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Received October 17, 1987

Summary. A well developed extra-adrenal chromaffin body with an axis of 200–400 μm was found in seven out of thirty adult male Wistar rats under a stereomicroscope. All seven bodies were located between the left and right kidneys. Blood vascular beds of the five bodies were reproduced with a methacrylate casting medium and observed with a scanning electron microscope. It was revealed that the extra-adrenal chromaffin body contained remarkably numerous capillaries, which anastomosed with each other to form a conglomerated network. The blood capillaries were of small and uniform caliber and did not represent swollen sinusoids as in the adrenal medulla. The capillary network was denser than that in the adrenal medulla and had no direct vascular linkage with the adrenal cortex or an extra-adrenal cortical body. Histological examination of the two bodies treated with dichromate containing fixatives confirmed that they mainly consisted of chromaffin cells. These findings suggest that in the rat, extra-adrenal chromaffin bodies survive throughout life, actively producing catecholamines.

As is well known, the adrenal gland develops from two components: a mesodermal portion which forms the adrenal cortex, and a neuroectodermal portion which forms the adrenal medulla (Keene and Hewer, 1927; Bachmann, 1954; Gardner, 1975). It is also well known that some parts of the mesodermal and neuroectodermal portions can remain outside the adrenal gland and survive as the extra-adrenal cortical and chromaffin bodies, respectively (Wrete, 1927; Watzka, 1943; Bachmann, 1954; Coupland, 1960; Hervonen, 1971; Hervonen et al., 1978). However, the three-dimensional organization of these capillaries has not yet been revealed. The present study demonstrates the blood vascular architectures of extra-adrenal chromaffin bodies in the adult rat by the corrosion casting/scanning electron microscope method (Murakami, 1971), and discusses the functional significance of these bodies in the production and storage of catecholamines.
MATERIALS AND METHODS

Thirty adult male Wistar rats weighing 300–400 g were anesthetized with ethyl ether, and their thoracic and abdominal cavities were opened. The exposed abdomen was carefully dissected with sharpened needles under a stereomicroscope (SMZ-10, Nikon) with a tungsten illuminator. By this dissection, an extra-adrenal chromaffin body could be identified in seven rats and an extra-adrenal cortical body in eighteen rats (see below). After this identification, all but four of the animals (two with an extra-adrenal chromaffin body, and two with an extra-adrenal cortical body) (see below) were perfused through the thoracic aorta with Ringer’s solution (40 ml) and with a low viscosity methacrylate casting medium (20 ml) (Murakami, 1975).

The methacrylate-perfused animals were placed in a hot water bath (60°C) for 3 h or longer, corroded in a hot 10% NaOH solution (60°C) overnight, washed in running tap water for 8 h or longer, and air-dried. Blood vascular casts of the five extra-adrenal chromaffin bodies and eighteen extra-adrenal cortical bodies thus prepared were isolated together with their affiliated vessels under the stereomicroscope, sputter-coated with gold in a vacuum evaporator (IB-3, Eiko), and observed with a scanning electron microscope (HHS-2R, Hitachi) using an acceleration voltage of 5 kV. After this observation, four specimens of the extra-adrenal chromaffin body were microdissected with sharpened needles, with one specimen being freeze-cut with razor blades. These microdissected or freeze-cut samples were also observed with the scanning electron microscope. This series of microdissection (or freeze-cutting) and scanning electron microscopy of the samples was repeated several times to observe completely the inner structures of the samples. Similar microdissection and scanning electron microscopy were also carried out on the cast specimens of the extra-adrenal cortical bodies.

Two extra-adrenal chromaffin bodies and two extra-adrenal cortical bodies isolated from the four rats (see above) were immersed in Orth’s or Helley’s fixative containing potassium dichromate, embedded in paraffin, cut into sections, stained with hematoxylin or by the Masson-Goldner method, and observed with a transmission light microscope (BH-2, Olympus).

RESULTS

The extra-adrenal chromaffin body in the anesthetized or living rat was slightly pink under the stereomicroscope with a tungsten illuminator. This body was thus easily discriminated from the dark-brown extra-adrenal cortical body. Both the chromaffin and cortical bodies, as observed by stereomicroscopic dissection, were embedded retroperitoneally in whitish-yellow adipose tissue between the left and right kidneys. They were found in seven and eighteen out of the thirty adult rats, respectively; in three cases, the extra-adrenal chromaffin and cortical bodies were only narrowly separated (Fig. 2). The extra-adrenal chromaffin and cortical bodies were ovoid in shape, and had long diameters of 200–400 and 200–700 \(\mu\)m, respectively.

Fixation of the extra-adrenal chromaffin and cortical bodies with Orth’s or Helley’s solution allowed examination of the chromaffin reaction of the cells in these bodies under the light microscope (Fig. 1).

Fig. 1. A light micrograph of a section of an extra-adrenal chromaffin body in an adult male Wistar rat. This body, treated with the Orth’s fixative and stained with hematoxylin, was located near the origin of the superior mesenteric artery. Note that the body contains many cells reactive with potassium dichromate (arrowheads). \(\times1,200\)
Fig. 2. A survey scanning electron micrograph of the blood vascular beds of an extra-adrenal chromaffin body (C) and an extra-adrenal cortical body (ectopic adrenal cortex, COUPLAND, 1960) (E), which were found near the origin of the superior mesenteric artery in an adult male Wistar rat and reproduced with a methacrylate casting medium. Note that the capillaries of the extra-adrenal chromaffin body (C) are thinner than the winded capillaries of the extra-adrenal cortical body (E). Also note that the extra-adrenal chromaffin body receives two afferent vessels (CA1 and CA2) from a branch (D) of the left inferior adrenal artery and emits an efferent vessel (CE1) continuous with a venous branch (V1). This extra-adrenal chromaffin body emits one more efferent vessel (see Figures 4-5, CE2). The arrowhead indicates the origin of the CA1 afferent vessel, a closer view of which is shown in the inset. Note in this inset that the origin of the afferent vessel is markedly constricted (arrowheads). The extra-adrenal cortical body (E) receives a single afferent vessel (EA) and emits three efferent vessels (EE1, EE2, and EE3) continuous with the V2 and V3 venous branches. The V2 and V3 branches drain, together with V1 branch, into the left renal vein. F vessels supplying the adipose tissue, Z leaked resin masses, ca1 a branch of the CA1 afferent vessel. ×145, Inset: ×580.
Injection of 20 ml low viscosity methacrylate casting medium through the thoracic aorta reproduced the whole blood vascular beds of the preliminarily identified extra-adrenal chromaffin and cortical bodies, as well as their affiliated vessels, with a little leakage of the injected resin and a few discontinuities of the injected capillaries (Figs. 2–6). Stereomicroscopy of the cast specimens without any dissection allowed observation of the parent vessels of the afferent and efferent vessels of the bodies. Scanning electron microscopy of the isolated, microdissected or freeze-cut casts allowed a detailed analysis of the vascular beds of the bodies and their connections to the affiliated vessels (Figs. 2–6). The present descriptions are concentrated on the findings in the extra-adrenal chromaffin bodies. Those of the extra-adrenal cortical bodies will be reported elsewhere.

Light microscopic observation of the tissue samples treated with Orth’s or Helley’s fixative and stained with hematoxylin or by the Masson-Goldner’s
The trichrome method revealed that the extra-adrenal chromaffin body was surrounded by a thin connective tissue, extensions of which penetrated the body and integrated with the parenchymal cells. The parenchymal cells were of two types: main (chief) and accessory (supporting) cells. The main cells occupied a large portion of the body. They were irregular in shape with a round or oval nucleus, and showed a conspicuous chromaffin reaction (Fig. 1). The accessory cells were small in number, and sparsely scattered among the main or chromaffin cells. They were more irregular in shape than the chromaffin cells, and contained an elongated or indented nucleus. The accessory cells showed no chromaffin reaction. Neither a nerve cell nor a nerve bundle was observed in the extra-adrenal chromaffin body. The blood capillaries were small in caliber and not sinusoidal in nature. They were richly distributed among the main

**Fig. 4.** A reversed and closer view of the extra-adrenal chromaffin body shown in Figure 1. Note that a newly exposed efferent vessel (CE2) collects other efferent rootlets (e) of the body. This micrograph, together with Figure 2, shows that the extra-adrenal chromaffin body in Figure 1 receives two afferent vessels (CA1 and CA2) and emits two efferent vessels (CE1 and CE2). The CE2 efferent vessel drains, together with the CE1, EE1, EE2 and EE3 vessels (see Figures 2, 3), into the left renal vein. Also note that, even on this reversed side, the afferent rootlets (a) course through in the surface layer of the body to continue into the capillaries (c) of the body. Some discontinuities (incomplete filling of resin into the capillaries) are observed (arrowheads). F vessels of the adipose tissue, ca1 a branch of the CA1 afferent vessel, ca2 a branch of the CA2 afferent vessel, t a terminal of the afferent rootlet. ×400
and accessory cells. On the average, three or four main or accessory cells were inserted between adjacent capillaries.

During the isolation of the cast extra-adrenal chromaffin bodies under the stereomicroscope, it was observed that each of the bodies received one or two afferent vessels from a branch of the right inferior diaphragmatic artery (one case), left inferior adrenal artery (one case), left first lumbar artery (two cases), or superior mesenteric artery (one case), and emitted one or two efferent vessels which drained, together with the left or right adrenal vein, into the left or right renal vein. More precisely, two bodies measured 200-250 $\mu$m in long diameter and possessed one afferent and one efferent vessels; one body measured 300 $\mu$m in long diameter and possessed one afferent and two efferent vessels; and two bodies measured 300-400 $\mu$m in long diameter and had two afferent and two efferent vessels. Regardless of the sizes of the bodies or the numbers of the afferent and efferent vessels, each afferent vessel gave off a few branches which supplied the adjacent adipose tissue, while
each efferent vessel received a few venous branches from the adjacent adipose tissue (Figs. 2, 3).

Scanning electron microscopy allowed the clear observation of the vascular casts, including their microdissected or freeze-cut forms. In all five cases observed, the origin of the afferent vessel regularly showed a marked circular constriction (Fig. 2, Inset). In each case, the afferent vessel branched, near or on the surface of the body, into a few afferent twigs which were further subdivided on the surface of the body into a few afferent rootlets (Figs. 3-6). A few terminals of the afferent rootlets were sometimes well developed, running deep into the body where they branched into capillaries (Fig. 6). Regardless of their position, the capillaries repeated divisions and anastomoses to form a rich and dense capillary network (Figs. 3-6). The capillaries were rather small and uniform in caliber. The course of each capillary was rather straight. Thus, neither wound nor looped capillaries were observed. In the freeze-cut samples, the distance between adjacent capillaries averaged about 10 µm.

The capillary network of the extra-adrenal chromaffin body converged into several efferent rootlets which were distributed beneath the surface of the body (Figs. 3-6). These rather short efferent rootlets...
gathered beneath the surface of the body into a common trunk, the afferent vessel (Figs. 3-6). No efferent rootlets creeping deep into the body were noted. The efferent vessel was as thick as the afferent vessel (Figs. 3-6). Even in those cases with two afferent and two efferent vessels, the afferent and efferent vessels showed similar calibers (Figs. 3-6). In the case of one afferent and two efferent vessels, one efferent vessel was as thick as the afferent vessel, and the other efferent vessel was markedly thin. A similar pattern may be seen in Figure 8.

Each efferent vessel immediately left the body and continued into its parent vein continuous with the renal vein (see above). The efferent vessel always maintained a certain distance from the afferent vessel. Thus, no anastomosis was detected between either vessel; neither was anastomosis found between the afferent and efferent rootlets. The surface of the efferent vessels was rather smooth. Thus, such a constriction as observed in the origin of the afferent vessel was never noted in any segment of the efferent. Also no valvular impression was observed in the efferent vessel.

The findings obtained from the cast samples, especially those with one afferent and one efferent vessels, are schematically diagrammed in Figure 7.

**DISCUSSION**

The term “extra-adrenal chromaffin body” used here may be replaced by terms such as the “sympathetic paraganglion” (Kohn, 1903; Ivanoff, 1925), “chromaffin tissue” (Hollinshead, 1937; Coupland, 1960), “epithelio-neural body” (Van Campenhout, 1946), “para-aortic body” (Coupland, 1952, 1954), “pre-aortic paraganglion” (Brundin, 1966) and “extra-adrenal chromaffin organ” (Mascorro et al., 1984). In any terminology the body can be defined as an encapsulated collection of chromaffin cells.

The present histological examination showed that the slightly reddish body between the left and right kidneys in the adult rat contains numerous chromaffin cells, and confirmed that this body is an extra-adrenal chromaffin body. The histological features of this body are similar to those of the extra-adrenal chromaffin bodies along the abdominal aorta or near the superior mesenteric artery in the fetal or newborn rat, mouse, hamster and dog (Lempinen, 1964; Mascorro and Yates, 1970, 1977; Mascorro et al., 1984).

The present scanning electron microscopy of vascular casts clearly showed that the extra-adrenal chromaffin body of the adult rat contains a rich blood vascular bed, which consists of freely anastomosing capillaries with homogeneous distribution. This dense capillary network is shown to receive one or two efferent vessels and to emit one or two efferent vessels with calibers similar to those of the afferent vessels; the capillaries inserted between the afferent and efferent vessels are all small in caliber except for short efferent rootlets. Watzka (1943) described in the human newborns how the capillaries of the extra-adrenal chromaffin bodies along the abdominal aorta or around the origin of the inferior mesenteric artery were rather large in caliber. In newborn dogs, Mascorro et al. (1984) demonstrated blood vessels in the

**Fig. 7.** A schematic diagram showing the vascular arrangement of the adult rat extra-adrenal chromaffin body (C). Arrowheads indicate the original circular constriction of the afferent vessel. A branch of an afferent vessel (CA), CE an efferent vessel, F vessels of the adipose tissue, d deep terminals of the afferent rootlets.
extra-adrenal chromaffin bodies along the abdominal aorta representing what deserved to be called sinusoids. Such thick sinusoidal capillaries were neither reproduced in casts nor observed in tissue sections in the present materials. LEMPINEN (1964) and MASCORRO and YATES (1970) observed histological sections of the extra-adrenal chromaffin bodies along the abdominal aorta or near the superior mesenteric artery in fetal or newborn rats, mice and hamsters. Although they omitted descriptions of blood vessels, the capillaries shown in their light micrographs are thin and not sinusoidal in nature (Figs. 1–8 in LEMPINEN, 1964; Fig. 1 in MASCORRO and YATES, 1970). Also, little information is available on the blood vasculatures of the extra-adrenal chromaffin bodies in adult animals and adult humans. COUPLAND (1956, 1960), who observed histological sections of extra-adrenal chromaffin bodies near the superior mesenteric artery in adult guinea pigs and rabbits, omitted a description of the blood vessels. HEBEL and STROMBERG (1976) in their text book described the extra-adrenal chromaffin body beneath the origin of the superior mesenteric artery in adult rats as being highly vascularized. HERVONEN et al. (1978) reported that, in the adult humans, well-vascularized extra-adrenal chromaffin bodies were widely distributed throughout the retroperitoneal spaces.

It has been widely accepted in humans that the
extra-adrenal chromaffin bodies mainly develop as the Zuckerkandl's organ around the origin of the inferior mesenteric artery, and that most of them, including those distributed along the abdominal aorta, disappear or disintegrate during childhood when the adrenal medulla is approaching full development (Iwanoff, 1925; Coupland, 1952, 1954; Weiner, 1975; Thompson and Gosling, 1976). In contrast to this view, Hervonen et al. (1978) have proved by the formaldehyde vapor-fluorescence method that, in human adults, many extra-adrenal chromaffin bodies with diameters of up to 1.00 mm are distributed throughout the retroperitoneal spaces, though the main body, i.e., Zuckerkandl's organ, disappears. In lower mammals, some different findings have been reported. Coupland (1956, 1960), Lempinen (1964) and Mascorro and Yates (1977) determined that in the dog, rabbit, guinea pig, rat and mouse the extra-adrenal chromaffin bodies are mainly distributed in the upper abdomen near the origin of the superior mesenteric artery, and that in the dog, rabbit and guinea pig, these main bodies persist throughout life, while in the rat and mouse, they begin to disintegrate around 3-4 weeks after birth. In the present study, we were able to demonstrate a well-developed extra-adrenal chromaffin body between both kidneys in seven out of the thirty adult rats. Thus, it is evidenced that, even in the rat, the main extra-adrenal chromaffin bodies distributed in the upper abdomen can survive throughout life.

It was considered that our stereomicroscopic dissection of living animals might have overlooked many extra-adrenal chromaffin bodies. To avert this, we carefully observed with a stereomicroscope and a scanning electron microscope twenty-three cast specimens obtained from adult rats in which a chromaffin body had failed to be identified by dissection. Through this observation we found one to three conglomerated capillary networks (100-400 \( \mu \)m in long diameter) in eighteen out of the twenty-three cast specimens. Each of these additionally found networks corresponded in structure and location to the extra-adrenal chromaffin bodies. One example is shown in Figure 8. This supplementary discovery may extend the above finding and suggest that in the rat, the extra-adrenal chromaffin bodies are widely distributed in the retroperitoneal spaces, to survive throughout life.

The adrenal medulla comprises chromaffin cells and capillaries. The present observations of cast samples show that the capillaries in the extra-adrenal chromaffin bodies are smaller and more uniform in caliber than those in the adrenal medulla, which are, as is well known, irregularly configurated and large in caliber. Moreover, the capillary network of the extra-adrenal chromaffin body is denser than that of the adrenal medulla. Our recent measurement of cast samples has shown the distance of adjacent capillaries in the adult rat adrenal medulla to average 15 \( \mu \)m. In the present materials, adjacent capillaries showed an average distance of 10 \( \mu \)m. This may suggest that in the adult rat, the extra-adrenal chromaffin body functions more actively than the adrenal medulla in the production or storage of catecholamines. By autoradiography, Coupland et al. (1982) studied the extra-adrenal chromaffin body and adrenal medulla in young rabbits and guinea pigs, and disclosed that the extra-adrenal chromaffin body showed a minimal decrease in labeled amine (DOPA) and that the amine content and concentration in this body doubles that in the adrenal medulla.

It has been well accepted that the biosynthesis of adrenaline from noradrenaline (or activation of N-methyl transferase) needs the aid of glucocorticoids (Wurtman and Axelrod, 1966; Coupland and McDougall, 1966; Pohorecky and Wurtman, 1971). This suggests that the extra-adrenal chromaffin body without direct contact with the adrenal cortex preferentially produces or stores noradrenaline. In fact, Coupland and Weakley (1970), observing tissue sections of a newborn rabbit with a transmission electron microscope, showed that the chromaffin cells in the extra-adrenal chromaffin body contained mainly (90% or more) noradrenaline-storing granules. Similar results were obtained in adult humans by Hervonen et al. (1978, 1979), who used the formaldehyde vapor-fluorescence method. Phillippe (1983) proved biochemically that the major catecholamine produced by the extra-adrenal chromaffin body in human fetuses is noradrenaline. Black (1978) showed in newborn dogs that exogenous steroids retarded the involution of chromaffin cells and increased the total number of these cells. Bohn et al. (1982) proved by immunooassay that the chromaffin cells in the extra-adrenal body of newborn rats, like those in the superior sympathetic cervical ganglion, contained N-methyl transferase. These data support the view that glucocorticoids secreted from the adrenal cortex can act, via general circulation, upon the extra-adrenal chromaffin cells producing adrenaline.

The circular constriction demonstrated at the origin of the afferent vessel of the extra-adrenal chromaffin body probably represents an endothelial cushion, which may either control the inflow of blood into the body or inhibit the retrograde blood flow from the body. A more typical circular constriction has been
observed at the origin of the afferent vessel of the rat carotid body (Taguchi, 1986) or the primary plexus of the rat hypophyseal portal system (Murakami et al., 1987). In contrast to the dense capillary networks of the extra-adrenal chromaffin bodies, their efferent vessels are rather thin, and not thicker than the afferent vessels. This may cause some congestion of blood in the bodies, giving them a reddish coloration under the stereomicroscope (see above). This congestion or slow blood flow may be useful for the chromaffin cells in the bodies to take up sufficient materials from the blood in order to synthesize the amines and neuropeptides such as enkephalins and somatostatin (Saito et al., 1982; Martin, 1985).

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