目的：观察短时脑缺血再灌后大鼠海马CA1神经元自发放电活动的改变以及地昔帕明(Des)对其放电频率的影响. **方法**：短暂性脑缺血(10 min, 4-VO法) 3 d后，细胞外记录CA1神经元单位放电的变化，尾静脉给药. 实验结束后对海马切片进行形态计量检查. **结果**：再灌d 3海马CA1细胞放电活动明显增加. Des (0.2 & 0.4 mg·kg^{-1}, iv)能显著减弱CA1区升高的放电频率，其最大抑制率分别在给药后6 min (58 % & 85 %)至9 min (69 % & 94 %)期间，与生理盐水对照值相比差异显著(ANOVA, $P<0.01$). 组织学显示该区约50 %锥体细胞呈缺血坏死. **结论**：Des能颉抗海马缺血后的高兴奋性活动.

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Effects of nitrendipine on capacity of calcium binding of erythrocyte membrane and total intraerythrocyte calcium content in SHR rats

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KEY WORDS nitrendipine; erythrocyte membrane; calcium; inbred SHR rats; inbred WKY rats

AIM: To study the effect of nitrendipine (Nit) on the capacity of calcium binding of erythrocyte membrane and total intraerythrocyte calcium content in spontaneous hypertensive rats (SHR). **METHODS**: Systolic blood pressure (SBP) of the consciouse rats was monitored by tailcuff method with a BP and HR recorder for MRS-Ⅱ rat. Erythrocyte membrane was prepared according to modified Bing's method. Calcium binding of membrane and total intraerythrocyte calcium content was determined by an automatic absorption spectrophotometer. The membrane protein was determined with a colorimetric methed. **RESULTS**: Nit (ig 10 mg·kg^{-1} *qd* ×20 d) induced a significant reduction in total intraerythrocyte calcium content (169±18 *vs* 87±14 μmol/L cell, $P<0.01$) accompanied by a marked fall of SBP (27.1±2.5 *vs* 16.7±1.0 kPa, $P<0.01$) but exerted no influence on the capacity of calcium binding of erythrocyte membrane under incubation in $CaCl_2$0 (basal calcium binding) or 40 mmol·L^{-1} (maximal calcium binding) (21.9±2.3 *vs* 22.7±2.1 and 55±14 *vs* 53±23 μmol/g protein, respectively, $P>0.05$). **CONCLUSION**: The antihypertensive effect of Nit is related to the reduction of intracellular calcium and possibly have no direct relation to the capacity of calcium binding of cell membrane.

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Abnormalities of calcium metabolism were found in essential hypertensive patients[1] and in SHR[2] with elevation of intracellular free calcium in erythrocyte and reduction of calcium binding to the erythrocyte[3-5]. Nitrendipine (Nit) is a calcium channel blocker and used in the treatment of hypertension[6]. The present study was designed to evaluate the effects of Nit on calcium binding of erythrocyte and total intraerythrocyte calcium content in SHR.

MATERIALS AND METHODS

Reagents Nit was purchased from Nanjing Pharmaceutical Co. Other reagents were of AR grade. All solutions were prepared with deionized distilled water. Containers were treated with 9.1 % nitric acid for 48 h, and washed with deionized water.

Rats SHR and Wistar-Kyoto (WKY) rats of 12-wk old, both sexes, were provided by the Animal Breeding Center of Fuwai Hospital.

The SHR rats were randomly divided into 2 groups. For the treated group, Nit 10 mg·kg^{-1} was given daily by gavage for 20 d. The control rats and WKY received only solvent of the same volume. All rats were maintained on the pellet food and tap water *ad lib*. Systolic blood pressure (SBP) of the conscious rats was monitored regularly

by tailcuff method with a BP and HR recorder for MRS-Ⅱ rat (Shanghai Hypertensive Research Institute).

Erythrocyte membrane At the end of the treatment period, 5 mL of blood were drawn from abdominal aorta of the rats under pentobarbital (ip 40 mg·kg^{-1}) anesthesia. Erythrocyte membrane was prepared according to modified Bing's method[7]. The heparinized blood was sedimented by centrifugation at 300×g for 10 min at 15 ℃ with phosphate buffer saline (5 mmol·L^{-1} sodium phosphate 150 mmol·L^{-1} sodium chloride, pH 8). After removal of the suspension and white blood cells, the red blood cells were lysed with Tris-HCl buffer (5 mmol·L^{-1} pH 8, 0 ℃) and centrifugated (20 000×g, 30 min, 0 ℃). The pellet was washed (×3) with Tris-HCl buffer. The final resuspension was used[8].

Calcium binding of erythrocyte membrane and total intraerythrocyte calcium content Calcium binding of membrane was determined by an automatic absorption spectrophotometer (Hitachi 180－80). The membrane protein was determined with a colorimetric method[9]. The total intraerythrocyte calcium content was measured[10].

RESULTS

SBP SBP in SHR was higher than that in WKY (27.1±2.5 *vs* 16.0±1.2 kPa, $P<0.01$). At the end of treatment, SBP in Nit-treated SHR was lower than that of the control (16.7±1.0 *vs* 27.1±2.5 kPa, $P<0.01$) (Tab 1).

Tab 1. Effect of Nit ig on calcium binding to erythrocyte membrane in SHR. $n=6$, $\bar{x}\pm s$. $^{a}P>0.05$ *vs* **untreated SHR**, $^{d}P>0.05$, $^{f}P<0.01$ *vs* **WKY**, $^{i}P<0.01$ *vs* **untreated SHR.**

Rats	Nit/ mg·kg^{-1}	SBP/ kPa	Calcium binding (μmol/g protein) $CaCl_2$ 0 mmol·L^{-1}	$CaCl_2$ 40 mmol·L^{-1}
WKY	0	16.0±1.2	33±8	164±24
SHR	0	27.1±2.5^{i}	22.7±2.1^{f}	53±23^{d}
SHR	10	16.7±1.0^{i}	21.9±2.3^{a}	55±14^{a}

Erythrocyte membrane calcium binding In the absence of $CaCl_2$, calcium binding capacity of erythrocyte membrane of SHR was significantly reduced as compared with that in the control normotensive WKY rats ($P<0.01$). No signifficant difference was found between SHR and WKY in the presence of $CaCl_2$ 40 mmol·L^{-1} ($P>0.05$). The control SHR and Nit-treated SHR had the same calcium binding capacity in the presence of $CaCl_2$ 40 mmol·L^{-1} as well as in its absence ($P>0.05$) (Tab 1).

Total intraerythrocyte calcium content The total intraerythrocyte calcium content was higher in untreated SHR than that in WKY ($P<0.01$), whereas in Nit-treated SHR, it was markedly reduced ($P<0.01$) (Tab 2).

Tab 2. Effect of Nit ig on total intracellular calcium in SHR. $n=6$, $\bar{x}\pm s$. $^{f}P<0.01$ *vs* **WKY**, $^{i}P<0.01$ *vs* **untreated SHR.**

Rats	Nit/ mg·kg^{-1}	SBP/ kPa	Intracellular calcium content (μmol /L cell)
WKY	0	16.0±1.2	82±18
SHR	0	27.1±2.5^{f}	169±18^{f}
SHR	10	16.7±1.0^{i}	87±14^{i}

DISCUSSION

The present study indicated that treatment of SHR with Nit caused a significant reduction of total intraerythrocyte calcium content and a lowered SBP. However, Nit did not exert any significant influence on the erythrocyte membrane calcium binding capacity.

Erythrocytes were chosen for this study because of its availability and a preparation of erythrocyte membrane was simple. Besides, it also possessed the unique property of having both the transport systems for active and passive ion transportation and 90 % of the exchangable calcium within the same depot. Abnormalities of vascular smooth muscle are primarily related to disturbances of calcium metabolism. Elevation of intracellular calcium content plays an important role in the induction of blood pressure elevation[11]. There are many factors influencing the intracellular calcium content. In erythrocytes of essential hypertensive patients and SHR[5,7], the calcium binding to the inner side of the plasma membrane and the calmodulin dependent component of calcium pump activity are reduced and passive calcium influx into ATP-depleted cell is accelerated.

Calcium channel blockers decreased the intraerythrocyte calcium content by influencing either passive influx or efflux of calcium[12]. Previous studies[13] reported that nifedipine could

stimulate the activity of Ca-ATPase which was reduced in SHR and essential hypertensive patients. It was by stimulating the efflux as well as by decreasing the passive calcium influx besides calcium channel blockade.

Results of the present study provided further evidences to substantiate the importance of intracellular calcium in the induction and development of hpertension and demonstrated that the antihypertensive effect of Nit was related to the lowering of intracelluair calcium and could not show direct relation to the capacity of calcium binding of cell membrane.

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尼群地平对自发性高血压大鼠红细胞膜钙结合力和红细胞内总钙含量的作用

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关键词 尼群地平；红细胞膜；钙；近交系 SHR 大鼠；近交系 WKY 大鼠

目的：研究尼群地平(Nit)对自发性高血压大鼠红细胞膜钙结合力和红细胞内总钙含量的作用. **方法**：应用尾套法测清醒大鼠收缩压，按改良 Bing 氏法制备红细胞膜，用原子吸收光谱法检测红细胞膜钙结合力及红细胞内总钙量. **结果**：尼群地平(ig 10 mg·kg^{-1} qd×20 d)能显著降低自发性高血压大鼠红细胞内总钙含量(169±18 *vs* 87±14 μmol/L cell, $P<0.01$)，同时使收缩压明显下降(27.1±2.5 *vs* 16.7±1.0 kPa, $P<0.01$)，但对 $CaCl_2$ 0 mmol·L^{-1}的基础膜钙结合力及 $CaCl_2$ 40 mmol·L^{-1}的最大膜钙结合力均无影响. **结论**：Nit 的抗高血压作用可能与其降低细胞内钙含量有关，而与细胞膜钙结合力没有直接关系.