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Nicardipine enhances cytoplasmic calcium concentration in mouse thymocytes *in vitro*[1]

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AIM: To study the effect of nicardipine (Nic) on the cytoplasmic free calcium concentration ($[Ca^{2+}]_i$) in thymocytes and on the proliferation of thymus lymphocytes from mice *in vitro*. **METHODS**: The $[Ca^{2+}]_i$ was measured by loading cells with the fluorescent dye Fura-2. The lymphocyte proliferation was determined by [^{3}H]thymidine incorporation. **RESULTS**: Nic 1 — 30 $\mu mol \cdot L^{-1}$ elevated $[Ca^{2+}]_i$ of resting thymocytes with the percentages of its increase 43 %— 192 % and 12 %—148 % in the presence and absence of extracellular Ca^{2+} respectively. Mitogen Con A 5 $mg \cdot L^{-1}$ also released Ca^{2+} from intracellular stores. Nic decreased the enhancement of $[Ca^{2+}]_i$ induced by Con A. The IC_{50} ($\mu mol \cdot L^{-1}$) was 26.8 (95 % limit: 24.8—29.0) and approximated to 10 in the presence and absence of extracellular Ca^{2+} respectively. Nic 1—30 $\mu mol \cdot L^{-1}$ did not stimulate the proliferation of resting lymphocytes from mouse thymuses ($P>0.05$), but inhibited the proliferation when the lymphocytes had been stimulated by Con A ($P<0.01$). **CONCLUSION**: Nic elevated $[Ca^{2+}]_i$, which decreased the response of lymphocytes to mitogen.

KEY WORDS nicardipine; thymus gland; cytoplasm; calcium; lymphocyte transformation; cultured cells

Calcium is critically important to lymphocyte activation[1]. After lymphocytes are stimulated by mitogens the cytoplasmic free calcium concentration rises[2]. Calcium channel blockers inhibit functions of immune cells[3,4]. Blocking voltage-depended calcium channel is thought to be the pharmacological basis of calcium channel blockers, but lymphocytes lack the voltage-dependent calcium channel[5]. Therefore, the questions whether and how calcium channel blockers influence cytoplasmic free calcium concentration in lymphocytes should be answered. In the present study, we observed the influence of calcium channel blocker nicardipine (Nic) on the cytoplasmic free calcium and lymphocyte proliferation in mouse thymocytes *in vitro*.

MATERIALS AND METHODS

Cells Kunming mice of either sex, 6 — 8 wk old, weighing 20.3 ± *s* 2.1 g, were killed by cervical dislocation. The thymocytes were prepared[6].

Ca^{2+} measurements Intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) were measured by loading cells with the fluorescent dye Fura-2[7]. The final concentration of Fura 2-AM was 3 $\mu mol \cdot L^{-1}$. The cell viability as assessed by the trypan blue exclusion test was more than 95 %. The fluorescence measurements were performed at 37 C in buffer, containing (mmol·L^{-1}) NaCl 145, KCl 5, Na_2HPO_4 1, $CaCl_2$ 1, $MgSO_4$ 0.5, glucose 5, HEPES 10, pH 7.4, the buffer was prepared in 3 distilled water. The Ca^{2+}-free buffer was prepared by omitting $CaCl_2$ with addition of egtazic acid 0.3 mmol·L^{-1}. The fluorescence of R_{max} and R_{min} were determined by addition of 0.1 % Triton X-100 and 5 mmol·L^{-1} egtazic acid (final concentrations). A Hitacb F-4010 spectrofluorometer was used at excitation wavelength of 340, 380 nm and an emission

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wavelength of 500 nm.

Lymphocyte proliferation Thymocytes were suspended at 1×10^{10} cells·L^{-1} in RPMI 1640 medium supplemented with 10 % fetal calf serum, penicillin G 100 kU·L^{-1}, and streptomycin 100 mg·L^{-1} (pH 7.4). The cells were plated duplicately in 1×10^5 cells/well in a 96-well flat bottom plate. Cells were incubated at 37 ℃ in humidified incubator with 5 % CO_2, 95 % air for 48 h. Eight hours before the conclusion of culture, [^{3}H]thymidine 37 MBq·L^{-1} was added to each well. Cells were subsequently harvested onto fiberglass filters by using a multiple-channel cell harvester. Samples were counted by liquid scintillation counter (LKB-1217/1218). At the conclusion of culture, Cell viability as assessed by the trypan blue exclusion test was more than 90 % in the absence and presence of Nic.

Chemicals Fura 2-AM (Sigma); nicardipine (Dongfing Pharmaceutical Factory, Zhangjiakou, China) was dissolved in Me_2SO to 20 mmol·L^{-1} and diluted with buffer. The final concentrations of Me_2SO was <0.15 %. HEPES (Merck); Concanavalin A (Con A, Sigma); RPMI 1640 medium (Gibco); [^{3}H]thymidine (Institute of Atomic Energy, Chinese Academy of Sciences). All other chemicals were AR.

RESULTS

$[Ca^{2+}]_i$ In the Ca^{2+}-free medium, the basal $[Ca^{2+}]_i$ was markedly lower than that in Ca^{2+}-containing medium. In the resting thymocytes Nic per se induced an evident increase of $[Ca^{2+}]_i$ in the presence, but less in the absence, of extracellular Ca^{2+}, and sustained for >10 min (Tab 1). The antagonists of calcium channels, nimodipine, nifedipine, and agonist Bay k 8644 at 0.1 — 50 μmol·L^{-1} also produced enhancements of $[Ca^{2+}]_i$ in resting thymocytes (data not shown). Depolarization of thymocytes with high $[K^+]_o$ (KCl 50 mmol·L^{-1}) did not elicit a rise of $[Ca^{2-}]_i$.

When Con A 5 mg·L^{-1} was added to the cell suspensions, the $[Ca^{2+}]_i$ was raised to a peak level within 1—3 min, in the presence or absence of extracellular Ca^{2+} (Tab 1), indicating that Con A also induced release of Ca^{2+} from intracellular stores. Con A 5 mg·L^{-1} was added 4 min after Nic to cells suspensions, as a result Nic inhibited the increase of $[Ca^{2+}]_i$ induced by Con A in the presence or absence of external Ca^{2+} in a concentration-dependent manner (Tab 2).

Tab 1. Influence of Nic and Con A on $[Ca^{2+}]_i$ of mouse thymocytes in media with/without Ca^{2+}. n = tubes of cell suspension (each was pooled from 2 mice). $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$.

Drug	$[Ca^{2+}]_i$/nmol·L^{-1}					
	$+Ca^{2+}$	n	Increase	$-Ca^{2+}$	n	Increase
	72±13	29		23±3	29	
Con A/ 5 mg·L^{-1}	129±16^c	11	79 %	52±13^c	9	126 %
Nic/μmol·L^{-1}						
1	103±12^c	8	43 %	27±4^a	4	12 %
10	137±24^c	4	90 %	39±4^c	7	70 %
30	210±44^c	6	192 %	57±9^c	9	148 %

Tab 2. Inhibitory effect of Nic on the increase of $[Ca^{2-}]_i$ induced by Con A (5 mg·L^{-1} added 4 min after Nic) in mouse thymocytes. n = tubes of cell suspension (each was pooled from 2 mice). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* absence of Nic.

Nic/ μmol·L^{-1}	Increase of intracellular Ca^{2-}/nmol·L^{-1} $+Ca^{2+}$	n	Inhibition	$-Ca^{2+}$	n	Inhibition
0	62±12	9		25±9	8	
1	51±4^a	4	18 %			
10	38±4^b	3	39 %	11±4^c	8	56 %
30	30±10^c	6	52 %	9±4^c	5	64 %
IC_{50}/μmol·L^{-1}	26.8			<10		
95 % limit of IC_{50}:	24.8—29.0					

Lymphocyte proliferation Nic did not stimulate much the proliferation of resting lymphocytes. But when lymphocytes had been stimulated by Con A (5 mg·L^{-1}), Nic inhibited significantly lymphocyte proliferation response (Tab 3),

Tab 3. Effect of Nic on proliferation of lymphocytes from mouse thymus. n = wells of cell suspension (each was taken from 1 mouse and assayed in duplicate). $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.01$ *vs* control; $^dP>0.05$, $^fP<0.01$ *vs* Con A.

Nic/ μmol·L^{-1}	Con A/ mg·L^{-1}	n	[^{3}H]thymidine incorporation/dpm
0	0	6	253±58
1	0	6	264±59^a
10	0	6	271±80^a
30	0	6	316±74^a
0	5	5	32 344±5 279^b
1	5	4	26 413±4 927^{ad}
10	5	4	13 625±1 606^{af}
30	5	5	420±79^{af}

DISCUSSION

Our experiments showed that Nic elevated $[Ca^{2+}]_i$ not only in the Ca^{2+}-containing medium, but also in the Ca^{2+}-free medium, which indicated that Nic released Ca^{2+} from intracellular stores. It is a problem that as a calcium antagonist, why Nic can increase $[Ca^{2+}]_i$ and where Nic releases Ca^{2+} from. In our previous study, we found that Nic at 5—10 mg·kg^{-1} × 4 d suppressed immune functions of mice and simultaneously influenced the mitochondrial structure of lymphocytes, macrophages, hepatocytes, and myocytes. There was decrease of electron-density of mitochondrial matrix, and the cristae became thinner and even formed some plaques[8]. Therefore, we can believe that Nic probably influences Ca^{2+} storage of mitochondria. In an experiment of mitochondria from rat liver Nic induced Ca^{2+} release of mitochondria and reduced its Ca^{2+} uptake[9], hence a rise of plasmic free Ca^{2+}. In the present study our results in intact cells consist with that in mitochondria. In rat myocytes, the protective effect of Nic against reoxygenation injury was considered to be a result of Nic preventing mitochondrial calcium loading[10]. In addition, the mechanisms by which dihydropiridine compounds nimodipine, Bay k 8644 and nifedipine enhanced $[Ca^{2+}]_i$ are not clear. Recent researches have shown that mitochondrial membranes possess dihydropyridines binding sites[11,12]. We may infer that the binding sites deal with pharmacological effects of these drugs including that on the Ca^{2+} transportation of mitochondria.

In general, when lymphocytes are stimulated by mitogens, the rises of $[Ca^{2+}]_i$ are due to the result of Ca^{2+} influx from extracellular medium[13]. But in our experiment, Con A released Ca^{2+} from intracellular stores, which is consistent with observation with rat thymocytes[14], and the mobilization of intracellular Ca^{2+} by Con A induced was inhibited by Nic, in spite of no excluding inhibition of Nic on the extracellular Ca^{2+} mobilization in the present experiment.

At the doses of evoking $[Ca^{2+}]_i$ rise Nic could not induce significantly proliferation of resting lymphocytes but inhibited proliferation stimulated by Con A. This suggested that Nic interfered with calcium homeostasis of cells, thereby decreased the cellular responsiveness to Con A.

In summary, Nic increases $[Ca^{2+}]_i$ by releasing Ca^{2+} from intracellular stores, the target of this act is probably the mitochondria.

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489-492

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尼卡地平升高小鼠胸腺细胞胞浆钙浓度[1]

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目的: 研究尼卡地平 (nicardipine, Nic)对小鼠胸腺细胞胞浆钙浓度 ($[Ca^{2+}]_i$)及增殖的影响. **方法**: 用 Fura-2 掺入细胞的荧光测定法测定 $[Ca^{2+}]_i$; 用[^{3}H]thymidine 掺入法测定胸腺淋巴细胞的增殖. **结果**: 无论在含 Ca^{2+} 或无 Ca^{2+} 介质中, Nic 1—30 $\mu mol \cdot L^{-1}$, 以浓度依赖的方式升高静息胸腺细胞的 $[Ca^{2+}]_i$. 丝裂原 Con A 5 $mg \cdot L^{-1}$ 也从细胞内库释放 Ca^{2+}, 而 Nic 抑制 Con A 引起的 $[Ca^{2+}]_i$ 升高. 在上述升高 $[Ca^{2+}]_i$ 的浓度中, Nic 不刺激静息胸腺淋巴细胞增殖, 但显著抑制 Con A 的增殖反应. **结论**: Nic 升高 $[Ca^{2+}]_i$, 干扰了细胞 Ca^{2+} 稳态, 因而抑制淋巴细胞对丝裂原的反应.

关键词 尼卡地平; 胸腺; 细胞质; 钙; 淋巴细胞转化; 培养的细胞

细胞培养