

Histological Structure of Area Postrema in Brain of Rat (Rutts Rutts)

THEKRA ATTA IBRAHIM¹

Department of Biology, College of Education for Pure Science
Diyala University, Diyala
Iraq

NAHLA A. AL-BAKRI

Department of Biology
College of Education for Pure Science-Ibn Al-Haitham
Baghdad University, Baghdad
Iraq

ANAM RASHEED AL-SALIHI

Institute of Embryo Researchers and Infertility Treatment
Al-Nahrain University, Baghdad
Iraq

Abstract:

Area postrema is a part of circumventricular organs which are lacking of Blood Brain Barrier (BBB). It has great importance where it represented as chemical reception area, allows the passage of toxics from the blood and working as motivate vomiting center. To study the histological structure of area postrema in adult rat brain 10 adult rats is conducted and incubate in appropriate environmental conditions. The histological structure was studied using paraffin section stained by hematoxylin and eosin. The results Area postrema composes of histological mass like a dome covers center channel and consists of two parts; vascular part include vascular capillaries and epithelial part include flattened ependymal cells contain microvilli. It is characterized with existence of many of abscess structures are called cystic. The results of this study refer to that the area postrema composes of single structure lie in the mid line under the fourth ventricle and it has high

¹ Corresponding author: thekraattaibrahim@gmail.com

vascular structure while the surface of the ependymal cells are free of cilia.

Key words: area postrema, fourth ventricle, circumventricular organs and blood brain barrier.

Introduction

Area postrema (AP) is a circumventricular organ of the brain in mammals. In primates it is located in the floor of the fourth ventricle as a bilateral structure that protrudes into the caudal floor of the ventricle on either side of the obex (1). In rodents and lagomorphs, the AP forms a single mound of tissue in the midline overlaying the central canal (2) The AP contains two principal components: a vascular Part A highly vascular structure, AP contains sinusoid vessels that loop toward the ventricular surface and are surrounded by distended per vascular spaces (Virchow–Robin spaces; (3). The vessels differ from the vast majority of cerebral vessels in that they lack a blood brain barrier These characteristics allow blood-borne substances that would otherwise be prevented from coming in contact with neurons to affect AP neurons directly (4) and a cellular part composed of flattened ependymal cells, small neurons, and glial cells (5) The afferent projections of the AP have been described as components of the vagus and glossopharyngeal nerves (6), whereas its efferent connections reach the nucleus of the tractus solitarius and the parabrachial nucleus (7). The AP and adjacent caudal-medial portion of the nucleus of the tractus solitarius are involved in the nausea and vomiting syndrome as well as in regulation of body weight (8). The functions of the AP are also related to the effect of angiotensin II (9) and vasopressin in cardiovascular regulation (10). The aim of this study is studying the histological structure of the area postrema in brain of the rat (Rutts rutts).

Methods

Animals Care and Breeding

This study was performed on the sexually mature laboratory breed Sprague-Dawley albino rats (*Rattus norvegicus*) about 1.5-2 months age as a mammalian model, the animals were obtained from the animal house of the High Institute of infertility Diagnosis and Assistant Reproductive Technology/ university of Al-Nahrain.

Histological preparation

The preparation of histological sections depends on the standard methods of (11) as follows:

Fixation 1.

The Bouin's fluid fixative was used to fixation the brain tissue (24 hours)

2. Washing

Samples were washed with 70% ethanol to remove the yellow color of the Bouin's fixative .

3. Dehydration

Samples were passed through progressive increasing concentrations of ethanol (70%, 80%, 90%, 95%, and 100%) with one hour interval .

4. Clearing

Done by immersing the samples in xylene or toluene for 30 min. the translucency was used as a guide to indicate that tissue was cleared .

5. Infiltration

Tissues were embedded in a melted paraffin wax (melting point 60°C) in automatic electric oven for 12-18h .

6. Embedding & Sectioning

The traditional method in the production of the wax block using "L" shape pieces to produce the moulds. Molten paraffin wax at two or three degrees above the melting temperature was dispensed into the moulds and the tissue introduced by warmed

forceps in the correct oriented plane. Serial sagittal sections were cut at 6 μm in thickness by the semi-computerized rotary microtome.

7. Mounting

Several slides should be cleaned, smeared with a drop of Mayer's egg albumin. Fixing the sections on the slide by using a hot plate (45c)

8. Staining

The brain sections were stained with (H&E) stain. All the sections were examined under light microscope at 40 \times , 100 \times and 1000 \times and take the images by digital camera.

Results

Histological Structure of Area Postrema in Fourth Ventricle in Adult Rat (Rutts rut)

The results of this study show that the area postrema in fourth ventricle of adult rat (Rutts rutts) be in form of histological mass like a dome covers center channel and has single structure lie in the mid line in the bottom of fourth ventricle directly under the cerebellum (as shown in Figure 1). It has been observed in this study that there are Two types of capillaries were observed in the rat area postrema: fenestrated capillaries with wide diameter, sinuous course and large perivascular spaces in the caudal part of the organ and continuous capillaries, smaller in diameter, with reduced or absent perivascular spaces in the rostral parts of the area postrema (as shown in Figure 2). The area postrema contains flattened ependymal cells with microvilli and cilia were absences in this organ. the lining epithelium of area postrema consist of tanocytes cells. The area postrema characterized with existence of Supraependymal cells, especially in the tail part where existence either single or arranged in groups (as shown in Figures 3). This study shows that the area postrema consist of small astrocytes cells and glial cells and little number

of oligodendroglia cells (as shown in Figure 4). The area postrema in adult rat is characterized with existence of many cyst-like structures named cysts, located both in the ependyma and deep in the parenchyma of the caudal area postrema (Figure 5).

Discussion

The results of this study show that the general morphological features of area postrema in adult rat (Rutts rutts) were similar to that features in various types of mammalian described by many researchers (12, 13). Its shape and position, area postrema lie on the dorsal surface as a histological mass like a dome covers center channel and has single structure lie in the mid line in the bottom of fourth ventricle directly under the cerebellum. This result is agreement with (14) in their studies about area postrema in rodents and lagomorphs. In contrast to this result, (15) show that area postrema in other mammalian lie in the dorsal surface of medulla oblongata in obex of fourth ventricle neighboring of nucleus of the solitary tract. (16) Show that area postrema of mink consist of symmetrical histological heights on the two sides of tail part of fourth ventricle and the two regions of area postrema coalesce towards the dorsal side of tail part of central canal entrance, giving a winged shape to the organ.

The results show the area postrema of rat contains flattened ependymal cells with microvilli and cilia were absences in this organ. Epithelium of area postrema consists of tancytes cells. This result is in agreement with (5) in our studies on the area postrema of cut and monkey where they mentioned that the surface of ependymal cells in area postrema contains number of microvilli and the later appears in flat position on the surface of ependymal cells. There is no evidence about any secretions inside CFS and the functional significance for microvilli arrangement still mysterious. Abundant microvilli

in on the surface of ependymal may be as electric receptors. The above result disagreement with (16) that shows that the ependyma didn't cover all area postrema due to winged shape and the epithelial lining of the caudal part was restricted to the protrusion of the organ into the fourth ventricle, whereas a basement membrane erived from the pia mater covered the rest of the structure.

The results of this study show that the ependyma related with supraependymal cells were appeared either isolated or arranged in clusters. This result is agreement with (17 and 5). The results of this study also show that the surface of ependymal cell of area postrema doesn't contain cilia, but only small microvilli. This result is agreement with (18) in their study on human area postrema and disagreements with (19) where they refer in their study on the domestic birds to that the area postrema has a single cilia and appear as smooth surface free of small microvilli. The present study show that the area postrema consist of neural cells and glial cells and this result disagreement with (20) whose refer in their study on the area postrema in the rat its doesn't contain neural cells. (21) Refer by their study that area postrema of the rabbit was composed of neuronal and neuroglial elements similar to those found in ordinary cerebral tissue, except for the blood capillaries surrounded by a special perivascular space.

The parenchymal cells of the area postrema included small neuronal cells and neuroglial cells. The neuroglial cells might be classified as specific astrocytes, which contained small round dense nuclei but showed a cytoplasmic structure similar to that of ordinary astrocytes. The researcher (13) pointed out in their study on the ultrastructure of the courtyard of the background using Scanning Electron Microscopy (SEM) of monkey that some neurons were located immediately beneath the attenuated ependymal cells. It is possible that the neurons may detect the changes of the cerebral spinal fluid in the fourth ventricle. (13) Show that the Neurons cell bodies are enveloped

by the astrocytic glial processes which also surround the external basal lamina of perivascular space.

The close structural relation would suggest a nutritive as well as a supportive role for the neurons. The present study showed that the area postrema is characterized by abundant blood vessels and arteries that arise from the bottom of the rear posterior arteries cerebellum. This result is in agreement with (17) and (22). It has been observed in this study that there are two types of blood capillaries in the area postrema of the rat, namely: The first type was blood capillaries with large-diameter, warped shape and characterized by abundant perivascular space at the tail of the organ, the second type was the blood capillaries with a diameter smaller than the blood capillaries vessels in the first type, with a small number of prevascular spaces in rostral portions of area postrema. This result is agreement with (16 and 22) Area postrema in rat was characterized by the presence of cyst-like structures located both in the ependyma and deep in the parenchyma of the caudal The cysts were spherical in shape and variable in size, and consisted of thin cytoplasmic projections which surrounded the cystic The cystic lumen seemed to be confined within a single cell, as no junctional structure was found around the cytoplasm that constituted the cystic wall.

Cysts were never seen in direct contact with perivascular spaces or the ventricular lumen. This result is agreement with (23) who pointed to the presence of cysts within the installation area postrema that described in monkeys, cats and rats, but they have only been studied in detail in rats by (12). The latter authors found them in adult rats but not in new born rats, a fact that led them to conclude that the frequency of cysts seemed to be related to the age of the animals'. However, they did not investigate the precise moment of their appearance in the rat AP. While (16) noted in his study on occurrence of cysts in the AP of 8-month-old minks (and that they were a common finding in older animals. Their presence does not seem

to be related to the physiological state of the animals, since their number and size did not show evident modifications among groups, but rather to the age of the minks in agreement with the theory put forward by (12).

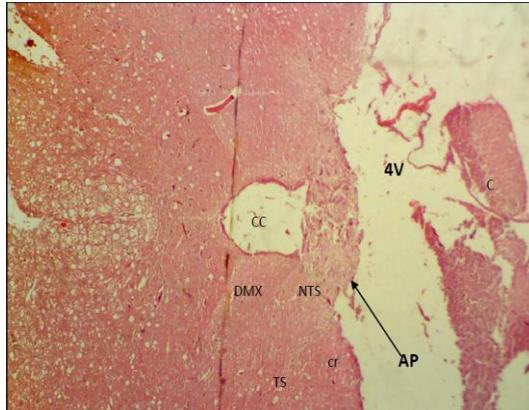


Figure 1 Coronal section in area postrema in fourth ventricle of adult rat. AP Area postrema, NTS Nucleus of tractus solitaries, CR Gracile nucleus, CC center channel, C Cerebellum, 4V Fourth ventricle. Staining with H&E, 40 \times

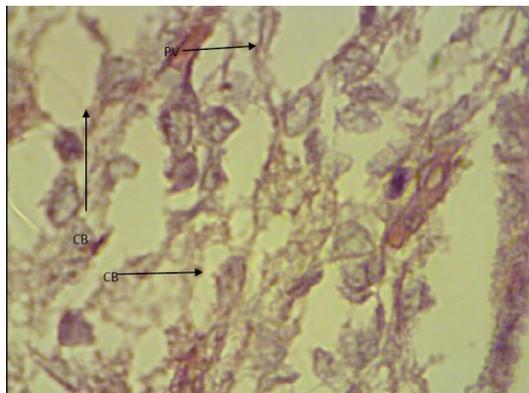


Figure 2 Coronal section in the middle of area postrema explain the dispersion of blood capillaries in adult rat. PV perivascular spaces, CB capillaries. Staining with H&E, 1000 \times

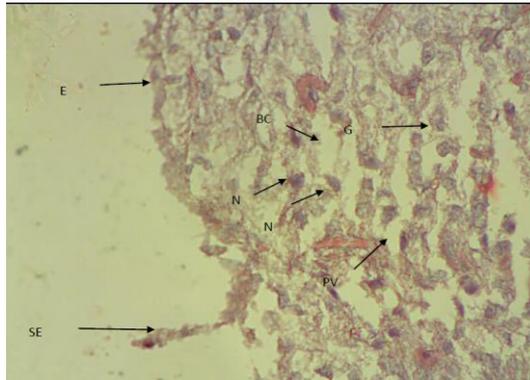


Figure 3 Coronary section in area postrema in fourth ventricle explain the ependymal cells and of adult rat. SE Supraependymal, N Neuron, G Glial cell, E Ependyma, BC capillaries, PV perivascular spaces. Staining with H&E, 400 \times

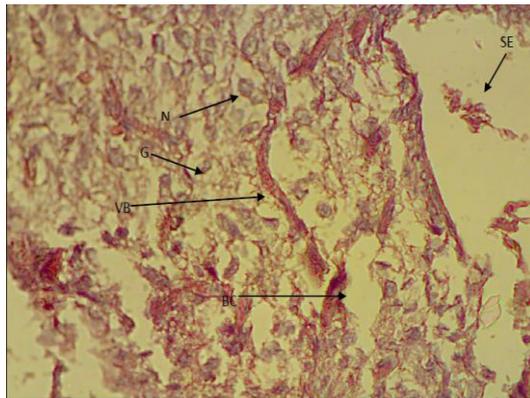


Figure 4 Coronary section in area postrema in fourth ventricle explain the glial cells of adult rat .N neuron, G Glial cell, VB perivascular spaces, BC capillaries, SE Supraependymal. Staining with H&E, 400 \times

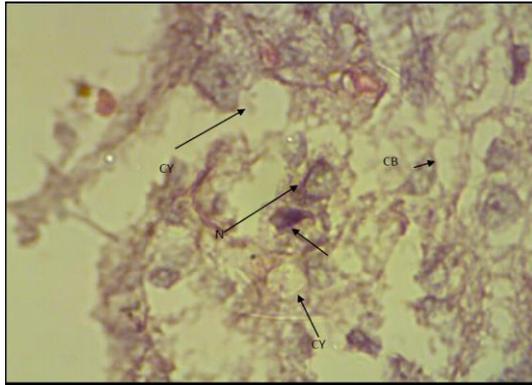


Figure 5 Coronary section in area postrema in fourth ventricle explains cyst-like structures in the ependyma of adult rat. CY Cysts, N Neuron, CB capillaries. Staining with H&E, 1000 \times

REFERENCES

- Alema, N., Cerutti, P., and Guerrero, F. 2002. "Morphological changes in the mink area postrema during growth and under different stages of sexual activity." *Histol Histopathol* 17: 55-64. <http://www.ehu.es/histol-histopathol> [16]
- Allen, D.C. and Cameron, R.I. 2004. *Histopathology specimens: clinical, pathological and laboratory aspects*. London: Springer. [11]
- Brizzee, K. R. and Klara, P. M. 1984. "The structure of the mammalian area postrema." *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 43: 2944–2948. [5]
- Buller, K.M. 2001. "Circum-ventricular organs: gateways to the brain. Role of circumventricular organ in pro-inflammatory cytokine-induced activation of the hypothalamic–pituitary–adrenal axis." *Clin. Exp. Pharmacol. Physiol.* 28: 581–589. [1]
- Cammermeyer, J. 1973. "Hypendymal cysts adjacent to and over circumventricular regions in primates." *Acta Anat.* 84: 353-373. [23]

- Duvernoy, H.M. and Risold, P.Y. 2007. "The circumventricular organs: an atlas of comparative anatomy and vascularization." *Brain Res. Rev.* 56 (1): 119–147. [3]
- Fodor, M., Palkovits, M., and Gallatz, K. 2007. "Fine Structure of the Area ubpostrema in Rat. Open Gate for the Medullary Autonomic Centers." *Ideggyogy Sz* 60(3–4):83–88. [22]
- Fry, M., Hoyda T. D., and Ferguson A. V. 2007. "Making Sense of It: Roles of the Sensory Circumventricular Organs in Feeding and Regulation of Energy Homeostasis." *Exp Biol Med* 232:14–26. [15]
- Gotow, T. and Hashimoto, P.H. 1980. "Fine structure of ependymal cystsin and around the area postrema of the rat." *Cell Tissue Res.* 206: 303-318. [12]
- Hirunagi, K. and Yasuda, M. 1979. "Scanning electron microscopic analysis of the linings of the fourth ventricle in the domestic fowl." *Cell and Tissue Research* 197(1): 169-173. [19]
- Ingves, M. V. 2009. "The Area Postrema: A Potential Site for Circadian Regulation by Prokineticin." Department of Physiology, Queen's University Kingston, Ontario, Canada. [4]
- Kenney, N.J., J.N. Kott, N. Tomoyasu, A.J. Bhatia, A.S. Ruiz, and M.M. Mcdowell. 1989. "Body weight of rats following area postrema ablation: Effect of early force-feeding. *Am. J. Physiol.*, 256; *L. Regul. Intergr. Comp. Physiol.* 251:R939-R945. [8]
- Ling, E.A. and Wong, W.C. 1987. "Ultrastructure of the area postrema of the monkey, *Macaca fascicularis*." *Histol. Histopathol.* 2: 39–48. [13]
- McKinley, M.J., McAllen, R.M., Davern, P., Giles, M.E., Penschow, J., Sunn, N., Uschakov, A., and Old, B.J. 2003. "The sensory circum-ventricular organs of the mammalian brain." *Adv. Anat. Embryol. Cell Biol.* 172 III-122. [17]

- Michelini, L.C., K.L. Barnes, and C.M. Ferraro. 1986. "Area postrema lesions augment the pressor activity of centrally administered vasopressin." *Clin. Exp. Theor. Pract.* A8r1107-1125. [10]
- Miselis, R.R., R.E. Shapiro, and T.H. Hyde 1987. "The area postrema in Circumventricular Organs and Body Fluids." P.M. Gross, ed. CRC, Boca Raton, FL, Vol. 2, pp. 185-207. [6]
- Olfield, D.J. and McKinley, M.J. 1995. "Circumventricular organs." In *The rat nervous system*. 2nd ed., edited by G. Paxinos, 391-403. New York: Academic Press. [14]
- Poberai, M., Karcsu, S., and Csillik, B. 1971. "Relationship of glia secretion to specific function in the area postrema." [trans.] *Acta histoeltem.* 39(1): 1-11. [20]
- Price, C. J., Hoyda, T. D., and Ferguson, A. V. 2007. "The Area Postrema: A Brain Monitor and Integrator of Systemic Autonomic State." *Neuroscientist* doi: 10.1177/1073858407311100. [2]
- Rabin, B.M., W.A. Hunt, A.C. Bakarich, A.L. Chedester, and J. Lee. 1986. "Angiotensin 11-induced taste aversion learning in cats and rats and the role of the area postrema." *Physiol. Behav.* 36:1173-1178. [9]
- Scott, D. E., Kozlowski, G. P., Paul, W. K., Ramalingam, S., and Dudeley, G. K. 1973. "Scanning electron microscopy of the human cerebral ventricular system." *Zeitschrift für Zellforschung und Mikroskopische Anatomie* 139(1): 61-68. [18]
- Shimizu, N. and Ishii, S. 1964. "Fine structure of the area postrema of the rabbit brain." *Z. Zellforsch* 64: 462-473. [21]
- Strain, S.M., D.G. Gwyn, J.G. Rutherford, and B.J. Losier. 1990. "Direct vagal input to neurons in the area postrema which project to the parabrachial nucleus: An electron microscopic-HRP study in the cat." *Brain Res. Bull.* 24:457-463. [7]