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肼酞嗪对蛋白激酶 A 和蛋白激酶 G 的体外抑制作用

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目的：研究血管扩张药肼酞嗪和 KRN2391对蛋白激酶的直接作用以探讨其作用机制. **方法**：采用层析纯化的蛋白激酶进行体外活性测定. **结果**：肼酞嗪可以抑制 PKA 和 PKG 的活性(0.01—10 mmol·L^{-1})，其 IC_{50}分别为1.2和2.5 mmol·L^{-1}，而对 PKC 作用很小. KRN2391 (1—1000 μmol·L^{-1})对 PKA，PKG 和 PKC 活性均无明显影响. **结论**：肼酞嗪对 PKA 和 PKG 的直接作用可能是其扩张血管的机制之一.

关键词 肼肽嗪；KRN2391；cAMP 依赖性蛋白激酶类；蛋白激酶类；蛋白激酶 C；cGMP 依赖性蛋白激酶类

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Evoked tensions in rabbit aorta by emptying intracellular Ca^{2+} stores with cyclopiazonic acid, thapsigargin, and ryanodine

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AIM: To study the increase of plasma membrane Ca^{2+} permeability in response to depletion of intracellular Ca^{2+} stores. **METHODS**: In Ca^{2+}-free medium, 2 selective inhibitors of sarcoplasmic reticulum (SR) Ca^{2+} pump ATPase, cyclopiazonic acid (CPA) and thapsigargin (Tha), and an activator of Ca^{2+}-induced Ca^{2+} release channel (CICR), ryanodine (Rya), depleted intracellular Ca^{2+} stores sensitive to both caffeine and phenylephrine in rabbit aortic rings and caused sustained tensions when Ca^{2+} reintroduction. These tensons were taken as the increase of plasma Ca^{2+} permeability by depletion of intracellular Ca^{2+} stores. **RESULTS**: The extracellular Ca^{2+}-dependent tensions caused by Tha and Rya 3 μmol·L^{-1} and CPA 30 μmol·L^{-1} were 0.94, 1.1, and 0.14 g, respectively, and the tension caused by Rya was not inhibited by CPA. **CONCLUSION**: (a) Besides the depletion of intracellular Ca^{2+} stores, an activated state of Ca^{2+} release channels in SR may also mediate the activation of Ca^{2+} influx from plasma membrane in rabbit aorta; (b) Rya needs caffeine to fully open CICR channel in SR.

KEY WORDS cyclopiazonic acid; thapsigargin; ryanodine; thoracic aorta; caffeine; phenylephrine

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Sarcoplasmic reticulum (SR) of vascular smooth muscle plays a pivotal role in the maintenance of intracellular Ca^{2+} concentra-

tion $[Ca^{2+}]_i$. Upon various triggers, it induces contractions by releasing Ca^{2+} but acts as a Ca^{2+} buffer when $[Ca^{2+}]_i$ rises[1,2]. In 1986, a "capacitative" coupling model was proposed that the plasmalemmal Ca^{2+} permeability could be mediated by depletion of inositol 1, 4, 5-trisphosphate (ITP)-sensitive intracellular Ca^{2+} store[3]. This model was evident in a variety of non-excitable cells and aortic smooth muscle cells[4,5]. Since no report was seen on the basis of contractile measurements, we were interested in expanding this model to the intact vascular smooth muscle by using 2 structurelly unrelated inhibitors of SR Ca^{2+} pump ATPase, the cyclopiazonic acid (CPA) and thapsigargin (Tha), and an activator of calcium-induced Ca^{2+} release (CICR) channel, the ryanodine (Rya).

In the present study, we conducted caffeine- and phenylephrine (Phe)-induced contractions of rabbit aorta in Ca^{2+}-free medium to detect the filling state of SR and measured tensions induced by the 3 drugs via depletions of the CICR and ITP-induced Ca^{2+} release (IICR) Ca^{2+} stores and sequent reintroduction of Ca^{2+}.

MATERIALS AND METHODS

Japanese white rabbits, ♂, weighing 2.5±*s* 0.6 kg were anesthetized by iv pentobarbital sodium 35 mg·kg^{-1}. Thoracic aorta was excised, cleaned, and cut into rings 2—3 mm in length. After the endothelial layer was removed by rubbing the luminal surface, the rings were suspended in organ baths containing 10 mL physiological saline solution (PSS) at 37 C, gassed with 100 % O_2. The PSS contained: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, glucose 11.5, and HEPES 5 mmol·L^{-1}, pH 7.4. High-K^+ PSS was made by substituting NaCl with an equimolar KCl and Ca^{2+}-free PSS was made by omitting $CaCl_2$ but adding egtazic acid 2 mmol·L^{-1}. Isometric tension was measured with a transducer (T7-30-240 Orientec, Japan), a carrier amplifier (6M81, NEC, Japan), and a potentiometric recorder (Servocorder SR 6211, Japan).

After a 2-h equilibration under a resting tension of 1 g, the muscle was challenged by high-K^+ PSS or Phe 1 μmol·L^{-1} until a steady contractile response to the 2 agonists was obtained. After a 50 min incubation in PSS and 10 min in Ca^{2+}-free PSS, the rings produced a phasic contraction either by caffeine 20 mmol·L^{-1} or Phe 1 μmol·L^{-1} as control. Then, the tissues were incubated with CPA, Rya, or Tha for 10 min, 40 min, and 5 h, respectively in PSS and were challenged with caffeine or Phe in Ca^{2+}-free PSS for 10 min to deplete the CICR or IICR Ca^{2+} store. During the next 50-min PSS incubation in the presence of CPA, Tha, or Rya, the rings produced a sustained tension which was taken as an increase of Ca^{2+} membrane permeability caused by emptying of Ca^{2+} stores for during this 50-min period: (1) The caffeine or Phe had been completely washed out after 25 min; (2) In Ca^{2+}-free PSS incubation for 50 min in the presence of CPA, Tha, or Rya, there was no tension observed; (3) CPA, Tha, and Rya exhibited no direct effect on stimulating the Ca^{2+} entry into cells[4-6]. Then, the PSS was changed to Ca^{2+}-free PSS for 10 min and incubated with caffeine or Phe to assess the filling state of the Ca^{2+} stores and thus referred to inhibition of Ca^{2+} reloading in SR by the 3 drugs (Fig 1). These inhibitory effects were expressed as % of contractile responses to either caffeine or Phe in controlled contractions and total inhibition was presumed to be complete depletion of the store.

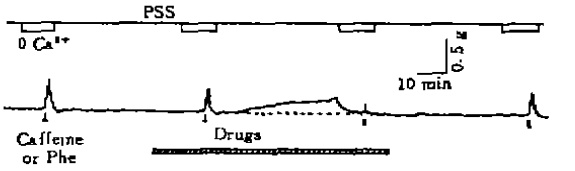

Fig 1. Experimental protocol for caffeine or phenylephrine.

CPA, Tha, and HEPES were purchased from Sigma. All other drugs and agents were bought from Wako Co, Japan. Statistic analysis referred to *t* test.

RESULTS

Tensions induced by CPA, Tha, and Rya

after depletion of Ca^{2+} stores After pretreatment of the rings with CPA, Tha, Rya, and then caffeine or Phe, a sustained tension was seen, but not after vehicles (H_2O and Me_2SO), upon Ca^{2+} reintroduction (Fig 2,3). Rya caused a tension after caffeine, but not after Phe.

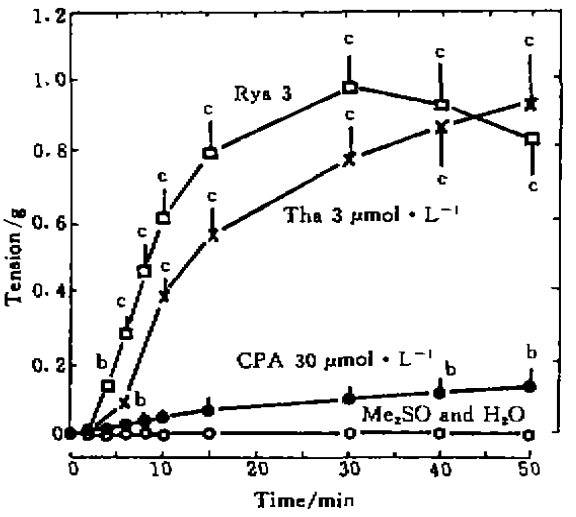

Fig 2. Tensions induced by CPA, Tha, and Rya in rabbit aortic rings after depletion of caffeine-sensitive Ca^{2+} store. $n=6$ rabbits, $\bar{x}\pm s$, $^bP<0.05$, $^cP<0.01$ *vs* Me_2SO and water.

Effects of CPA, Tha, and Rya on reloading of Ca^{2+} to intracellular stores CPA and Tha produced a concentration-dependent inhibition on the phasic contraction induced by caffeine or Phe (Fig 4).

The inhibition was almost complete with CPA 30 μmol·L^{-1} but around 60 % with Tha at the highest concentration, while Rya completely inhibited the caffeine-induced contraction and around 30 % inhibition of Phe-induced contraction (data not shown).

When the tissues were incubated with Rya for 40 min and then exposed to both Phe and caffeine 20 mmol·L^{-1} for 10 min, 1 h later a phasic contraction induced by Phe was completely abolished and an increase of tension after Ca^{2+} reintroduction was seen (Fig 3,4).

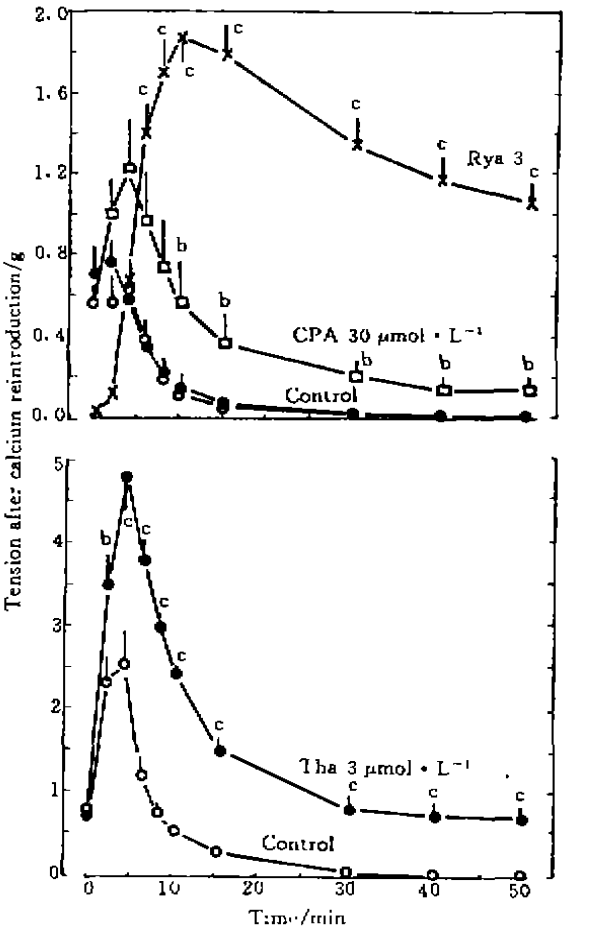

Fig 3. Tensions induced by CPA, Rya, and Tha in rabbit aortic rings after depletion of Phe-sensitive Ca^{2+} store. $n=6$ rabbits, $\bar{x}\pm s$, $^bP<0.05$, $^cP<0.01$ *vs* Me_2SO or water (●).

These results indicated that caffeine was essential to the effect of Rya on CICR. All the 3 drugs were hard to be totally washed out, especially Tha and Rya. When CPA was washed out, the inhibitory effects and the induced tension was no longer noticed.

Effects of CPA and Tha on contractions induced by high-K^+ and Phe in PSS Both the highest concentrations of CPA and Tha inhibited the contraction induced by high-K^+ PSS (Tha also inhibited the contractile response to

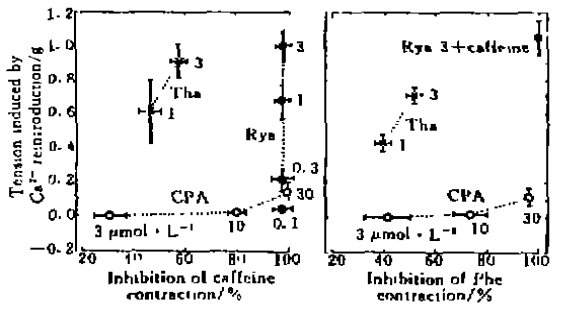

Fig 4. Relationships between inhibitions of contractile responses to caffeine and Phe in Ca^{2+}-free media and tensions produced after Ca^{2+} reintroduction with PSS by CPA, Tha, and Rya.

Phe 1 $\mu mol \cdot L^{-1}$) in PSS medium (Tab 1). The tension caused by Rya upon reintroduction of Ca^{2+} was not influenced by CPA 30 $\mu mol \cdot L^{-1}$ (n= 6 animals).

Tab 1. Inhibitory effects of CPA and Tha on contractions induced by high K^+ and Phe. n=6 animals, $\bar{x} \pm s$. $^bP<0.05$ *vs* Me_2SO.

$\mu mol \cdot L^{-1}$	Contraction/% high K^+	Phe
Me_2SO 30 μl	102.0±2.0	102.0±2.0
CPA 30	88.7±2.2^b	98.9±1.5
Me_2SO 30 μl	117.0±1.0	117.0±1.0
Tha 3	99.0±0.9^b	99.5±1.0^b

DISCUSSION

In this study, we have shown that 2 selective inhibitors of SR Ca^{2+} pump ATPase, CPA and Tha, and a CICR activator Rya produced a sustained tension after depletion of SR Ca^{2+} stores in the intact aortic smooth muscle. With each drug used the tension was extracellular Ca^{2+}-dependent and in a concentration-dependent manner. These observations were in agreement with the findings in a variety of isolated cells[4,5,7,8]. However, considerable differences among the tensions were observed with the 3 drugs. Though Rya and CPA (at the highest concentration) shared the ability of depleting Ca^{2+} stores completely, the tension due to Rya was much greater than that due to CPA. The interesting results were also seen with Tha (Fig 4) and had been reported in other studies[4,7,9,10].

Due to CPA and Tha were showed no inhibitory effect on Ca^{2+} pump ATPase activity of plasmalemma membrane from porcine aorta (to be published), the more $[Ca^{2+}]_i$ induced by Tha could not be different Ca^{2+} pump ATPase activity of cell membrane. Since Rya also produced an almost same tension even in the presence of CPA, and CPA showed no greater inhibitory effect on contraction induced by high-K^+ or Phe in PSS medium than Tha did (Tab 1). The suggestion that CPA may possess some inhibitory effect on Ca^{2+} influx[4] may not be the crucial point to explain this difference. Alternatively, Rya does not appear to affect the contractile apparatus or the sarcolemmal calcium transport mechanisms[6], instead it keeps the CICR channel in an open state and deplets this store. CPA also has no influences on the contractile proteins[11-14].

In sum, we suggested: (a) In addition to depletion of intracellular Ca^{2+} stores, an activated state of Ca^{2+} release channel may also be a messenger mediating the Ca^{2+} influx in rabbit aortic muscle; (b) Rya needs caffeine to fully open the CICR channel in SR.

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280-284

家兔主动脉细胞内钙池耗竭所致的基础张力变化

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目的：研究动脉平滑肌细胞内钙池耗竭所致浆膜钙通透性的增加。 **方法**：在无钙条件下，采用选择性肌浆网(SR)钙泵抑制剂环匹阿尼酸(CPA)和 Thapsigargin (Tha)及 SR 钙释放剂 Ryanodine (Rya)耗竭家兔主动脉环咖啡因和苯肾上腺素敏感的细胞内钙池，而当外钙加入时，以上三药均增大基础张力，此张力变化做为内钙耗竭后所致的浆膜钙通透性增加。 **结果**：3 $\mu mol \cdot L^{-1}$ Tha 和 Rya 及30 $\mu mol \cdot L^{-1}$ CPA 分别增加张力为0.94，1.1和0.14 g，Rya 的张力增加不受 CPA 抑制。 **结论**：(1)提示除细胞内钙耗竭外，SR 钙通道开放也增加浆膜钙通透性；(2) Rya 完全开放钙通道需咖啡因的存在。

关键词 环匹阿尼酸；Thapsigargin；ryanodine；胸主动脉；咖啡因；苯福林