Aging-related Changes of Microglia and Astrocytes in Hypothalamus after Intraperitoneal Injection of Hypertonic Saline in Rats*

WANG Xiaoli (王小丽)[1], XU Yun (许 耘)[1], WANG Fang (王 芳)[1], TANG Lihua (唐立华)[1], LIU Zhilong (刘子龙)[2], LI Honglian (李宏莲)[1], LIU Shenghong (刘胜洪)[1△]
Department of Histology and Embryology, [2]Institute of Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Summary: To examine the aging-related changes of microglia and astrocytes in hypothalamus of rats after intraperitoneal injection of hypertonic saline in rats, old- and young-aged rats were injected with hypertonic saline solution into peritoneal cavity. Lectin histochemical techniques using Ricinus communis agglutinin-1 (RCA-1) and immunocytochemical method employing antibody against glial fibrillary acidic protein (GFAP) were used to demonstrate microglia and astrocytes in the hypothalamus of the rats, and the positively-stained cells were analyzed by computer-assisted image analysis system. Our results showed that the numbers of microglia and astrocytes were significantly increased in the hypothalamus of old-aged rats. After intraperitoneal injection of hypertonic saline, the number of microglia was significantly decreased in the hypothalamus of both young- and old-aged groups. After introperitoneal injection of hypertonic saline, the number of GFAP positive cells was significantly increased in the hypothalamus of young rats, but the number of GFAP positive cells did not show significant change in the hypothalamus of old rats. It is concluded that in the hypothalamus of old-aged rats, the increase of microglia may be related with the aging or degeneration of neurons, and the increase of astrocytes may provide more nourishment required by the aged neurons. The microglia and astrocytes in the hypothalamus of the two group rats may be affected by hypertonic saline, and the response of these cells to the stimuli is characterized by some aging-related changes.

Key words: microglia; astrocyte; aging; hypertonic stimulation

Hypothalamus is a very important area in central nervous system, which takes part in the regulation of osmotic pressure. There are many kinds of neurons in this location that are involved in the synthesis and the release of vasopressin and maintain the balance of inner osmotic pressure by regulating the function of kidneys. It was reported that many neurons in hypothalamus showed aged-related change as a result of the alteration of osmotic pressure. But it remains unclear whether the neuroglial cells also show the change in stress to the hypertonic stimulation and whether the changes are aged-related. Recently, the relationship between neurons and neuroglial cells has become a hot topic in neuroscience field, but the data about the relationship between neuroglial cells and hypertonic stimulation is skimpy. In our research, lectin histochemistry with Ricinus communis agglutinin-1 (RCA-1) and immunocytochemistry with antibody against glial fibrillary acidic protein (GFAP) were used to demonstrate microglia and astrocytes on the hypothalamus of rats, and show the response to hypertonic stimulation and aging alteration. The objective is to examine the ability of neuroglial cells to respond to the hypertonic stimulation and the pattern of the age-related alteration.

WANG Xiaoli, female, born in 1960, Associate Professor
△Corresponding author

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1 MATERIAL AND METHODS

1.1 Animals and Grouping

Male Sprague-Dawlay rats (from the Center of Experimental Animals, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China), with body weight of the rats ranging from 250—300 g. The rats were anaesthetized by injection of pentobarbital sodium into abdomen and perfused by 4 % polyformaldehyde (in 0.1 mol/L phosphate buffer, pH=7.3) through heart for fixation. The rats were stored at 4 ℃ for 3 h, and then hypothalmus were taken out and post-fixed in the same fixative for another 3 h. The tissues were dehydrated in ascending alcohols, cleared and embedded in paraffin. Tissues were cut in the thickness of 5 μm, mounted on slide for immunocytochemistry and lectin histochemistry.

1.2 GFAP Immunocytochemistry

Paraffin sections were dewaxed in xylene and rehydrated in descending alcohols. Afterwards the sections were in turn treated by 30 g/L H_2O_2, normal goat serum. The tissues were incubated with GFAP antibody at 4 ℃ for 24 h. Then the sections were successively incubated with biotinized goat anti-rabbit IgG and avidin-biotin-complex (ABC) at room temperature for 45 min each. Finally, the sections were demonstrated with Tris-Hcl buffer containing 3 mg/L H_2O_2 and 5 mg/L diaminobenzidine. The sections were thoroughly washed with 0.01 mol/L PBS (pH=7.3) after each step except

normal goat serum. Upon the immunocytochemical reaction, the sections were processed as normal histological procedure.

For control sections, all steps were almost the same as above except GFAP antibody substituted by normal goat serum.

1.3 RCA Lectin Histochemistry

Paraffin sections were also dewaxed in xylene and rehydrated by descending series alcohols. Afterwards the sections were treated as follows: (1) 30 g/L H_2O_2 for 10 min; (2) normal goat serum, for 20 min at room temperature; (3) RCA-labeled with biotine (Sigma Co., USA) at 4 ℃ for 24 h; (4) incubation with avidin-biotin-complex (ABC) at room temperature for 45 min; (5) demonstration with Tris buffer containing 3 mg/L H_2O_2 and 5 mg/L DAB for 8—10 min. The remaining processes were the same as above-mentioned immunocytochemistry.

1.4 Computer-assisted Image Process

Three sections (with a interval of 50 μm) were taken from RCA histochemstry and GFAP immunocytochemistry respectively. 8 visual fields were randomly selected from the hypothalamus of each rat. Computer-assisted image analysis system was used for cell counting and measurement of optic density of RCA and GFAP positive reaction cells. The data were statistically processed with student *t*-test.

2 RESULTS

In young rats, few RCA-positive microglia were found to be randomly distributed in hypothalamus (fig. 1 and table 1). Compared with the rats of young-aged group, the number of RCA-positive cell in hypothalamus of old-aged rats was significantly increased (fig. 3 and table 1). The processes of RCA positive cells attached to or surrounded the neuron. After administration of hyperosmotic saline, the number of RCA-positive cells was decreased (fig. 2 and table 1) and the reaction of RCA was also significantly decreased (table 1). The number of RCA-positive cells and RCA reaction of lectin histochemistry in hypothalamus of old-aged rat were decreased after administration of hyperosmotic saline (fig. 4).

GFAP-positive astrocytes were randomly distributed in the hypothalamus of young-aged age rats, but many of them were located near brain ventricle (fig. 5 and table 2). Compared with the rats of young-aged group, the number of GFAP-positive cells in hypothalamus of old-aged rats was significantly increased and so was the GFAP reaction (fig. 3 and table 1). After administration of hyperosmotic saline, the number of GFAP-positive cell was significantly increased in hypothalamus of young-aged rats (fig. 6 and table 1) and the reaction of GFAP was also significantly increased (table 1). But no significant change was found in the number of GFAP-positive astrocytes in hypothalamus of old-aged rats after the administration of hyperosmotic saline (fig. 8).

In control sections, reactions of RCA and GFAP were negative in hypothalamus of both young- and old-aged rats.

Table 1 The number and optical density of RCA and GFAP positive cells ($\bar{x}\pm s$)

Groups		Mean optical density		Mean cell number / field	
		Young	Old	Young	Old
Normal	(RCA)	0.263±0.032	0.336±0.034#	46.3±6.1	58.5±6.4#
Hyperosmolarity	(RCA)	0.167±0.02*	0.252±0.05*	34.1±5.2*	44.2±5.5*
Normal	(GFAP)	0.1169±0.025	0.2108±0.11#	23.3±4.1	39.9±12.5#
Hyperosmolarity	(GFAP)	0.2773±0.07*	0.2382±0.06	49.3±9.12*	37.7±9.1

* $P<0.05$ as compared with normal group, $P<0.05$ as compared with young-aged group

3 DISCUSSION

In recent years, researches indicated that communication between neurons and non-neural cells called glia can affect neuronal excitability and synaptic transmission. At the different ages and under different stimulations, the activation of neuroglial cells and the changes of their function can reflect the regulative ability of nervous system[2].

We found in our experiment that the microglia in hypothalamus was increased in old-aged rats, but the number of microglia and the positive reaction of RCA were decreased as a result of hypertonic stimulation in both young- and old-aged groups. The aged-related increase of microglia may be related to the treatment of degenerated neurons of old rats[3,4]. With the increase of age, apoptotic rate of aged neurons goes up, which can induce the proliferation and activation of microglia for the purpose of engulfing and cleaning the remains of apoptotic neurons, processes or myelin sheath[5]. Therefore, the increase of microglia may be very important in maintaining the homeostasis of nerve tissue.

Hypothalamus is one of the most important areas for regulating osmotic pressure, in which some neurons may respond to hypertonic stimulation and release some hormones to maintain the homeostasis, but few of researches were previously reported concerning the effect of hypertonic stimulation on glial cells. Lawson found the increase of

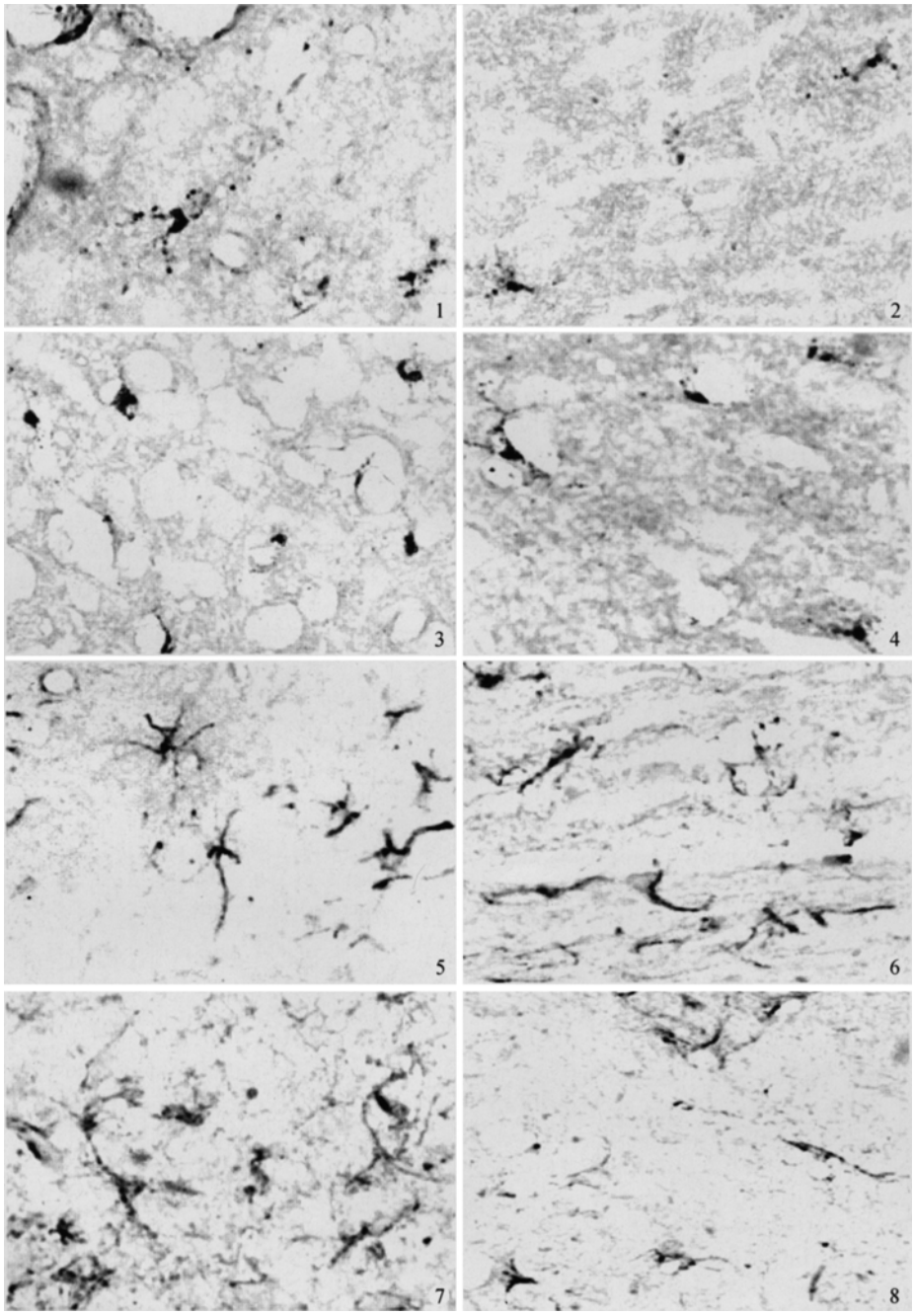

Fig. 1 RCA-positive microglias were randomly distributed in the hypothalamus of young-aged rat (RCA lectin histochemistry×40)

Fig. 2 After Intraperitoneal injection of hypertonic saline, the number of RCA positive microglias was decreased in the hypothalamus of young-aged rat (RCA lectin histochemistry×40)

Fig. 3 Compared with the young-aged rat, the number of RCA positive microglias was increased in the hypothalamus of old-aged rat (RCA lectin histochemistry×40)

Fig. 4 After intraperitoneal injection of hypertonic saline, the number of RAC-positive microglias was also decreased in the hypothalamus of old-aged rat (RCA lectin histochemistry×40)

Fig. 5 GFAP-positive astrocytes were randomly distributed in the hypothalamus of young-aged rat (SABC×40)

Fig. 6 After intraperitoneal injection of hypertonic saline, the number of GFAP positive astrocytes was significantly increased in the hypothalamus of young-aged rat (SABC×40)

Fig. 7 By comparing with young-aged rat, the number of GFAP positive astrocytes was increased in the hypothalamus of old-aged rat (SABC×40)

Fig. 8 After intraperitoneal injection of hypertonic saline, the number of GFAP positive astrocytes did not show increase in the hypothalamus of old-aged rat (SABC×40)

DNA synthesis and activation of microglia in neurohypophysis after hypertonic stimulation, but failed to provide any morphologic evidence[6]. We found that the hypertonic stimulation caused de-

crease in the number of microglia and RCA reaction in both young- and old-aged rats. The results were not coincident with that reported by Lawson. Why hyperosmotic stimulation results in decreased number of microglia awaits further study. We speculate that during hypertonic stimulation, the function of neurons in hypothalamus may be activated to regulate osmotic pressure, but correspondingly, microglia may be down-regulated in this process. It profits(benefit) for the function of neuron because the activated microglia will give rise to more free radicals that can result in the damage of the neurons[7]. On other hand, the difference may be related to brain area. Compared with neurohypophysis, the responsibility(response) of microglias in hypothalamus to hypertonic stimulation may be different from that of neurohypophysis, but this needs to be confirmed by further study.

GFAP is a specific protein produced by astrocytes. Its expression was related to the action of astrocytes and was increased with aging of rats[8]. Mouton *et al* found that the number of astrocytes in the hippocampus of aged mouse significantly increased up to 20 % compared with that of young mouse. Our study is coincident with their study. By using immunohistochemical study, Berciano *et al* found that the increased GFAP immunoreactivity and the morphometric changes of superoptic nuclear of old rats might reflect the up-regulation of cellular activity with age, resulting in hypertrophy of glial cell processes[10]. Because astrocytes play an important role in the nutritional supply for neurons, these changes may compensate for the nutritional deficiency of aged neurons.

Matsunaga *et al* found that hyperosmotic administration (9 % NaCl) may activate microglia in the posterior pituitary of rats[11]. The results between us were similar but not completely coincident. In the hyperosmotic stress of our experiment, the number of astrocytes and GFAP expression were increased in the hypothalamus of young rats, but they were decreased in that of old rats. It indicated that the response of astrocytes to hyperosmotic stress varied in the hypothalamus of different aged rat. The function of astrocytes is to provide nutrients and support for neurons, therefore, the increase in the number of astrocytes and GFAP expression in the hypothalamus of young rat would benefit nerve tissue to respond to the stress, and the decrease of astrocytes in the hypothalamus of the old rats indicated that the regulating ability of the astrocytes to stress were restricted, even induced by hyperosmotic stress. In summary, we conclude from this study that the response of the glial cell to hyperosmotic stress does not only have close relationship with the nerve activation in hypothalamus, but is also affected by age. The response of the glia may provide homeostasis for neuron activation in hyperosmotic stress. Although the changes of glia have been observed in hypertonic stimulation, the molecular mechanism still needs further investigation.

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