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多巴胺 D_2/血管紧张素 AT_1 嵌合受体：D_2 受体第三细胞内环影响受体与配基结合以及与 G 蛋白偶联的特性

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关键词 多巴胺 D_2 受体；血管紧张素受体；多巴胺激动剂；多巴胺拮抗剂；嵌合体蛋白类；聚合酶链反应；磷脂酰肌醇；G蛋白类

偶联

目的：研究受体第三细胞内环（IL_3）的长度对受体与配基结合及与 G 蛋白偶联特性的影响。方法：用目前已知的 G 蛋白偶联受体中 IL_3 最短的血管紧张素 II AT_1 受体的 IL_3 替换野生型 D_2 受体较长的 IL_3，组成 D_2/AT_1 嵌合受体。结果：与野生型 D_2 受体相比，D_2/AT_1 嵌合受体与拮抗剂的亲和性均降低，与激动剂的亲和性有的增高，有的降低。嵌合受体失去与 G 蛋白偶联的能力，也不能产生磷酸肌醇水解。结论：受体的 IL_3 对受体配基结合位点和空间构象有一定影响；受体与 G 蛋白的偶联不仅与 IL_3 有关，而且还受非 IL_3 区域的影响，而 IL_3 的长度是决定这两方面影响的因素之一。

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Effect of saponins of *Panax notoginseng* on synaptosomal ^{45}Ca uptake

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KEY WORDS ginseng; saponins; synaptosomes; calcium radioisotopes; nimodipine

AIM: To explore the calcium uptake antagonism of saponins of *Panax notoginseng* (PNS). **METHODS:** Synaptosomes were prepared from rat cerebral cortex by using differential Ficoll gradients. The effects of PNS on synaptosomal ^{45}Ca uptake were measured *in vitro* or after acute treatment. **RESULTS:** PNS 50 - 800 mg·L^{-1} produced a concentration-rated inhibition of Ca^{2+} uptake [IC_{50} = 111 (46 - 176) mg·L^{-1}]. Both initial and maximal uptake were inhibited. Similar effect was obtained after acute PNS treatment with 200 mg·kg^{-1} ip. The blocking effect of PNS was reversed by calcium in media. **CONCLUSION:** PNS is a calcium channel blocker in neurons.

Saponins of *Panax notoginseng* (PNS) protected brain or primary cultured myocytes from anoxic injuries[1-3]. Balance perturbation of calcium in the cell constitutes the final common pathway of cell damage during ischemia/reperfusion[4]. PNS blocked calcium into vascular smooth muscles and cultured myocardial cells[5,6]. In this study the effects of PNS on synaptosomal calcium uptake were tested.

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MATERIALS AND METHODS

Drugs and chemicals PNS was extracted and purified by Dept of Phytochemistry, Institute of Medicinal Plant Development, purity 94.5 %. $^{45}CaCl_2$ (148 GBq·L^{-1}) was purchased from Beijing Institute of Atomic Energy. Ficoll400 was obtained from Pharmacia and ATP-Na_2 was purchased from Sigma. All other chemicals were AR grade.

Preparation of synaptosomes Synaptosomes were obtained from rat cerebral cortex scraped free of as much white matter as possible before homogenization[7]. After centrifugation at 65 000 × g for 50 min, the material between the 7.5 % and 12 % Ficoll interface (synptosomes)

was removed and recentrifuged at 110 000 × g for 30 min. The pellet was separated and resuspended in a solution containing (final concentration): $CaCl_2$ 0.1, $MgCl_2$ 3, ATP-Na_2 3, and Tris 50 mmol·L^{-1}, pH 7.4.

Measurement of ^{45}Ca influx Aliquots with PNS or nimodipine were preincubated at 37 ℃ for 5 min. $^{45}CaCl_2$ (4.6 MBq·L^{-1}) was added. At fixed intervals (0.5, 1, 2, 4, and 10 min), reactions were terminated by filtering through glass fiber filters on a multiple cell harvest (DYQ, Zhejiang). The filters were washed thrice with cold stopping solution (NaCl 100, $MgCl_2$ 3, $CaCl_2$ 0.1, and Tris 50 mmol·L^{-1}, pH 7.4). The amount of ^{45}Ca in the synaptosomes was determined by scintillation spectrometry (Wallac 1409/1411).

Mouse treatment Mice weighing 20 ± s 1 g of either sex were injected ip PNS 200 mg·kg^{-1} or nimodipine 100 μg·kg^{-1} 30 min before sacrifice for preparation of synaptosomes. The control group was given equal volume of saline.

Protein assay Protein concentration was measured using bovine serum albumin standards[8].

Statistics Results were expressed as $\bar{x} \pm s$ and analyzed with t-test.

RESULTS

Effect of PNS on synaptosomal ^{45}Ca uptake Incubation of the synaptosomal suspensions with $^{45}CaCl_2$ solution resulted in an initial phase of rapid uptake for about 2 min, then a slower phase which reached a plateau in about 4 min. PNS 400 mg·L^{-1} reduced $^{45}CaCl_2$ uptake. The decrease was seen in both the initial rate and the maximal level which was reduced to 84.2 % of control (Fig 1).

The most prominent action on maximal ^{45}Ca uptake produced by PNS was a concentration-dependent inhibition with IC_{50} = 111 (46 − 176) mg·L^{-1} (Tab 1).

Effect of $[Ca^{2+}]_o$ on PNS action At various concentrations of external calcium in the presence and absence of PNS 400 mg·L^{-1}, increased $[Ca^{2+}]_o$ decreased synaptosomal $^{45}CaCl_2$ uptake and attenuated the blocking function of PNS (Tab 2).

Effect of $^{45}CaCl_2$ concentration on PNS blockade of ^{45}Ca uptake Though the blocking actions of PNS 400 mg·L^{-1} with $^{45}CaCl_2$ 10 μmol·L^{-1} to 0.1 mmol·L^{-1} were enhanced, ^{45}Ca uptake in synaptosomes alone shot up more. The result suggested that the effect of PNS was blunted by calcium in media (Tab 3).

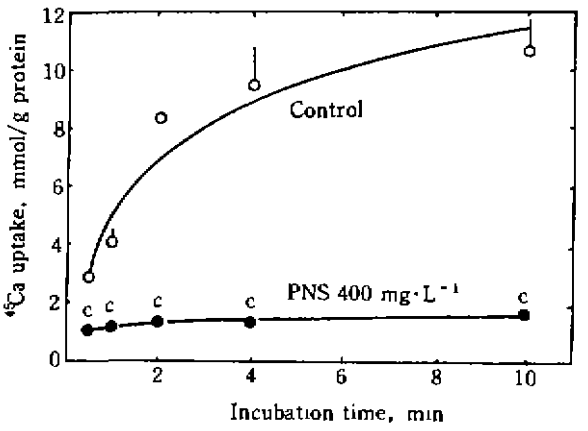

Fig 1. Effect of PNS on synaptosomal ^{45}Ca uptake. n = 6, $\bar{x} \pm s$. $^cP<0.01$ *vs* control.

Tab 1. Effect of PNS on ^{45}Ca uptake in synaptosomes. n = 6, $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Drugs	Concn, mg·L^{-1}	^{45}Ca uptake, mmol/g protein	Rate of inhibition, %
Control	0	9.55 ± 2.40	
PNS	50	5.74 ± 0.69^a	40
	100	4.67 ± 0.67^b	51
	200	2.54 ± 0.19^c	73
	400	1.70 ± 0.27^c	82
	800	1.12 ± 0.10^c	88
Nimodipine	4.2	1.93 ± 0.12^c	80

Tab 2. Effect of $[Ca^{2+}]_o$ on synaptosomal ^{45}Ca uptake. n = 6, $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control.

PNS mg·L^{-1}	Calcium concentration in media/mmol·L^{-1} 0.01	0.10	1.00
0	6.46 ± 1.64	2.60 ± 0.27	1.17 ± 0.09
400	1.80 ± 0.24^c	1.21 ± 0.12^c	0.99 ± 0.03^b

Effect of acute PNS treatment on ^{45}Ca uptake PNS applied ip inhibited ^{45}Ca uptake in mouse synaptosomes. PNS depressed markedly both initial and maximal uptake whereas only the initial uptake was affected by nimodipine (Tab 4).

DISCUSSION

The effect of PNS on the uptake of calcium *in*

Tab 3. Effect of external $^{45}CaCl_2$ concentration on calcium uptake into synaptosomes. $n = 6$, $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ vs control. $^fP<0.01$ vs relative value of increased ^{45}Ca uptake in synaptosomes, alone.

PNS	External ^{45}Ca concentration/μmol·L^{-1}			
mg·L^{-1}	0.1	1.0	10	100
0	0.37±0.04	0.88±0.09	4.24±0.18	18.8±0.6
400	0.28±0.03^b	0.41±0.07^{df}	1.23±0.12^{cf}	4.4±0.4^{cf}

Tab 4. Effect of acute PNS and nimodipine treatment on mouse synaptosomal ^{45}Ca uptake (nmol/g protein). $n = 5$, $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ vs saline.

Treatment	1 min	10 min
Saline 10 mL·kg^{-1}	3.03±0.48	11.7±1.6
PNS 200 mg·kg^{-1}	1.84±0.28^c	8.8±2.2^b
Nim 100 μg·kg^{-1}	1.77±0.25^c	10.5±1.6

vitro and *in vivo* was studied in animal cerebral synaptosomes. The results clearly indicate that synaptosomal calcium uptake can be blocked in a concentration-dependent manner by PNS *in vitro* or after acute PNS treatment. Elevation of $[Ca^{2+}]_o$ antagonized the inhibition of ^{45}Ca uptake produced by PNS. It appears reasonable to consider that PNS may realize the antagonism by competing with calcium the synaptosomal calcium-binding sites. Our results are consistent with the findings on myocardiac cells[6].

Increased calcium entry via the voltage-sensitive and receptor-operated channels was found a major reason for calcium overload associated with brain damage[4]. Though it was assumed that activation of the Na^+-K^+-ATPase and the resultant inhibition of Na^+/Ca^{2+} exchange was one of the mechanisms of calcium antagonism, blocking effect induced by PNS on synaptosomal calcium uptake suggests another facet which probably plays an important part in the anti-cerebral ischemia damage mentioned above.

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三七总皂甙对突触体钙摄取功能的影响

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R285.5

关键词 人参；皂甙类；突触体；钙放射性 三七同位素；尼莫地平

目的：研究三七总皂甙(PNS)的钙拮抗作用.
方法：大脑皮层突触体通过 Ficoll 密度梯度离心获取. 用同位素测定的方法，观察体内或体外给药时，PNS对突触体钙摄取功能的影响.
结果：在 50-800 mg·L^{-1}浓度范围内，PNS对钙摄取的抑制作用呈现量-效关系，IC_{50}=111 (46-176) mg·L^{-1}，并且对摄取过程的起始和最大反应相均有作用. PNS ip 200 mg·kg^{-1}具有类似反应. 介质中的钙离子浓度影响 PNS 的上述作用.
结论：PNS是神经元钙通道的阻滞剂.