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Effects of dipfluzine on cortical somatosensory evoked potentials and amino acid contents in ischemic rat brain

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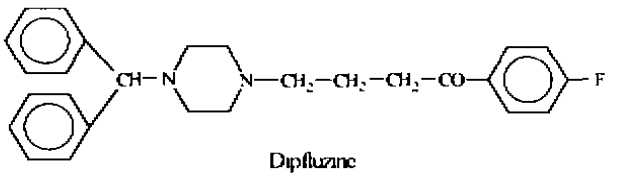
KEY WORDS dipfluzine; microdialysis; amino acids; cerebral ischemia; somatosensory evoked potentials; calcium channel blockers

AIM: To evaluate the effects of dipfluzine {1-diphenyl-methyl-4-[3-(4-fluorobenzoyl)]-piperazine, Dip} on the intra- and extra-cellular contents of the amino acids in brain and the cortical somatosensory evoked potentials (SEP) in rats with cerebral ischemia. **METHODS**: Amino acids in micro-dialysates and brain tissue homogenates in ♀ Wistar rats with bilateral carotid artery ligation (BCAL) were measured by HPLC and SEP was measured by the electrophysiological technique. **RESULTS**: Dip 50 mg·kg^{-1} ip prevented SEP from prolonging of the latency and overactivity of lowered amplitude, markedly lowered the elevation in extracellular level of glutamate (Glu), aspartate (Asp), and glycine (Gly) in intracerebral microdialysates, and alleviated the decrement of intracellular contents of Glu, Asp, Gly, taurine (Tau), and GABA in brain tissue. **CONCLUSION**: Dip reduced the disturbance of cortical function and the imbalance between excitatory and inhibitory amino acids in ischemic brain, therefore provided a further evidence for its protective effect on ischemic cerebral damage.

The excitatory amino acids (EAA) released during ischemia play a crucial role in the development of ischemic neuronal damage, and the inhibitory neurotransmitters or neuromodulators are also released massively during ischemia and may compensate for the release of EAA to protect brain against excitotoxicity[1-4]. Somatosensory evoked potentials (SEP) is useful for the serial monitoring of cortical function after brain ischemia[5,6]. Dipfluzine {1-diphenyl-methyl-4-[3-(4-fluorobenzoyl)]-piperazine, Dip}, a novel calcium channel blocker developed first by Department of Chemistry, Beijing University in China, alleviated the ischemic brain edema and the reduction of cerebral blood flow induced by bilateral carotid artery ligation (BCAL) in ♀ Wistar rats[7]. The goal of the present study was to determine the effects of Dip on the alterations to extra- and intra-cellular concentrations of amino acids in the brain tissue and to SEP in ♀ Wistar rats after BCAL.



Dipfluzine

MATERIALS AND METHODS

Chemicals Dip, synthesized in Department of Chemisty, Beijing University, was dissolved in 2 % tartaric acid solution containing 20 % dimethylacetamide (solvent) and injected ip 50 mg·kg^{-1}. Same amount of the solvent was used as control. Glutamate (Glu), aspartate (Asp), glycine (Gly), taurine (Tau), and GABA were the products of Sigma. Their mixed stock solution was diluted before use.

Surgical preparation Wistar rats, ♀, weighing 251 ± s 43 g were anesthetized with ip 25 % urethane 0.75 g·kg^{-1}. The bilateral carotid arteries were exposed and loosely encircled with silk thread for BCAL. The rats were placed in a stereotactic frame and two burr holes (2 mm in diameter) were drilled on the right and left sides of parietal bone 1.5 mm posterior and 2.5 mm lateral to bregma, into which a recording electrode of SEP and a microdialysis probe were inserted, respectively. After the probe and the electrode were implanted, the rats were allowed for stabilization about 60 min and the two baseline values of SEP and dialysate were collected at 50 and 35 min before BCAL. Then, 2 doses of Dip or solvent were injected ip, respectively, at 30 min before and 2.5 h after BCAL. SEP was measured at 1, 5, 15, 30, 60, and 120 min after BCAL and dialysates were collected at 15, 30, 60, and 90 min after BCAL for measuring extracellular concentrations of amino acids. At 48 h after BCAL, the rat was decapitated and the brain tissues were immediately placed in liquid nitrogen for measuring intracellular contents of amino acids. Six Wistar rats, ♀, were used as sham-operation control only for measuring brain tissue contents of amino acids.

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SEP recording The SEP induced[65] by an electronic stimulator (SEN-3201) were amplified 1000 times and filtered at 300—1000 Hz by AB-621G bioelectric amplifier connected to polygraph (RM-6000). Through an A-D converter, 64 responses were averaged, displayed, and analyzed on a microcomputer using a program designed by our department. The latency (ms, from stimulus artefact to first positive peak) and amplitude (mV, from first positive peak to first negative peak) were measured with a cursor and were evaluated as the primary indicators of SEP. The amplitude of SEP was expressed as a percentage of the average of two amplitudes before BCAL.

Microdialysis procedure A microdialysis probe (CMA/12, Carnegie Medicin, Stockholm, Sweden, tubular dialysis membrane 4 mm long and 0.5 mm outer diameter, molecular weight cutoff of 20 kDa) was stereotactically implanted from left parietal cortex to the CA_1 region of hippocampus. The probe was perfused with Ringer's solution of following composition ($mmol \cdot L^{-1}$): NaCl 155, KCl 5.5, $CaCl_2$ 2.3, pH 7.4, at 3.2 $\mu L \cdot min^{-1}$ using a microinfusion pump (607-A, Harvard, USA). Dialysate samples were obtained at 15 min intervals and collected in sampling tubes in an ice bath.

Amino acid analysis Amino acids in dialysates and brain tissue homogenates were measured by high performance liquid chromatography (HP-1050 system, USA) after automatic precolumn derivatization with *o*-phthalaldehyde (OPA). Briefly, the standard solution of amino acids, the supernatant of brain tissue homogenate, or the brain dialysate was automatically mixed with the borate buffer (pH 10) and OPA solution by a HP-79855A autosampler with injector program function to proceed precolumn derivatization for 2 min. The derivatives were injected onto the column (5-μm Hypsil ODS column, 125 × 4.6 mm, USA) at 40 ℃. Amino acids were separated with a linear gradient elution program, detected using a DAD UV detector (HP-1040M) at 338 nm, and quantified using a HP-300 DOS chemstation based on peak area.

RESULTS

SEP in BCAL rats The SEP of rats consisted of positive and negative waves (Fig 1).

The latency of SEP was prolonged at 5 min after BCAL, which was not recovered up to 120 min after BCAL. The amplitude of SEP was markedly decreased at 1 min after BCAL and restituted within 30 min, and then exceeded the baseline level at 60—120 min after BCAL. Dip 50 $mg \cdot kg^{-1}$ ip prevented the latency from prolonging and restrained the excessive increase in the amplitude (Tab 1).

Amino acids in dialysate In control group, the concentrations of Glu and Asp in dialysate

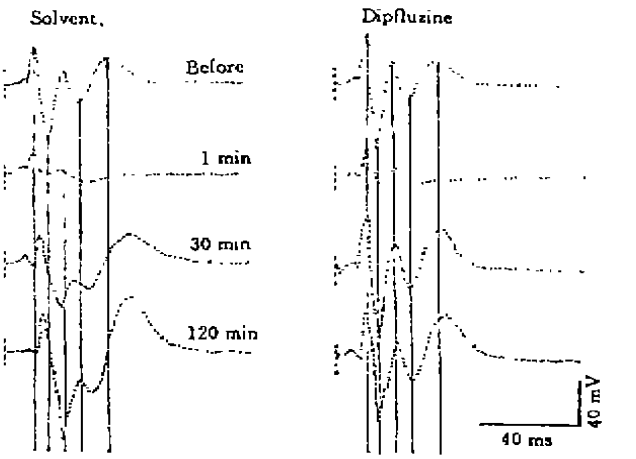

Fig 1. Effects of dipfluzine on cortical somatosensory evoked potentials in rats before and after carotid artery ligation. Upward deflection denotes positivity of potential.

Tab 1. Effects of Dip 50 $mg \cdot kg^{-1}$ on amplitude and latency of somatosensory evoked potentials in cortex of rats before and during bilateral carotid artery ligation. $n=6$, $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* 0 min.

Ischemic time/min	Solvent		Dipfluzine	
	Latency /ms	Amplitude /%	Latency /ms	Amplitude /%
0	12.1±0.8	100±0	12.4±0.6	100±0
1	12.7±1.0[a]	44±18[c]	12.8±0.5[a]	47±31[c]
5	13.2±0.8[b]	56±20[c]	12.9±0.8[a]	58±19[b]
15	13.2±0.9[b]	78±19[c]	13.2±1.1[a]	80±23[c]
30	13.4±1.2[b]	91±22[a]	12.9±0.6[a]	86±17[a]
60	13.7±1.2[b]	124±27[b]	12.8±0.7[a]	91±32[a]
120	13.8±0.9[b]	142±33[b]	12.8±0.7[a]	107±30[a]

after BCAL, were higher than those before BCAL, while the level of Gly or Tau was not obviously changed. In Dip group, the concentrations of 4 amino acids were not changed as compared with those before BCAL, but the concentrations of Asp at 15 min, of Glu at 15—90 min, and of Gly at 30—90 min after BCAL were less than those in control. The level of Tau after BCAL tended to increase ($P>0.05$) (Tab 2). The concentration of GABA in both groups was too low to be measured reliably.

Brain amino acids At 48 h after BCAL, the contents of Asp, Glu, Gly, Tau, and GABA in control group were lower than those in Dip-treated and sham-operated groups (Tab 3).

DISCUSSION

The depression of SEP may reflect ischemic

Tab 2. Effect of Dip 50 mg·kg^{-1} ip on amino acid contents in cerebral dialysate before and during bilateral carotid artery ligation in rats. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* 0 min, $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* solvent.

Group	Ischemia time/min	Amino acid contents/μmol·L^{-1} Aspartate	Glutamate	Glycine	Taurine
Solvent	0	5.1±0.8	12.0±5.0	34.1±6.3	78.9±25.4
	15	11.0±3.9^c	22.3±6.2^b	35.3±13.1^a	88.2±27.5^a
	30	9.0±2.9^c	19.8±3.3^c	40.8±8.1^a	90.2±25.9^a
	60	8.7±3.2^b	18.8±3.3^b	41.6±8.9^a	99.8±33.7^a
	90	9.3±4.0^b	17.5±4.7^a	41.1±7.4^a	97.9±34.6^a
Dip	0	5.6±2.4^d	13.9±2.6^d	29.8±6.9^d	85.9±19.8^d
	15	5.3±1.8^{de}	11.2±2.4^{be}	28.7±7.3^{de}	90.9±40.4^{de}
	30	6.1±2.2^{de}	11.4±4.3^{be}	29.5±5.0^{ce}	98.2±20.7^{de}
	60	5.5±1.9^{de}	13.4±4.0^{ce}	28.3±5.0^{fe}	110.9±36.4^{de}
	90	5.7±1.3^{de}	12.1±3.5^{ce}	30.6±7.6^{ce}	110.5±31.1^{de}

Tab 3. Effects of dipfluzine 50 mg·kg^{-1} ip on amino acid contents in rat brain tissue 48 h after bilateral carotid artery ligation (BCAL). $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Group	Content of cerebral amino acid/mmol·kg^{-1} Asp	Glu	Gly	Tau	GABA
BCAL+Solvent	3.0±0.4	2.0±0.4	1.94±0.22	2.5±0.4	4.2±0.7
BCAL+Dipfluzine	4.7±0.7^c	3.2±0.8^c	2.37±0.34^b	3.2±0.5^b	5.3±0.7^b
Sham-operation	7.7±0.7^c	4.8±0.8^c	3.14±0.24^c	3.7±0.7^c	6.1±0.6^c

damage of the cortical function and the excessive increase in amplitude of SEP may imply the development of the cerebral edema[5,6]. Our results indicated that BCAL could prolong the latency of SEP and result in an initial decrease and a delayed increase of SEP amplitude, and that Dip prevented prolonging of the latency and restrained excessive augmentation in SEP amplitude in BCAL rats, indicating that Dip may accelerate the restoration of the cortical disturbance induced by cerebral ischemia.

Excessive release of EAA is thought to play a major role in the ischemic neuronal damage by activating EAA receptors, which gate cation channels and promote the influx of extracellular Na^+ leading to osmolysis and Ca^{2+} leading to delayed neuronal injury[8]. Our results showed that Dip could not only markedly lower the elevation in concentrations of Glu and Asp in the dialysates, but also attenuate their decrement in brain tissue at 48 h after BCAL, suggesting that Dip alleviated the increase of interstitial EAA level during ischemia by enhancing the release and/or decreasing the uptake of EAA. The effect of Dip might be attributed to its calcium antagonist property[9] so as to inhibit Ca^{2+}-dependent EAA release, or to its improvement of energy metabolism so as to accelerate ATP-dependent EEA uptake, with resultant attenuation in calcium overload subsequent to EEA release. These results may in part explain the facts that Dip reduces the disturbance of SEP and alleviates experimental brain edema[7] in BCAL rats.

In addition, Dip also decreased the elevation of Gly that could increase the affinity of Glu for EAA receptor and augment excitotoxic effect of EAA[2]. It would be beneficial to protect brain from ischemic injury.

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双苯氟嗪对大鼠缺血脑皮层诱发电位和氨基酸含量的影响

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关键词 双苯氟嗪；微透析；氨基酸；脑缺血；体感诱发电位；钙通道阻滞剂

目的：测定双苯氟嗪(Dip)对大鼠缺血脑细胞内外氨基酸含量及皮层体感诱发电位(SEP)的影响。方法：在结扎双侧颈总动脉雌性 Wistar 大鼠，用 HPLC 法测定脑透析液和脑组织中的氨基酸含量，用电生理技术测定 SEP。结果：Dip ip 50 mg·kg^{-1}可防止缺血所致 SEP 潜伏期的延长及其幅度的过分增大，降低脑透析液中的谷氨酸、天冬氨酸和甘氨酸浓度以及减轻脑组织中谷氨酸、天冬氨酸、甘氨酸、牛磺酸和 γ-氨基丁酸的消耗。结论：Dip 能够改善脑缺血所致的皮层功能紊乱和脑内兴奋性与抑制性氨基酸释放失调，为其抗缺血性脑损伤作用提供了进一步的实验证据。

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Influences of ginsenosides Rb_1 and Rg_1 on reversible focal brain ischemia in rats[1]

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KEY WORDS ginseng; saponins; cerebral ischemia; cerebral infarction; calcium; potassium

AIM: To study the influences of ginsenosides Rb_1 and Rg_1 (active components of the total saponins of *Panax ginseng*) on the brains against ischemia-reperfusion injury. **METHODS**: Rat focal cerebral ischemia was induced by reversible middle cerebral artery occlusion (MCAO) without craniectomy. The influences of ginsenoside Rb_1 and Rg_1 on infarct size (IS), neurologic deficit (ND) and the contents of calcium and potassium in the infarct were observed. **RESULTS**: In a 2-h ischemia, Rb_1 10－40 mg·kg^{-1} iv 30 min before MCAO decreased IS by 20 %－49 % and ND score from 5.1 to 4.1－2.3, and inhibited Ca accumulation and K loss by 22 %－50 % and 18－37 %, respectively; Rb_1 10－40 mg·kg^{-1} iv immediately after MCA was recanalized decreased IS by 12 %－35 % and ND score from 5.2 to 4.3－3.3, and inhibited Ca accumulation and K loss by 10 %－40 % and 17 %－30 %, respectively. In permanent ischemia, Rb_1 40 mg·kg^{-1} iv reduced IS, ND, Ca accumulation and K loss. However, Rg_1 40 mg·kg^{-1} iv did not show effect on both permanent and 2-h MCAO. **CONCLUSIONS**: Rb_1 protected brain from ischemic and reperfusion injuries.

Ginsenosides could protect the brains against ischemia and decrease the infarct size (IS) produced by middle cerebral artery occlusion (MCAO)[1-2]. Ginsenosides are composed of many different monoginsenosidic saponins. That

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