly and lightly stained cells in the segmented SPBs are divided (fig. 2B) and the cellular clusters from

them start to be shared by nerve fibers. Some nerve sprouts from those segmented cellular clusters are observed in relation to blood vessels. Parts of the clusters are distributed between the aorta and the kidney behind the renal veins, and in front of and behind the renal arteries or their branches. Some are located in the renal sinus (figs. 2B and C).

Fetuses of 11-13 weeks

The segmented SPBs are observed between the aorta and both the kidneys and suprarenal glands. They are fragmented and nerve sprouts emerge from their cellular clusters towards the locations of the arteries. There are smaller cellular clusters between the aorta and both the renal sinus and adrenal gland. In the renal sinus, the renal arteries or their branches and nerve fibers retain their relationship. At the level of the renal arteries, the cellular clusters are located between the veins and arteries. However, caudal to the renal arteries, some ganglionic masses are disposed in front of the renal veins. Although some nerve fibers from the clusters reach the future inferior vena cava and renal veins, they do not accompany the renal veins to the kidneys (figs. 2D and E).

Fetuses of 14-16 weeks

The cellular components of the SPBs are shared by bundles of nerve fibers. The cellular clusters are mainly located behind the future inferior cava vein and renal veins, lateral to the aorta. The renal venous collar is still present. Small cellular clusters and nerve fibers are related to the arterial and venous

segmental vessels. No nerve fibers follow the renal veins (fig. 2F), but they are clearly associated with the renal arteries.

DISCUSSION

The renal plexus is part of the abdominal autonomic plexus and its development is related to the definitive renal arteries, renal veins and the development of autonomic nervous structures.

In the embryos examined, the sympathetic lumbar trunk was initially observed at embryonic stage 15 (33 days) and the first sympathetic chromaffin para-aortic bodies were seen at stage 17 (41 days). The connection between the nerve fibers of the sympathetic cord and para-aortic sympathetic chromaffin tissue is established during this period. To the best of our knowledge, no other authors have mentioned this.

The para-aortic sympathetic chromaffin bodies are composed of chromaffin and sympathetic cells, both of which have a common origin from the primitive sympathetic anlage (Coupland, 1952; Shtukmaster et al., 2013). From this anlage, the cells migrate to the dorsal aorta, where they acquire their catecholaminergic and neuronal characteristics (Reissmann et al., 1996). In the early fetal period, sympaticoblasts and chromaffin cells are located in the SPBs, which are associated with the adrenal, aortic, renal and gonadal plexuses (Coupland, 1952, 1954, 1956).

Although we first observed the cellular para-aortic masses at embryonic stage 17, these masses did not emit nerves towards the renal artery and its branches until they were extended and segmented between the aorta and kidneys to become autonomic ganglia during the early fetal period. It seems that as the renal nerves start to form, the sympathetic ganglia are not totally developed (Sisu et al., 2007, 2012). The para-aortic bodies are already present when the renal artery starts to grow towards the renal sinus, and this could explain the typical relationship among the ganglia, nerves and renal arteries in adults. To the best of our knowledge, this has not previously been described.

The initial renal nerves emerge as nerve sprouts from the cellular clusters that become ganglia during early development. They grow towards the renal artery and its branches, as demonstrated in this study. However, although the sympathetic nerves take arterial routes in response to factors expressed by the arteries (Manousiouthakis et al., 2014), and there is an intimate relationship between the renal artery and the nerves in the postnatal renal plexus (Petit-Dutaillis and Flandrin, 1923; Legueu and Flandrin, 1923; Mitchel, 1950 a, b; Atherton et al., 2011; Sakakura et al., 2014, Mompeo et al., 2016), we found no temporal coincidence between the prenatal appearances of the renal arteries and the renal nerves. The renal nerves started to grow two weeks after the formation of the definitive renal artery.

The renal artery emerged from the aorta towards the metanephros at stage 19, around 48 days of development, which is consistent with the findings of Hinata et al. (2015). On the other hand, Williams and Warwick (1992) described the

appearance of the definitive renal artery in the third month. They consider that the definitive renal artery is the most caudal suprarenal artery and represents a mesonephric artery. We observed no arterial vessels from the aorta running directly to the metanephros before stage 19. Therefore, the results of this work do not support the origin of the renal artery from one of the mesonephric arteries of the classically accepted ladder hypothesis (Felix, 1912). This hypothesis is currently widely discussed (Salama et al., 1982; Isogai et al., 2010; Hinata et al., 2015; Munro et al., 2017).

It was not until the early fetal period (nine weeks) that the nerve sprouts from the segmented sympathetic para-aortic bodies grew and extended along with the renal arteries to the kidneys, some of them arriving at the renal sinus. The smallest segmented cellular masses would probably become the origins of the small ganglia of the renal plexus and the small groups of cells on the nerve route along the renal artery and its branches (Mitchel, 1950 a, b; Mompeo et al., 2016). Once the nerve sprouts started, there was a rapid formation of nerve branches towards the renal sinus or directly to the renal parenchyma along with the definitive renal arteries and their branches or polar arteries.

A consistent ganglion was identified in relation to the suprarenal gland. The cellular cluster emits nervous sprouts to the suprarenal gland and to the nerves along the renal artery. We believe this specific cluster could correspond to the suprarenal ganglion in development and create the nerve connections between the suprarenal gland and the renal plexus at the level of the two distal thirds of the renal artery, as observed in adults (Testut, 1931; Mitchel, 1950 a, b).

The initial nerve sprouts from the ganglia are temporally coincident with the formation of the definitive renal veins, which were first observed at nine weeks. From our point of view, this would suggest some kind of association between the development of the renal veins and that of the renal nerves, as described in mouse and rat hearts (Nam et al., 2013; Manousiouthakis et al., 2014). The latter authors suggested a two-step process responsible for sympathetic innervation in the developing heart (Nam et al., 2013). In the first step, the factors are secreted by veins, and in the second, factors secreted by arteries intervene in the sympathetic innervation of the heart in rats. This could also happen during renal plexus formation and renal innervation. We observed that although the cellular clusters could sometimes be observed in front of the renal veins and some fibers could be directed towards them, those nerves did not follow the renal veins towards the kidneys.

In conclusion, the renal plexus starts to form during the early fetal period, which coincides with the establishment of the definitive renal veins, two weeks after the definitive renal arteries are identified. The disposition of the renal vessels and renal nerves in adults could be explained by the disposition and progress of the structures during prenatal development.

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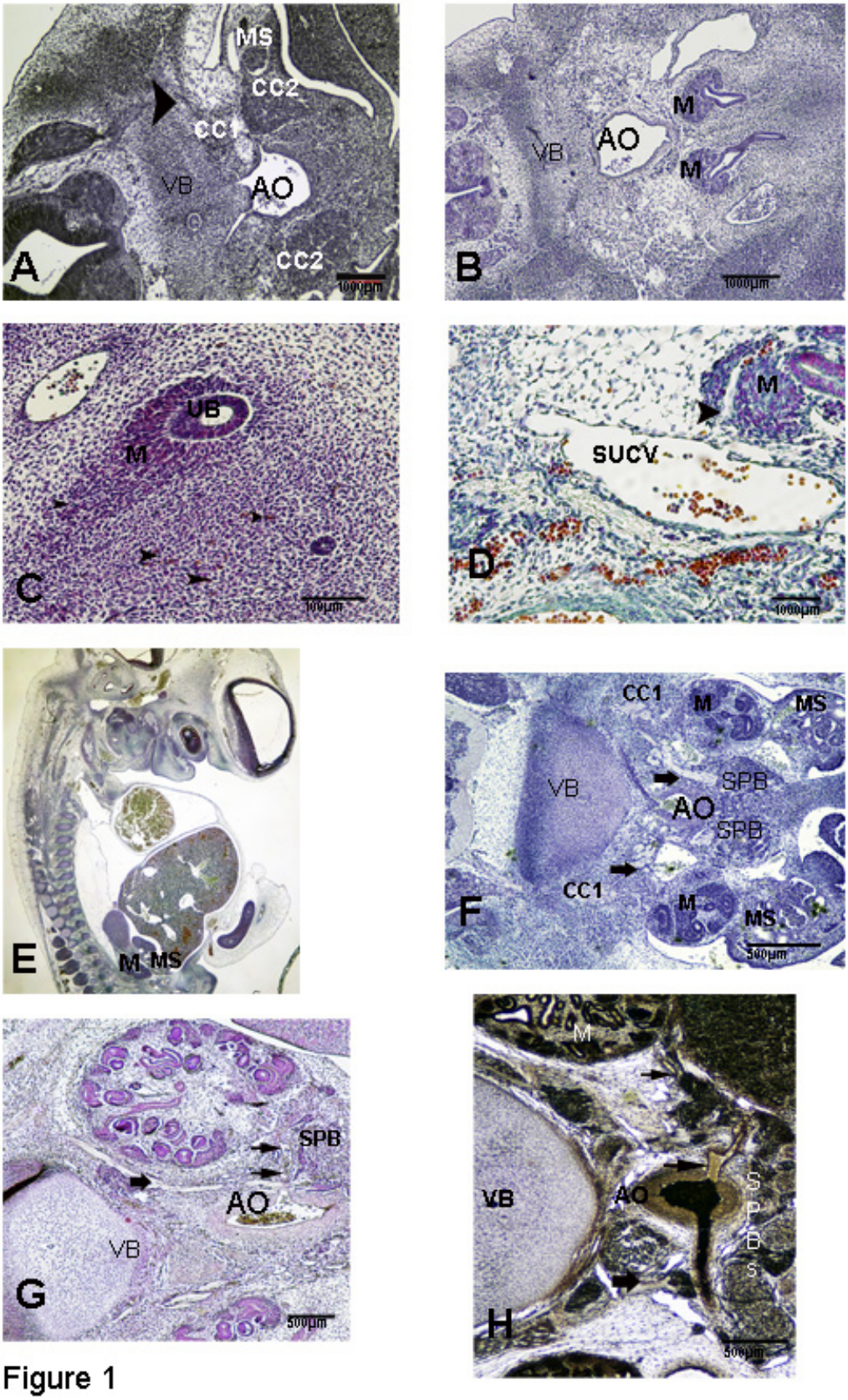

Figure 1

Figure 1

Embryonic sections at stages 15-16 (A, B, and C), stages 17-18 (D), 19-20 (E and F), and 21-23 (G and H). It shows the pattern of the vascularization of the metanephros during the corresponding Carnegie stages. The arrowheads show the blood cells and small vessels in the mesenchyme that surround the metanephros (C), and thin arrows show the renal artery and its branches (F, G, and H). The evolution of the prevertebral and para-aortic sympathetic cellular clusters and their connecting tracts are also showed (wide arrows in A, D, F, G, and H).

Abbreviations: AO, aorta; M: metanephros; MS: mesonephros; SUCV: subcardinal vein; PCA: posterior cardinal vein; vv: venous vessel; ST: sympathetic trunk; SPBs, sympathetic para-aortic bodies; and VB: vertebral body.

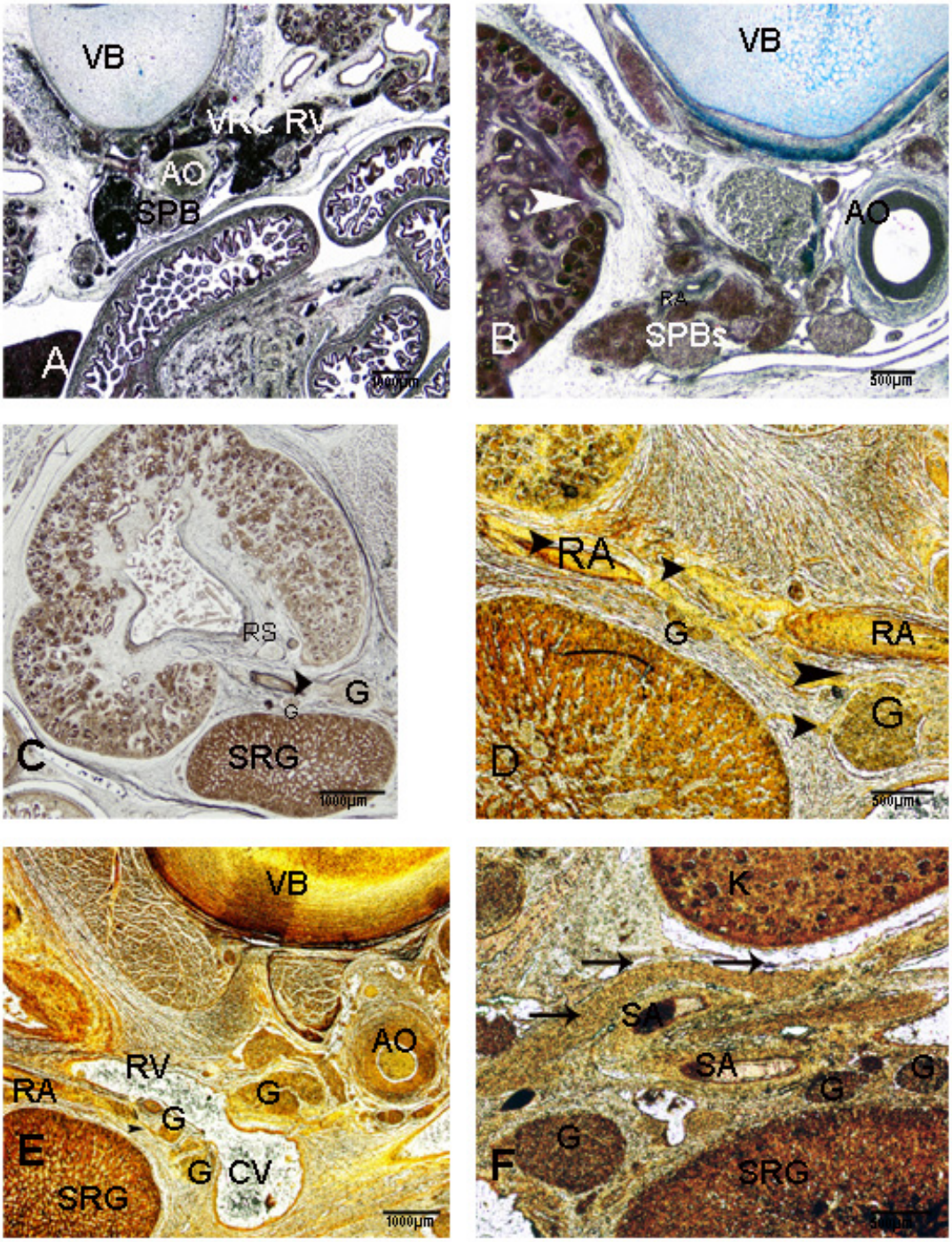

Figure 2

Figure 2

Fetus at 9-10 weeks (A, B, and C), fetus at 11-13 weeks (D and E), and fetus as 14-16 weeks (F) Renal veins run to the renal sinus from the renal venous collar (A). The renal artery and its branches are surrounded by cellular clusters from fragmented Sympathetic Bodies (SPBs), and these SPBs emit branches towards the kidney in relation with the arteries (arrowheads). In B, a nerve directly breaks through the renal parenchyma away from the renal hilum, along with a blood vessel. Nerve branches from the ganglia are observed between them and towards the renal artery (black arrowheads) (B, C, D, E, and F). A ganglion with nerves towards the suprarenal gland and the renal artery and its branches (black arrowheads) are noted in D. Some nerve fibers from the ganglia to the cava or renal veins are observed in panel E (black arrowheads). The renal nerves are in relation with the segmental arteries (black arrows) are noted in F. Abbreviations: VB: vertebral body; AO: aorta; SRG: suprarenal gland; K: kidney; SPB: sympathetic para-aortic body; G: ganglion; RS: renal sinus; CV: cava vein, RV: renal vein' RA: renal artery and branches; SA: segmental arteries; and VRC: venous renal collar.