

[Review Article]

Cholinergic Receptor-Mediated Secretion Mechanism in Adrenal Chromaffin Cells during Development

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[Abstract]

This article reviews when the cholinergic receptor-mediated secretion mechanism of developing adrenal chromaffin cells is expressed and becomes functional, morphological changes. Sympatho-adrenal progenitor cells constituted a major lineage among neural crest derivatives. These give rise to sympathetic neurons and endocrine chromaffin cells. Adrenal chromaffin cells are homologous to sympathetic ganglia, which are innervated by preganglionic cholinergic nerves. Developing adrenal chromaffin cells synthesized, first noradrenaline alone in the cells, subsequently noradrenaline (NA) and adrenaline (A) in single cell (mixed cell), finally NA in NA cells and A in A cells, respectively. Cholinergic marker proteins (acetylcholinesterase, choline acetyltransferase and vesicular acetylcholine transporter) reactive nerve fibers were no or very few in the adrenal medulla during embryonic development, and increased dramatically during early postnatal development. Developing intra-adrenal cholinergic nerve fibers contained, first no or very few clear vesicles, subsequently numerous clear vesicles and a few dense-cored vesicles. Cholinergic stimulation induced a secretion of catecholamines from chromaffin cells by exocytosis via muscarinic- and nicotinic-receptor. Developing adrenal chromaffin cells after cholinergic stimulation showed no response, subsequently appeared first nicotinic-receptor mediated secretion and then, muscarinic receptor- and nicotinic receptor-mediated secretion or nicotinic receptor-mediated alone secretion. Taken together, morphological changes of the developing chromaffin cells and the intra-adrenal cholinergic nerve fibers were related to the expression of a cholinergic receptor-mediated secretion mechanism and that this mechanism via a nicotinic receptor-mediated secretion preceded the muscarinic receptor-mediated one during development.

[Key words] cholinergic innervation, cholinergic receptor, adrenal chromaffin cells, development, secretion

Introduction

Sympatho-adrenal precursor cells originate from the neural crest (Hammond and Yntema, 1947; LeDouarin, 1984) and give rise to sympathetic ganglia and to endocrine chromaffin cells (Landis and Patterson, 1981). Thus, chromaffin cells have common morphological and physiological characteristics with sympathetic ganglia (Hervonen 1971; Fenwick et al. 1978; Coupland et al. 1980; Carmichael and Winkler

1985).

In most mammals adrenal chromaffin cells synthesize noradrenaline (NA) and adrenaline (A) as hormones. NA and A are derived from common precursor molecules (**Fig.1a**). These two different types of adrenal chromaffin cells are involved in the synthesis of NA and A, i.e. the NA and A cells. Previous cytochemical, electron microscopic and biochemical studies have shown the evidences for two cell types. The immunohistochemistry by using

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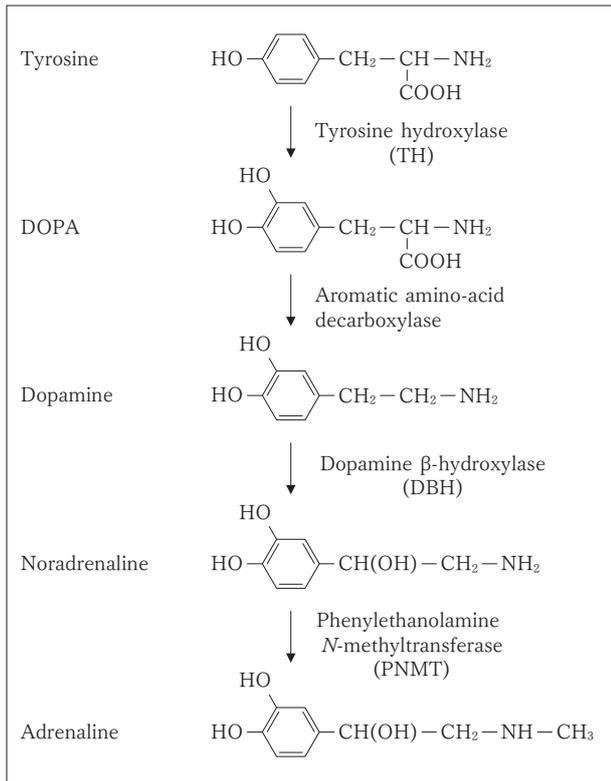


Fig.1a Biosynthetic pathway of noradrenaline and adrenaline in the adrenal medulla. Four enzymes are involved : tyrosine hydroxylase (TH), aromatic amino-acid decarboxylase (AADC), dopamine β -hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT).

specific antibodies for NA, A and related synthesizing enzymes tyrosine hydroxylase (TH), aromatic amino-acid decarboxylase (AADC), dopamine beta-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) have been used and examined in the prenatal and postnatal development of NA and A cells in various mammals (Teitelman et al., 1979; Verhofstat et al. 1979, 1985, 1989; Bohn et al., 1981; Dagerlind et al., 1993; Oomori et al., 1989a,b, 1991a, 1994, 1998; Iwasa et al., 1999a). Furthermore, previous immunohistochemical studies have demonstrated various transmitters and synthesizing enzymes of vasoactive intestinal polypeptide, neuropeptide-tyrosine, enkephalin, substance P, somatostatin, calcitonin gene related peptide, neurotensin, 5-hydroxytryptamine (serotonin), histamine, nitric oxide synthase, glutamate decarboxylase and gamma-aminobutyric acid in the chromaffin cells, intra-adrenal ganglion cells and nerve fibers of the various mammals by using specific antibodies (see review, Kondo, 1985; Bryant et al.,

1976; Schultzberg et al., 1978; Lundberg et al., 1979; Hökfelt et al., 1981; Rosenfeld et al., 1983; Varndell et al., 1984; Kuramoto et al., 1985, 1986; Höppölä et al., 1985; Ahonen et al., 1989; Oomori et al., 1993; 1994; Afework et al., 1994; Iwasa et al., 1998, 1999b; Murabayashi et al., 2007, 2009a,b).

Adrenal chromaffin cells produce and secrete a large amount of catecholamines under the control of preganglionic cholinergic nerves, whose cell somata are located in the intermediate gray matter of the lower thoracic and upper lumbar spinal cord (Coupland, 1965; Kesse et al., 1988). It is known that choline acetyltransferase (ChAT), vesicular acetylcholine transporter (VAcHT), and acetylcholinesterase (AChE) are concerned with acetylcholine synthesis, the filling with clear vesicles, and its hydrolysis, respectively (Fonnum, 1966; Roghani et al., 1994). Previous studies revealed cholinergic innervation to chromaffin cells in the prenatal and postnatal development of adrenal medulla by using electron microscopy, AchE-histochemistry, ChAT- and VAcHT-immunohistochemistry and acetylcholine biochemistry (Allen et al., 1958; Henion and Landis, 1990; Tomlinson and Coupland, 1990; Ahonen, 1991; Holgert et al., 1994; Arvidsson et al., 1997). Acetylcholine from cholinergic nerve fibers is a physiological stimulus which activates acetylcholine receptors in adrenal chromaffin cells. Muscarinic and nicotinic cholinergic agonists combine with muscarinic or nicotinic receptors in the chromaffin cell membrane to induce the release of the catecholamines in the secretory granules by exocytosis (Carmichael and Stoddard, 1993). In the secretion of the catecholamines from chromaffin cells by exocytosis, an increase in the cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_c$) after stimulation with cholinergic agonists plays a key role in regulating secretion from the adrenal chromaffin cells (Douglass, 1968; Burgoyne, 1991). In the adrenal chromaffin cells, membrane depolarization by nicotine results in extracellular Ca^{2+} entry (Douglas et al., 1967) whereas muscarinic agonist mediates Ca^{2+} mobilization independent of Ca^{2+} entry (O'Sullivan et al., 1989; Burgoyne, 1991; Harish et al., 1987; Neely and Lingle, 1992). On the other hand, the effect of acetylcholine on the developing cells is likely to

depend on the subclass of acetylcholine receptor that is functionally expressed during the developmental stages. The expression of the specific receptors appears to be closely linked in time with the onset of certain functions during development (Roßner et al., 1993). However, it remains to be clarified when the cholinergic receptor-mediated secretion mechanism of the developing chromaffin cells is expressed and becomes functional or how muscarinic and nicotinic responses correlate with the developmental state of the chromaffin cells.

The present article reviews digital Ca^{2+} imaging techniques, histochemistry, immunohistochemistry and electron microscopy in the adrenal chromaffin cells and cholinergic nerve fibers in the course of developmental stages, and reports the ontogenetic maturation of muscarinic and nicotinic receptor-induced Ca^{2+} dynamics with reference to the secretion mechanism of the rat adrenal chromaffin cells.

I. Adult adrenal chromaffin cells and cholinergic innervation

1. Adult chromaffin cells

In the adrenal medulla of adult animals two different types of chromaffin cells could be distinguished synthesizing and storing NA and A respectively, i.e., the NA- and A-storing cells (Hillarp and Hökfelt, 1954; Klein and Kracht, 1957; Eränkö, 1960; Palkama, 1962; Coupland, 1971). The chromaffin cells have been divisible into NA and A cells in various mammals by immunohistochemistry using specific antibodies for NA, A and synthesizing enzymes, DBH, PNMT (**Fig.1b,c**) (Teitelman et al., 1979; Verhofstat et al. 1979, 1985, 1989; Bohn et al., 1981; Dagerlind et al., 1993; Oomori et al., 1989a,b, 1991a, 1994, 1998; Iwasa et al., 1999a). At electron microscopic level, in material treated sequentially with glutaraldehyde and dichromate, NA inclusion granules were highly electron-dense while A granules were of low electron density by comparison (**Fig.1d**) (Coupland and Hopwood, 1966; El-Maghraby and Lever, 1980). Among mammals, remarkable differences in the number and intramedullary distribution of the NA and A cells (ratio of NA cells/A cells: 5~30%)

have been described (Hökfelt, 1951; Eränkö, 1955; Palkama, 1962; Coupland, 1975; Verhofstat et al., 1985). Furthermore, evidences of exocytotic figures of omega profiles in the cytoplasmic membrane of the adrenal chromaffin cells and pheochromocytoma cells after stimulation of secretagogues by electron microscopic levels (Benedeczky and Smith, 1972; Benedeczky and Somogyi, 1975; Brooks and Carmichael, 1987; Oomori et al., 1991b, 1998; Wick et al., 1997). Physiological studies have been confirmed that muscarinic and nicotinic agonists induced catecholamine release from the adrenal chromaffin cells of various mammals including the rat (Feldberg et al., 1934; Douglas and Poisner, 1965; Role and Perlman, 1983; Wakada and Wakada, 1983; Cheek et al., 1989; Warashina et al., 1989). We also found the omega profiles indicative of exocytosis in the cell membrane of the adult rat chromaffin cells after carbamylcholine stimulation and in the isolated rat chromaffin cells after stimulation with methacholine and nicotine (**Fig.1f**) (Oomori et al., 1998). Thus, muscarinic receptor-mediated Ca^{2+} signal transduction pathway related to the catecholamine secretion in the rat chromaffin cells is as important as the nicotinic one. However, nicotine induced a rise of $[\text{Ca}^{2+}]_c$ and catecholamine release in bovine adrenal chromaffin cells whereas muscarinic agonist induced a rise of $[\text{Ca}^{2+}]_c$, without effecting catecholamine release. It has been assumed that the modest elevation of $[\text{Ca}^{2+}]_c$ elicited by muscarinic drugs is insufficient to trigger the exocytotic machinery (Cheek et al. 1989).

Previous physiological studies have shown an induced rise of $[\text{Ca}^{2+}]_c$ in chromaffin cells after stimulation by muscarinic and nicotinic agonists (O'Sullivan et al., 1989; Burgoyne, 1991; Harish et al., 1987; Neely and Lingle, 1992; Busik et al., 1994). Nicotine stimulation induced mainly an influx of extracellular Ca^{2+} via voltage-dependent calcium channel of chromaffin cells whereas muscarinic agonist induced mainly a release of Ca^{2+} from the intracellular stores via inositol 1,4,5-triphosphate (IP_3) receptor (Burgoyne, 1991). Chromaffin cells have an IP_3 -sensitive calcium store (Stoehr et al. 1986; Cheek et al., 1991). Furthermore, a previous study has suggested that the $[\text{Ca}^{2+}]_c$ in chromaffin

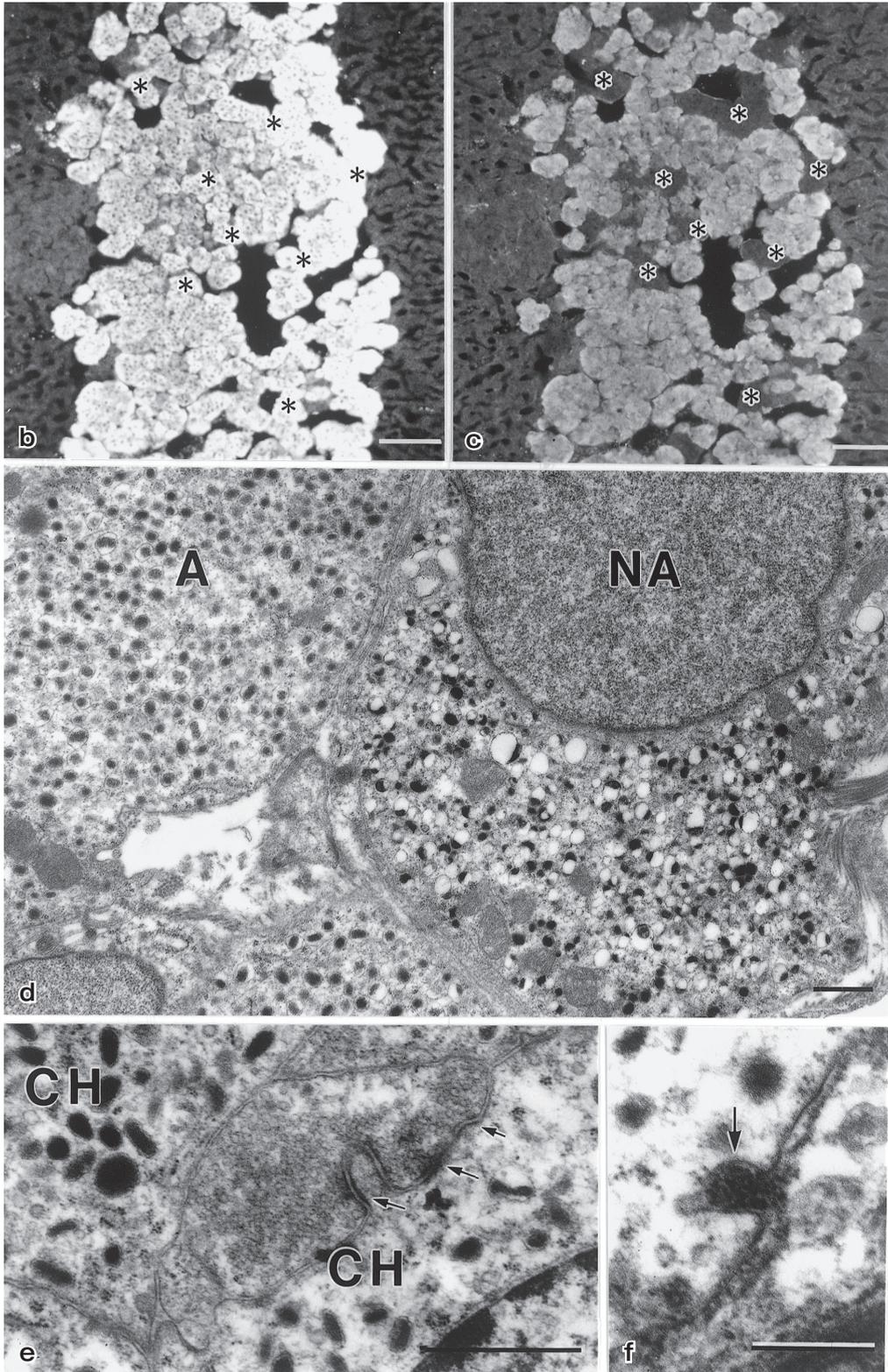


Fig.1b-f. Postnatal-8-week adult rat adrenal medulla. Immunofluorescent micrographs of dopamine β -hydroxylase (DBH) immunoreactivity (b) and phenylethanolamine N-methyltransferase (PNMT) immunoreactivity (c) in the same section. Both DBH and PNMT immunoreactivities reveal adrenaline (A) cells, whereas only DBH immunoreactivity reveals noradrenaline (NA) cells (asterisks)..

d Adrenaline cell (A) contains numerous granules with intermediate electron-density. Noradrenaline cell (NA) contains granules of highly electron-density in variably-sized vacuoles. **e** Nerve fiber containing many clear vesicles and a few dense-cored vesicles is closely apposed to the chromaffin (CH) cells. Increased membrane density (arrows) between nerve fiber and chromaffin cell. **f** Exocytotic omega profile (arrow) in membrane of the chromaffin cell. X200 (b,c,b), X10000 (d), X23000 (e), X40000 (f), Bar:50 μ m (b,c) Bar:1 μ m (d,e), Bar:0.5 μ m (f)

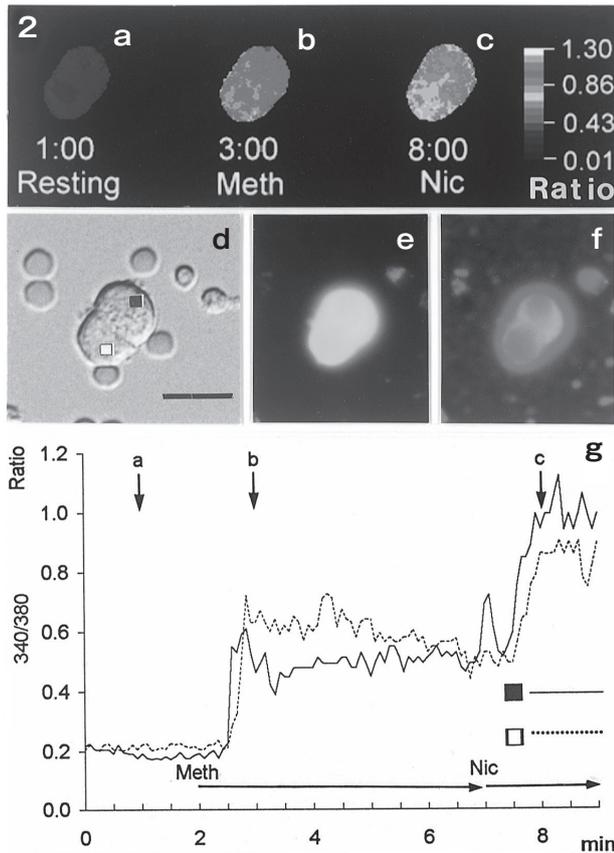


Fig.2a-g Postnatal-8-week (adult) rat adrenal chromaffin cells stimulated by 200 μ M methacholine (Meth) and 10 μ M nicotine (Nic). Pseudocolor ratio images of Ca^{2+} dynamics, (a-c), differential interference-contrast image (d), and immunofluorescent images of DBH (e) and PNMT (f). g Time course of $[\text{Ca}^{2+}]_c$ in selected regions (white square and black square in d) of the chromaffin cells (a 1min 00s, b 3min 00s, c 8min 00s). Methacholine and nicotine induce moderate and high rises of $[\text{Ca}^{2+}]_c$ in chromaffin cells showing both DBH and PNMT immunoreactivities. X500. Bars:20 μ m

cells response to methacholine may consist of two phases: an initial phase of Ca^{2+} elevation, probably due to Ca^{2+} release from intracellular stores, and an ensuing phase due to Ca^{2+} entry (Busik et al.1994).

We uncovered two response patterns of $[\text{Ca}^{2+}]_c$ in the adult adrenal chromaffin cells of rat after stimulation with muscarinic and nicotinic agonists (Oomori et al., 1998). Most cells showed a changes of $[\text{Ca}^{2+}]_c$ after stimulations by methacholine and nicotine, while a few cells showed a change of $[\text{Ca}^{2+}]_c$ after nicotine stimulation alone (Figs.2, 3) (Oomori et al., 1998). Our immunohistochemical study showed that these chromaffin cells, although showing different response patterns of $[\text{Ca}^{2+}]_c$ after

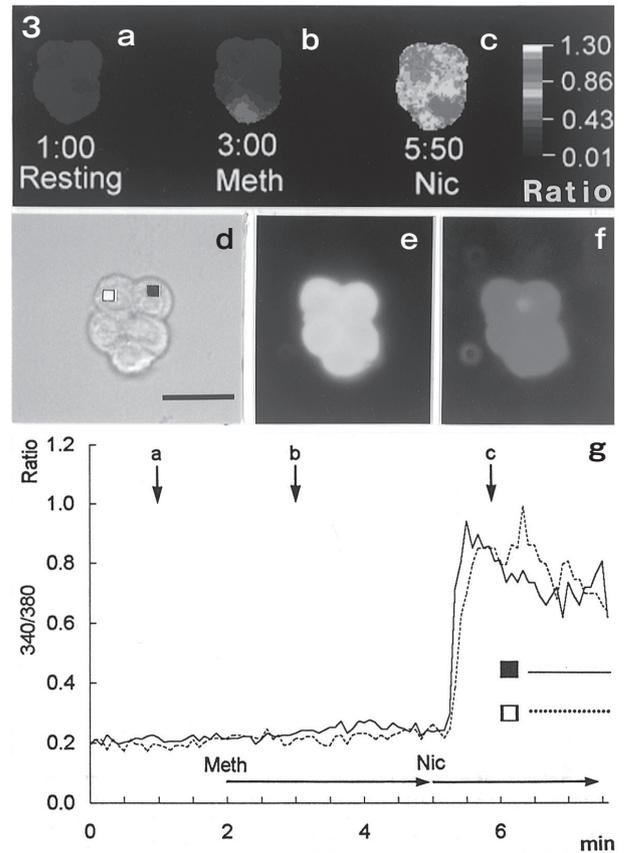


Fig.3a-g Postnatal-8-week (adult) rat adrenal chromaffin cells stimulated by 200 μ M methacholine (Meth) and 10 μ M nicotine (Nic). Pseudocolor ratio images of Ca^{2+} dynamics, (a-c), differential interference-contrast image (d), and immunofluorescent images of DBH (e) and PNMT (f). g Time course of $[\text{Ca}^{2+}]_c$ in selected regions (white square and black square in d) of the chromaffin cells (a 1min 00s, b 3min 00s, c 5min 00s). Methacholine induces no rise of $[\text{Ca}^{2+}]_c$, and nicotine alone induces a high rise of $[\text{Ca}^{2+}]_c$ in chromaffin cells showing both DBH and PNMT immunoreactivities. X500, Bars:20 μ m

the stimulation, were immunoreactive for both DBH and PNMT and thus identified as A cells (Figs.2, 3) (Oomori et al., 1998). These results suggest that muscarinic and nicotinic receptors-mediated Ca^{2+} signal transduction pathway may function in most A cells, while a few A cells may lack muscarinic receptor or, if present, contain immature receptors or an immature transduction pathway. As mitoses have been shown in a few chromaffin cells of adrenal medulla, including that of the rat (Malvaldi et al. 1968; Tischler et al. 1988), it is possible that A cells with nicotinic receptor alone are newly generated chromaffin cells. We could not analyse the change in $[\text{Ca}^{2+}]_c$ in NA cells. Although we observed very

few NA cells with DBH-immunoreactivity alone after digital imaging, the cell number was less than 1% of the total from postnatal 1 week onwards (Oomori et al., 1998). It may thus be more difficult to isolate NA cells than A cells with the collagenase procedure.

Regarding cholinergic receptor-related catecholamine secretion in chromaffin cells, activation by muscarine or pilocarpine leads to a preferential secretion of A in the cat adrenal gland, whereas nicotine causes a release in both NA and A cells (Douglas and Poisner, 1965). These findings suggested the possibility that muscarinic receptors are preferentially expressed on A cells, whereas nicotinic receptors are distributed on A and NA cells. In contrast, by binding experiments using tritiated quinuclidinyl benzylate (muscarinic agonist) to partially purified membranes from NA cells and A cells, it was learned that muscarinic receptors are present in both cell types, and NA cells contained a 2- to 3-fold greater density of these receptors (Michelena et al. 1991). Although we showed two types of chromaffin cells immunohistochemically in the cryostat sections of the adult adrenal medulla, we could not determine which receptors were present in the NA cells (Oomori et al., 1998). The kind of cholinergic receptors present in the NA cells needs further investigation by digital imaging and immunocytochemistry.

To date, 17 nicotinic acetylcholine receptor subunits have been identified, which are divided into muscle-type and neuronal-type subunits. Of these 17 subunits, $\alpha 2$ - $\alpha 7$, and $\beta 2$ - $\beta 4$ have been cloned in humans, the remaining genes identified in chick and rat genomes (Graham et al, 2002). Based on reverse transcription-polymerase chain reaction results, subtypes of nicotinic receptor including $\alpha 1$ - $\alpha 5$, $\alpha 7$, $\beta 2$ and $\beta 4$ were present in human, rat, mouse adrenal medulla (Mousavi et al. 2001; Wu et al. 2010). For muscarinic receptor, all subtypes of muscarinic receptor (M1-M5) transcripts were present in mouse (Wu et al. 2010). The mRNA transcripts provide evidence for expression of M1, M2 and M4 subtypes in bovine chromaffin cells (Fernando et al. 1991; Aguilar et al. 1992) and for the expression of M3 and M4 in rat adrenal glands (Fernando et al. 1991; Barbara et al. 1998).

2. Adult cholinergic innervation

In the adult adrenal medulla, AChE-active, ChAT- and VChAT-immunoreactive nerve fibers were numerous and densely contacted the chromaffin cells (Allen et al., 1958; Holgert et al, 1994; Oomori et al., 1994; Arvidsson et al., 1997; Iwasa et al., 1999; Murabayashi et al., 2009a). By using formaldehyde-induced fluorescence (FIF) method, AChE-active, ChAT- and VChAT-immunoreactive nerve fibers contacted NA cell areas showing FIF were more numerous than those contacted A cell areas in the rat and mouse adrenal medulla (**Figs.17, 22**) (Iwasa et al., 1999a; Murabayashi et al., 2009a). The number of varicosities of ChAT-immunoreactive nerve fibers in NA cell areas had more approximately twice than that of the nerve fibers in A cell areas (Oomori et al., 2013). AChE-positive nerve fibers, ChAT-, VChAT- and neurocalcin immunoreactive nerve fibers densely contacted NA cell groups compared to A cell groups (Allen et al., 1958; Eränkö 1959; Lewis and Shute 1968; Iino et al., 1997; Iwasa et al., 1999a; Murabayashi et al., 2009a). These findings showed that A cells and NA cells differ in degree of innervation and type of nerves. It has been demonstrated that distinct preganglionic neurons innervate NA cells and A cells in the adrenal medulla (Edwards et al. 1996). Nicotine and high K⁺ cause a greater secretion of NA than A (Douglas and Poisner 1965; Maley and Livett 1987). Thus, these differential secretions of A and NA may be differential cholinergic innervation between A and NA cells. Furthermore, electron microscopic studies showed that most intra-adrenal nerve fibers contained numerous clear vesicles and a few dense-cored vesicles and contacted chromaffin cells in various mammals (**Fig.1e**) (Tomlinson and Coupland, 1990; Oomori et al., 1998). The synaptic endings showed asymmetrical membrane thickenings (Tomlinson and Coupland, 1990; Oomori et al., 1998). The mean number of synapses per cells at postnatal 12 week and 22 week rats was 5.4/5.4, 4.7/5.4 on A and NA cells, respectively (Tomlinson and Coupland, 1990).

II. Developing adrenal chromaffin cells and cholinergic innervation

1. Developing chromaffin cells

The immunohistochemical and biochemical data presented seem to indicate that during histogenesis of the adrenal medulla three main phases can be distinguished in a gradual developmental process: (1) synthesis and storage of almost exclusive NA; (2) synthesis and storage of high concentrations of NA as well as A in a single cell type (“mixed cell type”); (3) synthesis and storage of NA and A in separate cell types (**Fig.4a-h**) (Verhofstat et al., 1979, 1985; Oomori et al., 1998).

Our digital imaging analysis showed that no change $[Ca^{2+}]_c$ of almost all chromaffin cells at the embryonic day 16 after stimulation of methacholine and nicotine (**Fig.7**) (Oomori et al., 1998). These results suggest that embryonic chromaffin cells may have immature receptor sites or immature signal transduction pathway. However, we also observed very few exocytotic figures in the plasma membrane of embryonic cells with or without stimulation of carbamylcholine stimulation, few contacts between the nerve fibers and chromaffin cells, and few clear and dense-cored vesicles in the nerve terminals (**Fig.5a,b**). Previously, the exocytosis in the chromaffin cells was observed on every embryonic day (Millar and Unsicker, 1981). So few exocytotic figures in the chromaffin cells suggest that the embryonic chromaffin cells with sparse granules had discharged spontaneously by exocytosis despite the immature state of neural transmission. Under these conditions, the secretion mechanism mediated via cholinergic receptors and the cholinergic transmission and synaptogenesis between chromaffin cells and nerve fibers may indicate functional immaturity.

Our digital analysis also showed that nicotine stimulation alone induced a rise of $[Ca^{2+}]_c$ in numerous cells at the embryonic day 19 (**Fig.8**) (Oomori et al., 1998). This suggests that a nicotinic receptor-mediated signaling pathway appears first in development. The expression of nicotinic receptors may reflect the homology of chromaffin cells and sympathetic

neurons during ontogeny. Sympathoadrenal precursor cells originate from the neural crest (Hammond and Yntema, 1947; LeDouarin, 1984) and give rise to the sympathetic ganglia and to endocrine chromaffin cells (Landis and Patterson, 1981). In fact, strong expression of neurofilament protein (NFP), growth associated protein-43 and AChE as neuron-specific markers was observed in embryonic adrenal chromaffin cells (Millar and Unsicker, 1981; Anderson and Axel, 1986; Ahonen, 1991).

At postnatal day 18, we observed by electron microscopy an increase number of exocytotic figures in the chromaffin cells after carbamylcholine stimulation, and an increase in clear and dense-cored vesicles in the nerve fibers, and release of neurotransmitters from the nerve terminals (**Fig.6a-c**) (Oomori et al., 1998). As in embryonic day 18-20, the presence of preganglionic nerve fibers on, and the appearance of exocytotic granule profiles in, adrenal medullary cells were taken as indications of secretory stimulation and release (El-Maghraby and Lever, 1980). These findings suggest that a catecholamine-secretion mechanism via nicotinic-receptor mediated Ca^{2+} signaling pathway in the chromaffin cells occurs in this period and becomes functional with the development of synaptogenesis and neurotransmission. On the other hand, pharmacological evidence based on the reflex response to insulin hypoglycaemia suggested that splanchnic nerve transmission in the rat adrenal gland does not become functional until the end of the 1st week of life (Lau and Slotkin, 1979; Slotkin, 1986). In contrast, our electron-microscopic study revealed the appearance of postsynaptic-like membrane thickenings of the chromaffin cells at embryonic day 18 (Oomori et al., 1998). Also a previous ultrastructural study revealed mature synapses with post- and pre-synaptic membrane thickenings at embryonic day 18.5 and an increased number of small pits and exocytosis in the chromaffin cells of the 18.5- to 21.5-day-old rat fetus after insulin injection (Daikoku et al. 1977). These findings suggest that cholinergic transmission from the splanchnic nerve in the adrenal gland and exocytosis via cholinergic receptors-mediated secretion mechanism in the adrenal chromaffin cells occur in this period.

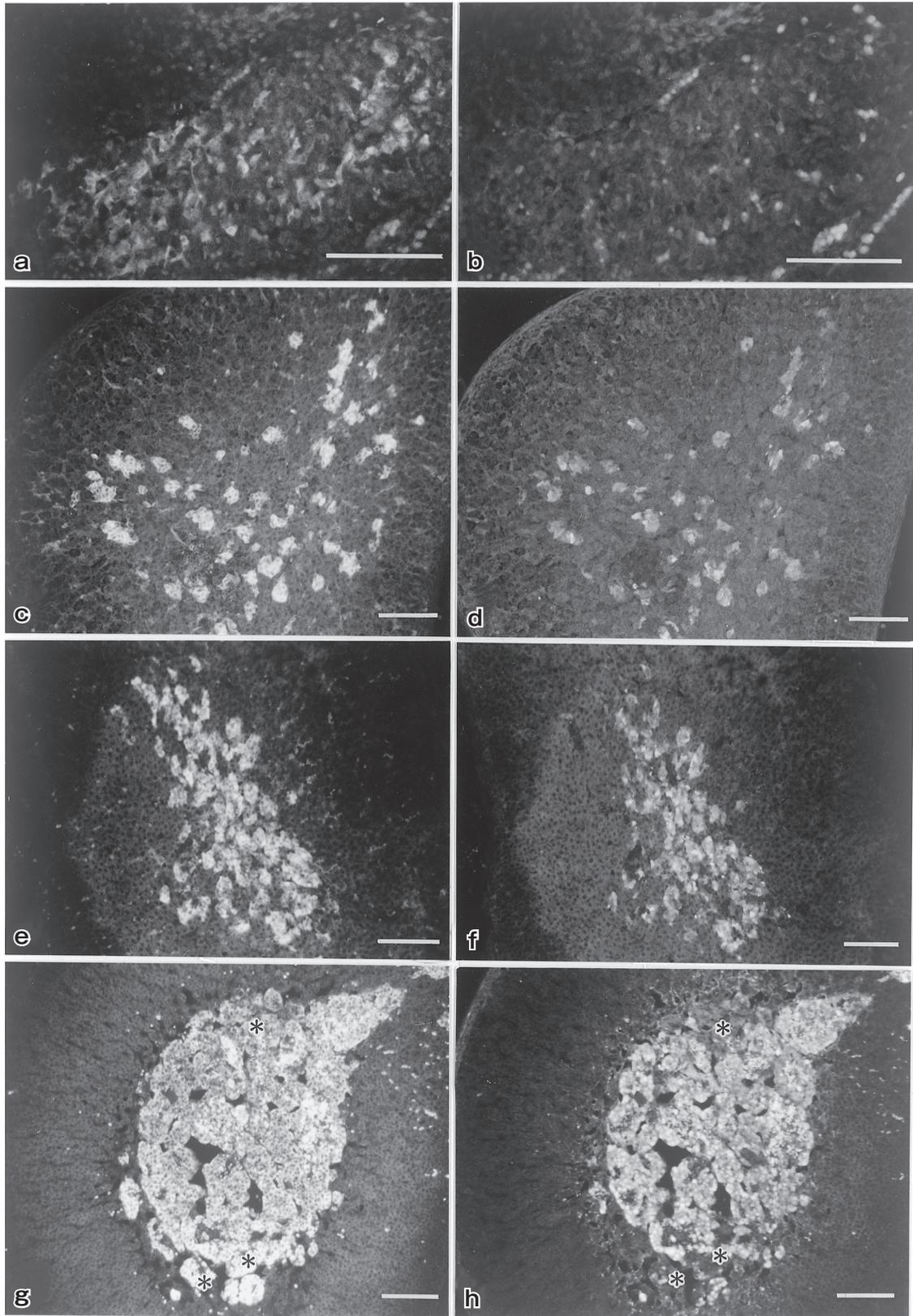


Fig.4a-h. Immunofluorescent micrograph of DBH (a,c,e,g) and PNMT (b,d,f,h) immunoreactivities in the same sections of developing rat adrenal gland. At embryonic day 14 (a,b), adrenomedullary cells are immunoreactive for DBH alone. At embryonic day 16 (c,d), adrenomedullary cells form small groups of chromaffin cells which show both immunoreactivities. Some groups are positive for DBH and weakly positive for PNMT. At embryonic day 20 (e,f), adrenomedullary cells are located in the center of the gland and show both immunoreactivities. At postnatal-1-week (g,h), adrenomedullary cells are located in the center of the gland and are for the most part reactive to both DBH and PNMT. Some cells are immunoreactive to DBH alone (asterisks). X400 (a-h), Bars: 100µm (a,b), 50µm (c-h).

We discovered by digital imaging that methacholine induced a low to moderate rise of $[Ca^{2+}]_c$ in many chromaffin cells from postnatal day 0 to postnatal-1-week, and nicotine induced a high rise of $[Ca^{2+}]_c$ in the cells in this periods (**Figs.10-12**), and that the number of chromaffin cells showing no response to either cholinergic agonists decreased after postnatal week 2 (Oomori et al., 1998). These findings suggest that the nicotinic receptor-mediated Ca^{2+} signaling pathway at postnatal day 0 reaches to the adult level, whereas the muscarinic receptor-mediated Ca^{2+} signaling pathway began to appear in the cells at postnatal day 0 and was gradually completed by the postnatal week 1. Our electron-microscopic study showed numerous putative exocytosis in the chromaffin cells after carbamylcholine stimulation, postsynaptic-like membrane density of chromaffin cells, and release of transmitter from intra-adrenal nerve terminals in these stages (**Fig.9a-c**) (Oomori et al., 1998). These findings suggest that a nicotinic receptor-mediated secretion pathway is already completed and that a muscarinic receptor-mediated secretion pathway in the cells begins to appear and is completed by the postnatal-1-week. The expression of muscarinic receptors in the chromaffin cells is probably related to intrinsic and extrinsic factors as well as to nicotinic receptors. In these stages, we showed immunohistochemically the differentiation of NA and A cells (**Fig.4g,h, Fig.9a**), which is in accord with the previous data (Verhofstad et al.1985), and changes of size, number and density in chromaffin cell granules (Oomori et al., 1998). Elimination or reduction of neurofilament protein (NFP), AChE and growth associated protein-43 as neuronal markers in the embryonic chromaffin cells (Ahonen, 1991; Grant et al., 1994; Holgert et al., 1994), and reduction of enkephalin, calcitonin gene-related peptide, neurotensin and neuropeptid-Y (in contrast to the increase of nerve fibers) in the chromaffin cells from postnatal day 0 to the postnatal week 1 have been reported (Kent and Coupland, 1989; Henion and Landis, 1990, 1992; Holgert et al., 1994). As the enkephalin content of the adrenal medulla is known to be regulated transsynaptically (Schultzberg et al., 1978), the maturation of transsynaptic impulse

activity may suppress synthesis of enkephalin and other peptides by chromaffin cells during this period. Furthermore, previous studies have also reported corticoids secretion in adrenal cortex during these periods. Corticoids induce chromaffin-specific genes such as PNMT gene (Bohn et al., 1981; Teitelman et al., 1982), and the extinction of neuron-specific genes such as NFP, AChE and GAP-43. This stage of expression of muscarinic receptor in developing chromaffin cells may be the timing of differentiation from sympathetic neuron-like cells into the endocrine-like cells. Furthermore, we showed the release of neurotransmitters, a numerical increase of number of intra-adrenal nerve fibers and an increase of clear and dense-cored vesicles in nerve fibers during this period (**Fig.9b,c**) (Oomori et al., 1998). The innervation density of adrenal medullary cells at postnatal day 8 increased significantly compared with neonates (Dalnok and Men β en, 1986; Coupland and Tomlinson, 1989). Growth associated protein-43, a marker for axonal growth, was most intense in the nerve fibers of the rat adrenal medulla at postnatal day 2 and 6 and then decreased progressively (Holgert et al., 1994). These observations suggest that the synthesis, storage, and release of neurotransmitters in the nerve fibers and axonal growth in the adrenal medulla may occur more actively in these stages. In the central nervous system, the expression and number of muscarinic receptor rapidly increased during early postnatal development, a period associated with synaptogenesis (Fiedler et al., 1987; Heacock et al., 1987; Buwalda et al., 1995). The mechanism that coordinates transmitter and receptor expression were not clearly elucidated. One hypothesis is that presynaptic innervation influences the postsynaptic expression of receptors and consequently coordinates it with a presynaptic release of neurotransmitter (Role, 1988). In addition, other extracellular factors (nerve growth factor, leukemia inhibitory factor and ciliary neurotrophic factor) controlling the expression of muscarinic receptors in central and peripheral nervous systems have been reported (Eva et al., 1992, 1994; Ludlam and Kessler, 1993). Thus, the expression of the muscarinic receptor in developing chromaffin cells during these periods may be

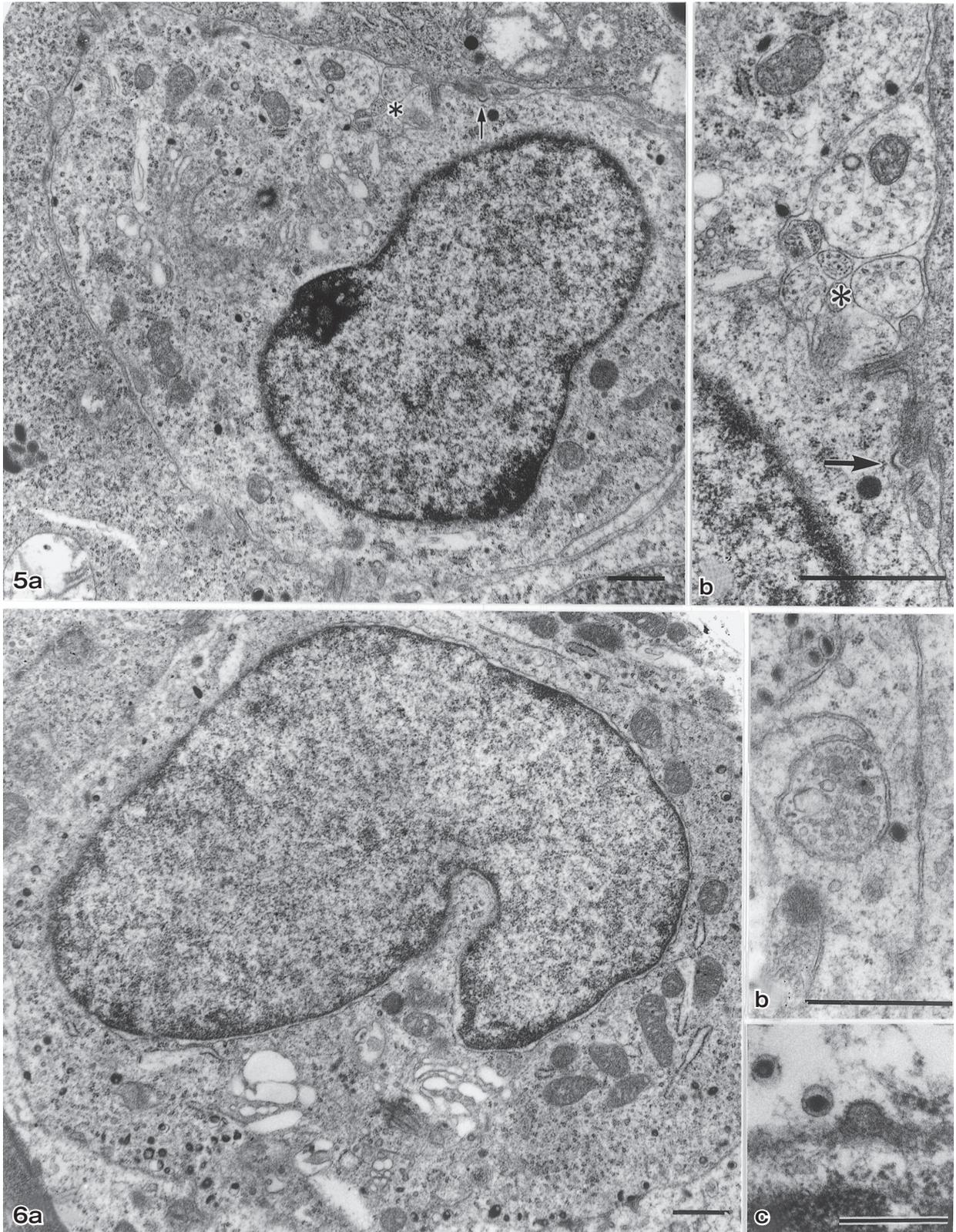


Fig.5a,b. Electron micrograph of rat adrenal chromaffin cells at embryonic day 16. **a** Chromaffin cells (*arrow*, omega profile) contains very few small granules of high electron density in the peripheral cytoplasm (*asterisk*, nerve fibers). **b** Enlargement of nerve fibers (*asterisk*) and omega profile of chromaffin cell (*arrow*) in **a**. Nerve fibers, containing a few clear vesicles, are closely apposed to the chromaffin cell. X10000 (**a**), X23000 (**b**). Bars 1 μ m

Fig.6a-c Electron micrographs of rat chromaffin cell of the embryonic day 18. **a** Chromaffin cell contains relatively numerous granules with highly electron-density. **b** Nerve fiber, containing relatively numerous clear vesicles and a few dense-cored vesicles, is closely apposed to the chromaffin cell. **c** Exocytotic omega profile in membrane of the chromaffin cell. X10000 (**a**), X23000 (**b**), X40000 (**c**). Bars:1 μ m (**a,b**), Bars:0.5 μ m (**c**)

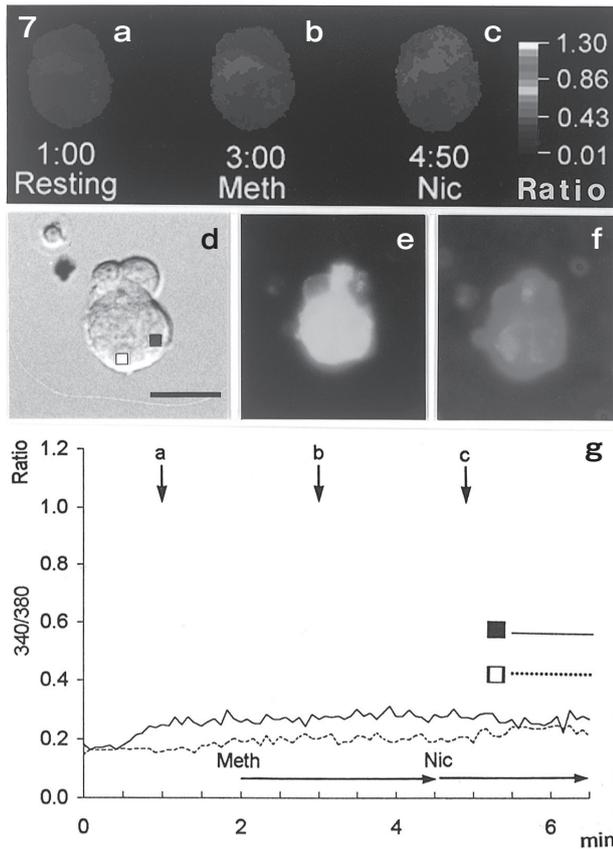


Fig.7a-g Embryonic-day-16 rat adrenal chromaffin cells stimulated by 200 μ M methacholine (Meth) and 10 μ M nicotine (Nic). Pseudocolor ratio images of Ca^{2+} dynamics, (a-c), differential interference-contrast image (d), and immunofluorescent images of DBH (e) and PNMT (f). **g** Time course of $[Ca^{2+}]_i$ in selected regions (white square and black square in d) of the chromaffin cells (a 1min 00s, b 3min 00s, c 4min 50s). Neither methacholine nor nicotine induces any change of $[Ca^{2+}]_i$ in the chromaffin cells showing both DBH and PNMT immunoreactivities. X500. Bars:20 μ m

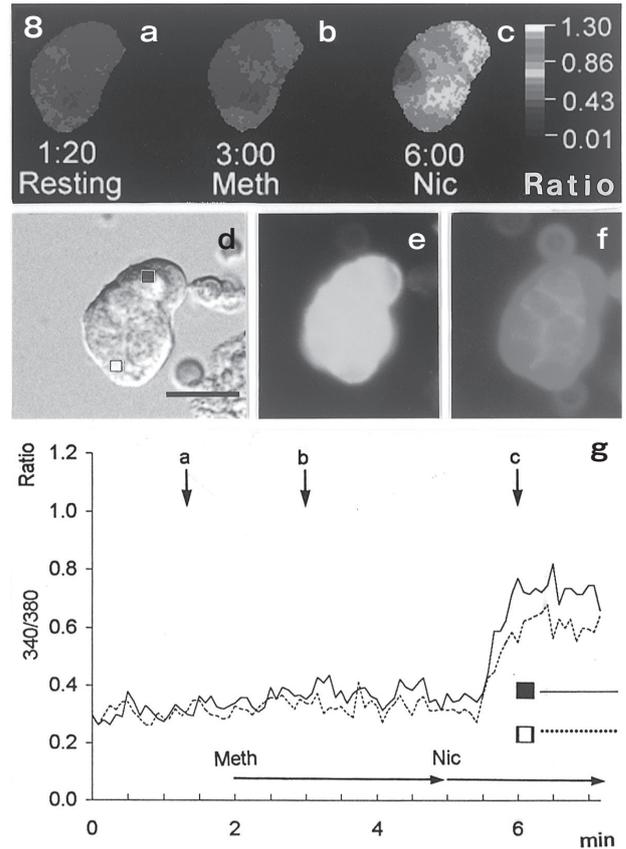


Fig.8a-g Embryonic-day-19 rat adrenal chromaffin cells stimulated by 200 μ M methacholine (Meth) and 10 μ M nicotine (Nic). Pseudocolor ratio images of Ca^{2+} dynamics, (a-c), differential interference-contrast image (d), and immunofluorescent images of DBH (e) and PNMT (f). **g** Time course of $[Ca^{2+}]_i$ in selected regions (white square and black square in d) of the chromaffin cells (a 1min 00s, b 3min 00s, c 6min 00s). Nicotine alone induces a relatively high rise of $[Ca^{2+}]_i$ in the chromaffin cells showing both DBH and PNMT immunoreactivities. X500. Bars:20 μ m

controlled by programmed factors in the cell itself as well as by extracellular factors in the millieu.

2. Developing cholinergic innervation

No ChAT-immunoreactive nerve fibers were observed in the rat adrenal medulla during embryonic development (Henion and Landis, 1990). On the other hand, in embryonic day 16.5 and 17.5, light AChE reaction product was seen in some fibers in the rat adrenal medulla (Ahonen, 1991). The synthesis and hydrolysis of acetylcholine in the adrenal medulla may not be fully activated during embryonic development.

In electron microscopic study, numerous unmyelinated nerve bundles were present in the

rat adrenal medulla, but no evidence was found of a synaptic relationship between nerves and parenchymal elements at the rat embryonic day 14 (El-Maghraby and Lever, 1980). We observed that few contact between nerve fibers and chromaffin cells, and few clear vesicles and dense-cored vesicles in the terminals from rat embryonic day 14 to 17 (**Fig.5a,b**) (Oomori et al., 1998). During this period, cholinergic transmission and synaptogenesis between chromaffin cells and nerve fibers may indicate functional immaturity. Synapses with thickening of pre-and post-synaptic membranes were first evidenced on the medullary cells of 15.5-day-old fetuses, and increased gradually in number with advancing age (Daikoku et al., 1977). Clear evidence of synaptic

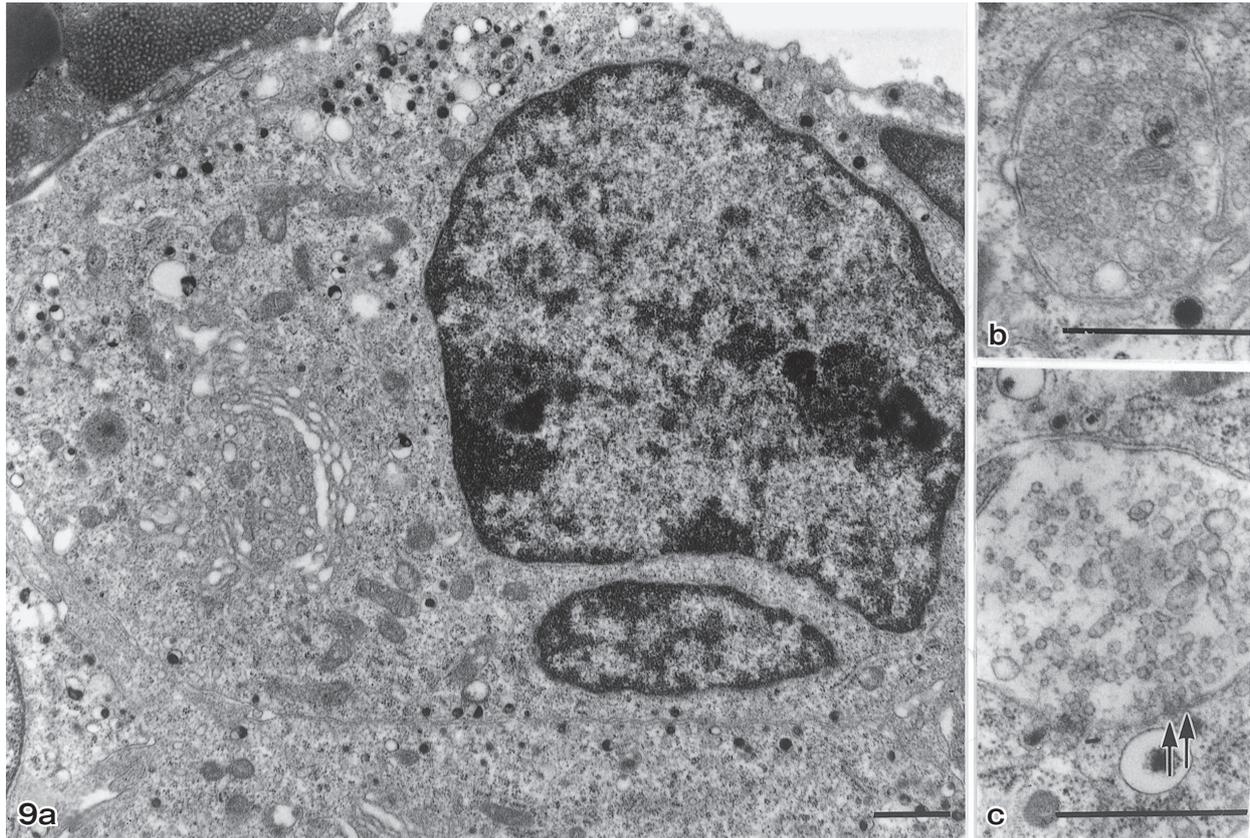


Fig.9a-e Electron micrograph of rat chromaffin cells at postnatal day 0. **a** Chromaffin cell contains relatively numerous granules of highly electron-density in variably sized vacuoles. **b** Nerve fiber ,containing numerous clear vesicles and a few dense-cored vesicles, is closely apposed to the chromaffin cell. **c** Exocytotic omega profiles (*arrows*) in membrane of the nerve terminal. X10000 (**a**), X23000 (**b**). *Bars:1μm*

Table 1 Morphological changes and calcium digital image analyses of adrenal chromaffin cells and nerve fibers during development (*ED*:embryonic day, *PD*:postnatal day, *PW*:postnatal week)

Developmental period	Immunoreactivity ^a		Chromaffin cells ^b (n)	Exocytotic profiles ^b (n)	Nerve fibers ^b (n)	Nerve fiber vesicles ^b (n)	[Ca ²⁺] _c after stimulation ^c	
	Dopamine β-hydroxylase	Phenylethanolamine N-methyltransferase					Methacholine	Nicotine
ED 14-17	++	-	+	-	+	+	-	-
ED 18-20	++	+	+++	+++	++	+++	-	-
PD 0-PW 1	++	++	++++	++++	++/+++	++++	+	+++
PW 2-8	++	++	++++	++++	++++	++++	++	+++

^a - no fluorescence, + moderate fluorescence, ++ intense fluorescence

^b - none, + very few, ++ a few, +++ many, ++++ very many

^c - no change, + low-to-moderate rise, ++ moderate rise, +++ high rise

contacts between cholinergic axons and developing medullary cells was apparent at the rat embryonic day 18 (El-Maghraby and Lever, 1980). For rat embryonic day 18 to 20, we found an increase in clear vesicles and dense-cored vesicles in the nerve fibers and release of neurotransmitters from the nerve terminals and increase of membrane density between chromaffin cells and nerve fibers (**Fig.6b**) (Oomori et

al., 1998). These results suggest that synaptogenesis and neurotransmission between chromaffin cells and nerve fibers may develop and become functional during this period.

Previous studies have reported that until postnatal day 2, a small number of AChE-immunoreactive or AChE-active nerve fibers are present in the rat or mouse adrenal medulla (Ahonen, 1991; Holgert et

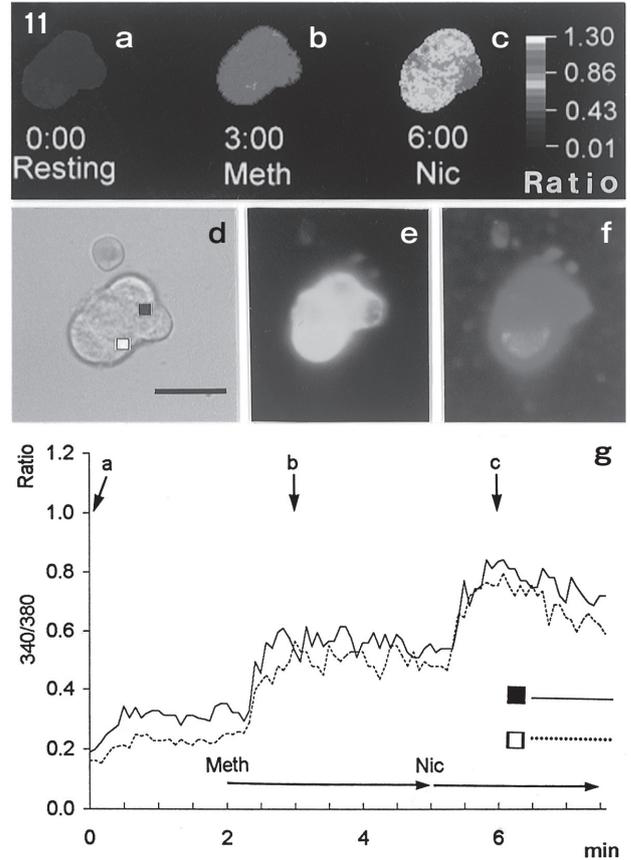
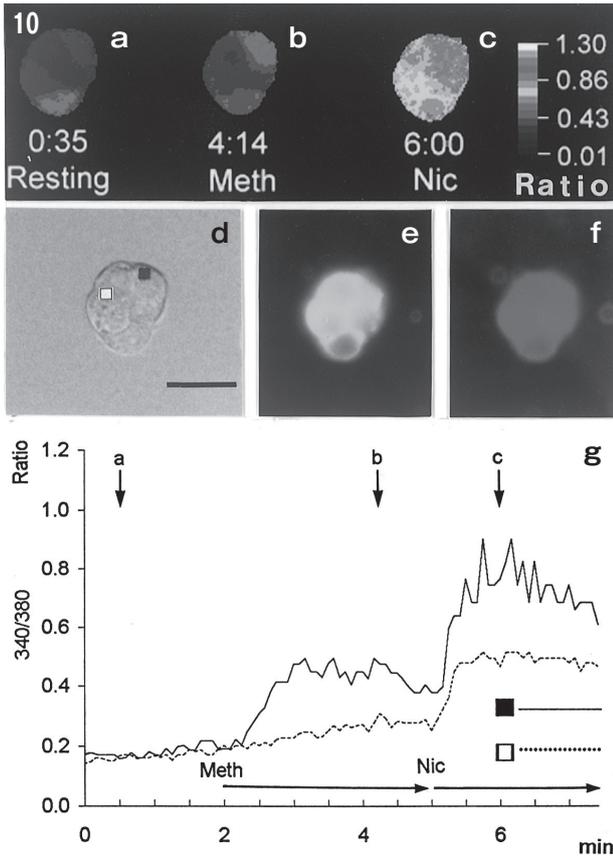


Fig.10a-g Postnatal-day-0 rat adrenal chromaffin cells stimulated by 200 μ M methacholine (Meth) and 10 μ M nicotine (Nic). Pseudocolor ratio images of Ca^{2+} dynamics, (a-c), differential interference-contrast image (d), and immunofluorescent images of DBH (e) and PNMT (f). **g** Time course of $[Ca^{2+}]_c$ in selected regions (white square and black square in d) of the chromaffin cells (a 0min 35s, b 4min 14s, c 6min 00s). Methacholine and nicotine induce low and high rises of $[Ca^{2+}]_c$ in the chromaffin cell showing both DBH and PNMT immunoreactivities, while nicotine alone induces a change of $[Ca^{2+}]_c$ in the these same cells X500. Bars:20 μ m

Fig.11a-g Postnatal-1-week (P1W) rat adrenal chromaffin cells stimulated by 200 μ M methacholine (Meth) and 10 μ M nicotine (Nic). Pseudocolor ratio images of Ca^{2+} dynamics, (a-c), differential interference-contrast image (d), and immunofluorescent images of DBH (e) and PNMT (f). **g** Time course of $[Ca^{2+}]_c$ in selected regions (white square and black square in d) of the chromaffin cells (a 0min 00s, b 3min 00s, c 6min 00s). Methacholine and nicotine induce moderate and high rises of $[Ca^{2+}]_c$ in the chromaffin cells showing both DBH and PNMT immunoreactivities. X500. Bars:20 μ m

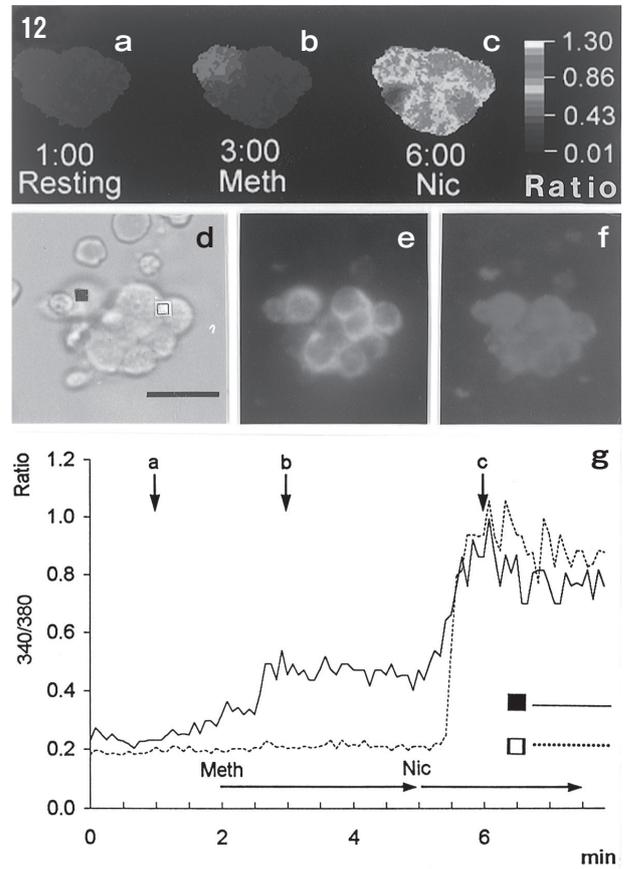


Fig.12a-g Postnatal-1-week rat chromaffin cells stimulated by 200 μ M methacholine (Meth) and 10 μ M nicotine (Nic). Pseudocolor ratio images of Ca^{2+} dynamics, (a-c), differential interference-contrast image (d), and immunofluorescent images of DBH (e) and PNMT (f). **g** Time course of $[Ca^{2+}]_c$ in selected regions (white square and black square in d) of the chromaffin cells (a 1min 00s, b 3min 00s, c 6min 00s). Methacholine and nicotine induce moderate and high rises of $[Ca^{2+}]_c$ in the chromaffin cells, while nicotine alone induce a change of $[Ca^{2+}]_c$ in the chromaffin cells showing both DBH and PNMT immunoreactivities. X500. Bars:20 μ m

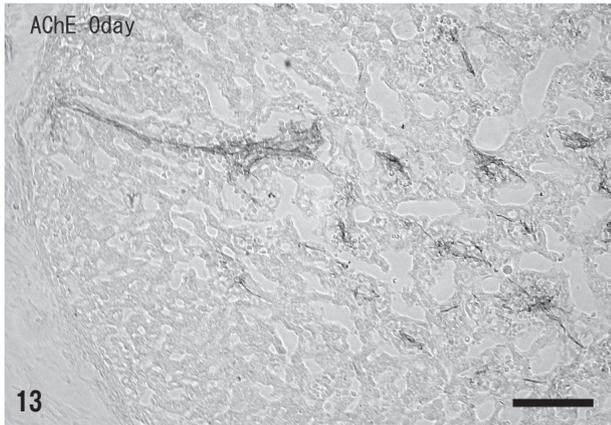


Fig.13 Light micrograph of acetylcholine esterase (AChE) activity in the rat adrenal gland at postnatal day 0. Thick AChE-active nerve bundle penetrates through the cortex into the medulla. A few AChE-active nerve fibers are seen in the medulla. Scale bar = 100µm

al., 1994; Iwasa et al., 1999a). We have demonstrated that a few AChE-active and ChAT-immunoreactive nerve fibers are present in the rat adrenal medulla between postnatal day 0 and postnatal day 2 (**Fig.13**) (Murabayashi et al., 2009a). It is well known that AChE and ChAT are synthesized in the neuronal cell body and are transported to the nerve endings (Hebb and Waites, 1956; Fukuda and Koelle, 1959; Kasa, 1968; Ekstrom and Emmelin, 1971; Kasa et al., 1973). Further, neurofilament immunoreactivity is found in the moderately dense network of the nerve fibers in the rat and mouse adrenal medulla at early postnatal stage (Holgert et al., 1994; Iwasa et al., 1999a). These results suggest that the synthesis and hydrolysis enzymes of the acetylcholine may not be fully transported to the nerve endings, although the network of nerve fibers has been formed in the rat adrenal medulla during early postnatal stage. In the adult animals, the endocytic reuptake of choline and synaptic vesicle membranes after release of and refilling of acetylcholine with clear vesicles are occurred in cholinergic nerve endings (Carrol and Nelson, 1978; Meyer and Cooper, 1982; De Camilli and Takei, 1996; Betz and Bewick, 1992). These results suggest that this acetylcholine recycle system may not be fully functional in the rat adrenal medulla between postnatal day 0 and postnatal day 2. Furthermore, we revealed that relatively numerous VAcHT-immunoreactive nerve fibers is present in the

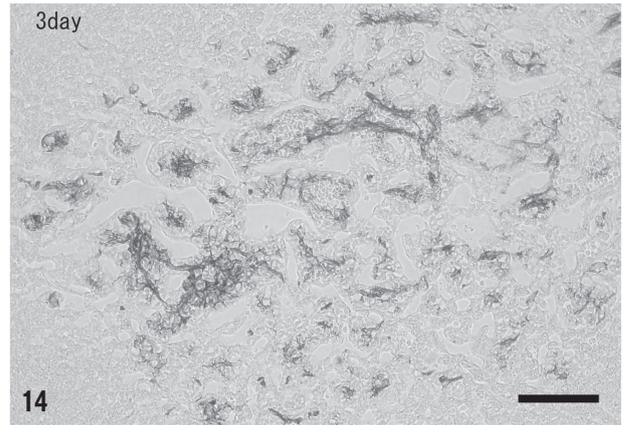


Fig.14 Light micrograph of AChE activity in the rat adrenal gland at postnatal day 3. Relative numerous AChE-active nerve bundles and fibers are found in the medulla. Some thinner AChE-active nerve bundles and nerve fibers are densely seen in certain areas of the medulla. Scale bar = 100µm

rat adrenal medulla at postnatal day 0, and showed the VAcHT-immunoreactivity in many small clear vesicles of the intra-adrenal nerve fibers (**Figs.18, 23**) (Murabayashi et al., 2009a). It has been previously shown that VAcHT-immunoreactivity in PC12 cell neurites is confined to the membrane of small synaptic vesicles (Weihe et al., 1996). Thus, these results indicate that in the rat adrenal medulla, between postnatal day 0 and postnatal day 2, relatively numerous clear vesicles have already been transported to the nerve endings by axonal flow. In fact, electron microscopic observations of rat adrenal medulla in the fetal stage and at birth demonstrated the small clear vesicles contained in synaptic nerve endings in contact with the chromaffin cells (Daikoku et al., 1977; Tomlinson and Coupland, 1990; Oomori et al., 1998). Contrastingly, physiological studies have shown that splanchnic control of adrenomedullary functions is absent at birth, appears during the first week and becoming fully mature by 10 days after birth (Slotkin et al., 1980, 1982). On the other hand, we demonstrated that the chromaffin cells have secretory activity to the cholinergic stimulations during the late fetal stage (Oomori et al., 1998). Electron microscopic observations demonstrated that the chromaffin cells showed exocytotic omega profiles and thickening of pre- and post-synaptic membranes was seen in the rat adrenal gland at late fetal stages (Daikoku et al., 1977; Oomori et al., 1998). These

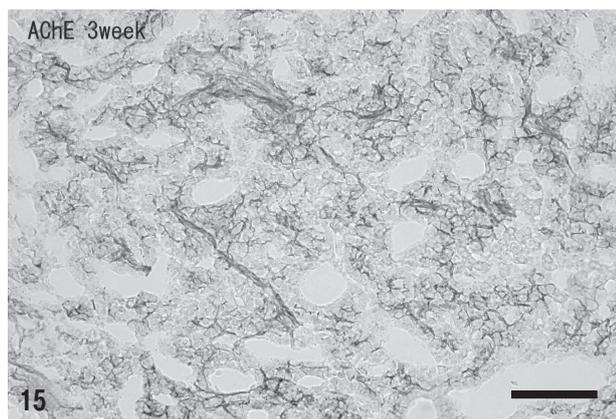


Fig.15 Light micrograph of AChE activity in the rat adrenal gland at postnatal week 3. Much abundant AChE-active nerve bundles and fibers are seen in the medulla. These nerve fibers are densely found certain areas in the medulla. Scale bar = 100 μ m



Fig.16 Light micrograph of AChE activity in the rat adrenal gland at postnatal week 8. Abundant thick and thinner AChE-active nerve bundles and fibers are seen in the medulla. These nerve fibers are densely visible certain areas in the medulla. Scale bar = 100 μ m

facts suggest that relatively numerous synaptic vesicles may be present in the cholinergic nerve endings and that acetylcholine is released from these vesicles. However, since the acetylcholine recycle system is not completely established until postnatal day 2, the endocytic reuptake of choline and synaptic vesicle membranes and refill of acetylcholine within the vesicles does not occur in the rat adrenal medulla until that time.

Previous studies have reported that the number of the AChE-active nerve fibers was rapidly increased in the mouse medulla at postnatal day 2 or postnatal day 3 and in the rat medulla at postnatal day 6 compared with that during early postnatal stage (Holgert et al., 1994; Iwasa et al., 1999a). In our study, as compared to the number of AChE-active and ChAT-immunoreactive nerve fibers at postnatal day 2, those between postnatal day 3 and postnatal week 1 were dramatically higher (**Fig.14**) (Murabayashi et al., 2009a). In addition, a biochemical study reported that the ChAT-activity in the rat adrenal gland has been identified from postnatal day 4 (Tomlinson and Coupland, 1990). These data suggest that the AChE and ChAT may reach the nerve endings and acetylcholine release may be rapidly activated in the rat adrenal medulla at postnatal day 3. Further, AChE-active and ChAT-immunoreactive nerve fibers were denser in certain areas (NA cell areas) of the medulla at postnatal day 3 (**Figs.17, 22**) (Murabayashi

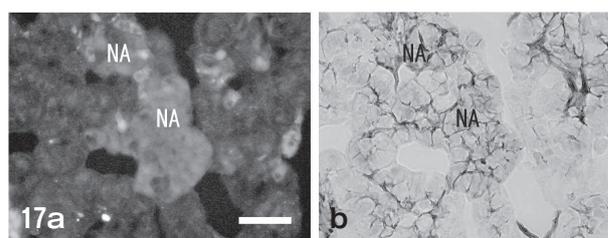


Fig.17a-b Fluorescence micrograph showing noradrenaline (NA) fluorescence (a) and histochemical micrograph of the same section showing AChE activity in the rat adrenal medulla at postnatal week 8. AChE-active (b) nerve fibers are more numerous in NA cells (a) than in adrenaline cells. Scale bar = 50 μ m

et al., 2009a). Previous studies have reported that chromaffin cells differentiated into NA cells and A cells at postnatal day 2 or postnatal day 3 in the rat and mouse adrenal gland (Verhofstad et al., 1985; Oomori et al., 1998; Iwasa et al., 1999a). These facts suggest that once the differentiation of NA cells and A cells occurred, the preferential innervation of cholinergic nerve fibers to the NA cells and A cells was immediately started in the rat adrenal medulla. Our study revealed that as compared to the number of VAcHT-immunoreactive puncta at postnatal day 2, that at postnatal day 3 was higher (**Fig.19**) (Murabayashi et al., 2009a). An electron microscopic study revealed that the majority of nerve endings were contained around the small clear synaptic vesicles in the rat adrenal medulla at postnatal day 4 (Tomlinson and Coupland, 1990). These results suggest that the

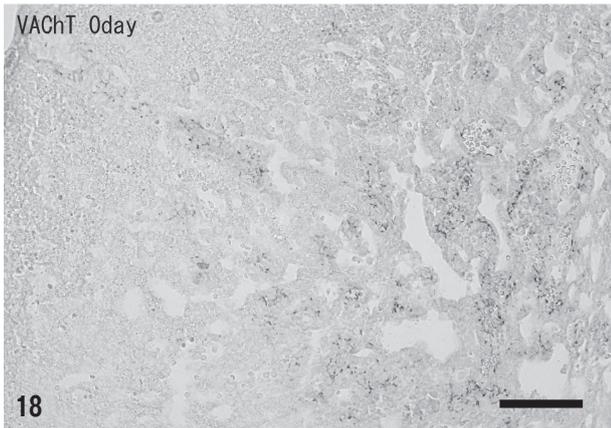


Fig.18 Light micrograph of vesicular acetylcholine transporter (VACHT) immunoreactivity in the rat adrenal gland at postnatal day 0. Moderate number of small VACHT-immunoreactive puncta in the nerve fibers is seen in the medulla. Scale bar = 100 μ m

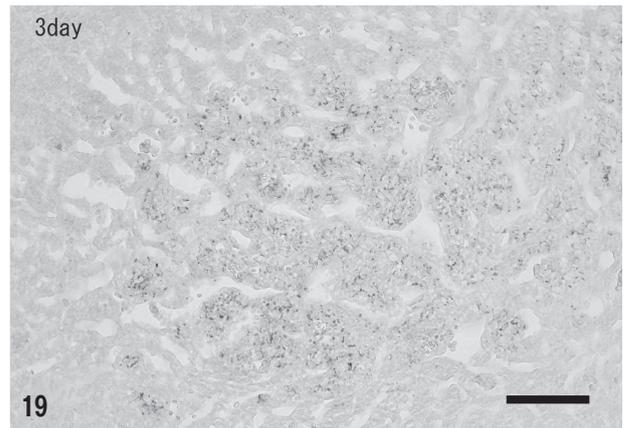


Fig.19 Light micrograph of VACHT immunoreactivity in the rat adrenal gland at postnatal day 3. Abundant small VACHT-immunoreactive puncta in the nerve fibers surround certain areas in the medulla. Scale bar = 100 μ m

synthesis and hydrolysis of acetylcholine may be dramatically activated and that recycle and refill of acetylcholine may be activated in the rat adrenal medulla during this period. Moreover, these data imply that catecholamine secretion from chromaffin cells may be activated by acetylcholine stimulation in the rat adrenal medulla during this period. In fact, we reported that nicotinic and muscarinic receptor-mediated catecholamine secretion pathway was completely established by postnatal week 1 (Oomori et al., 1998).

The abundance of AChE-active and ChAT-immunoreactive nerve fibers in the medulla was gradually increased from postnatal week 2, and finally reached adult levels at postnatal week 3 (**Figs.15, 16, 20-22**) (Murabayashi et al., 2009a). **Table2** shows the relative abundance of ACE active, ChAT and VACHT immunoreactive nerve fibers in the developing adrenal medulla. These facts suggest that acetylcholine synthesis and hydrolysis system may be completely established at the nerve endings in the rat adrenal medulla at postnatal week 3. Previous studies on the rat and mouse adrenal medulla showed that the number of AChE-active or -immunoreactive nerve fibers reached the adult levels at postnatal day 16 (Holgert et al., 1994) and postnatal week 3 (Iwasa et al., 1999a), respectively, and that biogenic ChAT-activity was rapidly increased to adult levels in the rat adrenal gland by postnatal day 26 (Tomlinson and

Coupland, 1990). We demonstrated that AChE-active, ChAT-immunoreactive and VACHT-immunoreactive nerve fibers densely surround the clusters of NA cells more densely than that of A cells (**Figs.17, 22**) (Murabayashi et al., 2009a). A similar preferential cholinergic innervation has been previously observed in the rat and mouse adrenal medulla (Holgert et al., 1994; Iwasa et al., 1999a). It has been demonstrated that different preganglionic neurons innervate the NA and A cells in the adrenal medulla (Edwards et al., 1996), and that nicotine and high K^+ cause a greater secretion of NA than A (Douglas and Poisner, 1965). These facts suggest that the preferential innervation of NA and A cells may cause differential secretion of NA and A.

In our study, the diameter of the VACHT-immunoreactive puncta in the nerve fibers in the medulla was gradually increased from postnatal day 0 to postnatal week 8, while the number of VACHT-immunoreactive puncta in the nerve fibers was maximal at postnatal week 2 and then decreased with aging (**Figs.18-21**) (Murabayashi et al., 2009a). These results suggest that the increase of the number of VACHT-immunoreactive small puncta and the decrease of VACHT-immunoreactive large puncta may be concerned with the developmental changes of the diameters of nerve fibers and of the axonal transport rate of small clear vesicles. In fact, it has been reported that the diameter of axons increases

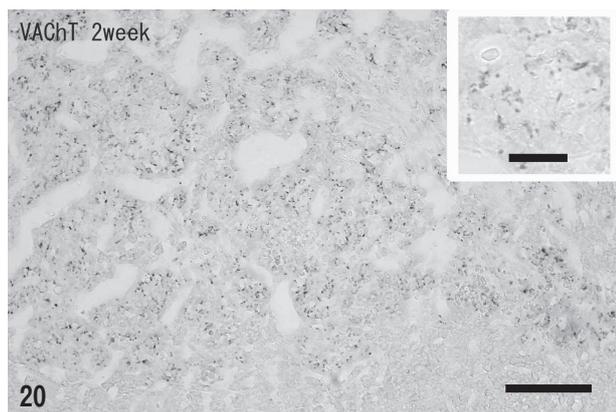


Fig.20 Light micrograph of VAcHT immunoreactivity in the rat adrenal gland at postnatal week 2. Very abundant small VAcHT-immunoreactive puncta in the nerve fibers are seen in the medulla. Inset shows high magnification of a part of the same micrograph. Scale bar = 100 μm and 20 μm (inset)

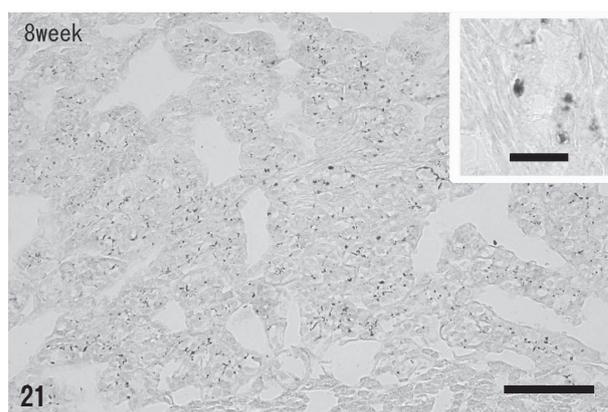


Fig.21 Light micrograph of VAcHT immunoreactivities in the rat adrenal gland at postnatal week 8. Moderate number of very large VAcHT-immunoreactive puncta in the nerve fibers is seen in the medulla. Inset shows high magnification of a part of the same micrograph. Scale bar = 100 μm and 20 μm (inset)

Table 2. Intensities of acetylcholinesterase (AChE) activity, and choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAcHT) immunoreactivities in the nerve fibers of rat adrenal medulla during postnatal development

	0 Day	3 Day	1 Week	2 Week	3 Week	4 Week to 8 Week
AChE	+	++	++	+++	+++	+++
ChAT	+	++	++	+++	+++	+++
VAcHT	++	+++	+++	++++	++++	++

+, a few; ++, moderate; +++, abundant; +++++, very abundant.

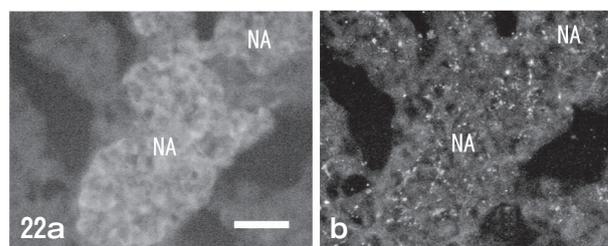


Fig.22a–b Fluorescence micrograph showing NA fluorescence (a) and immunohistochemical micrograph of the same sections showing VAcHT immunoreactivity (b) in the rat adrenal medulla at postnatal week 8. VAcHT-immunoreactive nerve fibers are numerous seen around NA cells compared with adrenaline cells. Scale bar = 50 μm

with age in the developing rat optic nerve (Hunter and Bedi, 1986), and that anterograde and retrograde vesicle transport rates are significantly slower in older rat axons (Viancour and Kreiter, 1993). Furthermore, we revealed that the VAcHT-immunoreactive deposits were seen in the clusters of small clear vesicles in the nerve fibers of the rat adrenal medulla by immunoelectron microscopy (**Fig.23**) (Murabayashi et al., 2009a). In our conventional electron microscopy, the diameter of the nerve fibers and the number of small clear vesicles in the nerve fibers of the medulla at postnatal week 8 increased about 1.6 times and about 1.5 times, respectively, compared to those at postnatal week 2 (**Figs.24, 25**) (Murabayashi et al., 2009a). A previous study showed that the vesicles were transported from cell body to nerve terminals by the motor protein (Yonekawa et al., 1998). The motor protein may transport the cluster of small clear

vesicles at regular intervals in the axon. With regard to the transport of small clear vesicles in the intra-adrenal nerve fibers, the number of motor proteins may increase, or a molecule of motor protein may increase the amount of small clear vesicles at a time during development. Further, the decrease of the speed of the motor proteins may be caused by the axonal flow's resistance produced by enlarged clusters.

Finally, in order to understand the secretion mechanisms of developing adrenal chromaffin cells, further investigation needs to clarify the genes and proteins related to developmental events of differentiation of sympatho-adrenal precursor cells, NA and A cells in the medulla, and them related to the functional maturation of secretion mechanism of the chromaffin cells and nerves in the adrenal medulla.

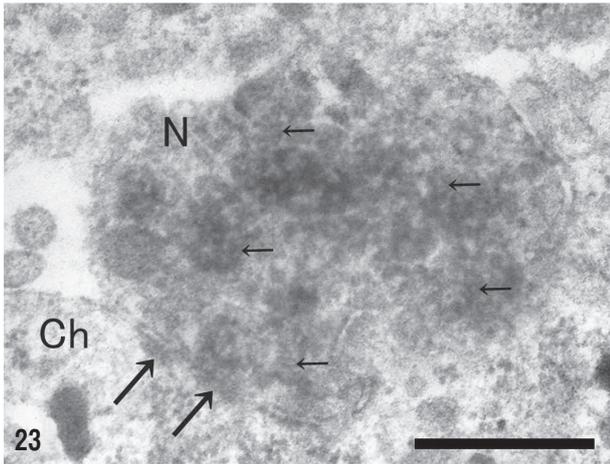


Fig.23 Immunoelectron micrograph of VAcHT-immunoreactive nerve fiber in the rat adrenal medulla at postnatal week 8. The immunoreactive-deposits are densely seen in numerous small clear vesicles (small arrows). The membrane specialization (large arrows) is visible in the chromaffin cell (Ch). Scale bar = 0.5 μ m

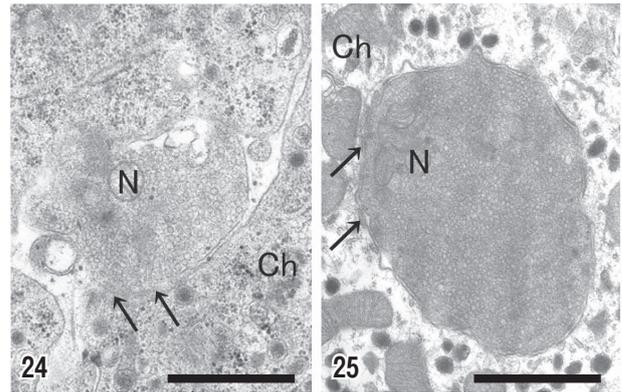


Fig.24 Electron micrograph of nerve fiber in the rat adrenal medulla at postnatal week 2. The intra-adrenal nerve fiber (N) contains numerous small clear vesicles and a few dense cored vesicles. The membrane density is increased (arrows) between the chromaffin cell (Ch) and nerve fibers. Scale bar = 1 μ m

Fig.25 Electron micrograph of nerve fiber in the rat adrenal medulla at postnatal week 8. The intra-adrenal nerve fiber (N) contains very numerous small clear vesicles and a few dense cored vesicles. The membrane density is increased (arrows) between the chromaffin cell (Ch) and nerve fibers. Scale bar = 1 μ m

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発達過程における副腎髄質細胞の コリナージック受容体関連分泌機構について

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【要 旨】

この総説では、発達中の副腎髄質細胞のコリナージックレセプター関連分泌がいつ頃発現し、いつ頃機能的になり、そしていつ頃形態変化を起こすのかについて述べている。交感神経—副腎髄質前駆細胞は神経堤由来細胞の中でおもな系統を構成している。これらの細胞から交感神経細胞と内分泌の副腎髄質細胞が分化している。副腎髄質細胞は交感神経と相同であり、節前神経性のコリナージック神経線維によって神経支配されている。発達中の副腎髄質細胞は、最初にノルアドレナリンのみを細胞内で合成し、その後にノルアドレナリン (NA) とアドレナリン (A) を同一細胞内で合成し (混合細胞)、最終的にノルアドレナリンはNA細胞、アドレナリンはA細胞でそれぞれ合成していた。コリナージック指標蛋白 (アセチルコリンエステラーゼ、コリンアセチル転移酵素、アセチルコリン小胞輸送蛋白) の反応神経線維は胎児期中の副腎髄質では無いかまたは極めて少なかったが、生後発達中に急激に増加した。発達中の副腎内のコリナージック神経は、最初は全く顆粒がないか極めて少数の無芯小胞を含んでいた、その後は多くの無芯小胞と少数の有芯小胞を含んでいた。アセチルコリン刺激では、副腎髄質細胞はニコチックとムスカリニックレセプターを介する開口放出によってカテコールアミンの分泌が起こった。発達中の副腎髄質細胞のコリナージック刺激では、最初は無反応で、その後まずニコチックレセプター関連分泌が出現し、その後にムスカリニックレセプター関連とニコチック関連分泌、あるいはニコチックレセプター関連分泌のみが起こった。これらのことを考え合わせると、発達中の副腎髄質細胞や副腎内のコリナージック神経の形態変化はコリナージック関連の分泌メカニズムの発現に関係し、さらに発達中の副腎髄質ではニコチックレセプター関連分泌を介したメカニズムが、ムスカリニックレセプター関連分泌に先んじて出現していた。

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