



ENDOCRINE SYSTEM PATHOLOGY



Katarina KATIĆ
Steva NAJMAN
Snežana CEKIĆ
Vladimira BOJANIĆ
Vuka KATIĆ

MEDICAL FACULTY, UNIVERSITY OF NIŠ, NIŠ, YUGOSLAVIA

Hypothalamic lesions in mice treated with monosodium glutamate

ABSTRACT

In newborn mice subcutaneous injections of monosodium glutamate induced acute neuronal gliosis, microglial nodules and neuronophagia in several region of the developing brain including hypothalamus. Twenty newborn C57BL/6J mice were injected daily with MSG (4,4 mg/g body wt) sc, on days 1-10 of life, for the first 5 postnatal days. Another group of 10 mice, which were injected with saline solution, 0,02 ml/g body wt, every 24 h for the first 5 postnatal days, served as controls. As adults, treated animals showed stunted skeletal development, marked obesity and sterility. Pathological changes were also found in several organs associated with endocrine functions. Studies of food consumption failed to demonstrate hyperphagia to explain the obesity. It is postulated that the adult syndrome represents a multifaceted neuroendocrine disturbance arising from the disruption of developing neural centers concerned in the mediation of endocrine function.

KEYWORDS: Sodium Glutamate; Obesity; Hypothalamus; Mice

INTRODUCTION

In an earlier reports from this laboratory, neonatal treatment with monosodium glutamate (MSG) was shown to cause a disruption of the hypothalamic-pituitary-gonadal axis which induced Cushing's obesity, letargy, hypogonadism and sterility (1-4), as well as spleen atrophy (2). The administration of monosodium glutamate (MSG) to mice in the neonatal period induces destructive lesions in the arcuate nuclei of the hypothalamus and results in a syndrome of obesity, stunting and hypogonadism (4). In spite of a destruction of the arcuate nucleus which plays a major role in neuroendocrine and autonomic integration, treated animals consume less food than controls (1,5,6). Corticosterone level is, however, high in the MSG-obese mouse, but the mechanism of this obesity is not clear (7).

Having in mind the contradictory data on hypothalamic lesions in obese mice, the aim of the present work was thus to study the MSG-effect on hypothalamus.

MATERIALS AND METHODS

Twenty newborn C57BL/6J mice were injected daily with MSG (4,4 mg/g body wt) sc, on days 1-10 of life, for the first 5 postnatal days. Another group of 10 mice, which were injected with saline solution, 0,02 ml/g body wt, every 24 h for the first 5 postnatal days, served as controls. Mice were killed at 4 months of life. Their hypothalamic tissue was fixed in 10% Formalin for 24 h.

After fixation, hypothalami were routinely processed and embedded in paraffin. Each section (5 μm in thickness) was stained with HE, Van Gieson and Gomori, and observed microscopically.

RESULTS

Treated animals were quite lethargic as adults, and they lacked the sleekness of body coat, seen in controls. Cushing-type of obesity was evident macroscopically. Basophil cells of pituitary gland were hyperplastic of large size and with foamy aspect. Cortical cells of the adrenal gland, symmetrically, were also hyperplastic.

Hypothalamic tissue. The arcuate nuclei were completely destroyed along with neuronal constituents in the median eminence. Instead these tissues, gliosis was found, the most important histopathologic indicator of CNS injury. So, astrocytes were hypertrophic and hyperplastic, their nuclei small and dark located in a dense net of processes (glial fibrils). Multifocal small foci of microglial nodules, associated with neuronophagia, were also observed.

DISCUSSION

The hypothalamic lesions induced by neonatal treatment with MSG are primarily in the preoptic and arcuate nuclei and in the median eminence. The arcuate nucleus plays a major role in neuroendocrine and autonomic integration (6), and lesions in this region of the hypothalamus result in disruption of the hypothalamic-pituitary-adrenal axis. Corticosterone level is high in the MSG-obese mouse suggesting that ACTH secretion is not reduced. Whether a regulatory mechanism affecting fat metabolism in the mouse can be localized to the arcuate nucleus, or to other brain areas selectively destroyed by MSG treatment (8), requires further study, especially the topography of acute lesions of hypothalamus. The question if MSG at the same time destroy peptidergic fibers responsible for SRIF synthesis (inhibitor of ACTH secretion) or not, also waits the answer.

CONCLUSION

Brain lesions, obesity, endocrine gland dysfunction and hypothalamic multifocal lesions in mice treated with MSG suggest a complex hypothalamo-pituitary-adrenal-gonadal disturbance.

REFERENCES

1. Katić V, Matijaš M, Najman S et al. Adrenal Gland in Glutamate-treated Rats. *Pathol Res Pract* **1995**;191:816.
2. Katić V, Najman S, Vukanović A et al. Spleen atrophy in Glutamate-treated Mice. New approach on Cushing's type of Obesity. *Int J Hematol* **1996**;64(Suppl.1):78.
3. Katić V, Matijaš M, Najman S et al. Čelije pankreasa u Kušingovom sindromu. Eksperimentalna studija. *Acta Medicae Medianae* **1996**;5:5.
4. Katić V, Najman S, Stanković T, Katić K. Testicular Function in mice Treated with MSG. *Pathol Res Pract* **1997**;193:385.
5. Katić V, Najman S, Đinđić B. New approach on Cushing's type of Obesity in mice treated with MSG. *Archives d'anatomie et de cytologie pathologiques* **1998**;(Suppl.5-6):356.
6. Poon T, Camron D. Measurement of oxygen consumption and locomotor activity in monosodium glutamate - induced obesity. *Am J Physiol* **1978**;234:E532.
7. Tokuyama K, Himms-Heagen J. Adrenalectomy prevents obesity in glutamate -Treated mice. *Am J Physiol (Endocrinol Metabol 20)* **1989**;257: E139.
8. Olney J. Brain Lesions, Obesity and Other Disturbances. *Science* **1969**;164:719.

Address correspondence to:
Dr Katarina Katić, Medical Faculty of Niš University, 18000 Niš, Yugoslavia

The manuscript was received: 17. 03. 2000.

Accepted for publication: 20. 04. 2000.

Slavica KOSTADINOVA - KUNOVSKA
Gordana PETRUŠEVSKA
Svetlana DUGANOVSKA
Mirjana TOLOVSKA
Pvetkovski CVETKOVSKI

INSTITUTE OF PATHOLOGY, MEDICAL FACULTY, SKOPJE, REPUBLIC MACEDONIA

Immunophenotypical characteristics of phaeochromocytomas

ABSTRACT

Pheochromocytoma is a rare tumor originating from the chromaffin cells in the adrenal medulla and at the extra-adrenal sites in the body (paraganglia). The purpose of this paper is to determine the immunophenotypical characteristics of pheochromocytomas in our bioptic material. Seven cases of phaeochromocytomas were analyzed, using standard histological, histochemical and a panel of immunohistochemical stainings. All 7 cases showed marked positivity with synaptophysin and chromogranin. Four cases from 4 stained were positive for NSE and NFP. All epithelial markers were negative in most of the cases, except in two cases where weak signal was noted. Three cases from three stained were positive for S-100. Stainings for LCA and GFAP were negative, and for bcl2, as a valuable prognostic antibody, were also negative. Most valuable were the immunohistochemical stainings with synaptophysin, chromogranin, neuron-specific enolase (NSE), neurofilament protein (NFP) and S-100 protein, which showed marked positivity in all stained tissue specimens.

KEYWORDS: Pheochromocytoma; Immunohistochemistry

INTRODUCTION

Pheochromocytoma is a rare tumor originating from the chromaffin cells in the adrenal medulla, and with less frequency, from the chromaffin cells at the extra-adrenal sites in the body (paraganglia). 10-20% of these cases have extra-adrenal localization. According to their biological behavior, pheochromocytomas are mostly benign tumors, with a distinctive clinical manifestation of paroxysmal hypertension crises, owing to the excessive production of a different quantity of adrenaline and noradrenaline from the chromaffin cells. Nevertheless, in less than 5% of the cases, the biological behavior of the tumor is malignant. The only valid criteria that leads to such diagnosis is the existence of metastatic deposits on sites where chromaffin cells are not usually present. The purpose of this paper is to determine the immunophenotypical characteristics of pheochromocytomas in our bioptic material.

MATERIALS AND METHODS

Address correspondence to:
Dr S. Kostadinova-Kunovska, Institute of Pathology, Medical Faculty, Skopje, Republic Macedonia

The manuscript was received: 10. 03. 2000.

Accepted for publication: 14. 04. 2000.

We present 7 cases of pheochromocytomas, 5 with adrenal and 3 with extra-adrenal localization, analyzed at the Institute of Pathology in the last 15 months. 2 of the patients were female and 5 were male, with an average age of 35,3 years. The received operative material was fixed in neutral formaldehyde solution, stained with standard histological HeEo staining, histochemical stainings (Van Gieson, Reticulin, Trichrome and PAS) and immunohistochemical stainings with a panel of monoclonal antibodies available at the Institute of Pathology (synaptophysin, chromogranin, neuron-specific enolase (NSE), neurofilament protein (NFP), S-100 protein, GFAP, CKWS, CK18, CK19, ESA, LCA and bcl2, all DAKO products), using the modified Avidin-Biotin Complex Immunoperoxidase Method of Hsu.

RESULTS

The analyzed operative material had dimensions varying from 22 to 200 mm, and the largest tumor mass weighed 1200 grams. The standard histological analyses showed tumor processes composed of chromaffin cells arranged around stromal blood vessels with abundant granular cytoplasm and large nuclei containing one or more nucleoli. The cellular and nuclear pleomorphism varied from case to case, as well as the presence and number of mitotic figures. In order to determine the immunophenotypical characteristics of the analyzed tumors, immunohistochemical analyses with a panel of the above mentioned antibodies were done. All 7 cases showed marked positivity with synaptophysin and chromogranin. Four cases from 4 stained were positive for NSE and NFP. All epithelial markers were negative in most of the cases, except in two cases where weak signal was noted. Three cases from three stained were positive for S-100. Stainings for LCA and GFAP were negative, and for bcl2, as a valuable prognostic antibody, were also negative. Since the general histological criteria of malignancy are not applicable in phaeochromocytomas and in the absence of any clinical information, none of these cases was pronounced to be malignant.

DISCUSSION

According to the available literature (1), the incidence of pheochromocytomas is 1 case in year on 1-2 million population, but other authors (2) consider that the frequency of this entity is higher. Our series contains 4 cases analyzed during 12 months on 2 million population. We can not explain this higher frequency and it could be a subject of a further more extensive study. In the retrieved literature, only a few articles treating the immunohistochemical properties of the pheochromocytomas were found. The following antibodies were used for routine diagnosis of these tumors: NSE, S-100, cytokeratins, HMB 45. Most of the cases were positive for NSE and S-100 protein (3,4). In a study of 102 patients, in 29 cases treated as malignant, expression of bcl2 protein was found, and the rest were negative (5). In our analyzed 7 cases, most of the cases were positive for synaptophysin and chromogranin, as well as NSE, NFP and S-100 protein, which means that the panel of antibodies useful for diagnosis of pheochromocytoma could be extended with the above mentioned. The positivity for chromogranin in tissue specimens corresponds to the found increased levels of plasma chromogranin detected by RIA (6,7).

CONCLUSION

Immunohistochemical analyses are most valuable in the diagnosis of the tumors of chromaffin cells originating from the adrenal medulla and extra-adrenal sites, and could be applied especially when their histological appearance is unclear, cells are highly anaplastic, and the tumor is necrotic.

REFERENCE

1. de Graeff J, Horak BJV. The incidence of phaeochromocytoma in the Netherlands. *Acta Med Scan* 1964;176:583.



2. Silverberg SG. Pathology of the Adrenal Medulla in Principles and Practice of Surgical Pathology. Vol 1. Churchill Livingstone, **1988:1475-8.**
3. Lloyd RV, Shapiro B, Sisson JC, Kalf V, Thompson NW, Beierwaltes WA. An immunohistochemical study of pheochromocytomas. Arch Pathol Lab Med **1984;108:541-4.**
4. Unger P, Hoffman K, Pertsemidis D, Thung S, Wolfe D, Kaneko M. S100 protein-positive sustentacular cells in malignant and locally aggressive adrenal pheochromocytomas. Arch Pathol Lab Med **1991;115:484-7.**
5. de Krijger RR et al. Prognostic value of p53, bcl-2 and c-erbB-2 protein expression in phaeochromocytomas. J Pathol **1999;188:51-5.**
6. Kimura N et al. Plasma chromogranin A in pheochromocytoma, primary hyperparathyroidism and pituitary adenoma in comparison with catecholamine, parathyroid hormone and pituitary hormones. Endocr J **1997;44:319-27.**
7. Stridsberg M, Husebye ES. Chromogranin A and chromogranin B are sensitive circulating markers for phaeochromocytoma. Eur J Endocrinol **1997;136:67-73.**

M. BUDEČ
V. KOKO
T. MILOVANOVIĆ
A. PETKOVIĆ

INSTITUTE FOR MEDICAL RESEARCH, BELGRADE, YUGOSLAVIA

Ethanol-induced changes in the adrenal cortex of ovariectomized rats

KEYWORDS: Ethanol; Naltrexone; Adrenal Cortex; Rats; Ovariectomy

We previously showed an increased level of progesterone in adult female rats following acute ethanol treatment. To determine whether this effect is the result of activation of adrenal glands we morphologically analysed the adrenal cortex in ovariectomized rats. In addition, a possible involvement of the opioid system in observed phenomenon was tested. Adult female Wistar rats were ovariectomized and three weeks after surgery treated intraperitoneally with: a) ethanol (4 g/kg), b) naltrexone (5 mg/kg) followed 45 min later by ethanol (4 g/kg) and c) naltrexone (5 mg/kg) followed 45 min later by saline. Untreated and saline-injected rats were used as controls. The animals were sacrificed 1/2 h after ethanol administration. Paraffin sections were stained with hematoxylin-eosin and analysed using stereological measurements. Morphometric analysis revealed that a single dose of ethanol significantly increased the volume density of zona glomerulosa and zona fasciculata and decreased the volume density of zona reticularis. Pretreatment with naltrexone prevented these ethanol-induced changes. The results indicate that the previously described increased level of progesterone following acute ethanol treatment, is a result of the activation of the adrenal glands and suggest the involvement of opioid receptors in this phenomenon. (This work was supported by the Ministry of Science and Technology of the Republic of Serbia.)



M. HAVELKA¹
I. PAUNOVIĆ²
S. TATIĆ¹
R. JANKOVIĆ²
A. DIKLIĆ²
H. BAŠIĆ³

¹INSTITUTE OF PATHOLOGY, SCHOOL OF MEDICINE, UNIVERSITY OF BELGRADE, YUGOSLAVIA

²CENTER OF ENDOCRINE SURGERY, INSTITUTE OF ENDOCRINOLOGY, DIABETES, AND METABOLISM DISORDERS, CLINICAL CENTER OF SERBIA, BELGRADE, YUGOSLAVIA

³INSTITUTE OF PATHOLOGY AND PATHOLOGICAL ANATOMY, SCHOOL OF MEDICINE, UNIVERSITY OF NIŠ, NIŠ, YUGOSLAVIA

The calcitonin and carcinoembryonic antigen expression in medullary thyroid carcinoma inside MEN II syndrome

KEYWORDS: Carcinoma Medullary; Thyroid Neoplasms; Multiple Endocrine Neoplasia; Immunohistochemistry; Calcitonin

The calcitonin (CT) content in medullary thyroid carcinoma (MTC) appears to be the best marker for tumor behavior. Carcinoembryonic antigen (CEA) is also a reliable marker of follow-up in patients with MTC. In this study 6 cases of MTC, belonging to MEN II syndrome, were analyzed. MTC were associated with pheochromocytomas. The patients were between 32 and 46 years of age, their average age being 38,6 and they were three males and three females. The expression of CT and CEA was investigated using the APAAP method, with appropriate antibodies (DAKO) being applied. The diameter of tumors varied from 1 to 4 cm. Histological appearance of MTC was classical, with plentiful stromal amyloid in 3 cases and trabecular, glandular, and carcinoid-like, respectively. Analysis of CT and CEA expression is done. All MTC showed CT immunopositivity, among them 4 MTC were moderately to intensely positive and in 2 MTC CT positivity was mild. Intensive CT positivity correlated with classical histologic pattern and infrequent mitotic figures. MTC with numerous mitoses were mildly CT immunopositive. There was no correlation between CT positivity and stromal amyloid content. All MTC showed intense CEA immunopositivity but without any correlation between CEA positivity and histologic pattern, stromal amyloid content, calcifications of mitotic figures, respectively. In this study the correlation between CT immunopositivity and histologic pattern and mitotic figures among MTC inside MEN II syndrome was found. There was no correlation between CEA positivity and histologic pattern, stromal amyloid content, calcifications or mitotic figures, respectively.

S. TATIĆ¹
M. HAVELKA¹
H. BAŠIĆ²
I. PAUNOVIĆ³
R. JANKOVIĆ³
A. DIKLIĆ³
V. BOŽIĆ³
M. OKLOBDŽIJA¹
D. BRAŠANAC¹
M. JANČIĆ-ZGURICAS¹

¹INSTITUTE OF PATHOLOGY, SCHOOL OF MEDICINE, UNIVERSITY OF BELGRADE, BELGRADE, YUGOSLAVIA

²INSTITUTE OF PATHOLOGY AND PATHOLOGICAL ANATOMY, SCHOOL OF MEDICINE, UNIVERSITY OF NIŠ, NIŠ, YUGOSLAVIA

³CENTER OF ENDOCRINE SURGERY, INSTITUTE OF ENDOCRINOLOGY, DIABETES, AND METABOLISM DISORDERS, CLINICAL CENTER OF SERBIA, BELGRADE, YUGOSLAVIA

Nonfunctional adrenocortical carcinoma - histomorphological and immunohistochemical study

KEYWORDS: Carcinoma, Adrenal Cortical; Immunohistochemistry; Keratin; Vimentin

The differential diagnosis between adrenocortical adenoma and well-differentiated adrenocortical carcinoma may be the challenge even for the experience pathologist. The aim of the study is to make histomorphological and immunohistochemical analysis of 27 nonfunctional adrenocortical carcinomas (ACC) obtained from Institute of Endocrinology, Clinical Center of Serbia, Belgrade in the 1986. to 1997. period. The patients were between 31 and 74 years of age, their average age being 54, and they were 16 males and 11 females. The expression of cytokeratin and vimentin was investigated using the PAP method, with appropriate antibodies (DAKO) being applied. The weight of the tumors in question was from 80 to 2350 grams, the average weight being 630.58 grams. The diameter of ACC was 5 cm and above. On gross examination the tumors proved to be ill-defined and showed numerous foci of necrosis, hemorrhage and cystic softening. The tumor histology (HE) revealed predominantly large, polygonal cells arranged in trabecular or alveolar patterns. Pleomorphism, atypia and numerous mitotic figures were present, as well as the invasion of the capsule. Cytokeratin reactivity was present in 12 cases (5 cases - weak immunopositivity, and 7 cases - moderate). 18 ACC were vimentin immunopositive (7-intense, 3-moderate, 8-weak). Immunohistochemistry is not of great value in confirming the diagnosis of ACC.



R. RADOSAVLJEVIĆ
J. HADŽI-ĐOKIĆ
S. MIČIĆ

CLINIC OF UROLOGY, CLINICAL CENTRE OF SERBIA, BELGRADE, YUGOSLAVIA

Pheochromocytoma- a case report

KEYWORDS: Pheochromocytoma; Hypertension; Adrenal glands

Pheochromocytomas are rare neoplasms, solitarily or as a part of Multiple Endocrine Neoplasms syndrome, von Hippel-Lindau disease and neurofibromatosis. It consists of chromotofile cells which secrete catecholamines and manifested as a paroxysmal hypertensive crisis. Most frequently it is unilateral, situated in the adrenal gland, rarely intraabdominal beneath the bifurcation of the aorta or in the wall of the urinary bladder. The authors reported one case of a woman who had been operated at the Clinic of Urology of Serbian Clinical Center. A 38 years old woman have paroxysmal hypertensive crisis, after clinical and biochemical tests was found a tumor in the hilus of the left kidney. She had been operated in February in 1999. at Clinic of Urology of Serbian Clinical Center in Belgrade when a total nephrectomy and adrenalectomy were performed. On gross inspection, the kidney was sized 12x6x5cm, with a tumor mass in the size of 13x10x7 cm in the hilus of the kidney, nodular and lobular, gray-brown on cut section, with focal necrosis, haemorrhages and small cysts. The material was pathohistologically analysed by histochemistry and immunohistochemistry methodes. Microscopically was found pheochromocytoma which consist of chromaffine tumor cells with granular cytoplasm. The tumor cells were arranged in trabecules and alveolar in form, with cellular and nuclear pleomorphism, lining the wall of capillaries and sinusoids. Rarely there were giant and bizzare cells and atypical mitotic figures. Deposits of tumor cells were found in the branches of the renal vein. The postoperative course was without complications, recidives and metastases. We conclude that pheochromocytoma may be a cause of hypertensive crisis, and its treatment is always surgical.