

Effects of hyperin on free intracellular calcium in dissociated neonatal rat brain cells

CHEN Zhi-Wu¹, MA Chuan-Geng

(Department of Pharmacology, Anhui Medical University, Hefei 230032, China)

KEY WORDS hyperin; brain; cultured cells; Fura-2; calcium; norepinephrine; sodium glutamate; serotonin

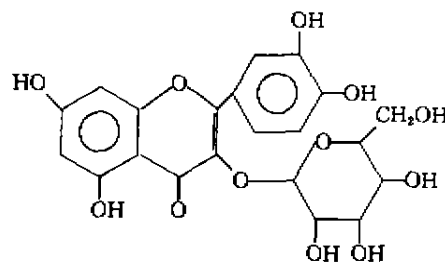
ABSTRACT

AIM: To study the effects of hyperin (Hyp) on free intracellular calcium concentration ($[Ca^{2+}]_i$) of brain cells. **METHODS:** The neonatal rat brain cells were dissociated. $[Ca^{2+}]_i$ in presence and absence of extracellular high K^+ , *L*-glutamic acid (Glu), 5-hydroxytryptamine (5-HT), and norepinephrine (NE) were assayed with Fura 2-AM. **RESULTS:** The resting $[Ca^{2+}]_i$ in Hanks' solution ($CaCl_2$ $1.3 \text{ mmol} \cdot L^{-1}$) was $(208 \pm 12) \text{ nmol} \cdot L^{-1}$ ($n = 17$). Hyp had no significant effects on the resting $[Ca^{2+}]_i$. Hyp 1.0 , 4.0 , and $16.0 \mu\text{mol} \cdot L^{-1}$ markedly inhibited the increase of $[Ca^{2+}]_i$ evoked by K^+ $50 \text{ mmol} \cdot L^{-1}$ in a concentration-dependent manner. Hyp $16.0 \mu\text{mol} \cdot L^{-1}$ inhibited the increases of $[Ca^{2+}]_i$ induced by NE 1 , 2 , 4 , and $8 \mu\text{mol} \cdot L^{-1}$. Hyp ($16.0 \mu\text{mol} \cdot L^{-1}$) also markedly attenuated 5-HT and Glu-induced increase of $[Ca^{2+}]_i$. **CONCLUSION:** Hyp possessed inhibitory effects on influx of Ca^{2+} in the neonatal rat brain cells.

INTRODUCTION

Hyperin (hyperoside: quercetin-3-*O*-galactoside; Hyp), a flavonal glycoside, was extracted from Chinese herb *Abelmoschus manihot*

L. Our previous studies showed that Hyp inhibited inward flow of calcium ion in cardiomyocyte^[1] and had protective effects against cerebral ischemia-reperfusion injury in mice^[2] and rats^[3]. It is obvious that Hyp possesses potential clinical values in preventing and treating ischemic-reperfusion injuries. It has been considered that its protective effects may be related to the reduction of free intracellular calcium concentration ($[Ca^{2+}]_i$). In the present study, effects of Hyp on $[Ca^{2+}]_i$ level in dissociated brain cells of neonatal rats were observed.



3,3',4',5,7-Pentahydroxyflavone-3-*O*-galactoside

MATERIALS AND METHODS

Hyp, a yellow powder, mp $230 - 231 \text{ }^\circ\text{C}$, purity $> 95 \%$ (Anhui Institute of Medical Science); dimethyl sulfoxide (Beijing Chemical Factory); RPMI-1640 medium (Gibco); Fura 2-AM (Shanghai Institute of Physiology, Chinese Academy of Sciences) dissolved in Me_2SO ; 5-HT (Sigma); *L*-glutamic acid (Glu, Sigma). All other chemicals were AR.

Preparation of brain cells^[4] Sprague-Dawley neonatal rats ($n = 120$, Grade II, Certificate No AHEA-01, 1-3-d old) were

¹ Correspondence to Dr CHEN Zhi-Wu. Phn 86-551-363-9026.

Fax 86-551-552-5461. E-mail aydxt@public.ah.hf.cn

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killed, the brain was isolated and rinsed with ice-cold Hanks' solution containing: NaCl 137; KCl 5.0; CaCl₂ 1.3; MgSO₄ · 7H₂O 0.8; Na₂HPO₄ 0.6; KH₂PO₄ 0.4; NaHCO₃ 3.0; glucose 5.6 mmol · L⁻¹; pH 7.4. Blood vessels and meninges were removed carefully. Following a wash step with ice-cold Hanks' solution, the brain was cut into about 1 mm³ fragments which were digested with trypsin 1.25 g · L⁻¹ at 37 °C for 15 min. Twice volume of ice-cold RPMI-1640 medium (containing 10 % fetal calf serum) was used to end the digestion. The brain cells were filtered through nylon mesh (200 mesh). The cells were centrifuged (300 × g, 5 min), washed twice and resuspended in RPMI-1640 medium. Trypan blue exclusion method showed that cellular viability rates were above 90 %.

Measurement of Ca²⁺_i (4,5) Fura 2-AM was added into the cell suspensions (final concentration = 5 μmol · L⁻¹). Having been incubated at 37 °C for 40 min to load Fura 2, the cells suspensions were preincubated with Hyp for 10 min and incubated at 37 °C for 3 min prior to measurement.

The fluorescence (*F*) was determined by a Hitachi 650 – 60 fluorescence spectrophotometer (λ_{ex}: 340 nm, λ_{em}: 500 nm). At the end of the analysis, the final concentration of 0.1 % Triton X-100 was added to measure the maximal fluorescence (*F*_{max}), the final concentration of egtazic acid 10 mmol · L⁻¹ was added to measure minimum fluorescence (*F*_{min}). The values of [Ca²⁺]_i were calculated: [Ca²⁺]_i = K_D × (*F* – *F*_{min}) / (*F*_{max} – *F*), the apparent dissociation constant (K_D) was 224 nmol · L⁻¹.

Statistical method All data were analyzed by *t*-test.

RESULTS

Effects of Hyp on the resting [Ca²⁺]_i. The resting [Ca²⁺]_i in dissociated neonatal rat brain cells was (208 ± 12) nmol · L⁻¹ in Hanks'

solution (CaCl₂ 1.3 mmol · L⁻¹); Hyp 1.0, 4.0, and 16.0 μmol · L⁻¹ had no significant effect on the resting [Ca²⁺]_i (Tab 1).

Tab 1. Effects of Hyp on the resting and KCl-induced increases of [Ca²⁺]_i in dissociated neonatal rat brain cells. Number of cell suspensions (each was pooled from 5 neonatal rats and assayed in triplicate) is in parentheses. $\bar{x} \pm s$.

^a*P* > 0.05, ^b*P* < 0.05, ^c*P* < 0.01 vs control; ^f*P* < 0.01 vs resting.

Group	Concentration/ μmol · L ⁻¹	[Ca ²⁺] _i / nmol · L ⁻¹	
		resting	K ⁺ 50 mmol · L ⁻¹
Control	–	208 ± 12(17)	517 ± 59(9) ^f
Hyp	1.0	209 ± 8(4) ^a	430 ± 31(4) ^b
	4.0	208 ± 8(5) ^a	347 ± 19(5) ^c
	16.0	206 ± 8(20) ^a	295 ± 42(8) ^c
Nim	5.0	204 ± 10(4) ^a	315 ± 38(4) ^d

Effects of Hyp on KCl-induced increase of [Ca²⁺]_i KCl 50 mmol · L⁻¹ increased the [Ca²⁺]_i in the brain cells, the net increase of [Ca²⁺]_i was 309 nmol · L⁻¹; Hyp (1.0, 4.0, and 16.0 μmol · L⁻¹) markedly inhibited this elevation in a concentration-dependent manner, its inhibitory rates were 28.2 %, 55.0 %, and 71.8 %, respectively. Similar results were obtained with nimodipine (Nim).

Effects of Hyp on NE-evoked increase of [Ca²⁺]_i Addition of NE 1, 2, 4, and 8 μmol · L⁻¹ in cells suspensions, the [Ca²⁺]_i was increased concentration-dependently. Hyp (16.0 μmol · L⁻¹) markedly attenuated the NE (1 – 8 μmol · L⁻¹)-induced increase of [Ca²⁺]_i, its inhibitory rates for 1, 2, 4, and 8 μmol · L⁻¹ NE were 46.4 %, 53.2 %, 44.0 %, and 51.2 %, respectively (Fig 1).

Effects of Hyp on sodium glutamate and 5-HT-induced increase of [Ca²⁺]_i Both of sodium glutamate (pH 7.8) 150 μmol · L⁻¹ and 5-HT 1.5 μmol · L⁻¹ markedly increased [Ca²⁺]_i (Tab 2).

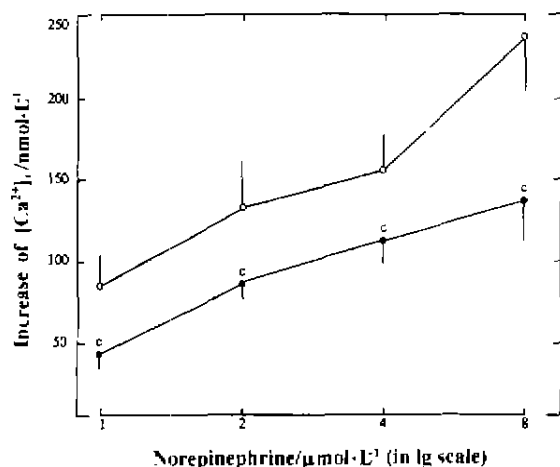


Fig 1. Effect of Hyp on NE-evoked increase of $[Ca^{2+}]_i$ in dissociated neonatal rat brain cells. $n = 5$ cell suspensions. $\bar{x} \pm s$. ^c $P < 0.01$ vs control.

Tab 2. Effects of Hyp on 5-HT and Glu-induced increases of $[Ca^{2+}]_i$ in dissociated neonatal rat brain cells. $\bar{x} \pm s$. n is the number of cell suspensions. ^c $P < 0.01$ vs control.

Group	Concentration/ $\mu\text{mol} \cdot \text{L}^{-1}$	n	$[Ca^{2+}]_i / \text{nmol} \cdot \text{L}^{-1}$	
			5-HT	Glu
Control	-	5	431 ± 27	440 ± 20
Hyp	16.0	5	321 ± 22 ^c	306 ± 25 ^c
Nim	5.0	4	340 ± 30 ^c	348 ± 19 ^c

Hyp $16.0 \mu\text{mol} \cdot \text{L}^{-1}$ inhibited 5-HT and Glu-induced increases of $[Ca^{2+}]_i$, the inhibitory rates were 49.3 % and 58.1 %, respectively. Nim ($5.0 \mu\text{mol} \cdot \text{L}^{-1}$) also inhibited 5-HT and Glu-induced increases of $[Ca^{2+}]_i$.

DISCUSSION

The resting $[Ca^{2+}]_i$ value of dissociated newborn rat brain cells in our study is close to the value of $[Ca^{2+}]_i$ ^[6]. Our results showed that Hyp ($1.0, 4.0, 16.0 \mu\text{mol} \cdot \text{L}^{-1}$) did not influence the resting $[Ca^{2+}]_i$ in neonatal rat brain cells.

High K^+ could cause depolarization of

membrane and open voltage-dependent calcium channel (VDC)^[4,7]. Hyp ($1.0 - 16.0 \mu\text{mol} \cdot \text{L}^{-1}$) markedly and concentration-dependently inhibited $50 \text{ mmol} \cdot \text{L}^{-1}$ KCl-induced $[Ca^{2+}]_i$ elevation in brain cells. This finding corresponded to our previous report that Hyp inhibited ⁴⁵Ca influx in atrium preparation in mice. The result suggested that Hyp blocked VDC to attenuate KCl-induced increase of $[Ca^{2+}]_i$ in nerve cells.

The regulation of G proteins on Ca^{2+} channels has been an active area of research. G proteins not only regulated Ca^{2+} channels indirectly via cytoplasmic second message, but also act directly on Ca^{2+} channels^[7]. Since G proteins couple a variety of receptors to Ca^{2+} channels, NE, 5-HT *etc* can activate the proper G protein-coupled receptors to open Ca^{2+} channels. Hyp inhibited NE and 5-HT-induced increase of $[Ca^{2+}]_i$. Results suggested that Hyp inhibited Ca^{2+} influx induced by activation of G protein-coupled receptors (5-HT and α -adrenergic receptors).

N-methyl-*D*-aspartate (NMDA) receptors found on neurons were important in the control of nerve activity. Activation of NMDA receptors led to the opening of Ca^{2+} channels^[8,9]. Although there are different subtypes of Glu-sensitive Ca^{2+} channels, the effects of NMDA receptor-linked Ca^{2+} channels on Glu-induced $[Ca^{2+}]_i$ elevation are critical. In our study, Glu caused $[Ca^{2+}]_i$ increase and Nim inhibited this elevation, the responses were similar to Lu's report^[10]. Hyp $16.0 \mu\text{mol} \cdot \text{L}^{-1}$ markedly inhibited Glu-induced increase of $[Ca^{2+}]_i$. These inhibitory effects suggested that Hyp could also block NMDA receptor-linked Ca^{2+} channels to decrease $[Ca^{2+}]_i$ increase in nerve cells.

In summary, Hyp possesses the inhibitory effects on influx of Ca^{2+} in neonatal rat brain cells.

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金丝桃苷对分离的新生大鼠脑细胞内游离钙浓度的影响

陈志武¹, 马传度

(安徽医科大学药理教研室, 合肥 230032, 中国)

关键词 金丝桃苷; 脑; 培养的细胞; Fura-2; 钙; 去甲肾上腺素; 谷氨酸钠; 血清素

目的: 研究金丝桃苷(Hyp)对新生大鼠的脑细胞内游离钙浓度的作用。方法: 分离新生大鼠的脑细胞; 用钙离子荧光指示剂 Fura-2 测定静息和激动剂存在时新生鼠脑细胞内游离钙浓度。结果: 在 $CaCl_2$ $1.3 \text{ mmol} \cdot L^{-1}$ 的 Hanks' 液中, 静息 $[Ca^{2+}]_i$ 为 $(208 \pm 12) \text{ nmol} \cdot L^{-1}$ ($n = 17$)。Hyp 对静息 $[Ca^{2+}]_i$ 无明显影响; Hyp $1.0, 4.0, 16.0 \mu\text{mol} \cdot L^{-1}$ 呈浓度依赖性显著抑制 KCl $50 \text{ mmol} \cdot L^{-1}$ 致 $[Ca^{2+}]_i$ 增高; Hyp $16.0 \mu\text{mol} \cdot L^{-1}$ 显著抑制去甲肾上腺素 $1, 2, 4$ 和 $8 \mu\text{mol} \cdot L^{-1}$ 诱发的 $[Ca^{2+}]_i$ 的增高; Hyp $16.0 \mu\text{mol} \cdot L^{-1}$ 还可显著抑制谷氨酸和 5-羟色胺致 $[Ca^{2+}]_i$ 增高。结论: Hyp 对新生大鼠脑细胞钙内流有阻滞作用。

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