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RESEARCH ARTICLE

Effects of special brain area regional cerebral blood flow abnormal perfusion on learning and memory function and its molecular mechanism in rats

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Abstracts To study the effect of special brain area regional cerebral blood flow (rCBF) abnormal perfusion on learning and memory function and its molecular mechanism, 64 adult male healthy Sprague-Dawley (SD) rats were randomly divided into two groups, the false operation group (control group) and the operation group (model group). After surgical operation, the operation group undertook bilateral common carotid artery permanent ligation, while the other group did not. Learning and memory function were measured by Y-maze at 4 h, 8 h, 24 h and 3 d after surgical operation, respectively. The rCBF of the right frontal lobe and hippocampus was also detected by the PerifluxPF model laser Doppler flowmetry, and the expressions of *c-fos* or *c-jun* or *Bcl-2* and *Bax* were also measured by immune histochemistry S-P method accordingly. Results showed that the rCBF of the right frontal lobe and hippocampus in the operation group was significantly lower than that in the false operation group ($P < 0.05$). The learning indexes, error number (EN), day of reach standard and total reaction time (TRT) in the operation group, were significantly higher than that in the false operation group ($P < 0.05$). However, the initiative evasion rate in the operation group was significantly lower than that in the false operation group. The study also found that the rCBF was relatively more, the indexes (EN, the day of reach standard and TRT) relatively fewer, but the initiative evasion rate and the memory keeping rate were relatively more. The positive expression and the average absorbency of Fos and Jun in the operation group were significantly higher than that in the false operation group ($P < 0.05$). Furthermore, Bax and Bcl-2 positive cells were all increased over time in the operation group, and the expression ratio of Bax/Bcl-2 in the operation group was significantly higher than that in the false operation group ($P < 0.01$). In conclusion, rCBF decrease can impair the learning and memory function in rats, which may be related to the increase of the expression ratio of c-*fos* or c-*jun* or *Bcl*-2 or *Bax* in the frontal cortex and hippocampus.

Keywords rCBF, learning memory function, correlation, frontal lobe, hippocampus, molecular mechanism

1 Introduction

It is known that cerebral ischemia can lead to the decline in learning and memory function. However, it is still not clear which parts of the brain are concerned with the function and how these parts participate in it. Up to now, there have been many studies on the mechanism of learning and memory and some results have been obtained. For example, some biochemical studies indicated that: AchE (acetylcholinesterase), GABA (γ-amino butyric acid), SOD (super oxide dismutase), and MDA (malondialchehyche) in the frontal lobe and hippocampus area as well as the change of content of some amino acids are associated with cerebral ischemia and the decline of the learning memory function. After cerebral ischemia, the synthesis of protein was obviously inhibited, but the expression of some genes was enhanced. Consequently, gene products such as glucose-binding proteins, calcium-binding protein D28 as well as immediate early genes *c-fos*, *c-jun*, *jun-B* and *jun-D,* etc., were all increasing simultaneously. At the early stage of impermanent cerebral ischemia, the synthesis of the protein Bcl-2 has been enhanced in the hippocampus (Gao et al., 2005; Han and Guo, 2004). However, there were also some opposing views (Krajewski et al., 1995).

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Thus, we carried out this study to further understand the effect of reduced rCBF (regional cerebral blood flow) on the learning and memory function as well as its molecular mechanism.

2 Study objects and methods

2.1 Study objects

During a Y-type electric labyrinth test, 64 healthy male SD rats were selected. The rats, with an average weight of (308 ± 54) g, were sensitive to electric stimuli and had a swift escape response. The 64 rats were randomly divided into two groups, the operation group (model group) and the false operation group (control group), and each group consisted of 32 individuals. The two groups were also randomly divided into A, B, C, D and A_0, B_0, C_0, D_0, respectively, and each group consisted of 8 individuals.

2.2 Establishing of animal models

All the selected rats were fasted for 12 h and banned from taking water for 4 h before the experiment. The dissection from the middle of their necks was performed under a ventral anaesthesia induced by pentobarbital (40 mg/kg) with a concentration of 1%. After that, both sides of the common carotid artery were exposed. For the operation group, cuts were sewed up with a silk thread of size "0", and the thread was imbedded subcutaneously. However, for the false operation group, the common carotid artery was opened, then the cuts were sewn up too.

2.3 Measure of learning and memory function

A measure of learning and memory function was carried out based on the protocol of Li et al. (1995). Indexes below were noted: training times, error number (EN), time to reach the mark, total reaction time (TRT), initiative evasion rate.

2.4 Measure of rCBF

After measuring the learning and memory function in each group, fiber probes of type Periflux PF3 LDP were inserted into the corresponding cerebral regions, and the orientation of the cerebral regions referred to the cerebral orientation atlas of the rat (Bao and Shu, 1991). Then, rCBF was continuously measured for 10 min in the frontal lobe and hippocampus of each rat, and the output signal of LDP was noted. The average rCBF of each rat was calculated by the disposure of PERISOFT procedure.

2.5 Detecting *c-jun*, *c-fos* and *Bcl-2*

2.5.1 Preparation of tissue slice

A ventral anaesthesia was induced by 1% pentobarbital (i.e., 40 mg/kg) in the rat. After that, the perfusion was carried out as follows: a duct was inserted from the left ventricle to the ascending aorta with the chest-opened, then, fleetly affused with NaCl (pH7.4) of about 50 mL in 5 min, followed by polyoxymethylene about 100 mL (confected by 0.1 mol/L PBS, pH7.4) at first for 10 min and then slowly for 20 min, the whole course was about 30–35 min. After perfusion, the cerebral tissue was excised and fixed in a 4% polyformaldehyde at 4C overnight, then placed in a 20% cane sugar (confected by 0.1 mol/L PBS, pH7.4, 4C). When the tissue was deposited, it was transferred into another 30% cane sugar (confected by 0.1 mol/L PBS, pH7.4, 4C) to deposit again, then sliced up continuously for coronary frozen sections, each slice 40 μm, and one slice was chosen in every four pieces. The selected slice was preserved in PBS (0.01 mol/L, pH7.4), the 4 sets of slices were collected, and the expression of genes *c-jun*, *c-fos*, *Bcl-2*, and *Bax* were detected.

2.5.2 Immunohistochemical detection of *c-fos* and *c-jun*

The immunohistochemical detection of c-fos and c-jun proteins as follows: (1) the slice was routinely dewaxed to water, immersed into 3% H_2O_2 for 10 min to inactive the endogenetic peroxidase. (2) Microwave antigen retrieval was carried out for 10 min in EDTA, then, the slice naturally cooled to room temperature. (3) A 10% natural sheep serum was dipped in the slice at room temperature for 30 min. (4) The slice with sheep serum was immersed into rabbit antiserum, anti-c-jun (1:200) and anti-c-fos (1:50), for 24 hours at 4C. (5) Then, according to the directions in the SP reagent box, the secondary antibody (consisted of 1:100 biotin) was dripped into the above slice and incubated for 120 min at room temperature, shaken and washed with PBS for 10 min, 3 times. After that, 1:100 streptavidin-preoxidase was dripped into, incubated for 60 min at room temperature, shaken and washed with PBS for 10 min, 4 times. Then the slice was completely stained under HP, pasted, dried, dehydrated, clarified, restained and enveloped, then imaged by a computer. PBS of 0.01 mol/L was used as the control.

c-fos and c-jun positive cells and quantificational measurement were as follows: The positive cells were randomly mensurated in 20 units of areas on each of the stained slice under the circumstances of amplifying 400 times (units of areas were the computer window areas), the positive cells in the 23 units were added and then the average as cells of c-fos and c-jun positive were obtained

in one unit of the sample. Then, 20 cells were randomly selected in each group to detect the average pigmentation absorbency with the MPIAS-500 multimedia color pathological image analytical system.

2.5.3 Detection of Bcl-2 and Bax

We routinely dewaxed the coronary slice to water and immersed into 3% H_2O_2 for 15 min, then washed with PBS and added the rabbit antibody of anti-rat Bcl-2 or Bax. To eliminate the non-special pigmentation, a part of the slices was replaced with PBS, and incubated for 2 h at 37C, then washed with PBS. The instant type II antibody was added in the slice, and incubated for 30 min at 37C, then washed with PBS, and stained with DAB, used a sapanwood element to restain, followed by dehydration and covered. Ten pieces of slices of every rat were randomly obtained. We counted the number of Bcl-2 or Bax positive cells per 100 cells in each hippocampus CA1 region of the same visual field under the microscope.

If the cell was positive, it would be stained yellow brown under an optical microscope.

2.6 Statistical analysis

All the data were presented with mean ± standard deviation ($\bar{x} \pm s_{\bar{x}}$) SSPS12.0 statistical software was applied to analyze the data. *t*-test was adopted to compare the mean between the two groups, and *F*-analysis was applied to the comparison of the mean between multi-groups.

3 Results

3.1 Effect of decreased rCBF on the learning and memory function

Learning and memory performance of the rats in A, B, C and D groups were lower than that of rats in A_0, B_0, C_0 and D_0 groups at 4 h, 8 h, 24 h and 3 d after operation. These indexes, training times, EN, time to reach the mark and TRT, were also increased gradually in A, B, C and D groups, but the initiative evasion rate (IER) decreased gradually (trend test $P < 0.05$). However, no difference of these indexes was detected between A_0, B_0, C_0 and D_0 groups (Table 1).

3.2 Comparison of rCBF in dexter frontal lobe and hippocampus between the two groups after operation

rCBF of dexter frontal lobe and hippocampus of rats in the operation group was significantly lower than that of rats in the false operation group at 4 h, 8 h, 24 h and 3 d after operation, and the difference between each group was significant ($P < 0.05$). The differences of rCBF within the operation group were significant at different times ($P < 0.05$). rCBF decreased gradually with time after operation (trend test $P < 0.05$). However, the differences of rCBF within the false operation group were not significant ($P > 0.05$) at different times after operation (Table 2).

Table 1 The comparison of learning memory performances between the operation and false operation groups at different times after operation

time	groups	number	training times	EN	days to reach the mark	TRT/s	IER/%
4 h	A	8	67.25 ± 9.08	42.09 ± 8.12	0.50 ± 0.12	13.42 ± 0.51	80.13 ± 5.01
	A_0	8	62.03 ± 7.65	29.46 ± 5.31	0.33 ± 0.05	11.98 ± 0.39	83.32 ± 4.87
8 h	B	8	75.91 ± 10.23	47.18 ± 6.43	0.55 ± 0.09	14.43 ± 0.45	77.32 ± 5.07
	B_0	8	60.12 ± 6.12	30.13 ± 4.09	0.34 ± 0.06	12.09 ± 0.33	82.14 ± 3.47
24 h	C	8	82.12 ± 13.09	54.16 ± 7.23	0.58 ± 0.21	16.13 ± 0.52	74.46 ± 6.43
	C_0	8	59.56 ± 8.76	28.98 ± 6.05	0.34 ± 0.07	12.23 ± 0.40	83.47 ± 5.09
3 d	D	8	90.92 ± 15.16	61.45 ± 8.07	0.62 ± 0.14	18.64 ± 0.58	70.57 ± 6.13
	D_0	8	63.87 ± 6.34	30.16 ± 6.43	0.35 ± 0.04	12.67 ± 0.37	84.05 ± 3.03

Table 2 The comparison of rCBF (mL/100 g.min) in dexter frontal lobe, hippocampus between operation and false operation groups at different time

time	number	dexter frontal lobe		*t*	*P*	dexter hippocampus		*t*	*P*
		false operation group	operation group			false operation group	operation group		
4 h	8	82.34 ± 9.97	39.46 ± 5.31	78.97	< 0.05	62.03 ± 7.65	43.21 ± 2.98	23.45	< 0.05
8 h	8	79.98 ± 11.01	34.09 ± 3.23	83.09	< 0.05	61.08 ± 8.12	38.32 ± 3.21	25.49	< 0.05
24 h	8	80.29 ± 9.08	29.98 ± 2.36	87.25	< 0.05	59.12 ± 7.26	32.07 ± 2.01	26.78	< 0.05
3 d	8	79.98 ± 11.01	25.09 ± 3.05	89.27	< 0.05	60.54 ± 6.43	28.59 ± 2.45	28.07	< 0.05
F		4.41	24.35			5.27	35.84		
P		> 0.05	< 0.05			> 0.05	< 0.05		

3.3 Effect of decreased rCBF on the expression of c-*fos*, *c-jun* in dexter hippocampus and frontal lobe

The pigmentation positive rate and the average absorbency of c-fos, c-jun in dexter hippocampus and frontal lobe in the operation group were higher than that of the false operation group at different times after operation ($P > 0.05$). Within the operation group, the differences were significant (trend test $P < 0.05$) between the pigmentation positive rate and the average absorbency of c-fos and c-jun at different times after operation, and increased with the time after operation. Within the false operation group, the differences were not significant ($P > 0.05$) between pigmentation positive rate and average absorbency of c-fos, c-jun at different times after operation (Tables 3, 4 and Figs 1, 4). It was obvious that the expression of c-*fos* and c-*jun* positive cells in the operation group was more than that of the false operation group.

3.4 The effect of decreased rCBF on the expression of Bcl-2 and Bax in dexter hippocampus and frontal lobe

Bcl-2 and Bax positive cells could be found in the operation group, and the expression of Bcl-2, Bax was different at different times after operation, i.e., the expression of Bcl-2 and Bax increased gradually with the time (trend test $P < 0.05$). The ratio of Bax/Bcl-2 protein expression was also increased along with the time after operation, but the difference in frontal lobe was not significant. In the false operation group, no expression of Bcl-2 and Bax positive cell could be found at each different times (Tables 5–6 and Figs. 5–8).

4 Discussion

The cerebral tissue needs an abundant blood supply, and operates an energetic metabolism; the oxygen consumption of the cerebral tissue accounts for 20%–30% of the whole body. Its energy mainly depends on the aerobic metabolism of sugar, and scarcely has any stored energy. Thus, it is sensitive to ischemia and anoxia. The sensitivity of the cerebral tissue to ischemia and anoxia differs between different regions. The most sensitive regions are nerve cells in the hippocampus and frontal lobe. Hence, in this study, the hippocampus and frontal lobe are selected as the main study regions.

This study showed that rCBF in the dexter frontal lobe and hippocampus of rats in the operation group was significantly lower than that in the false operation group. Furthermore, rCBF was gradually decreased with time after operation. The learning capability of rats in the operation group was also lower than that of rats in the false operation group, and similarly decreased as time extended. All of the above indicated that the model in this experiment was successful. It goes a step further to verify the learning and memory function and the

Table 3 The comparison of the expression of c-*fos*, c-*jun* in the dexter hippocampus and frontal lobe of each group of rats after operation

time	group	c-*fos*		c-*jun*	
		dexter frontal lobe	dexter hippocampus	dexter frontal lobe	dexter hippocampus
4 h	A	22.09 ± 2.02	23.52 ± 2.08	17.20 ± 1.96	24.12 ± 1.32
	A_0	2.96 ± 0.17	3.06 ± 0.13	3.61 ± 0.23	4.23 ± 0.46
8 h	B	27.17 ± 3.32	30.21 ± 3.32	22.45 ± 2.45	29.45 ± 1.09
	B_0	3.21 ± 0.20	2.97 ± 0.16	3.39 ± 0.36	4.97 ± 0.53
24 h	C	34.34 ± 4.36	35.13 ± 3.23	26.31 ± 2.17	34.05 ± 2.34
	C_0	3.13 ± 0.21	3.12 ± 0.18	3.94 ± 0.31	4.19 ± 0.41
3 d	D	43.76 ± 4.45	39.57 ± 4.02	34.25 ± 3.67	38.67 ± 3.32
	D_0	3.01 ± 0.19	3.17 ± 0.15	3.43 ± 0.22	4.64 ± 0.13

Table 4 The comparison of the average absorbency of c-fos, c-jun in the dexter hippocampus and frontal lobe of each group of rats after operation

time	group	c-fos/A/μm^2		c-jun/A/μm^2	
		dexter frontal lobe	dexter hippocampus	dexter frontal lobe	dexter hippocampus
4 h	A	0.32 ± 0.07	0.30 ± 0.03	0.29 ± 0.02	0.23 ± 0.01
	A_0	0.12 ± 0.03	0.13 ± 0.02	0.11 ± 0.01	0.13 ± 0.03
8 h	B	0.39 ± 0.02	0.37 ± 0.02	0.38 ± 0.03	0.29 ± 0.03
	B_0	0.13 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.14 ± 0.02
24 h	C	0.43 ± 0.05	0.41 ± 0.02	0.42 ± 0.01	0.38 ± 0.01
	C_0	0.12 ± 0.01	0.14 ± 0.03	0.12 ± 0.01	0.11 ± 0.02
3 d	D	0.49 ± 0.04	0.45 ± 0.03	0.47 ± 0.03	0.45 ± 0.04
	D_0	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.02	0.12 ± 0.03

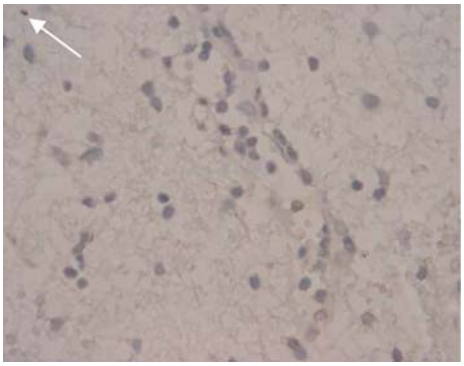

Fig. 1 The protein *c-fos* expression positive cell is rare in the hippocampus tissue of group A_0, SP stained serosity looks buffy (× 400).

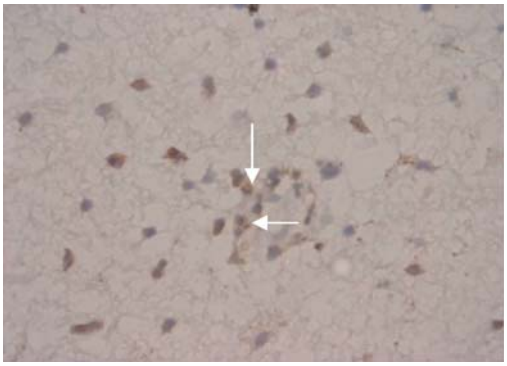

Fig. 2 The protein *c-fos* expression positive cells were significant in the hippocampus tissue of group A, SP stained serosity looks brown yellow (× 400).

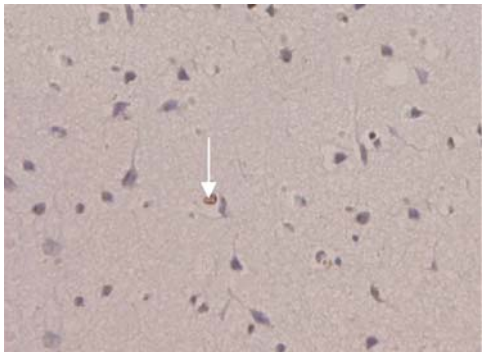

Fig. 3 The protein *c-jun* expression positive cell is rare in the hippocampus tissue of group A_0, SP stained karyon looks brown yellow (× 400).

relationship of rCBF between the hippocampus and the frontal lobe.

There are usually two sorts of ischemic cerebral tissue traumas: necrosis and apoptosis. Most apoptosis relates to genes and protein expression during cerebral ischemia. This indicates that the mechanism of apoptosis relates to ischemic trauma. However, the mechanism of neuron apoptosis after cerebral ischemia has not been known completely yet. Some studies considered that a neuron

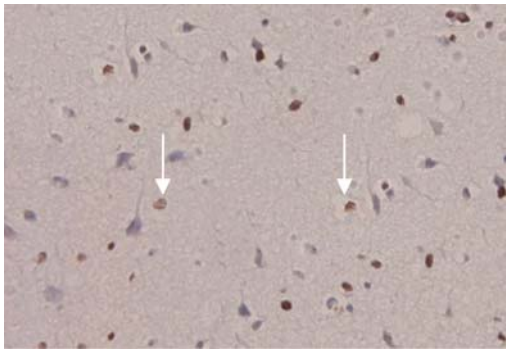

Fig. 4 There are a great deal of c-jun protein expression positive cells in the hippocampus tissue of group A, SP stained karyon looks brown yellow (× 400).

Table 5 The comparison of the expression of Bcl-2 and Bax in the dexter hippocampus and frontal lobe of the rats in operation groups

group	Bcl-2		Bax	
	frontal lobe	hippocampus	frontal lobe	hippocampus
A	6.03 ± 1.36	7.67 ± 1.12	7.28 ± 1.96	7.89 ± 1.32
B	10.87 ± 2.47	11.13 ± 2.23	14.15 ± 2.99	13.23 ± 2.45
C	21.15 ± 3.24	22.17 ± 3.35	28.34 ± 3.05	29.76 ± 3.21
D	29.05 ± 3.29	32.26 ± 3.09	39.65 ± 3.32	43.75 ± 3.42
F	28.91	19.17	26.82	23.05
P	< 0.05	< 0.05	< 0.05	< 0.05

Table 6 The comparison of Bax/Bcl-2 protein expression ratio in the dexter hippocampus and frontal lobe of the rats in operation groups

group	time	ratio of Bax/Bcl-2 in frontal lobe	ratio of Bax/Bcl-2 in hippocampus
A	4 h	1.21	1.03
B	8 h	1.30	1.19
C	24 h	1.34	1.32
D	3 d	1.36	1.36

Fig. 5 The expression of Bcl-2 positive cell is not found in the immunohistochemical slice of the hippocampus in group A_0 (SP × 400).

apoptosis may be related to the increase of oxygen radicals, calcium overloaded and the release of amino acid stimulants during cerebral ischemia. Nevertheless,

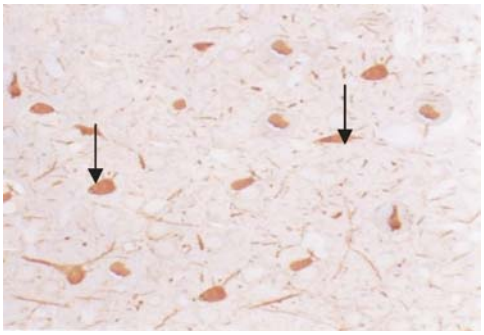

Fig. 6 The expression of Bcl-2 positive cells is clearly found in the immunohistochemical slice of the hippocampus in group A, the serosity looks yellow brown (SP × 400).

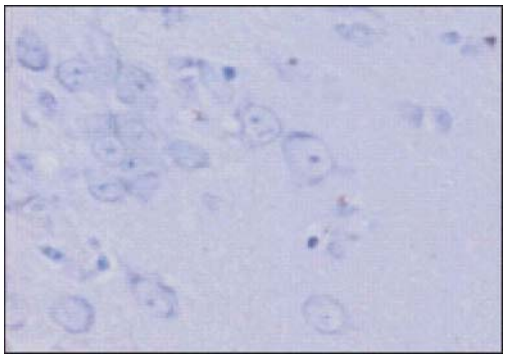

Fig. 7 The expression of Bax positive cell is not found in the immunohistochemical slice of the hippocampus in group A_0 (SP × 400).

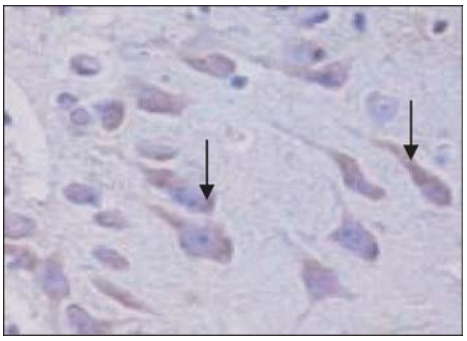

Fig. 8 The expression of Bax positive cells is clearly found in the immunohistochemical slice of the hippocampus in group A; the serosity looks yellow brown (SP × 400).

the more important and direct reason is the active expression of genes in the apoptosis cell. There are usually two sorts of apoptosis related genes. The first sort is apoptosis promoting genes like c-*fos*, c-*jun*, c-*myc*, *p53*, *Bax*, *fas* etc. The other sort is anti-apoptosis genes, such as *bcl*-2, *bcl*-*x*1 etc. Genes c-*fos* and c-*jun* can express fleetly when the cell is stimulated by a set of physical, chemical and biological factors, and simultaneously coded and produced the protein fos, jun. Therefore, the expression of c-*fos* and c-*jun* is considered as a good index of activation of the brain. Moreover, many references indicate that genes c-*fos* and c-*jun* in a broad area of the brain are activated during stress (Matsuda et al., 1996; Dragunow and Faull; 1989; Zhou et al., 2006; Sagar et al., 1988). This study showed that the pigmentation positive rate and the average absorbency of c-fos, c-jun in dexter hippocampus and frontal lobe in the operation group were higher than that of the false operation group at different times after operation ($P > 0.05$). Within the operation group, the differences were significant between pigmentation positive rate and average absorbency of c-fos, c-jun at different times after operation, and increasing along with the extending of time after operation (trend test $P < 0.05$). Within the false operation group, the differences were not significant ($P > 0.05$) between pigmentation positive rate and average absorbency of the c-fos, c-jun at different times after operation. It suggested that the expression of c-fos and c-jun had a negative correlation with the rCBF in memory region.

The enhanced expression of c-jun and c-fos caused by cerebral ischemia may be influenced by the following factors: c-fos codes for a kind of phosphoryl nucleoprotein which acts as a transcription factor to activate the expression of correlated genes. This couples the information in cells (caused by outside stimulation) with the long-term reaction because of gene mutation. When it lacks new or intense stimulation, the transcription level of the c-*fos* gene is low. However, the gene expresses swiftly when the neuron is excited or changing. In addition, it silences fleetly in 5–6 hours, and c-*fos* immunohistochemical method can mark the excited neuron in multisynaptic neural pathways (Herrera and Robertson, 1996). Thus, a high level of protein fos detected by immunohistochemical method can be regarded as the sign of changing of neuron activity. It is widely applied to the study of functional nerve anatomy and neural pathways. It deems that the decline of learning and memory function caused by cerebral ischemia may have a relationship with the enhanced expression of c-*fos* and c-*jun* in the hippocampus and frontal lobe caused by ischemia.

However, the mechanism of c-fos and c-jun promoting apoptosis has not been clarified yet, and it has a presumable relationship with AP-1 (Takeuchi et al., 1990). AP-1 is a product of apoptosis promoting genes c-*fos* and c-*jun*, nucleus DNA-binding protein, a heterogenous dimer joined by leucine zipper, and the structural base of transferring of apoptosis signals. TPA response element (TRE) is AP-1 DNA binding region, and binds to DNA by each of the regions of alkalescent amino acid to regulate the replication and transcription of DNA. At the same time, it induces the expression of genes (contains TRE) and acts as a gene-regulating protein to induce apoptosis or promote the cell into the division cycle. There is another point of view that fos protein (regarded as a sort of membrane protein) cross-links to the fos on fos expression cells and induces apoptosis (van der Borght et al, 2005). The basis of memory is the

neurons in the learning memory cerebral regions, so the enhanced expression of c-fos and c-jun can lead to the decline of learning and memory function.

Some study discovered that Bcl and Bcl-xl proteins of *bcl*-2 gene family have the effect of anti-apoptosis, but Bax and Bcl-xs proteins have the effect of promoting apoptosis. The excessive expression of *bcl*-2 together with *Bax* composes an isogenous dimmer, which may inhibits apoptosis. However, Bax-Bax isogenous dimer can promote apoptosis. This is because *Bax* is an apoptosis promoting gene. It causes the releasing of some molecules which lead to the activation of cysteine proteinase, and simultaneously, hastens apoptosis by inhibiting the protecting effect of Bcl-2 or induces apoptosis by starting a conversion of mitochondrial transparency (Runyan and Dash, 2005). In this study, it showed that, there were Bcl-2 and Bax positive cells expressed in the operation group and the expression of *Bcl-2* and *Bax* in the dexter hippocampus and frontal lobe was different at different times after operation. With time after operation, the expression of Bcl-2, Bax and the Bax/Bcl-2 protein expression ratio increased gradually (trend test $P < 0.05$). However, there was no expression of Bcl-2 and Bax positive cell in false operation group at differents time after operation. It illuminated that as the rCBF of the hippocampus and frontal lobe decreased, cerebral ischemia could enhanced the expression of Bcl-2 and Bax positive cells; especially, the enhanced expression ratio of Bax/Bcl-2 could promote the cerebral cell apoptosis or death (Gillardon et al., 1996), which ulteriorly caused the decline of learning and memory function. Thus, it is clear that the reduction of rCBF could cause the elevation of the expression of Bcl-2, Bax and Bax/Bcl-2 ratio, which lead to the decreased substance elements of learning and memory, and eventually a lower learning and memory function.

In conclusion, the mechanism of the decline of learning and memory function caused by decreased rCBF is very complex, it is related to not only the apoptosis of the above genes, but also other causes need to be further discussed.

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