Chromaffin Cells in the Adrenal Medulla of the Mouse: A Morphological Study of their Innervation

Faraj Bustami, [1] Salim Khraisha [1]*

Abstract

Objective: To study the synaptic nerve endings on the adrenaline(A), noradrenaline(NA) and small granular Chromaffin (SGC) cells of the mouse before and after puberty and the possible functional relationship between these cells.

Methods: Male albino mice bred at the animal house-Jordan University, which aged 5, 10 & 12 days and 5 weeks were used. After sacrifice, the adrenal glands were removed from the animals and processed for examination with a Philips 300 electron microscope.

The percentage of SGC cells and the number of synaptic endings on A and NA cells were calculated.

Results: All the nerve endings observed are typical in appearance with the cholinergic-type synaptic endings. No evidence of an adrenergic innervation of Chromaffin tissue of the adrenal medulla has been obtained during the present work, though the elongated processes of small granular Chromaffin cell could readily be misinterpreted as adrenergic nerve fibres.

The processes of these cells come close to other Chromaffin cells, both A, NA cell and form synapses. Frequent exocytotic figures related to these cells were observed.

Conclusion: The present findings suggest that the SGS cells form an intrinsic system that regulates the activity of other chromaffin cells, particularly in the early postnatal period when the extrinsic innervation of the chromaffin cells is not yet fully developed.

Keywords: Chromaffin cells, synaptic nerve endings, exocytosis.

(J Med J 2009; Vol. 43 (2):91- 97)

Received	***Accepted***
March 30, 2008	*June 15, 2008*

Introduction

The preganglionic sympathetic nerve fibres of the splanchnic nerves have been implicated in the control of adrenal medullary catecholamine secretion since the late nineteenth and early twentieth centuries. [1,2,3,4]

Electron microscopic studies on the rat [5,6,7] indicated that the majority of the nerve fibres in the adrenal medulla are non-myelinated and that nerve endings, are, in appearance, typical of cholinergic-type synaptic endings.

1- Departments of Anatomy and Physiology, Faculty of Medicine, University of Jordan, Amman, Jordan.
**** Correspondence should be addressed to:***
Dr. Faraj Bustami
Faculty of Medicine, University of Jordan, Amman, Jordan.

Lewis and Shute reported that noradrenaline-storing (NA) cells had a denser innervation than adrenaline-storing (A) cells in the rat. Grynspan-Winograd described conformational differences in nerve endings on A and NA cells in the hamster.

In the rat, splanchnic control of adrenomedullary function is absent at birth and starts functioning by 10 days after birth. [10] According to Slotkin, [11] the unresponsiveness of adrenal chromaffin cells in the rat to neurogenic stimuli during the early postnatal period is due to relatively few nerve terminals.

The SGC cell was described by several authors as an intermediate type between the chromaffin cells and sympathetic neurons. [12,13,14] These authors reported that these cells store noradrenaline and dopamine.

In the present work, we sought to provide a descriptive and quantitative account on the innervation of the mouse adrenal chromaffin cells at different ages. A possible functional relationship between SGC cells and the A and NA cells is discussed.

Materials and Methods

Male albino mice bred at the animal house-Jordan University, which aged 5,10 and 12 days and 5 weeks were used. Five to nine animals were used in each age group. They were housed under 12 hours light, 12 hours darkness and have access to mouse pellets and water ad libitum. The animals were anesthetised with sodium pentobarbitone (60 mg/kg I.P). After thoracotomy they were fixed by perfusion through the left ventricle using a solution of gluteraldehyde 2.5% in 0.1 M cacodylate buffer, PH 7.4. the adrenal glands were then removed from the animals and cut with a razor blade into parallel slices approximately one millimeter thick, immersed in the above fixative for a further 3 hours, washed in cacodylate buffer, postfixed in 1% osmium tetroxide in millonig buffer, dehydrated and embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

Three randomly selected areas of adrenal medulla were examined from each specimen for synaptic contacts on chromaffin cells. The criteria of a synaptic contact was considered to be the presence of pre-and post-synaptic membranous thickenings together with the presence of clear and few dense cored vesicles in the pre-synaptic boutons.

The percentage of SGC cells was as follows: Three to six thin sections were obtained from different levels of each adrenal gland and the total number of SGC, while NA and A cells were calculated per grid-square of section. Then, all squares occupied by adrenal medulla were counted.

The percentage of SGC cells per adrenal medulla=

$$\frac{\textit{Total number of SGC cells per section}}{\textit{Total number of SGC, NA \& A cells per section}} \times 100$$

Results

In the first five days, the chromaffin cells were immature in form, size and appearance of their secretory granules (Fig.1). synaptic nerve endings of widely different profile diameters and appearance were observed together with zonal Pre- and post-synaptic membrane thickenings. They contained many small clear vesicles of about 50 nm diameter and some contained larger dense-cored vesicles (Fig. 2). The mean number of synapses per A and NA cell was l.2 and 2.5 per SGC cell (table 1). The latter formed 8.5% of the total chromaffin cells. SGC cells and their processes possessed mainly small amine storage granules (less than 150 nm core diameter) and few aggregations of synaptic-type vesicles (STV) (Fig. 3). Many of the small granules were aligned along the plasma membrane suggestive of an exocytotic process (Fig.4). Tapering extensions or processes of SGC cells ended adjacent to other chromaffin cells. A synaptic arrangement was suggested by desmosomal symmetrical densities of plasma membranes at

the contact zone between SGC process and an adjacent chromaffin cell (Fig. 5).

Between the 10-12 postnatal days distinct adrenaline (A), noradrenaline- storing (NA) and SGC cells were evident in the adrenal medulla (Fig.6). SGC cells and their processes showed an abundance of mitochondria. The synaptic nerve endings also showed a larger number of mitochondria as compared to those seen in the first week. Occasionally, a synaptic profile ends with both A or NA cells as well as with SGC cell (Fig. 7) The mean number of synapses per chromaffin cell and SGC cells was 2.8 for the A cell, 3.0 for the NA cell and 2.8 for the SGC cell (table 1). The SGC cells formed 6% of the total chromaffin cells (table 1).

By the end of the fifth week, the general appearance of the adrenal medulla was similar to that of the mature animal. The synaptic profiles have the appearance of a typical cholinergic nerve ending (Fig 8). Synaptic endings of the SGC cell as well as the exocytotic figures of this cell are still evident.

(Fig. 9, 10) The mean number of synapses per chromaffin cell was 3.1 and 3.3 for A and NA cells and 2.9 for the SGC cell (table 1). The SGC cells formed only 5% of the total chromaffin cells (table 1).

Table (1): Mean Numbers of synaptic profile on chromaffin cells.

Age (n)	*A cells*	*NA cells*	*SGC cells*
Day 5 (6)	*1.2 ± 0.2*	*1.2 ± 0.3*	*2.5 ± 0.9*
Day 11 (8)	*2.8 ± 0.5*	*3.0 ± 0.4*	*2.8 ± 0.2*
Week 5 (9)	*3.1 ± 0.4*	*3.3 ± 0.6*	*2.9 ± 0.3*

Results expressed as mean ± S.D; n= number of animals.

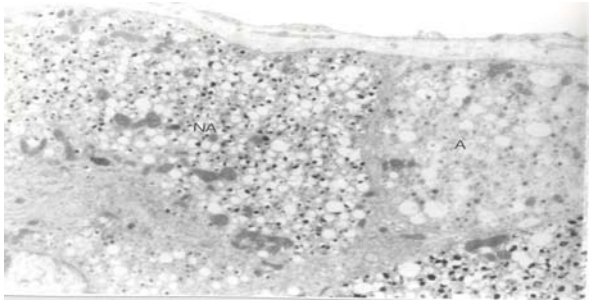

Fig. (1): Adrenal medulla of a 4 days old mouse. It shows part of an A and NA cells. (X 10.000).

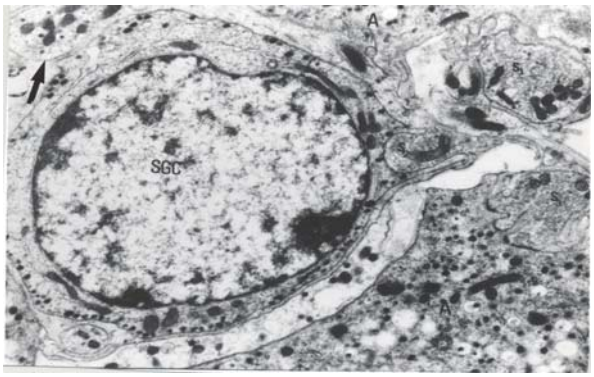

Fig. (2): Adrenal medulla of a 5 days old mouse. Three synaptic nerve endings (S1, S2, S3), can be seen. S2 on SGC cell and S3 on A cell. The contents of the synaptic endings are mostly clear vesicles and the membrane densities asymmetrical (arrow head). A fourth synaptic profile (arrow) with dense-cored vesicle can be seen (X 18.000).

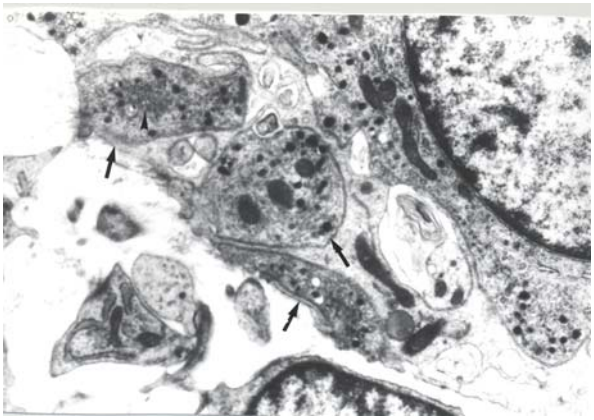

Fig. (3): Three processes of SGC cells (arrows) can be seen, two of them have clusters of synaptic-type vesicles (arrow heads)(X 20.000).

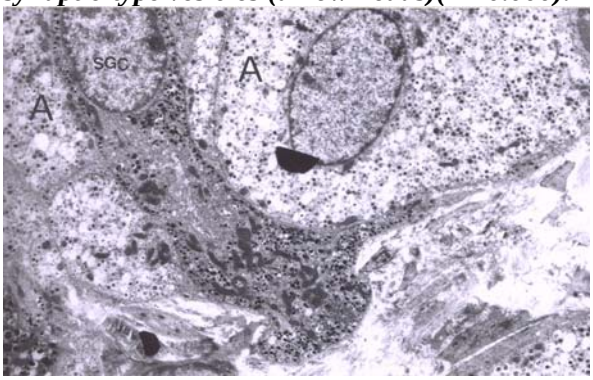

Fig. (4): A SGC cell appears between two A cells. The secretory granules of SGC cells are aligned along the plasma membrane (X 8.000).

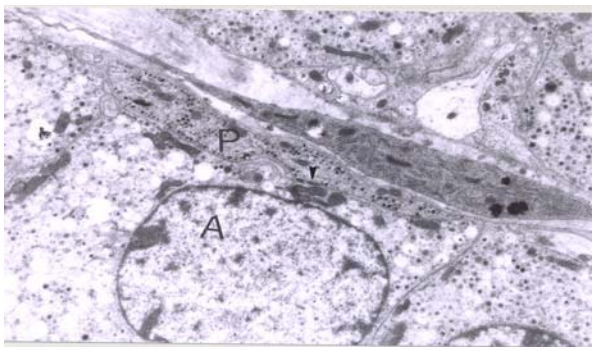

Fig. (5): Adrenal medulla of 5 days old mouse showing a process (P) of SGC cell makes a synapse with an A cell-Alignment of the secretory granules along the plasma membrane and symmetrical densities (arrow head) can be seen (x 9 500).

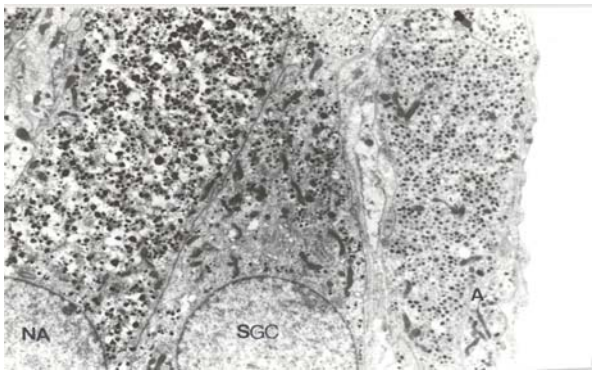

Fig. (6): Adrenal medulla of II days old mouse showing A, SGC and NA (X 6000).

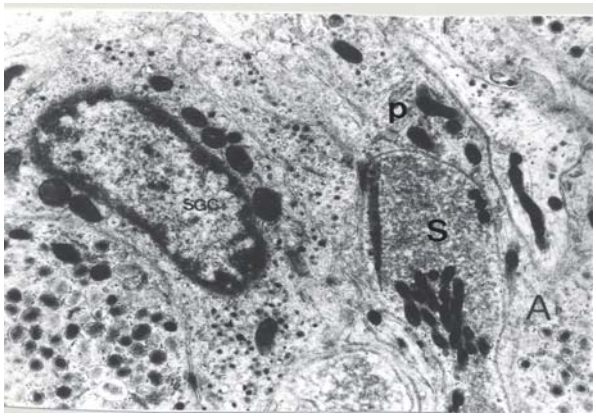

Fig. (7): Adrenal medulla of 12 days old mouse showing a large synaptic nerve ending (S) associated with part of an A cell (A) and the process (P) of an SGC cell. The synaptic ending contains clear vesicles and large number of mitochondria. Part of SGC cell (SGC) can be seen with large number of mitochondria (X 18.000).

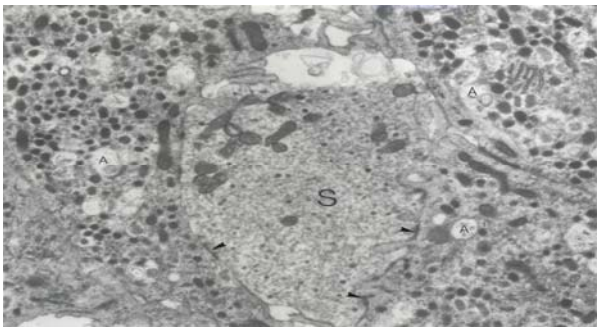

Fig. (8): Adrenal medulla of 5 weeks old mouse showing a large synaptic nerve ending (S) associated with part of three A cell (A). The contents of synaptic ending are mostly clear vesicles with some dense-cored vesicles. The cell membranes show symmetrical densities (arrow heads) (X 22.000).

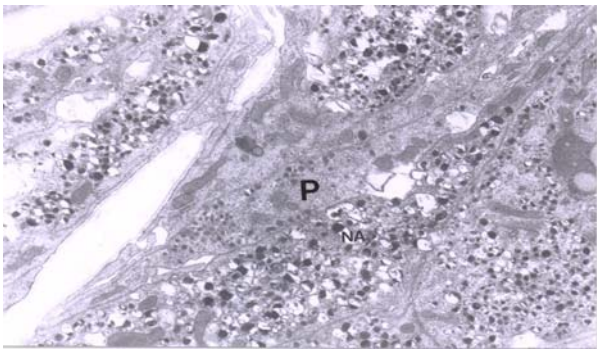

Fig. (9): Adrenal medulla of five weeks old mouse showing the process (P) of an SGC cell synapsing on an NA cell (NA) (X 10.000).

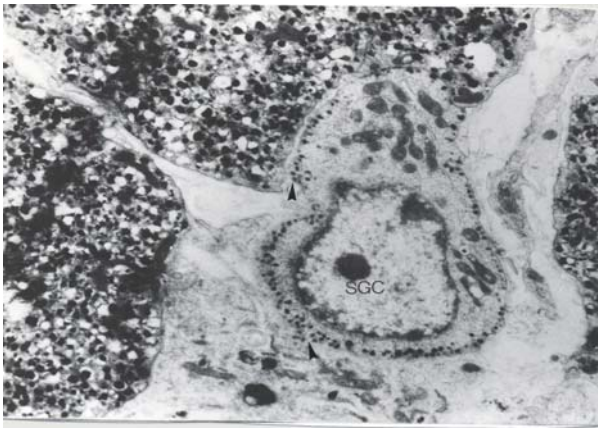

Fig. (10): Adrenal medulla of five weeks old mouse showing an SGC cell surrounded by a group of NA cells. Some of the secretory granules appear just outside the cell membrane (X 10.500).

Discussion

The present findings indicate that all the three types of chromaffin cells in the adrenal medulla of the mouse receive almost similar cholinergic synaptic endings. The only morphological difference between these endings is in the relative amount of the mitochondria which were more numerous in the synapses on the NA and SGC cells. Except for the first few days after birth, no significant difference was observed in the number of the synaptic endings on the A, NA and SGC cells. These observations are in keeping with previous work in the rat [7] but they differ from those reported on the hamster. [14]

The greater number of the synaptic endings on the SGC cells at the early postnatal period may be related to the function of these cells. The bodies and processes of these cells extend between the A and NA cells and synapse on the bodies of these cells. The SGC cells may play a role in regulating the functions of the A and NA cells especially during the early postnatal period, at a time when the splanchnic control of the adrenomedullary function is absent. [10,11]
The physiological findings of Smith, Slotkin and Mills[10] and Mills and Smith[11] has demonstrated the unresponsiveness of the adrenal chromaffin cells of the rat to neurogenic stimuli during the first ten days after birth, although catecholamines were detected in the blood coming out of the adrenal gland.

In addition to the sympathetic preganglionic innervation to the adrenal medulla, a significant spinal sensory innervation of the adenal gland has been demonstrated in the guinea pig[12] and rat. [13] The possibility exists that as with enteric neurons, SGC cells may act through local (short) or long reflexes.

Although the present work does not explain the cause of the decrease in the percentage of SGC cells to 5% of the total chromaffin cells by the age of five weeks; the cells that persist still show evidence of synapse on A and NA cell as well as exocytosis.

The phenomenon of exocytosis as the discharge mechanism for the secretion of catecholamines is largely accepted, although morphological evidence is rarely obtained. [6,14- 17] In the present work, most of the steps of the exocytotic process were observed including the alignments of the secretory granules along the cell membrane of the SGC and the appearance of these granules on the outer surface of the cell membrane.

No evidence of an adrenergic innervation of chromaffin tissue of the adrenal medulla has been obtained during the present work, though the elongated process of SGC cell could readily be misinterpreted as adrenergic nerve fibres.

Recently, Tischler et al. [18- 20] using reserpine has reported that the chromaffin cell proliferation is mediated by the interaction of neurogenic and hormonal signals. The same authors suggested that the rat pheochromocytomas may be derived from a distinctive stem cell such as the SGC cell. [18] These physiological and pharmacological observations suggest the possibility that chromaffin cells might be propagated in vitro for use in basic biological studies or in transplants for the treatment of Parkinson's disease.

The authors would like to thank Miss Joulliete Shamieh for the excellent typing of the manuscript.

References

1. Dreyer GP. On secretory nerves to the suprarenal capsules. American Journal of Physiology 1899; 2: 203-219.
2. Elliott TR. The control of the suprarenal glands by the splanchnic nerves. Journal of Physiology 1912; 44: 374-409.
3. Elliott TR. The innervation of the adrenal glands. Journal of Physiology 1913; 44: 285-290.
4. Feldberg W, Minz B & Tsudzimura H. The mechanism of the nervous discharge of adrenaline. Journal of Physiology 1934; 81: 286-304.
5. Coupland RE. The Natural History of the Chromaffin Cell. London: Longmans Journal of Physiology 1965; a.

6. Coupland RE. Electron microscopic observations on the structure of the rat adrenal medulla: I. The ultrastructure and organization of chromaffin cells in the normal adrenal medulla. Journal of Anatomy 1965 b; 99: 231-254.
7. Tomilinson A and Coupland RE. The innervation of the adrenal gland. IV, Innervation of the rat adrenal medulla from birth to old age. A descriptive and quantitative morphometric and biochemical study of the innervation of chromaffin cells and adrenal medullary neurons in Wistar rats. J Anat 1990; 169: 209-236.
8. Leweis PR & Shute CCD. An electron microscopic study of cholinesterase distribution in the rat adrenal medulla, Journal of Microscopy 1969; 89: 181-193.
9. Grynspan-Winograd O. Adrenaline and noradrenaline cells in the adrenal medulla of the hamster A morphological study of their innervation., Journal of Neurocytology 1974; 3: 341-361.
10. Slotkin TA. Development of the sympathoadrenal axis. In Developmental Neurobiology of the Autonomic Nervous System (ed. P.M. Gootman) 1986; 69-96. Clifton, NH: Humana Press.
11. Slotkin TA, Chantry CJ & Baratolome J. Development of central control of adrenal catecholamine biosynthesis and release. Advances in Bioscience 1982; 36: 95-102.
12. Mohamed AA, Parker TL & Coupland RE. The innervation of the Adrenal gland. II. The source of spinal afferent nerve fibres to the guinea-pig adrenal gland. Journal of Anatomy 1988; 160: 51-58.
13. Afework M. The sensory innervation of the rat adrenal gland: a retrograde tracer study. M. Phil thesis; 1988, University of Nottingham.
14. Accord F. The chromaffin cells of the siren lacertian, cytological characteristics and evidence of exocytosis, 1988; 150:169-176.
15. Coupland RE. Electron microscopic observations on the structure of the rat Adrenal medulla. II Normal innervation. Journal of Anatomy 1965; 99: 255-272.
16. Grynszpan-Winograd O. The adrenaline and noradrenaline cells in the adrenal medulla of the hamster: a morphological study of their innervation. J.Neurocytol.1974; 3: 341-361.
17. Carmichael SW & Winkler H. The adrenal chromaffin cell. Scientific American 1985; 253: 40-49.
18. Tischler AS, Delellis RA, Allen JM et al. Spontaneous proliferative lesions of the adrenal medulla in aging Long-Evans rats: Comparison of PC 12 cells, small granule-containing cells and human adrenal medullary hyperplasia. Lab Invest 1985; 53: 486-498.
19. Tischler AS, McClain RM, Childers H, and Downing J. Neurogenic signals regulate chromaffin cell proliferation and mediate the mitogenic effect of reserpine in the adult rat adrenal medulla. Lab invest. 1991; 65: 347-376.
20. Tischler AS, James FP, Jeffery Z, Downing J and McClain M. Comparative studies of chromaffin cell proliferation in the adrenal medulla of Rats and Mice. 2002; Fundamental & Applied Toxicology 35: 216-220.

الخلايا أليفة الكروم في نخاع الغدة الكظرية للفأر: دراسة تشريحية حول تعصيب هذه الخلايا

فرج البسطامي، سليم خريشا
قسم التشريح والأنسجة، كلية الطب، الجامعة الأردنية، عمان، الأردن

الملخص

الهدف: دراسة نهايات الأعصاب التشابكية على أجسام الخلايا أليفة الكروم المفرزة للأدرينالين والنورأدرينالين، والخلايا أليفة الكروم الصغيرة في الفأر، وكذلك دراسة علاقة وظيفية محتملة بين هذه الخلايا.

الطرق: استخدمت أعمار مختلفة من ذكر حيوان الفأر الأبيض، الموجود في بيت الحيوانات، الجامعة الأردنية. وقد تراوحت أعمار الحيونات المستخدمة بين خمسة وعشرة واثني عشر يوماً وخمسة أسابيع. وبعد قتل هذه الحيوانات فُحصت شرائح من نخاع الغدة الكظرية باستخدام المجهر الإلكتروني، كما أُحصيت النسبة المئوية للخلايا أليفة الكروم الصغيرة وعدد التشابكات العصبية على أجسام الخلايا أليفة الكروم المفرزة للأدرينالين والنورأدرينالين.

النتائج: كانت جميع النهايات العصبية التشابكية من النوع المفرز للأستيل كولين، ولم نلحظ وجود أيّ من النهايات العصبية التشابكية من النوع المفرز للنورأدرينالين بالرغم من أن زوائد الخلية أليفة الكروم الصغيرة تشبه في محتواها النهايات العصبية المفرزة للنورأدرينالين. ولوحظ اقتراب زوائد هذه الخلية من أجسام الخلايا أليفة الكروم المفرزة للأدرينالين والنورأدرينالين مشكلة ما يشبه التشابك العصبي مع هذه الخلايا، وكذلك لوحظ أن هذه الخلايا الصغيرة تظهر باستمرار إفرازاً خلويا تسريبا.

الاستنتاج: تشير نتائج البحث إلى احتمال قيام الخلايا أليفة الكروم الصغيرة بدور خلايا من داخل نخاع الغدة الكظرية تعمل على تنظيم وظائف الخلايا أليفة الكروم المفرزة للأدرينالين والنورأدرينالين، وخاصة في الأيام الأولى بعد الولادة، التي يكون فيها تكوين الأعصاب المنبهة لإفراز هذه الخلايا غير مكتمل.

الكلمات الدالة: الخلايا أليفة الكروم، التشابك العصبي، إفراز خلوي تسربي.