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CLOSE RELATIONSHIP BETWEEN CORTICAL AND MEDULLARY CELLS IN THE FETAL MOUSE ADRENAL: MORPHOLOGICAL EVIDENCE AND FUNCTIONAL IMPLICATIONS

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In adult adrenal glands, the close cellular contact between the cortical and medullary cells has been proposed as a morphological basis for the paracrine regulations between these two endocrine tissues. To address this issue in the fetal adrenal gland at late gestation, we characterized the cellular contact of cortical and medullary cells in the fetal mouse adrenal by electron microscopy. From embryonic day 16 (E16) to E18, isolated medullary cells or islands of medullary cells could often be detected in the cortical area and strands or clusters of cortical cells were found intermingled with medullary cells in the centralized medullary region. In these regions, cortical and medullary cells were in direct contact without intervening fibrous tissue or vessels. We also found that the secretory vesicles in the medullary cells positioned close to the cell membrane opposing the cortical cells, and that filopodia from the cortical cells extended to the medullary cells, suggesting direct intercellular message exchange. These findings suggest a paracrine interaction between the cortical and medullary cells in the fetal adrenal gland similar to that in the adult.

Key words : adrenal cortex / adrenal medulla / mouse embryos / ultrastructure / paracrine

The adrenal cortex and medulla are derived from separate embryonic origins and have conventionally been regarded as two functionally independent endocrine systems. Recently, however, the evidence is increasing concerning the functionally bi-directional interactions and crosstalk between these two functional units (1-7).

Adrenocortical steroids are known to regulate the medullary enzymes synthesizing catecholamines (1). Also, glucocorticoids may directly affect the secretion of adrenaline (2, 3). More strikingly is the postulated influence of the medullary cells on the adrenal cortex (4-7). Previous publications have addressed both stimulatory and inhibitory effects of different catecholamines on adrenocortical functions (4-7). β-adrenergic receptors have demonstrated to be within the rat adrenal cortex (6). This suggests that these catecholamines act on the adrenocortex through β-adrenergic receptors. Moreover, experimental evidence suggests that cytokines such as IL-1, IL-6 and TNFa in the medulla may regulate functions of the cortex (7).

How products from the each adrenal part could reach the other has been an intriguing question. It has been suggested that corticosteroids reach the medulla via the venous effluent from the cortex and, thus, may influence medullary functions at least partially via the humoral regulation (8). On the other hand, there is no morphological evidence supporting a humoral effect of the adrenal medulla on the cortex via the vascular system. Recently, it has been proposed that the medullary and cortical cells influence each other in a paracrine manner (7, 9). Investigations have demonstrated that there are close cellular contacts of the medulla cells with the cortical cells in many species (9-12). These data suggest that this intimate contact is prerequisite for such a paracrine regulation.

However, to date, this issue has been mainly investigated in adults (9-12) and has not yet been addressed in the fetal stage. In our previous study, after continuous ACTH stimulation (13), we observed that bromo-deoxyuridine (BrdU)-positive cells significantly increased in number and the

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expression of tyrosine hydroxylase appeared to decrease in the adrenal medullary tissues, and smooth endoplasmic reticulum (SER) became swollen in the adrenal cortical cells. These findings suggest that the pituitary-adrenal axis may affect the medulla and, conversely, the changed medulla may also influence the cortex in the fetal adrenal gland (13). We also showed some preliminary observations of close contact between the cortical and medullary cells at the fetal stage (13). To gain further insight into this issue, we investigated the cellular contact between the adrenal cortical and medullary cells in late-gestation fetal mice by transmission electron microscopy (TEM).

MATERIALS AND METHODS

Jcl:ICR mice (CLEA Japan, Tokyo) between 10 and 16 weeks of age were used. The presence of a vaginal plug on the morning after mating indicated embryonic day 0 (E0). Adrenal glands of embryos on E16 to E18 (two to three embryos from each group) were dissected and fixed by immersion into 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Specimens were rinsed in 0.1 M phosphate buffer containing 5% sucrose, postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in epoxy resin. Ultrathin sections (70 nm) were double-stained with uranyl acetate and lead citrate and examined with a JEOL JEM-1200EX TEM.

RESULTS

The cortical cells were characterized by the typical mitochondria with vesicular cristae, ample smooth endoplasmic reticulum and lipid droplets (Figs. 1, 2 and 3). The medullary cells were identified by their characteristic rough endoplasmic reticulum (RER) and electron-dense secretory vesicles which are supposed to correspond to chromaffin vesicles by histochemistry (Figs. 1, 2 and 3).

During the late gestation (E16 to E18), ongoing migration of medullary cells could still be observed (Fig. 2a). The migrating medullary cells were scattered in the cortical area and isolated medullary cells or islands of the medullary cells intermingled with the cortical cells (Fig. 2a). In the centralized medullary area, cortical cells intermingled or interlocked with the medullary cells (Fig. 2b).

In the investigated area, cortical and medullary cells always contacted each other directly without intervening fibrous tissue or vessels (Fig. 1). The close distances between the membranes of two types of cells were 24.7 ± 5.8 nm in average (10

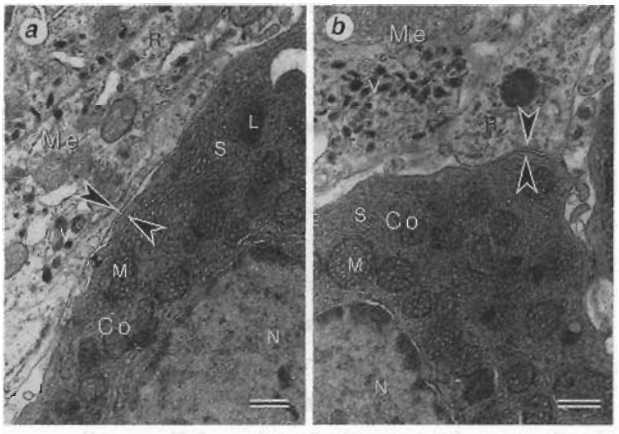

Fig. 1. Electron micrographs of the fetal adrenal on E17 (a) and E18 (b). The cortical cells and medullary cells directly contact each other without intervening fibrous tissue or vessels (arrowheads). Co: cortical cell; Me: medullary cell; M: mitochondria; S: SER; L: lipid droplet; R: RER; V: secretory vesicle; N: nucleus. Bars = 500 nm.

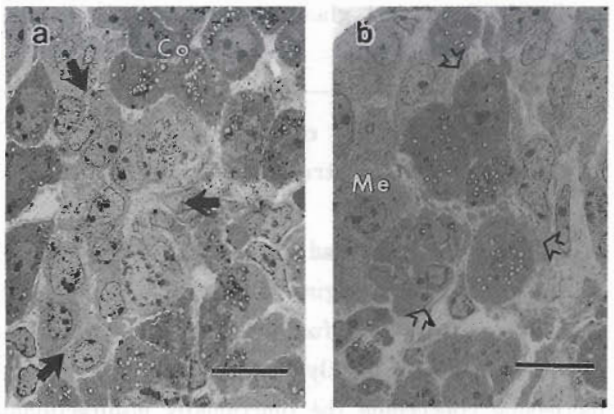

Fig. 2. Electron micrographs of the mouse fetal adrenal on E17. a. Migrating medullary cells (dark arrows) in the cortical area. b. Cortical cells (open arrows) interlocked with medullary cells within the centralized medullary area. Co: cortical cell; Me: medullary cell. Bars = 2 μm.

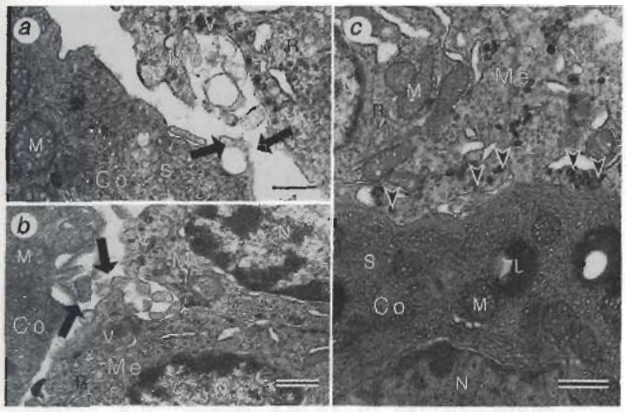

Fig. 3. Electron microscopy of the fetal adrenal on E16 (a), E17 (b) and E18 (c). Filopodia (arrows) from the cortical cells extend to medullary cells (a and b) and some secretory vesicles (arrowheads) are positioned close to the cell membrane opposing the cortical cells (c). Co: cortical cell; Me: medullary cell; M: mitochondria; S: SER; L: lipid droplet; R: RER. Bars = 500 nm.

samples, arrowheads in Fig. 1).

Filopodia (arrows in Fig. 3a and b) from cortical cell extended to medullary cells and some secretory vesicles (arrowheads in Fig. 3c) were positioned close to the cell membrane opposing the cortical cells.

DISCUSSION

The present study demonstrates that the cellular contact between cortical and medullary cells does occur in the fetal adrenal gland at late gestation and also suggests some possible functional implications.

In adults, the major contact between the two types of cells have been found in the medulla although isolated single medullary cells or islets of medullary cells can also be observed sometimes in the cortex (10-12). In fetal stage, since the migration of medullary cells, which are of neural crest origin, to the center part of the gland, the final site of the medulla, are underway, anatomical relationship between cortical and medullary cells could be fallen into two categories. 1) The medullary cells which were still migrating to the center part of the gland scattered in the cortical area and contacted with cortical cells. 2) Strands of residual cortical cells at the center part of the gland intermingled with medullary cells which had reached the center medullary region. However, the mode of cellular contact, as deduced by TEM and described below, looked similar in both cases.

TEM studies clearly show that cortical cells and medullary cells contact each other directly without fibrous tissues or vessels. The close contact of these two different types of cells in the fetal adrenal gland may suggest the mutual regulation of its function. Interestingly, we found that the secretory vesicles positioned close to the cell membrane opposing the cortical cells and filopodia from the cortical cells extended to the medullary cells. These findings further imply a possible paracrine action between the cortical and medullary cells in the fetal adrenal gland as that in adult one (9-12).

Up to now, the intimate close contact between the cortical and medullary cells have been reported in the adult rat, bovine, porcine and human adrenal gland (9-12), but there has been no relevant report in mice or in embryos. Our findings in mice may be important since mice have become an important experimental animal species for molecular genetic studies in mammalian development due to feasibility of conducting genetic manipulations such as transgenic and knockout techniques, and also due to much more extensive genetic and molecular information in this species than in other mammalian species (14). Thus, we have provided the basic information for our further studies of the fetal adrenal gland in molecular genetic aspects (15).

The fetal adrenal gland performs a series of functions for maintaining intrauterine homeostasis, preparing fetuses for birth and postnatal adaptation (16, 17). Unlike that of adults, the knowledge of crosstalk between the adrenal cortical and medullary cells at the fetal stage is limited. Our findings offer morphological basis for functional crosstalk between the fetal adrenocortical and medullary cells, and suggest that the colocalization of catecholamine-secreting and steroid-producing cells under a common capsule may, via their paracrine regulations, coordinate to the series of actions during this important stage of life.

REFERENCES

1) Wurtman RJ and Axelrod J (1996) Control of enzymatic synthesis of adrenaline in the adrenal medulla by the adrenal cortical steroids. *J Biol Chem* 241: 2301-2305

2) Wurtman RJ and Axelrod J (1965) Adrenaline synthesis: control by the pituitary gland and adrenal glucocorticoids. *Science* 150: 1464-1465

3) Ungar A and Philips JH (1983) Regulation of the adrenal medulla. *Physiol Rev* 63: 787-843

4) Ehrhart-Bornstein M, Bornstein SR and Trzeciak WH (1991) Adrenaline stimulates cholesterol side chain cleavage cytochrome P450 mRNA accumulation in bovine adrenocortical cells. *J Endocrinol* 131: R5-R8

5) Guse-Behling H, Ehrhart-Bornstein M, Bornstein SR, Waterman MR, Bockmann M, Scherbaum WA and Alder G (1992) Regulation of adrenal steroidogenesis by adrenaline: Expression of cytochrome P450 genes. *J Endocrinol* 135: 229-237

6) Shima S, Komoriyama K, Hirai M and Kouyama H (1984) Studies on cyclic nucleotides in the adrenal gland. XI. Adrenergic regulation of adenylate cyclase activity in the adrenal cortex. *Endocrinology* 114: 325-329

7) Hinson JP (1990) Paracrine control of adrenocortical function: a new role for the medulla? *J Endocrinol* 124: 7-9

8) Vinson GP, Pudney J and Whitehouse BJ (1985) The mammalian adrenal circulation and the relationship between adrenal blood flow and steroidogenesis. *J Endocrinol* 105: 285-294

9) Gallo-Payet N, Pothier P and Isler H (1987) On the presence of chromaffin cells in the adrenal cortex: their possible role in adrenocortical function. *Biochem Cell Biol* 65: 588-592

10) Bornstein SR, Gonzalez-Hernandez JA, Ehrhart-Bornstein M, Adler G and Scherbaum WA (1994) Intimate contact of chromaffin and cortical cells within the human adrenal gland forms the cellular basis for important intraadrenal interactions. *J Clin Endocrinol Metab* 78: 225-232

11) Bornstein SR, Ehrhart-Bornstein M, Usadel H, Bockmann M and Scherbaum WA (1991) Morphological evidence for a close interaction of chromaffin cells with cortical cells within the adrenal gland. *Cell Tissue Res* 265: 1-9

12) Bornstein SR and Ehrhart-Bornstein M (1992) Ultrastructural evidence for a paracrine regulation of the rat adrenal cortex mediated by the local release of catecholamine from chromaffin cells. *Endocrinology* 131:3126-3128

13) Zhang H, Hatta T, Udagawa J, Ma L, Naora H, Hashimoto R and Otani H (1998) Fetal adrenocortex-medulla interactions and changes of pituitary in mouse embryos with an ectopic corticotrophic tumor. *Endocr Res* 24: 947-951

14) Marth JD (1996) Molecular medicine in genetically engineered animals. *J Clin Invest* 97: 1999-2002

15) Zhang H, Hatta T, Udagawa J, Moriyama R, Hashimoto R and Otani H (1998) Induction of ectopic corticotropic tumor in mouse embryos by *exo utero* cell transplantation and its effects on the fetal adrenal gland. *Endocrinology* 139: 3306-3315.

16) Lanman JT (1977) Parturition in nonhuman primates. *Biol Reprod* 16: 28-37

17) Pepe GJ and Albrecht ED (1995) Actions of placenta and fetal adrenal steroid hormones in primate pregnancy. *Endocr Rev* 16: 608-648