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Research Article

Factors Affecting The Differentiation Of Small Granular Chromaffin (SGC) Cells Of The Mouse Adrenal Medulla

Faraj Al Bustami And Islam Al Tarawneh*

Department Of Anatomy And Histology, Faculty Of Medicine –University Of Jordan , Amman – Jordan

*Corresponding author: *fbustami@ju.edu.jo*

Abstract

SGC cells have been suggested as being intermediate cell types between chromaffin cells and sympathetic neurons. At the EM level , in gluteraldehyde /osmium tetroxide fixed material , the characteristic features of SGC cells in the mouse are : 1. the presence of small chromaffin granules of variable electron density , 2. the occurance of synaptic-type vesicles (STV). 3. a high nucleus-to-cytoplasm ratio and 4. a relatively well developed rough endoplasmic reticulum . SGC cells tend to lie peripherally in the adrenal medulla , adjacent to noradrenaline storing (NA) cells and may send processes between adjacent chromaffin and cortical cells (into the X-zone in young mice). Recent observations on the post-natal development of SGC cells have shown that SGC cells appear to achieve their greatest complement of secretory granules including STV at the time when X-zone of the mouse shows maximal development. Thereafter the size of aggregates of STV and number of secretory granules decrease and STV are few in number or apparently absent in SGC cells of post –pubertal animals 9 or more weeks old. However, STV reappear following post –pubertal castration as a secondary X-zone develops and decline in number following parenteral testosterone proprionate which precipitates the disappearance of the X-zone. This effect may be due to either a direct effect of testosterone on the adrenal medulla and/or to its effect on the hypothalamus-pituitary leading to a reduction in output of luteinising hormone (LH) and /or degeneration of the X-zone.

Keywords: Mouse adrenal medulla – SGC cells – X-zone – Chromaffin cells- Catecholamines- STV.

Introduction

The close anatomical association of the adrenal cortex and the medulla in mammals has for some time stimulated speculations that the two zones might be functionally related.The secretory products of chromaffin tissue , catecholamines, are synthesised from either tyrosine or dopa (3,4-dihydroxyphenylalanine). Tyrosine is converted by tyrosine -hydroxylase to dopa ,then by dopa decarboxylase to dopamine. Nor-adrenaline is subsequently formed by the action of dopamine -hydroxylase, and adrenaline from noradrenaline by the action of the methylating enzyme phenylethanolamine *N*-methyl transferase (PNMT).All the previous enzymes need the presence of locally high concentration of corticosteroids produced by the adrenal cortex (Kirshneir and Goodall ,1957; Wartman and Axelrod ,1965; Margolis et al ,1966; Coupland and Macdougall,1966; Mueller ,R.A1970; Unsicker et al ,1978 ,2013). Electron microscopy revealed the fine structural features of the chromaffin cells ; after initial fixation of the adrenal medulla in glutaraldehyde, the A(adrenaline) cells were found to contain the secretory granules with moderate electron density, whereas the cores of secretory granules in the NA(noradrenaline) cells were highly electron –dense (Wood and Barrnett,1964; Coupland et al,1964) . A third type of chromaffin cell was described by several authors. Unsicker (1973,1976) has reported the presence of of small granule chromaffin cells (SGC cells) in the adrenal medulla of birds and reptiles. The

SGC cells of birds are morphologically quite similar to the small granule containing cells or small intensly fluorescent cells (SIF cells) in sympathetic ganglions of several mammals (Eranko and Harkonen, 1969; Bjorklund et al., 1970 ; Eranko and Eranko ,1971). Unsicker (1973) considers the SGC cells in birds to be ganglion cells, which are morphologically identical to those in sympathetic ganglions. The SGC cells of reptiles and mice, however , were regarded as a special type of chromaffin cells different from the A and NA cells (Unsicker,1976; Kobayashi and Coupland, 1977,1993) . The mouse adrenal gland is unusual in so far as it has a characteristic X-zone in the cortex that is largly under the control of androgens , progesterone and gonadotrophines(Jones 1949,1952;Holmes and Dickson 1971) and which regress after puberty , quickly in the male and slowly in female unless pregnancy supervenes .

In view of the experimental importance of chromaffin cells as primary catecholaminergic sites, and the application of this type in brain transplantation, this study was conducted to identify a possible association between the presence of an X-zone and the stage of differentiation of SGC cell.

Materials and Methods

Albino mice (strain CS1) were supplied by Jordan university medical school animal house from closed colonies maintained under standard conditions of tempreture and lighting (12 hours light , 12 hours darkness).

Animals were allowed free access to water and a normal diet.

Male and female mice have been examined at different ages bridging the period in which the X-zone of the cortex begins to degenerate (4 weeks in males, 6 weeks in females ; Howard ,1927) ;(Hirokawa and Ishikawa,1974).

Two groups of animals were used ,each comprising 12 male and 12 female mice.

Experiments to study the effect of postpubertal castration.

12 mature animals (9 week old) were castrated and afterwards animals were sacrificed at a weekly interval postoperatively for 5 weeks. Castration of male mice were performed under Nembutal anesthesia (0.4 ml per Kg body weight); a small incision was made in the scrotal skin and by gentle pressure over lower abdomen , the testes bulged out , the spermatic cords were ligated proximal to ther testes which removed by cutting the cords just distal to ligation points. The skin of the scroyum was closed by one or two stitches.

Experiments with testosterone proprionate

Twelve male mice (4 weeks old) were used. Testosterone proprionate dissolved in corn oil was administered by subcutaneous injection at a single daily dose of 5 mg per animal and for 5 consecutive days. Twelve animals were used as control and were injected with corn oil.

All animals were sacrificed between 11.00 and 12.00 h to minimize the effects of circadian changes. They were anesthetized intravenously with_Nembutal and with minimum stress before the removal of adrenal glands or blood or perfusion with fixative for morphological examination.

Adrenal vein corticosterone concentration

As soon as the animals were unconscious, the abdomen was opened ,a 27 gauge needle was passed through the left renal vein into the adrenal vein .

Blood was withdrawn slowly over a 5-minute period into a 1 ml syringe.0.5 ml of serum were added to 1 ml ethanol and the clear supernatant fraction was removed after centrifugation in a bench centrifuge for 10 minutes. The ethanol extracts were dried down and corticosterone concentrations determined by the displacement of [^{3}H] corticosterone from the binding globulin (Delong and Van der Molen ,1972; Brindly et al., 1979). [^{3}H] corticosterone (51 Ci /mmol) was obtained from the radiochemichal center ,Amersham, Bucks, U.K.

For electron microscopy

As soon as the animals were unconscious , the heart was exposed via a thoracotomy and the animal perfused through the left ventricle with a mixture of 3% glutaraldehyde in 0.1 M-phosphate buffer (PH 7.2). One hour after perfusion , the adrenal glands were removed , any fat was dissected of and the glands

were cut into approximately 1 mm slices and then fixed for a further 4 hours in glutaraldehyde phosphate. The slices were washed overnight in buffer , immersed for 1 hour in 1% aqueous osmium tetroxide ,rinsed in distelled water, dehydrated in a graded ethanol series ,embedded in araldite and sectioned on sorval MT-2B microtome. Thin sections were stained with alkaline lead citrate and examined in a Zeis EM10B microscope. After glutaraldehyde fixation few glands were immersed in potassium dichromate (Wood and Barrnet ,1963),and processed as above .Semi –thin sections were stained with methylene blue and were examined by light microscopy.

Results

In this work three types of SGC cells have been recognized by ultrastructural characteristics:

Type I " early SGC cell "
Type II " typical SGC cell "
Type III " late SGC cell "

Ultrastructural characteristics of SGC Cells

Certain features are common to the three types of SGC cell in the mouse adrenal including their position peripherally near the cortico-medullary junction, adjacent to noradrenaline cells where they exist either as a single cell or in small clusters.

A high nucleus-cytoplasm ratio is easily recognized in the three types SGC cell.

In addition to these common features, each type shows additional characteristics features:

TYPE I (Early SGC Cell)

This type of SGC cell (Fig.1+ 2) contains only one variety of typical secretory granules which lie mostly at the periphery of cell beneath the cell membrane, they are of variable size and density, but mostly show a moderate electron density. The cell shows larger aggregates of rough endoplasmic reticulum than normally observed in the A or NA cells ; it appears particularly well developed late in the third week. By this time the X-zone is often found interdigitating with the medulla to a considerable extent and is much thicker in females than in males.

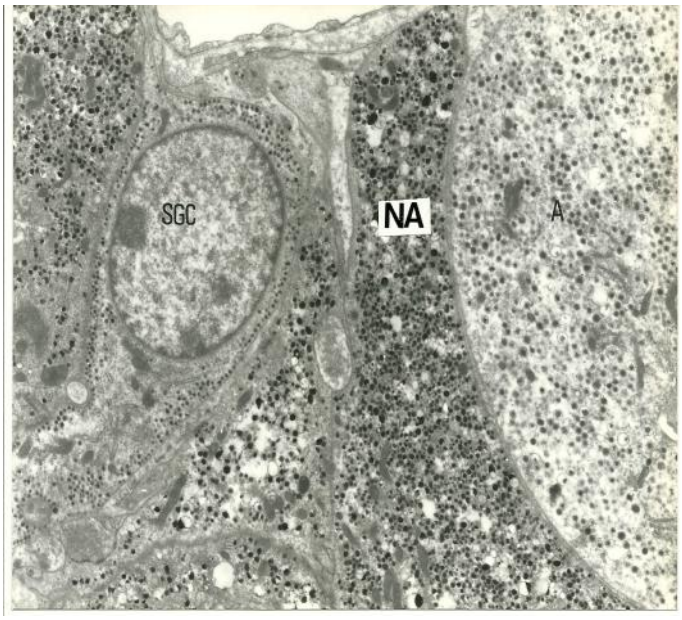

Fig.1 Adrenomedullary section from a normal 3 week-old male mouse .SGC = type I SGC cell, A= adrenaline storing cell, NA = noradrenaline storing cell.X 5800

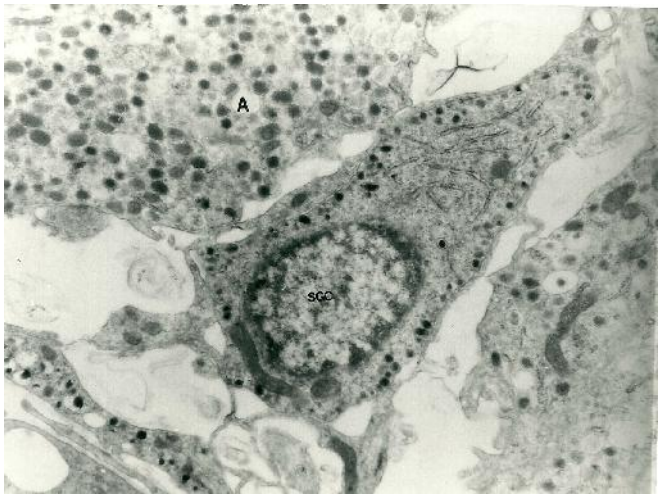

Fig.2 Adrenomedullary section from a normal 3 week-old male mouse. Type I SGC cell can be seen with one type of peripherally situated secretory granules , A= adrenaline storing cell. X 12600

TYPE II (Typical SGC Cell)

The most characteristic feature of typical SGC cell is the appearance of a second population of cytoplasmic microvesicles, in addition to the already present secretory granules (Fig.3). These synaptic-like vesicles (STV) are round in shape and measure 40-70 mm in diameter. They are either scattered among the large secretory granules or from clusters (Fig.4). After glutaraldehyde /osmium tetroxide fixation they appear empty or have electron-dense contents that are usually eccentrically placed. However, using a modified method of fixation described by Tranzer and Richard (1976) which uses low concentrations of glutaraldehyde (1 percent) and the addition of chromate/ dichromate mixture in all steps of fixation, the STV appear with electron dense cores occupying most of the vesicle cavity (Fig.5). This observation suggests that these vesicles contain either dopamine or adrenaline , both substances do not react with glutaraldehyde in a way similar to noradrenaline and are displaced from their binding to the ATP and chromogranin protein in the chromaffin granules (Coupland and Hopwood,1966a).

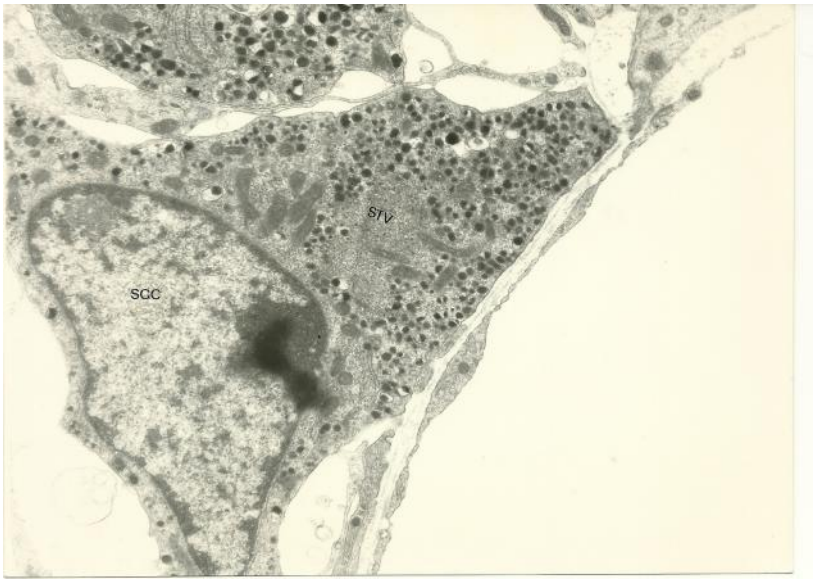

Fig. 3 Section through adrenal medulla from a normal 4 week-old male mouse .Type II (typical) SGC cell can be seen with a cluster of STV in addition to the typical secretory granules . X 14600

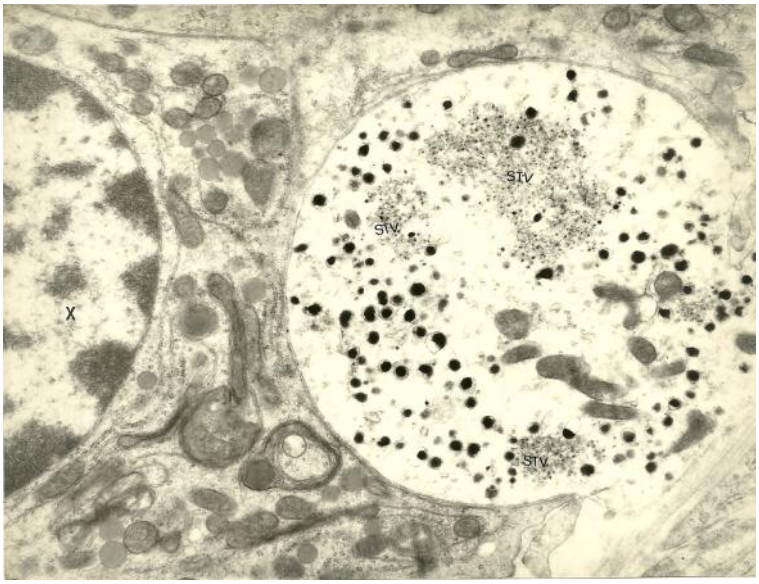

Fig. 4 Corticomedullary junction from a normal 4 week-old male mouse. Part of a typical SGC cell type II can be seen surrounded by X-zone cells . Note the large clusters of STV , in addition to typical secretory granules. X 20400

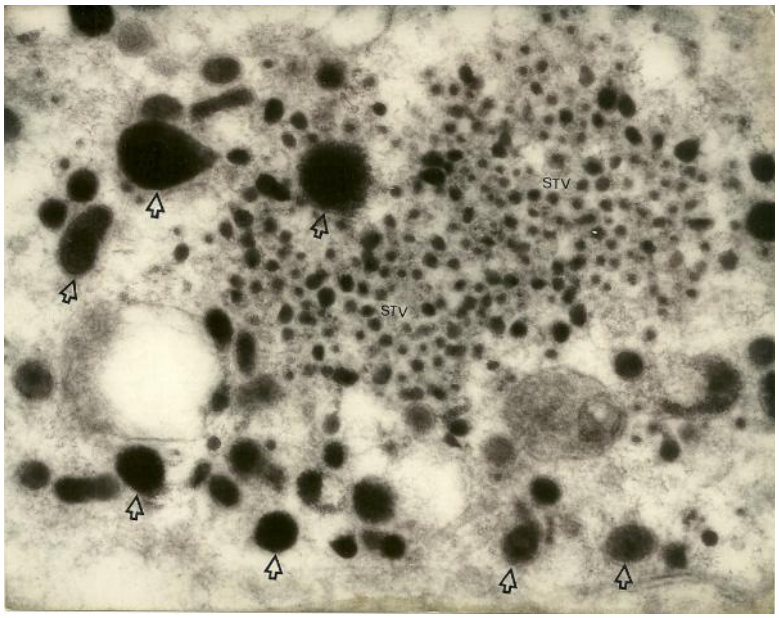

Fig. 5 Adrenomedullary section from a normal 6 week-old female mouse. Part of a typical SGC cell type II can be seen with clusters of STV and scattered typical secretory granules (arrows) . The section was fixed in a mixture of glutaraldehyde(1%) and dichromate (2.5%) and postosmicated. X 42000

The synaptic-type vesicles show a progressive decrease in number at the time of puberty in the male mouse which coincides with time of degeneration of the X-zone. Their decrease is associated with a concomitant increase of the large secretory granules and this marks the change to the next type; consequently they show a change to the next type at an earlier time in males than in females, this is probably due to a much earlier collapse of the X-zone in male mice.

A further distinctive feature of the typical SGC cell is the presence of one or more cytoplasmic processes of variable thickness and length, profiles of latter are mostly seen adjacent to their mother cell separating it from an NA or A cell or running in the interval between a SGC cell and Satellite Schwann cells. Those processes running adjacent to an NA or A cell either have a direct close relation to such cells or are separated from them by a thin process of schwann cell.

Some of them appear long enough to reach cortical cells situated at a considerable distance from the SGC cell (Fig. 6). The fine structure in the cytoplasmic processes are similar to those seen in the paranuclear cytoplasm and secretory granules and STV appear less well developed than in typical SGC cell.

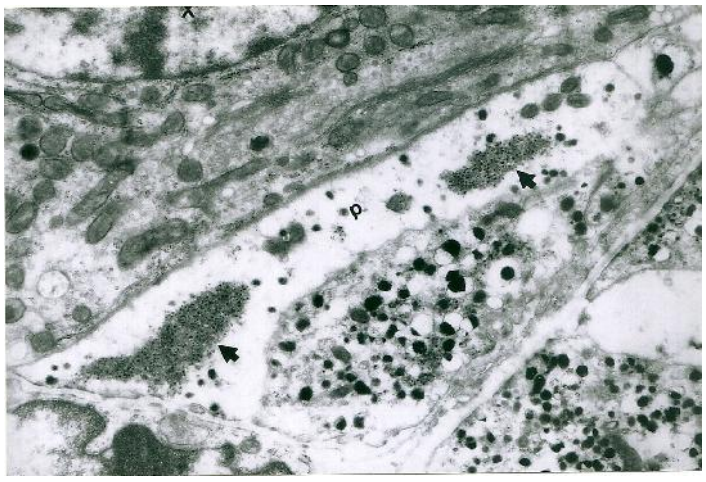

Fig. 6 Adrenomedullary section from a normal 5 week-old female mouse. A process of type II (typical) SGC cell (P) can be seen adjacent to cortical cell. Note the large clusters of STV (single arrow) . X 20400.

TYPE III OR (Late SGC Cell)

This type forms majority of SGC cells present after collapse of X- zone by natural (at puberty in males) or induced (administration of testosterone) means . consequently it tends to be present in older adult male and female mice. SGC cell gradually takes an appearance more or less similar to the early type. It can be induced to differentiate into typical SGC cell by castration of the adult male mouse which is known to induce the formation of secondary X-zone.

Effect Of Testosterone Proprionate Administration

After 2 doses of testosterone proprionate (5mg per dose) X-zone degeneration was observed (Fig. 7) and it was accompanied by progressive disappearance of STV.

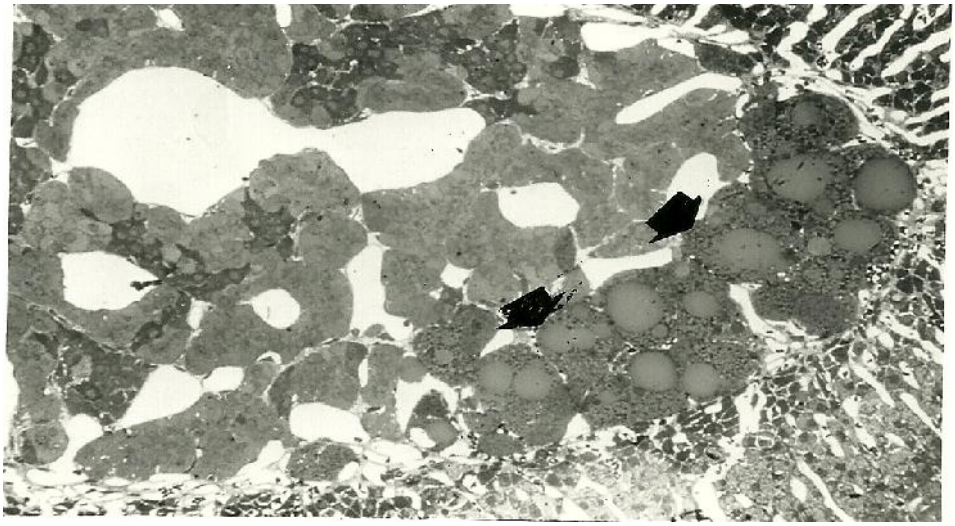

Fig. 7 Section of the adrenal gland from a 5 week-old male that received 2 doses of testosterone proprionate. X-zone degeneration can be seen (arrows). X 140

Giving further doses of testosterone results in disappearance of typical secretory granules as well and SGC cells take the appearance of small sympathetic neurons (Fig.8).

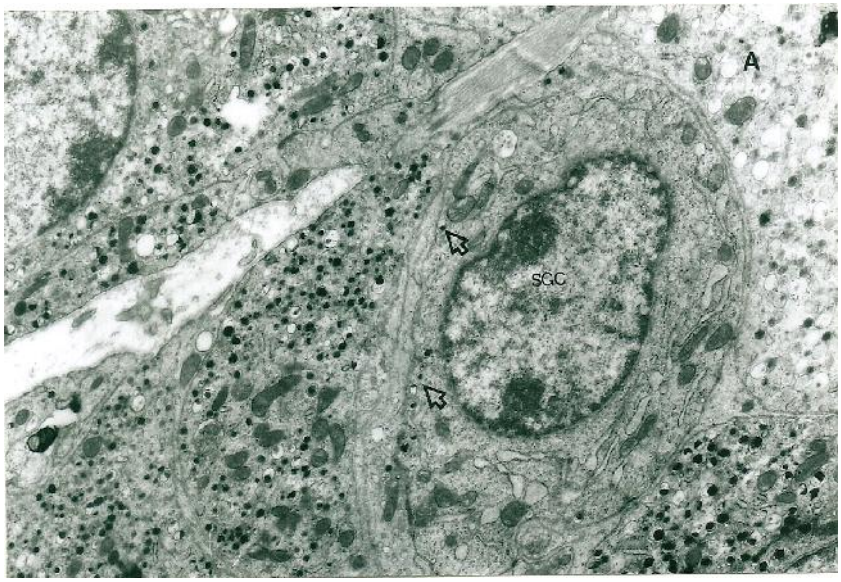

Fig. 8 Section from a 10 week-old male mouse that had been castrated 5 weeks before. Type III (late) SGC cell can be seen with few peripherally situated typical secretory granules (single arrows). The appearance of the cell is suggestive of a gradual change into a sympathetic neuron. A= adrenaline storing cell with depleted secretory granules. X 12300

Effect Of Postpubertal Castration

A secondary X-zone starts to appear 2-3 weeks following postpubertal castration , however typical SGC cells appear only after the fourth postoperative week, at a time when the X-zone cells showed the whorled pattern of smooth endoplasmic reticulum (SER)(Fig. 9).

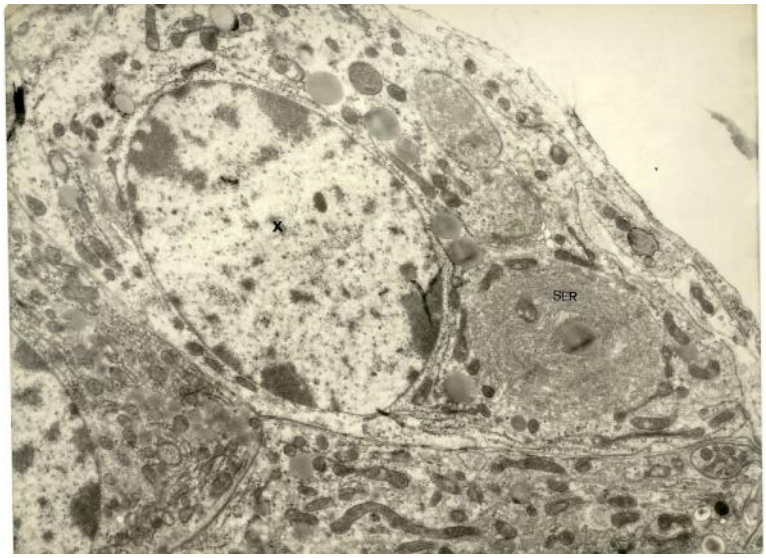

Fig. 9 Adrenocortical section from a male mouse which was postpuberally castrated 4 weeks before. Typical secondary X-zone can be seen with the characteristic whorled -pattern of smooth endoplasmic reticulum (SER). X=X-zone X 12600

Adrenal Vein Corticosterone Concentration

The serum corticosterone concentration of 3 weeks old male mice which were used as control was 225± 21 nmol/L . In postpubertal and testosterone –treated mice , corticosterone_levels were 164 ± 15 and 135 ± 13 nmol/L respectively and hence , both groups showed a highly significant fall as compared with normal age –matched animals.

Discussion

It is now generally accepted that the glucocorticoids produced mainly by the zona fasciculata of the adrenal cortex have important role in the maintenance and function of the adrenal chromaffin cells (Coupland , 1953; Wurtman and Axelrod, 1966; Wurtman, 1966; Coupland and Macdougall ,1966; Coupland ,1968; Doupe et al.,1985; Gut et al.,2005; Unsicker et al.,2013).

So far, no work has reported any effect of juxta-medullary zone and its product on the structure and function of the adrenal medulla. In the 3 week-old mouse , the adrenal medulla is composed of islands of cells surrounded by and interspersed with X-zone cells. The latter are well differentiated by this time and in particular show a well –developed whorled pattern of SER and bizarre-shaped mitochondria (Hirokawa and Ishikawa, 1974).Chester jones (1955) reported that the growth of X-zone is under control by luteinzing hormone (LH) of the adenohypophysis , consequently in the presence of a well- developed X-zone, LH production by the pituitary is expected to be high. Testosterone administration induces degeneration of X-zone which is believed to be due to a direct action of this androgen as well as its suppressing effect on pituitary LH (Jones ,1955).

In this work three types of SGC cells have been described : early, typical and late SGC cells. We believe that X-zone with its unknown secretory product induce an early cell to differentiate into the typical SGC cell.The presence of high concentrations of LH in the animals circulation at this time cannot be ignored as a pssible directly acting factor in this differentiation , however, the following observations suggest that the X-zone is primarily involved :

1) Its close physical relationship to SGC cells in 2-4 week-old animals.
2) The processes of a typical SGC cell which lie adjacent to X-zone cell show an abundance of synaptic –type vesicles.
3) Typical SGC cells with synaptic –type vesicles are numerous in females than in males and this correlates with the better developed and more persistent X-zone in females.
4) Synaptic –type vesicles (STV) have only been observed in SGC cell in mice , the only species to show a prominent X-zone , although small granule chromaffin cells without synaptic vesicles have been observrd in many species.

The effect of exogenous testosterone on SGC cells seems to be a composite one. The early disappearance of synaptic –type vesicles following the administration of 2 doses of testosterone propionate (5 mg per dose) coincides with the degeneration of X-zone and most probably results as a secondary effect to the collapse of the zone; the appearance of STV and secretory granules at this time is suggestive that they are interconvertible, the latter could be the precursor of the former and vice versa.

Giving further doses of testosterone results in disappearance of typical secretory granules as well and SGC cells take on the appearance of small sympathetic neurons. This latter change may result from a direct effect of the drug on SGC cell or from further suppression of pituitary LH , a third possibility is an antagonistic effect of testosterone to ACTH secretion (Kitay,1963) and /or the inhibition of 11β-hydroxylation step in corticosteroids biosynthesis (Sharma et al,1963). If the last effect proved valid then it means that SGC cells are particularly sensitive to slight changes in ACTH and /or corticosterone concentration , possibly through suppression of certain enzymes involved in biosynthesis of its secretory granules e.g. tyrosine hydroxylase and PNMT. In favour of this last possibility is the widespread depletion of A cells which accompanies the disappearance of typical secretory granules of SGC cells. The marked drop in corticosteroids concentration of the adrenal vein blood following the administration of testosterone proprionate and the postpubertal castration ,provide evidence for a role of both androgens and corticosteroids in the differentiation of SGC cell. Following hypophysectomy in the mouse, coupland et al (1979) reported an increase in the number of SGC cell.

Postpubertal castration of adult male mice gives a clear example of the capacity of a well-developed X-zone to induce the differentiation of typical SGC cells from late types already present. Although proliferation of zona reticularis cells to form a secondary X-zone takes place in the first 2-3 weeks following postpubertal castration, typical SGC cells reappear only after the fourth postoperative week, at a time when well differentiated cells of the zona reticularis take on specific features of X-zone cells, in particular the whorled pattern of SER.

In conclusion the SGC cells in the adrenal medulla of the mouse might be interpreted as relatively immature elements that are not reached by factors, which are required for further differentiation. The high nucleus cytoplasm ratio and the abundance of ribosomes would be in line with an interpretation of SGC cells as immature chromaffin cells.

The long processes of SGC cells which have a direct close relation to cortical cells suggest that these cells may influence corticosteroids secretion. If this assumption proved correct , then the SGC cell like the other chromaffin cells is influenced by corticosteroids produced by the adrenal cortical cells and in addition has an influence on the function of the cells.

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