345－348 16

人参二醇组皂甙对烧伤大鼠心功能的保护作用

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目的：研究人参二醇组皂甙(PDS)对烧伤大鼠心功能的影响及其机制. **方法**：测定离体心功能，丙二醛(MDA)含量，超氧化物歧化酶(SOD)活力. **结果**：烧伤后，心率(HR)，心输出量(CO)，左室内压(LVP)及其最大变化率($\pm dp/dt_{max}$)，SOD 活力分别由206 bpm，92 mL min^{-1} g^{-1}，7 kPa，149 kPa s^{-1}，73 kPa s^{-1}，2.9 NU/mg 蛋白分别降低至 162，72，4，77，44，1.7，MDA 含量由0.77 nmol/mg 蛋白升至 1.35. PDS 30 mg kg^{-1} ip 使烧伤后降低或升高的上述指标恢复或接近正常.
结论：PDS 对烧伤心功能保护作用的机制与 MDA 含量降低以及 SOD 活性增高有关.

关键词 人参；皂苷类；烧伤；心脏；丙二醛；超氧化物歧化酶

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3,4-Diaminopyridine facilitates norepinephrine release in chick sympathetic neurons[1]

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AIM: To study the mechanism by which 3,4-diaminopyridine (DAP) facilitates electrically evoked [^{3}H]norepinephrine ([^{3}H]NE) release in sympathetic neurons from chick embryos. **METHODS**: The neurons were incubated with [^{3}H]NE or Fura-2. [^{3}H]NE release or $[Ca^{2+}]_i$ was determined. **RESULTS**: The electrically evoked [^{3}H]NE release and the elevation of $[Ca^{2+}]_i$ were inhibited completely by sodium channel blocker tetrodotoxin (TTX), strongly by the antagonist of N-type calcium channel ω-conotoxin GVIA (CTX), and slightly by the antagonist of L-type calcium channel (－)isradipine, but enhanced by the agonist of L-type calcium channel Bay k 8644. In the presence of DAP, the electrically evoked [^{3}H]NE release and $[Ca^{2+}]_i$ were facilitated, the inhibition of [^{3}H]NE release and $[Ca^{2+}]_i$ by CTX was attenuated, but that by (－)isradipine was enhanced, and Bay k 8644 was no longer effective. **CONCLUSION**: In the cultured chick sympathetic neurons DAP facilitates electrically evoked [^{3}H]NE release mediated by enhancement of influx of external Ca^{2+} through the L-type Ca^{2+} channel.

KEY WORDS sympathetic neuron; norepinephrine; 3, 4-diaminopyridine; calcium channel; ω-conotoxins; tetrodotoxin

The discovery that calcium is necessary for the release of neurotransmitter led to the calcium hypothesis of neurotransmitter release[1] in which release is initiated after an action potential by an increase in intracellular calcium concentration near the release sites.

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Through which calcium channels the extracellular calcium enters into nerve terminals? 3,4-diaminopyridine (DAP) evoked [^{3}H]norepinephrine ([^{3}H]NE) release from rat hippocampal slices and the influx of extracellular Ca^{2+} was mostly through the N- type calcium channel, but not the L-type calcium channel, in the process of DAP-induced [^{3}H]NE release [2]. In the present investigation we determined electric stimulation - evoked [^{3}H] NE release and intracellular calcium concentration ($[Ca^{2-}]_i$) in chick sympathetic neurons to compare the effects of N- and L-type calcium channels on the facilitation by DAP of electrically evoked [^{3}H]NE release.

MATERIALS AND METHODS

Chemicals 1-(7,8-^{3}H)norepinephrine ([^{3}H]NE, Amersham); DAP, tetrodotoxin (TTX), Fura 2-acetoxymethylester (Fura-2) (Sigma), ω-conotoxine GVIA (CTX, Peninsula, England); (−)isradipine (Isr), Bay k 8644 (Bayer), Rotiszint, Soluene-350, Ultima-Gold (Packard). TTX was dissolved in sodium citrate buffer, pH 4.85, Isr, Bay k 8644, and Fura-2 were dissolved in Me_2SO.

Cell culture The paravertebral sympathetic ganglia of 14-d-old chick embryo was collected in phosphate-buffer saline (NaCl 138, KH_2PO_4 1.5, Na_2HPO_4 3.9 mmol L^{-1}) and digested in Dulbecco's Modified Eagle Medium (DMEM) (DMEM 10, glucose 1.2 g L^{-1}, insulin 10, gentamycin 20, streptomycin 100, penicillin 100 mg L^{-1}), with trypsin (0.1 %), 36 C, 30 min. The neurons were planted on polystyrol discs (5 mm, covered by collagen) and kept in culture in DMEM with nerve growth factor, 36 C, 5 % CO_2 for 4 d.

[^{3}H] NE release Neurons preincubated with [^{3}H]NE 0.05 μmol L^{-1} (1.6 PBq mol^{-1}), 36 C, 60 min in a 5 % CO_2 incubator were transferred to small chambers between platinum electrodes (5 mm apart) and superfused with medium. Samples were collected every 4 min at 60 min after onset of superfusion. After 96 min the neurons were stimulated electrically (36 rectangular pulses, 3 Hz, 0.5 ms, 40 V/cm, 70 mA) to induce [^{3}H]NE release. Drugs tested were added to the superfusion medium from 12 min before stimulation onwards. Medium composition: NaCl 133, KCl 4.8, HEPES 10.0, NaH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2 \cdot 2H_2O$ 1.3, glucose 11 mmol L^{-1}, pH 7.4. Oxaprotiline 1 μmol L^{-1} was present throughout the superfusion to block the reuptake system of NE. At the end of superfusion the neurons were dissolved in Soluene-350 and added Rotoszint and the samples were added Ultima-Gold before determination of tritium content [14].

Measurement of $[Ca^{2+}]_i$ $[Ca^{2+}]_i$ concentration was determined [15] using a Ca^{2-} indicator Fura-2. The neurons were incubated with Fura-2 3 μmol L^{-1}, 36 C, 30 min, and then transferred to a closed chamber for superfusion with medium composed as same as that in the experiment of [^{3}H]NE release. Fluorescence measurements were made in a Ca^{2+} analyzer. λ_{ex} 340 and 380 nm, and λ_{em} 510 nm.

Calculation and statistics Stimulation-evoked outflow of ^{3}H was calculated by subtraction of the basal outflow from the total outflow of ^{3}H obtained from 4 samples after onset of stimulation. The basal outflow of ^{3}H was assumed to decline linearly from the sample before, to the 5th sample after the onset of stimulation. The evoked-^{3}H outflow was expressed as % of the ^{3}H of the neurons at the onset of the stimulation. $[Ca^{2+}]_i$ values were calculated from Fura-2 fluoresence ratios of 340 and 380 nm [15]. All results are shown as means ± *s*. The significance of differences between the groups were determined by Student's *t* test [17].

RESULTS

TTX, CTX, (−)isradipine, and Bay k 8644 on electrically evoked [^{3}H]NE release and $[Ca^{2+}]_i$ concentration TTX 0.3 μmol L^{-1} added to the superfusion medium 12 min before stimulation almost completely abolished the electrically evoked [^{3}H]NE release from 2.36±0.15 % to 0.13±0.09 % (inhibition of 95 %). $[Ca^{2+}]_i$ was also reduced from 130±10 nmol L^{-1} to 55±2 nmol L^{-1}. CTX 0.1 μmol L^{-1} added to the superfusion medium 12 min before stimulation inhibited [^{3}H]NE release to 0.43 %±0.09 % (inhibi-

tion of 82 %) and $[Ca^{2+}]_i$ to 70±3 nmol L^{-1}. [^{3}H]NE release was attenuated by Isr 1 μmol L^{-1} to 1.95±0.18 % (inhibition of 17 %). Bay k 8644 (Bay k) 1 μmol L^{-1} enhanced [^{3}H]NE release to 4.65 %±0.41 % and $[Ca^{2+}]_i$ to 375±15 nmol L^{-1} (Tab 1).

Tab 1. Effects of TTX, CTX, Isr, and Bay k 8644 on evoked [^{3}H]NE release and $[Ca^{2+}]_i$. $n=6-20$ discs, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$.

Drug/ μmol L^{-1}		Evoked out flow of ^{3}H/ % of tissue ^{3}H	n	$[Ca^{2+}]_i$/ nmol L^{-1}	n
—		2.36±0.15	20	130±10	6
TTX	0.3	0.13±0.09^c	10	55±2^c	6
CTX	0.1	0.43±0.09^c	10	70±3^c	6
Isr	1	1.90±0.18^c	6	—	
Bay k	1	4.65±0.41^c	6	375±15^c	6

DAP on evoked [^{3}H]NE release and $[Ca^{2+}]_i$ concentration DAP 10, 50, 100, and 200 μmol L^{-1} added to the superfusion medium 12 min before stimulation facilitated electrically evoked [^{3}H]NE release and elevation of $[Ca^{2+}]_i$ in a concentration dependent manner. [^{3}H]NE release was increased by 8 %, 53 %, 104 %, and 117 %, respectively, and $[Ca^{2+}]_i$ was increased by 13 %, 67 %, 167 % and 175 %, respectively (Tab 2).

Tab 2. Effect of DAP on evoked [^{3}H]NE release and $[Ca^{2+}]_i$. $n=6-14$ discs, $\bar{x}\pm s$.

Drug/ μmol L^{-1}		Evoked out flow of ^{3}H/ % of tissue ^{3}H	n	$[Ca^{2+}]_i$/ nmol L^{-1}	n
—		2.40±0.20	14	120±10	14
DAP	10	2.60±0.18	10	135±10	6
	50	3.68±0.25	10	200±12	6
	100	4.90±0.30	6	320±12	6
	200	5.20±0.32	6	330±14	6

In the presence of DAP, TTX, CTX, Isr, and Bay k 8644 on evoked [^{3}H]NE release and $[Ca^{2+}]_i$ concentration The neurons were superfused with medium containing DAP 100 μmol L^{-1}, the electrically evoked [^{3}H]NE release and the elevation of $[Ca^{2+}]_i$ concentration were augmented to 4.50 %±0.32 % and 320±15 nmol L^{-1}, respectively. TTX 0.3 μmol L^{-1} still strongly inhibited the evoked [^{3}H]NE release and $[Ca^{2+}]_i$. CTX 0.1 μmol L^{-1} decreased evoked [^{3}H]NE release to 2.35 %±0.12 % (inhibition of 48%) and $[Ca^{2+}]_i$ to 230±10 nmol L^{-1}. Evoked [^{3}H]NE release was reduced to 2.00 %±0.09 % (inhibition of 56 %) by Isr 1 μmol L^{-1}. Bay k 1 μmol L^{-1} added to the superfusion medium 12 min before stimulation was effective neither on [^{3}H]NE release nor on $[Ca^{2+}]_i$ concentration (Tab 3).

Tab 3. In the presence of DAP, effects of TTX, ω-conotoxin GVIA, Isr and Bay k 8644 on evoked [^{3}H]NE release and $[Ca^{2+}]_i$. $n=6-14$ discs, $\bar{x}\pm s$. $^aP>0.05$, $^cP<0.01$.

Drug/ μmol L^{-1}		Evoked out flow of ^{3}H/ % of tissue ^{3}H	n	$[Ca^{2+}]_i$/ nmol L^{-1}	n
—		4.50±0.32	14	320±15	14
TTX	0.3	0.11±0.09^c	10	58±3^c	6
CTX	0.1	2.35±0.12^c	10	230±10^c	6
Isr	1	2.00±0.09^c	6	—	
Bay k	1	4.54±0.40^a	6	325±14^a	6

DISCUSSION

The experimental results with TTX, CTX, Isr, and Bay k indicate that following depolarization of the membrane of neuron processes, the influx of extracellular Ca^{2+} was mostly through the N-type calcium channel during the electrically evoked [^{3}H] NE release.

We have previously reported[9] that DAP evoked [^{3}H]NE release from rat hippocampal slices and in the NE release process also N-type calcium channels, but not L-type calcium channels, played an important role. In the present study, it was observed that DAP facil-

itated electrically evoked [^{3}H]NE release and [Ca^{2+}]$_i$ concentration. Under the conditions, the inhibition of electrically evoked [^{3}H]NE release and [Ca^{2+}]$_i$ concentration by CTX was relatively reduced, and that by Isp was enhanced. These results suggest that in the presence of DAP the component of L-type calcium channels in the process of evoked [^{3}H]NE release was increased. This suggestion is strongly supported by the experiments in which the agonist of L-type Ca^{2+} channels, Bay k, became ineffective on evoked [^{3}H]NE release and [Ca^{2+}]$_i$ concentration in neurons superfused with medium containing DAP.

All the parallel changes in the electrically evoked [^{3}H]NE release and [Ca^{2+}]$_i$ concentration led to the conclusion that L-type calcium channels are involved in the mechanism of the facilitatory effect of DAP on the electric stimulation-evoked [^{3}H]NE release.

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348-351

3,4-二氨基吡啶易化鸡胚交感神经元去甲肾上腺素释放[1]

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目的：用鸡胚交感神经元研究3,4-二氨基吡啶（DAP）易化电刺激诱发[^{3}H]NE 释放的机制。

方法：用[^{3}H]NE 或 fura-2孵育神经元，测[^{3}H]NE 释放或[Ca^{2+}]$_i$。

结果：电刺激诱发[^{3}H]NE 释放和 [Ca^{2+}]$_i$升高被 ω-conotoxine GVIA（CTX）抑制，被（－）isradipine（Isp）减弱，被 Bay k 8644加强。当3,4-二氨基吡啶（DAP）存在时，电刺激诱发[^{3}H]NE 释放被易化，这时 CTX 的作用减弱，Isp 的作用增强，Bay k 8644不再显示作用。

结论：DAP 对电刺激诱发[^{3}H]NE 释放的易化作用，可能是通过 L-型 Ca^{2+}通道而实现的。

关键词 交感神经元；去甲肾上腺素；3,4-二氨基吡啶；钙通道；ω-水蜗牛毒素；河豚毒素