Effects of ethanol extract of "Rokujo," pilose antler, on uptake and release of noradrenaline in cerebral cortical slices of rats

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Abstract

The effects of ethanol extract of pilose antler, "Rokujo," (EEPA) on the active uptake and release of L-[^{3}H]noradrenaline (NA) were studied in rat cerebral cortical slices. EEPA (0.1－1 mg/ml) significantly inhibited the L-[^{3}H]NA uptake and caused L-[^{3}H]NA release from the slices in a concentration-dependent manner. The release was also observed in the Ca^{2+}-free medium, although 20 mM KCl-evoked release was abolished in the medium of Ca^{2+}-deficiency. It is suggested that pilose antler increases NA level at synaptic sites by inhibiting the uptake and by causing release in NA nerve terminals of the brain.

Key words pilose antler, noradrenaline, uptake, release, brain slices.

Abbreviations EEPA, ethanol extract of pilose antler ; NA, noradrenaline ; Rokujo, 鹿茸.

Introduction

Pilose antler, generally termed "Rokujo," is one of Chinese traditional medicines which has long been used in the clinic of Chinese medicine as drugs for treatment for neurosis, enriching the vital energy, nursing the blood, strengthening the kidney and prolonging life.[1,2] A recent study from our laboratory showed anti-aging action of pilose antler in senescence-accelerated mice.[3] It has never been known, however, whether pilose antler affects function in the central nervous system and if so how. To know the effects of pilose antler on noradrenergic function in the brain, effects of ethanol extract of pilose antler (EEPA) on the uptake and release of noradrenaline (NA) were investigated in cerebral were cortical slices of rats.

Materials and Methods

Male Wistar adult rats (about 2 months old) were used. The animals were maintained at 23℃ under a 12 hr light/12 hr dark cycle.

Pilose antler is non-ossified deer horn of *Cervus nippon* TEMMINCK, which were purchased from Jilin (吉林) province of China. Water extract of pilose antler was prepared by extraction with boiled water from minced powder of deer horn and subsequent evaporation as a dry powder. Further extract from water extract

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with 30% ethanol was used as an ethanol extract (EEPA). EEPA were light yellow powder and easily dissolved in Krebs-Ringer solution. The Krebs-Ringer solution of EEPA was used in the experiments.

Levo-[ring-2,5,6-^{3}H]noradrenaline (48.4 Ci/mmol, New England Nuclear), and glycoletherdiaminetetraacetic acid (EGTA, Dohjin Lab.) were used.

Preparation of cerebral cortical slices and L-[^3H]*NA uptake* : Animals were decapitated, the whole brain was removed, and the cerebral cortices were dissected on ice. Slices (300×300×300 μm, 20 mg wet weight) prepared by the methods previously described[4] were suspended in 2 ml Krebs-Ringer bicarbonate buffer (pH 7.2, 126.5 mM NaCl, 2.4 mM KCl, 0.83 mM $MgCl_2$, 1.1 mM $CaCl_2$, 0.5 mM Na_2SO_4, 2.75 mM $NaHCO_3$, 0.5 mM KH_2PO_4 and 5.9 mM glucose) bubbled with 95% O_2-5% CO_2. Slices were then incubated in the Krebs-Ringer buffer containing 30 mM KCl replaced by equimolar concentration of NaCl at 37℃ for 10 min. The slices were washed with Krebs-Ringer solution. These slices were preincubated with EEPA the final concentration of which was 0, 0.1, 0.5, and 1.0 mg/ml in normal Krebs buffer containing 10 μM nialamide at 37℃ for 10 min. At the end of the incubation period, [^{3}H]NA (final conc. 20 nM) was added into the slices and the slices were further incubated at the same temperature for 15 min. After the incubation, the slices were washed by normal Krebs and were dissolved in 0.5 ml of 1 N NaOH for 1 hr and then neutralized by 0.5 ml of 1 N HCl. The radioactivity in the dissolved slices was estimated in 8 ml of scintillator containing toluene and Triton X-100 (2/1, v/v).

Release of L-[^3H]*NA from cerebral cortical slices* : Experiments of L-[^{3}H]NA release from slices loaded with the radioactive amine were carried out by the superfusion methods previously described.[5] The test drugs were perfused for 10 min. The release evoked by each drug (enhanced release) was determined by substracting the estimated amount of spontaneous release from the amount of total radioactive amine release during the perfusion of drug (Fig. 1). The amount of the total uptake of L-[^{3}H]NA referred to the sum of the total release and the residual on the filter.

The statistical significance of differences between control and test values was analyzed by Student's t-test.

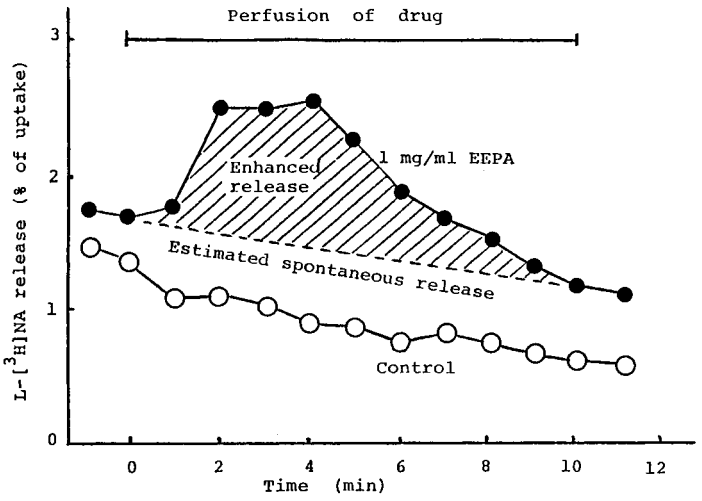

Fig. 1 A typical pattern of L-[^{3}H]noradrenaline release in the experiment of superfusion method.
Enhanced release of L-[^{3}H]noradrenaline by EEPA was determined by subtraction of the estimated spontaneous release from the amount of total release during the perfusion of EEPA.

Results

Effects of EEPA on L-[^3H]NA uptate

As shown in Table I, treatment with EEPA at 0.1−1 mg/ml resulted in a significant ($p<0.001$) inhibition of L-[^{3}H]NA uptake compared to the control.

Effects of EEPA on L-[^3H]NA release

EEPA at 0.1−1 mg/ml significantly ($p<0.01$) increased L-[^{3}H]NA release compared to spontaneous release (Table II). A high K^+ (20 mM KCl) also caused a significant enhancement in L-[^{3}H]NA release. EEPA at 1 mg/ml tended

Table I Effect of EEPA on L-[^{3}H]NA uptake into brain slice.

Concentration of EEPA (mg/ml)	[^{3}H]NA uptake	
	pmol/mg protein	% of control
Control	16.8±0.48 (6)	−
0.1	12.6±0.26*** (6)	75.0
0.5	8.0±0.86*** (5)	47.6
1.0	5.6±0.26*** (6)	33.3

Each value represents the mean±S.E.M. The number of experiments is shown in parentheses. Significance, ***$p<0.001$ vs. control.

Table II Effects of EEPA on L-[^{3}H]NA release from cerebral cortical slices of rats.

Perfused drug	L-[^{3}H]NA release (% of total uptake)	Enhanced release (% of total uptake)
0	10.88±1.14 (3)	—
EEPA 0.1 mg/ml	12.75±0.54 (3)	1.87±0.54 (3)
〃 0.5 〃	15.49±0.59* (3)	4.61±0.59 (3)
〃 1.0 〃	19.43±1.07** (4)	8.55±1.86 (4)
20 mM KCl	28.96±1.72** (5)	18.08±3.45 (5)
20 mM KCl plus EEPA 1.0 mg/ml	33.78±1.58** (5)	22.90±3.16 (5)

The experiment was carried out by the superfusion method as described in "Materials and Methods." Each drug was perfused for 10 min. Each value represents the mean±S.E.M. The number of experiments is shown in parentheses. Significance, *$p<0.05$, **$p<0.01$ vs. spontaneous release.

Table III Effects of Ca^{2+}-deficiency on EEPA-enhanced L-[^{3}H]NA release.

Drug	Enhanced L-[^{3}H]NA release (% of total uptake)		
	Normal	Ca^{2+} free	Ca^{2+} free/2 mM EGTA
EEPA 1 mg/ml	8.55±1.86 (4)	9.57±1.75 (3)	7.37±1.52 (3)
20 mM KCl	18.08±3.45 (5)	1.11±0.42** (3)	0.66±0.54** (3)
20 mM KCl plus EEPA 1 mg/ml	22.90±3.16 (5)	7.86±1.73** (3)	7.95±1.96** (3)

The EEPA-enhanced L-[^{3}H] NA release was examined in the normal-, Ca^{2+} free- and Ca^{2+} free/2.0 mM EGTA-containig Krebs-Ringer solution. Each value represents the mean±S.E.M. The number of experiments is shown in parentheses. Significance, **$p<0.01$ vs. normal.

to enhance the amount of the high K^+-evoked L-[^{3}H]NA release.

Effects of Ca^{2+}-deficiency on EEPA-enhanced L-[^{3}H]NA release

As shown in Table III, neither amount of EEPA-enhanced L-[^{3}H]NA release nor control release was significantly changed in Ca^{2+}-deficient Krebs-Ringer solution, although a high K^+ (20 mM KCl)-enhanced L-[^{3}H]NA release resulted in a significant ($p<0.01$) reduction. In Ca^{2+}-deficient Krebs-Ringer solution, the amount of release evoked by the high K^+ plus EEPA was significantly ($p<0.01$) reduced compared to that in the normal Krebs-Ringer one (Table III).

Discussion

The present results show that EEPA caused reduction in the L-[^{3}H]NA uptake and enhancement of the release. It seems unlikely that EEPA-induced NA release is secondarily induced by the result of inhibition of the uptake, since the present experiments regarding the release were carried out by the superfusion method in which the uptake of free amine occurs with difficultly. EEPA possesses both actions of uptake inhibition and release similar to that of reserpine.[6,7] The EEPA-induced L-[^{3}H]NA release was independent of Ca^{2+} in comparison with Ca^{2+}-dependence of high K^+-evoked L-[^{3}H]NA release. In addition, the effect of EEPA plus high K^+ was significantly reduced in Ca^{2+}-deficient media compared with that in the normal Krebs-Ringer solution, the ammount of NA released by high K^+ plus EEPA was nearly the same as that released by EEPA alone, indicating that Ca^{2+}-deficiency reduced only a fraction of NA release by high K^+.

EEPA seems to exert action on synaptic NA dynamism similar to the action of reserpine as mentioned above. It is presumable that EEPA induces NA release by displacing stored NA like methamphetamine.[8] In addition, cyclic AMP in presynaptic terminals seems to enhance NA release.[9] Therefore, EEPA may cause NA release by increasing cyclic AMP level in terminals. The chemical entity of EEPA underlying uptake inhibition and release of NA, influences of the extract on synaptic dynamism of dopamine and 5-hydroxytryptamine and detail mechanism of its action remain to be clarified in future.

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和文抄録

ラット大脳皮質切片における L-[^{3}H]ノルアドレナリン（NA）取り込みおよび遊離に及ぼす鹿茸エタノール抽出画分の，影響を検討した。本画分は，0.1，0.5および1.0 mg/mlで脳切片への L-[^{3}H]NA取り込みを濃度依存的に，有意に抑制した。また，本画分0.5および1.0 mg/mlは，予め脳切片に負荷した L-[^{3}H]NAを，Ca^{2+} 存在下でも非存在下でも有意に遊離させた。本結果より，鹿茸は，中枢NA神経系シナプスにおいて遊離型NAを増加させNA神経系機能を促進する可能性について考察した。

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