

Effects of ginsenosides on release of [³H]norepinephrine from rat vas deferens and portal vein¹

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ABSTRACT The effects of ginsenosides (G) on the release of [³H]norepinephrine ([³H]NE) from the isolated rat vas deferens (RVD) and portal vein (RPV) preloaded with [³H]NE were studied. G (100 µg/ml) did not affect the spon-

taneous or high potassium ($H-K^+$, 60 mmol/L)- and tyramine (Tyr 10 $\mu\text{mol/L}$)-evoked release of [^3H]NE, but obviously blunted the phentolamine (Phe 10 $\mu\text{mol/L}$)-induced increase in [^3H]NE release from RVD and enhanced the isoprenaline (Iso 0.1 $\mu\text{mol/L}$)-augmented [^3H]NE release from RPV evoked by $H-K^+$. It is still not known whether G can bind with adrenoceptors. We infer that G may act as a modulator in sensitizing both presynaptic α_2 - and β -receptors.

KEY WORDS ginsenosides; adrenergic receptors; neuroregulators; tyramine; phentolamine; isoproterenol; vas deferens; portal vein

Ginsenosides (G) was shown to blunt augmentation of the pressor response to spinal electric stimulation by yohimbine in pithed rats and serve as a presynaptic α_2 -receptor agonist⁽¹⁾. The aim of our work was to investigate the effects of G on transmitter release *in vitro*, and to search for the relationship between this action and adrenoceptors.

Materials and methods

Preparation of tissues Sprague-Dawley σ^7 rats weighing 180–200 g and roughly 10 wk of age were decapitated. Rat vas deferens (RVD) and portal vein (RPV) were incubated in 2.0 ml of Krebs-bicarbonate solution for 30 min at 37°C with 95% O_2 + 5% CO_2 aeration. The composition of the solution was: NaCl 119, KCl 4.7, CaCl_2 2.5, $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ 1.2, NaHCO_3 25, glucose 11, disodium EDTA 0.03, and ascorbic acid 5.7 mmol/L. After a 30-min equilibration period, the tissues were incubated for an additional 30 min with 185 kBq 7,8-[^3H]-*l*-norepinephrine (1.37 TBq/mmol, final concentration 0.14 $\mu\text{mol/L}$) and then washed every 15 min for a 90-min period with Krebs-bicarbonate solution. Cocaine 3.3 $\mu\text{mol/L}$ and hydrocortisone 20 $\mu\text{mol/L}$ were added to the solution to block the neuronal and extra-neuronal uptake of [^3H]NE, respectively.

The method of determining transmitter release described by Ceña was used^(2,3). Briefly, a 5-min sample was collected as spontaneous release of [^3H]NE at the end of the washout period. The tissue was then transferred into 5 ml Krebs solution containing different drugs and incubated for 5 min: the incubation solution was obtained for measurement of [^3H]NE. During the experiments the medium was bubbled continuously with 95% O_2 + 5% CO_2 . When the external K^+ was raised, NaCl should be subtracted from the Krebs solution in order to maintain isotonicity. Tissues were stimulated 2 or 3 times with $H-K^+$ or drugs during the 40–60 min period. There was at least a 10 min interval between 2 stimulations in order to allow the tissues time to recover from the secretory response to the stimuli. G was added to the solution 10 min prior to the next stimulation.

Measurement of [^3H]NE release For liquid scintillation counting, aliquots of 1.0 ml of each sample were added to 7.0 ml of triton-based solution which was a 2:1 (vol/vol) mixture of toluene and triton X-100 containing PPO 5.5 g/L and POPOP 150 mg/L. The radioactivity was counted in a Beckman LS 1801 liquid scintillation counter. Counting efficacy was determined using an internal standard, and all samples were adjusted for the amount of quench by quench curves.

At the end of the experiment, the tissue was placed in a vial containing 0.2 ml formic acid, 0.2 ml H_2O_2 , and a drop of octanol, then dissolved by heating at 70–80°C for 2 h. The radioactivity was then determined in a liquid scintillation counter.

The release of [^3H]NE was expressed as the % fractional release and was represented by the amount of [^3H]NE released into the incubation solution divided by the amount of [^3H]NE present in the

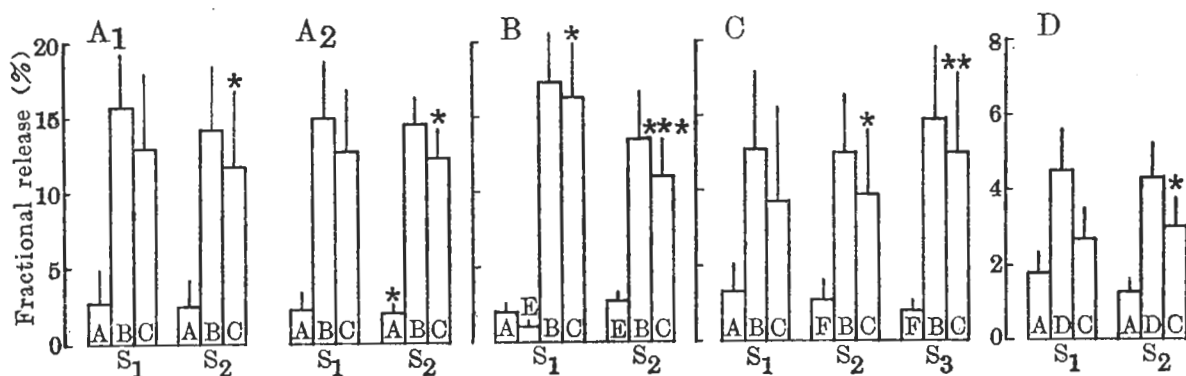


Fig 1. Effects of ginsenosides (G, 100 µg/ml) on the release of [³H]NE from rat vas deferens and portal vein. A₁) No drug was added before and during 2 consecutive stimuli (S₁ and S₂) with high potassium (H-K⁺, 60 mmol/L). **P*>0.05 S₂-C vs S₁-C. A₂) G was added 10 min before and during S₂ with H-K⁺. **P*>0.05 either S₂-A vs S₁-A or S₂-C vs S₁-C. B) G was added 10 min before and during S₂ with phentolamine (10 µmol/L) + H-K⁺. **P*>0.05 S₁-C vs S₁-C in A₂. ****P*<0.01 S₂-C vs S₁-C. C) 3 consecutive stimuli (S₁, S₂, S₃) with H-K⁺ only, H-K⁺ + isoprenaline (Iso, 0.1 µmol/L), and H-K⁺ + Iso + G were shown, respectively. **P*>0.05 S₂-C vs S₁-C. ***P*<0.05 S₃-C vs S₂-C. D) G was added 10 min before and during S₂ with tyramine (10 µmol/L). **P*>0.05 S₂-C vs S₁-C. A₁: spontaneous release; B: H-K⁺; C: net fractional release; D: tyramine-induced release; E: phentolamine; F: isoprenaline. n=4-6 rat. $\bar{x} \pm SD$.

tissue at the time of stimulation, and multiplied by 100.

Net fractional release of [³H]NE was calculated as the difference between the stimulus-induced % fractional release and the % fractional release just prior to the stimulation.

Drugs used 7,8-[³H]-1-norepinephrine (Amersham, UK), tyramine (Ciba-Geigy), and isoprenaline (Beijing Pharmaceutical Factory) were used. Ginsenosides was a gift from Prof. DENG Wen-Long of the Institute of Chinese Material Medica of Sichuan Province.

Statistics Statistical analysis for significance was calculated by the paired *t* test.

Results

Effects of G on spontaneous release and H-K⁺-induced release of [³H]NE RVD preloaded with [³H]NE were exposed to H-K⁺ (60 mmol/L) Krebs solution for 5 min twice (S₁ and S₂) with an interval of 20 min. The spontaneous releases of [³H]

NE before S₁ and S₂ (Fig 1 A₁) were 2.8 ± 2.4 and $2.7 \pm 1.8\%$, respectively. The net fractional releases were $13 \pm 5\%$ for S₁ and $12 \pm 7\%$ for S₂. Fig 1 A₂ is the result of a similar experiment, except G (100 µg/ml) was added 10 min before and during S₂. There were no significant differences between either the spontaneous or net fractional releases of [³H]NE, whether in the presence or absence of G.

Effect of G on the release of [³H]NE from Phe-treated RVD Fig 1 B shows the effects of G on the H-K⁺-evoked release of [³H]NE in RVD which were exposed to Phe 10 µmol/L 10 min before and during the stimuli. S₁ and S₂ represent two consecutive experiments without or with G, respectively. The net fractional releases of [³H]NE were $17 \pm 4\%$ for S₁ and $11 \pm 3\%$ for S₂. Phe showed a tendency to increase the release of [³H]NE evoked by H-K⁺ compared with the data from Fig 1 A₁ (*P*>0.05). G significantly reduced Phe-augmented [³H]NE release (*P*<0.01).

Effect of G on release of [³H]NE from Iso-treated RPV Fig 1 C shows the effects of G on the H-K⁺-evoked release of [³H]NE from RPV which were exposed to Iso 0.1 μmol/L 10 min before and during the stimuli. S₁, S₂, and S₃ represent 3 consecutive stimuli with no drug, Iso only, and Iso plus G, respectively. Iso tended to potentiate the release of [³H]NE evoked by H-K⁺ ($P > 0.05$) and G significantly augmented the facilitatory effect of Iso ($P < 0.05$).

Effect of G on the release of [³H]NE elicited by Tyr RVD preloaded with [³H]NE were exposed to Tyr (10 μmol/L) for 5 min twice (Fig 1 D) with an interval of 20 min. G (100 μg/ml) was added 10 min before and during S₂. G did not affect the increase in release of [³H]NE elicited by Tyr.

Discussion

G (iv) did not effect the pressor response to the exogenous NE, but did significantly attenuate it with respect to spinal electric stimulation⁽¹⁾. Yohimbine, a selective α₂-receptor blocker, augmented the pressor response of spinal electric stimulation while G blunted the augmentation. Thus, G may possess a presynaptic α₂-receptor activation function through which it depresses NE release from sympathetic terminals.

It is well known that the release of NE evoked by H-K⁺ involves a Ca²⁺-dependent process⁽⁴⁾. Tyr-induced release of NE is a Ca²⁺-independent process⁽⁵⁾. Our data indicate that G did not affect H-K⁺- and Tyr-induced release of [³H]NE from the RVD (Fig 1 A₂ and 1 D).

Phe, an α-receptor blocker, by itself does not affect [³H]NE release from RVD and brain synaptosomes^(2,6). However, it antagonizes the inhibitory effect of both

NE and adrenaline. We found that Phe enhanced the H-K⁺-evoked release of [³H]NE and that G significantly inhibited the increased release induced by Phe (Fig 1 A₂ and 1 B). These data imply that G may possess α₂-receptor activation and are consistent with the results reported in (1).

In addition, we found that G significantly enhanced the Iso-induced increased release of [³H]NE. Thus, it seems that G may activate or sensitize presynaptic β-receptors.

It is still unclear as to whether G can specifically bind to adrenoceptors, so we can not say definitely that G is an adrenoceptor agonist. If it is not, we believe that G may act only as a modulator.

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人参皂甙对大鼠输精管和门脉中 $[^3\text{H}]$ 去甲肾上腺素释放的作用¹

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提要 采用 $[^3\text{H}]$ NE 预标记的大鼠输精管(RVD)和门静脉(RPV)研究了人参皂甙(G)对递质释放的影响。结果表明, G(100 $\mu\text{g}/\text{ml}$)对 $[^3\text{H}]$ NE 自发释放、高 K^+ (60 mmol/L)和 Tyr(10 $\mu\text{mol}/\text{L}$)引起的释放均无明显影响, 但明显削弱 Phe(10 $\mu\text{mol}/\text{L}$)对高 K^+ 所致 $[^3\text{H}]$ NE 释放的增强作用。与此相反, G却加强 Iso(0.1 $\mu\text{mol}/\text{L}$)对高 K^+ 所致 $[^3\text{H}]$ NE 释放的促进作用。鉴于G未必能与肾上腺素受体特异地结合, 因此认为G可

能起调谐剂(modulator)样作用, 使突触前膜 α_2 和 β 受体敏化。

关键词 人参皂甙; 肾上腺素受体; 递质; 酪胺; 酚妥拉明; 异丙肾上腺素; 输精管; 门静脉

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