ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jun; 22 (6): 498 - 504
http://www.chinaphar.com

# Evidence against inhibition of sarcoplasmic reticulum $Ca^{2+}$-pump as mechanism of $H_2O_2$-induced contraction of rat aorta

SHEN Jian-Zhong, ZHENG Xiu-Feng[1], WEI Er-Qing, KWAN Chiu-Yin[2] (*Department of Pharmacology, School of Medicine, Zhejiang University, Hangzhou 310031, China*; [2]*Smooth Muscle Research Program and Department of Medicine, Faculty of Health Sciences, McMaster University, Ontario L8N 3Z5, Canada*)

**KEY WORDS** hydrogen peroxide; cyclopiazonic acid; thoracic aorta; smooth muscle; purinergic $P_2$ receptors; suramin; sarcoplasmic reticulum; $Ca^{2+}$-$Mg^{2+}$-ATPase; 2-aminoethoxydiphenyl borate

## ABSTRACT

**AIM**: To test whether inhibition of sarcoplasmic reticulum (SR) $Ca^{2+}$-pump is involved in $H_2O_2$-induced contraction of endothelium-denuded rat aorta. **METHODS**: Isometric tension recording of $H_2O_2$ and cyclopiazonic acid (CPA)-induced contractions of rat aortic rings were compared in the absence or presence of various pharmacological tools to discriminate their signaling pathways involved. **RESULTS**: Both $H_2O_2$ and CPA contracted rat aortic rings, but with different contractile patterns. $H_2O_2$ triggered a fast and phasic contraction, whereas CPA elicited a slow and sustained contraction. In $Ca^{2+}$-free medium, pretreatment of aortic rings with CPA 30 μmol/L but not with $H_2O_2$ 30 μmol/L nearly abolished phenylephrine (10 μmol/L)-induced contraction. In addition, upon the maximal contraction induced by thapsigargin 30 μmol/L, $H_2O_2$ but not CPA further contracted aortic rings. On the other hand, $H_2O_2$ (30 μmol/L)- but not CPA (10 μmol/L)-induced contraction could be inhibited by suramin and RB-2 (each 100 μmol/L), two $P_2$-purinoceptor antagonists. Furthermore, although pretreatment with 2-APB, a membrane permeable $IP_3$ receptor blocker, inhibited both $H_2O_2$- and CPA-induced contractions, only $H_2O_2$ (30 μmol/L)-induced contraction could be depressed, to different degree, by various inhibitors of receptor-coupled or downstream signaling enzymes, including PLC, PKC, $PLA_2$, COX, and protein tyrosine kinases. **CONCLUSION**: Inhibition of smooth muscle SR $Ca^{2+}$-pump is unlikely the mechanism responsible for $H_2O_2$-induced contraction of endothelium-denuded rat aorta.

[1] Correspondence to Prof ZHENG Xiu-Feng.
Phn 86-571-721-7269. Fax 86-571-721-7391.
E-mail shenjz@mail.hz.zj.cn
Received 2000-11-09 Accepted 2001-03-01

## INTRODUCTION

The discovery of NO as a signaling molecule in several biological systems including vasculature renders scientists refocus on the research area of free radicals other than NO. In particular, growing body of evidence now indicate that reactive oxygen species (ROS) including hydrogen peroxide ($H_2O_2$) as well as superoxide anion ($O_2^-$) may also act as signaling molecules in cellular signal transduction[1,2]. In cardiovascular system, the work done by Vanhoutte *et al*[3] is the first to propose ROS may serve as one of the endothelium-derived contracting factors (EDCF), thereby suggesting ROS, like NO, may be also a diffusible signaling molecule involved in vascular tone regulation. Another pioneer work by Finkel *et al*[4], however, showed that $H_2O_2$ was an intracellular signaling molecule required for platelet-derived growth factor (PDGF)-induced proliferation of vascular smooth muscle cells. This hypothesis has been readily confirmed now by many groups on different cell lines. For example, it was demonstrated that activation of many G-protein coupled or uncoupled cell surface receptors results in elevation of intracellular $O_2^-$ and/or $H_2O_2$[5,6]. In addition, the enzyme, called MOX1, which produces the intracellular $O_2^-$ and $H_2O_2$ upon receptor activation, has also been identified in vascular smooth muscle cells[7].

However, despite of these extensive studies, the potential cellular specific sites or molecules, which might be targeted by the generated ROS, are not well defined. Moreover, the actual species of intracellular ROS acting as a signaling molecule is also controversial. From vascular tone regulation view, our previous study had shown that the three different ROS including $H_2O_2$, $O_2^-$, and hydroxyl radical (·OH) all contract rat aorta, but with

different contractile patterns[8]. $H_2O_2$ evokes phasic contraction, whereas $O_2^-$ triggers dual phasic contraction. A sustained contraction is induced by the ·OH generating system ($Fe^{2+}$ plus vitamin C). The mechanism responsible for this difference is unclear. However, our ongoing study had shown that only $H_2O_2$-induced contraction could be specifically inhibited by suramin and RB-2, two widely used $P_2$-purinoceptor antagonists[9,10]. Based on these findings, we proposed that $P_2$-purinoceptor might be one of the targets modified and thereby activated by physiological concentration of $H_2O_2$[9].

At present, however, at least one of the confused factors which interfere our data interpretation is concerning the specificity of suramin and RB-2 on $P_2$-purinoceptors. On the other hand, ROS including $H_2O_2$ have been shown to inhibit sarcoplasmic reticulum (SR) $Ca^{2+}$-pump in coronary smooth muscles[11,12], which may play a role in the regulation of vascular tone, because it is well known that cyclopiazonic acid (CPA) and thapsigargin, two specific inhibitors of SR $Ca^{2+}$-pump, all contract vascular smooth muscles[13]. It is therefore possible that $H_2O_2$-induced contraction of rat aorta may be also the result of SR $Ca^{2+}$-pump inhibition. To address this important question, the present study aims to further compare $H_2O_2$ and CPA-induced contraction of rat aorta and their signaling pathways involved. In this work, we provided functional evidence to suggest that SR $Ca^{2+}$-pump inhibition played a minor (if any) role in $H_2O_2$-induced contraction of rat aorta.

## MATERIALS AND METHODS

**Drugs and chemicals** The following pharmacological tools were purchased from Sigma (St Louis, MO, USA): acetylcholine chloride (ACh), phenylephrine hydrochloride (PE), hydrogen peroxide ($H_2O_2$), egtazic acid, 2-nitro-4-carboxypheny-*N*, *N*-diphenylcarbamate (NCDC), indomethacin, mepacrine, 1-(5-isoquinolinysulfonyl)-2-methylpiper-azine (H7), suramin, reactive blue 2 (Cibacron blue 3GA, RB-2), genistein, cyclopiazonic acid (CPA), and thapsigargin. 2-Aminoethoxydiphenyl borate (2-APB) was from TOCRIS (MO, USA). All chemicals were dissolved in Krebs' solution or distilled water except for CPA, thapsigargin, and 2-APB in $Me_2SO$. The final bath concentration of $Me_2SO$ never exceeded 0.1 %. Indomethacin was first dissolved in 2 % $Na_2CO_3$, then diluted with Krebs' solution.

**Rats and tissue preparations** Male Sprague-Dawley rats (Grade Ⅱ, Zhejiang Medical Laboratory Animal Center, Certificate № 22-9601018, conferred by Animal Management Committee, Chinese Academy of Sciences), weighing 300 – 350 g, were stunned and killed by cervical dislocation. The thoracic aorta was isolated and placed in Krebs's solution at pH 7.4 containing (mmol/L): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $KH_2PO_4$ 1.0, $NaHCO_3$ 25, and glucose 11. Fat and connecting tissue were removed and the aorta was cut into 3 – 4 mm rings. In most rings, the endothelium was intentionally removed by gently rubbing against the teeth of a pair of forceps. The successful removal of endothelium was assessed by showing that ACh at 1 μmol/L failed to relax the rings pre-contracted by PE 1 μmol/L.

**Tissue-bath experiments** The aortic rings were mounted on a 3-mL organ bath, connected to a force transducer and a pen recorder. The organ bath containing Krebs' solution was bubbled continuously with 95 % $O_2$ + 5 % $CO_2$ at 37 ℃. For experiments in which $Ca^{2+}$-free Krebs' solution was used, $Ca^{2+}$ was omitted and egtazic acid 0.1 mmol/L was added. The solution in the bath was changed every 20 min. The rings were equilibration for 20 min before stretching them to approximate 2 g, and were allowed for further equilibration for 90 min. Before data collection, stimulation of the rings with KCl 60 mmol/L was repeated every 20 min until a reproducible contractile response was obtained. Then, the testing chemicals including $H_2O_2$ and CPA were added to the bath at different concentrations. Preliminary experiments showed that the contractile response to $H_2O_2$ was not reproducible when it was added in a cumulative manner, so a non-cumulative concentration-response curve for $H_2O_2$ and a cumulative concentration-response curve for CPA were constructed. Additionally, in most tests, each vessel ring was challenged with a single concentration of $H_2O_2$ or CPA.

In another series of experiments, the possible signaling pathways involved in the contractile response induced by $H_2O_2$ and CPA were analyzed in endothelium-denuded aorta. To test whether $H_2O_2$ and CPA-induced contractile responses were due to activation of putative receptors, endothelium-denuded rings were pretreated with suramin and RB-2, two $P_2$-purinoceptor antagonists[10], or with several inhibitors of receptor-coupled or downstream signaling enzymes including PLC, $PLA_2$, PKC, and protein tyrosine kinase for 20 min before addition of $H_2O_2$ and CPA. To determine whether intracellular $IP_3$ receptor

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jun; 22 (6)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

was involved in $H_2O_2$ and CPA-induced contractions, aortic rings were pretreated with 2-APB, a membrane-permeable $IP_3$ receptor blocker[15], before addition of $H_2O_2$ and CPA.

**Data analysis** All of the data were expressed as $\bar{x} \pm s$, taken from at least 3 rats. Contractile responses induced by $H_2O_2$ and CPA were expressed as percentage of the KCl (60 mmol/L)-induced contraction or as mg of the tension developed. One-way analysis of variance (ANOVA) was performed, followed by $t$-test.

## RESULTS

**Characteristics of $H_2O_2$- and CPA-induced contractions** In endothelium-denuded resting rings, both $H_2O_2$ and CPA concentration-dependently evoked contraction (Fig 1C), but with different patterns. $H_2O_2$ induced phasic contraction, whereas CPA triggered a slow and sustained contraction. In addition, after the first contraction induced by $H_2O_2$ 30 μmol/L, which subsequently washed out for 30 min, a second challenge of $H_2O_2$ failed to, but CPA 10 μmol/L could still evoke contraction of rat aorta (Fig 1A). The same tachyphylaxis phenomenon, however, was not happened for CPA-induced contraction (Fig 1B).

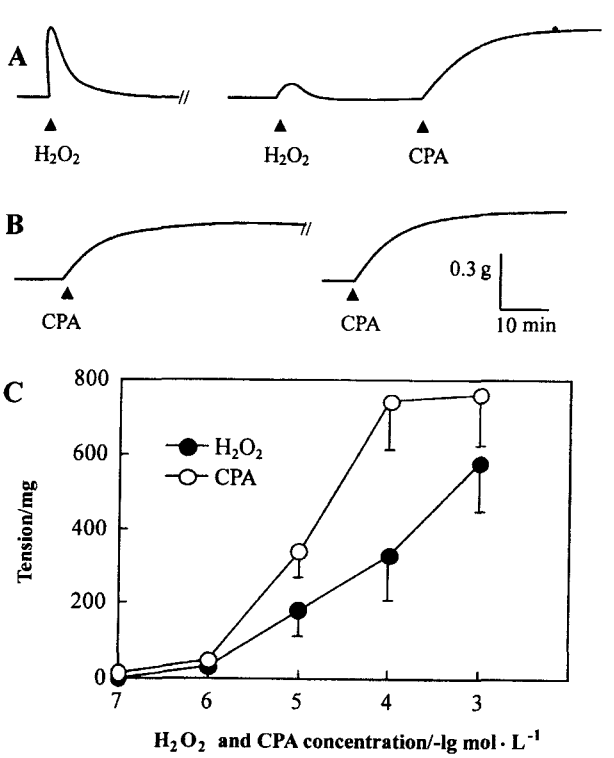


**Fig 1. Contractile responses induced by $H_2O_2$ and CPA in endothelium-denuded rat aortic rings. (A, B) Typical traces showing the phasic contraction evoked by $H_2O_2$ 30 μmol/L and tonic contractions by CPA 10 μmol/L. Test drug applied point is indicated by (▲), and (//) denotes washout for 30 min. Data in each tracing are representative of experiments using 5 individual aortic rings taken from at least 3 rats, and expressed in mg of tension developed. (C) The concentration-response curves were constructed in a cumulative manner for CPA (○) and non-cumulative way for $H_2O_2$ (●). $n = 5-7$. $\bar{x} \pm s$.**

Due to tachyphylaxis for $H_2O_2$-induced contraction, a cumulative concentration-response curve was not employed in the subsequent experiments. We selected a concentration of $H_2O_2$ 30 μmol/L and CPA 10 μmol/L which close to their $EC_{50}$ for further investigation.

**Effects of CPA and $H_2O_2$ on PE-induced contraction in $Ca^{2+}$-free medium** When rat aortic rings denuded endothelium were pretreated with $Ca^{2+}$-free Krebs' solutions for 10 min, PE 10 μmol/L evoked a phasic contraction (Fig 2A). This is consistent with the notion that in the absence of extracellular $Ca^{2+}$, receptor agonists, like PE, contract vascular smooth muscle via mobilization of intracellular $Ca^{2+}$. To deplete SR-stored $Ca^{2+}$, rat aortic rings were co-incubated with $Ca^{2+}$-free Krebs' solutions and a specific SR $Ca^{2+}$-pump inhibitor of CPA 30 μmol/L. This protocol nearly abolished PE (10 μmol/L)-induced contraction (Fig 2B and 2D). However, co-incubation with $Ca^{2+}$-free Krebs' solutions and $H_2O_2$ 30 μmol/L was no effect on PE (10 μmol/L)-induced contraction (Fig 2C and 2D).

**Effects of CPA and $H_2O_2$ on thapsigargin-induced maximal contractions** To achieve full inhibition of SR $Ca^{2+}$-pump in smooth muscles, rat aortic rings without endothelium were incubated with thapsigargin 30 μmol/L, another highly specific inhibitor of SR $Ca^{2+}$-pump[14]. Upon the maximal steady-state contraction triggered by thapsigargin, addition of $H_2O_2$ 30 μmol/L but not CPA 10 μmol/L produced further contraction of aortic rings (Fig 3A, B, and C).

**Effects of $P_2$-purinoceptor antagonists on $H_2O_2$- and CPA-induced contractions** Suramin and RB-2 (each 100 μmol/L), two widely used $P_2$-purinoceptor antagonists[10], all inhibited $H_2O_2$ (30 μmol/L)-induced contraction in endothelium-denuded preparations by more than 90 % (Fig 4A), in supporting our previous proposal that $H_2O_2$ might target $P_2$-purinoceptors[9]. The same concentration of suramin and RB-2, however, did not affect CPA (10 μmol/L)-induced contraction (Fig 4B).

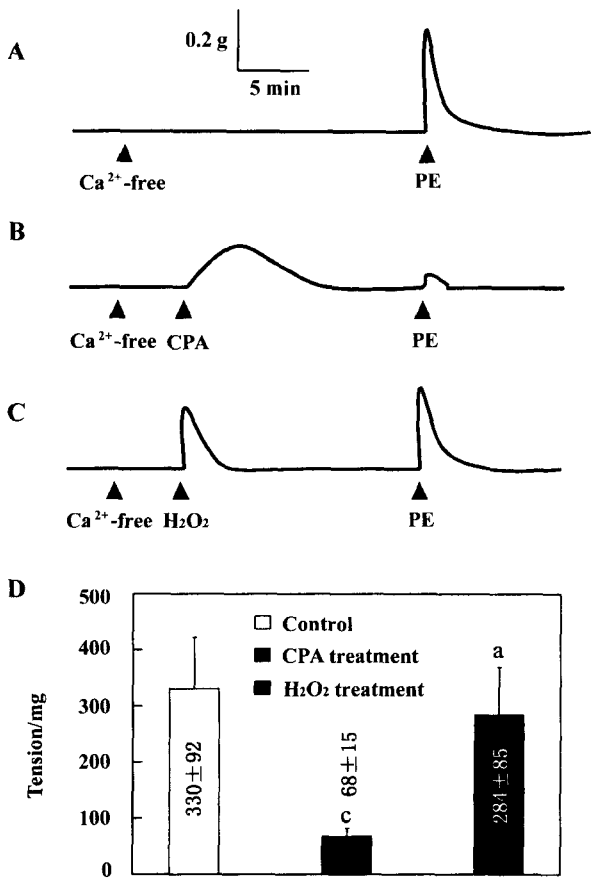


**Fig 2. Effects of CPA 10 μmol/L and $H_2O_2$ 30 μmol/L on PE (10 μmol/L)-induced contraction of rat aorta in the absence of endothelium and extracellular $Ca^{2+}$. (A, B, and C) Typical traces showing the phasic contraction of PE 10 μmol/L in $Ca^{2+}$-free medium, and the influence of CPA 10 μmol/L or $H_2O_2$ 30 μmol/L on PE (10 μmol/L)-induced contractions. (▲) denotes addition of drugs or treatment with $Ca^{2+}$-free Krebs' solution (containing egtazic acid 100 μmol/L). (D) Data summary. $n=6$. $\bar{x} \pm s$. $^aP>0.05$, $^cP<0.01$ *vs* control.**

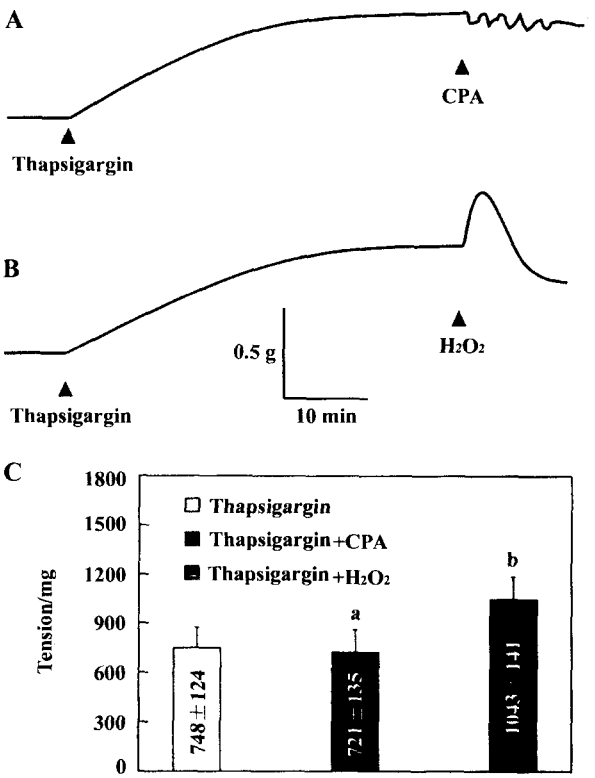


**Fig 3. Effects of $H_2O_2$ 30 μmol/L and CPA 10 μmol/L in the presence of maximal response to thapsigargin 30 μmol/L. (A, B) Typical traces showing $H_2O_2$ 30 μmol/L but not CPA 10 μmol/L evoked further contraction on top of the maximal response to thapsigargin. (▲) denotes addition of drugs. (C) Data summary. $n=6$. $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$ *vs* thapsigargin group.**

**Roles of receptor-coupled enzymes and $IP_3$ receptors in $H_2O_2$- and CPA-induced contractions**

In normal Krebs' solutions, $H_2O_2$ (30 μmol/L)-induced contraction was significantly depressed by NCDC 10 μmol/L, a PLC inhibitor; by H7 10 μmol/L, a PKC inhibitor; by genistein 30 μmol/L, an inhibitor of protein tyrosine kinases; or by mepacrine 50 μmol/L and indomethacin 3 μmol/L, inhibitor of $PLA_2$ and COX respectively (Fig 5A). However, pretreatment with the same concentration of NCDC, H7, genistein, mepacrine, and indomethacin had no effect on CPA (10 μmol/L)-induced contraction (Fig 5B). Interestingly, preincubation with 2-APB 50 μmol/L, a membrane-permeable $IP_3$ receptor blocker[15], not only inhibited $H_2O_2$ (30 μmol/L)- but also CPA (10 μmol/L)-induced contractions (Fig 5A and 5B), with no influence on KCl (30 μmol/L)-induced contraction (data not shown).

## DISCUSSION

This is the first study that systematically compared $H_2O_2$- and CPA-induced contraction of rat aorta in the absence of endothelium. We found that although both $H_2O_2$ and CPA all concentration-dependently contracted rat aorta, their contractile patterns were quite different. $H_2O_2$ evoked a fast phasic contraction, whereas CPA triggered a slow and tonic contraction. Furthermore, a tachyphylaxis phenomenon was observed for $H_2O_2$ but not for CPA-induced contractions. These results would give the first evidence not supporting the proposal that inhibition of SR $Ca^{2+}$-pump by $H_2O_2$ as reported by Grover *et al*[11,12] could be the mechanism of $H_2O_2$-induced

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jun; 22 (6)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

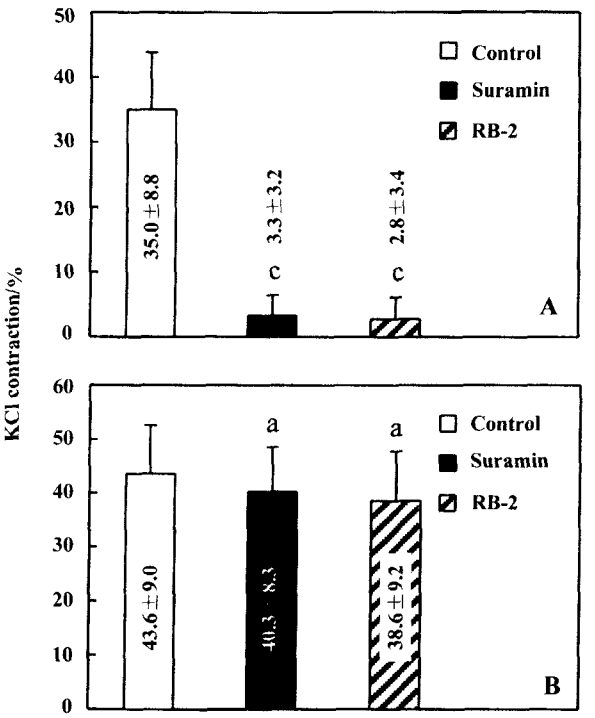


**Fig 4. Effect of suramin and RB-2 on the contraction induced by 30 μmol/L of $H_2O_2$ (A) or 10 μmol/L of CPA (B) in endothelium-denuded rat aortic rings. Each preparation was challenged with $H_2O_2$ or CPA only once after pre-incubation with either vehicle (control) or suramin and RB-2 (each 100 μmol/L) for 20 min. $n$ = 6-9. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control.**

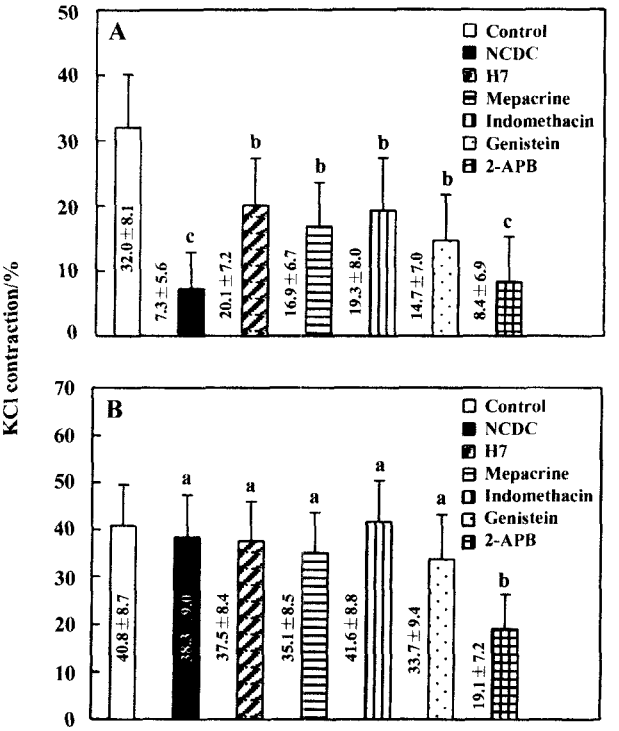


**Fig 5. Effects of NCDC 10 μmol/L, H7 10 μmol/L, mepacrine 50 μmol/L, indomethacin 3 μmol/L, genistein 30 μmol/L, and 2-APB 50 μmol/L on the contractions induced by 30 μmol/L $H_2O_2$ (A) or 10 μmol/L CPA (B) in endothelium-denuded aortic rings. Each preparation was challenged with $H_2O_2$ or CPA only once after pre-incubation with either vehicle (control) or the various inhibitors for 20 min. $n$ = 6 - 9. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.**

contraction of rat aorta. However, it may be argued that the fast onset and termination of $H_2O_2$-induced contraction as showed here may be due to its fast membrane diffusion and intracellular metabolism in vascular smooth muscle cells. This seems to be the reason of the different contractile patterns exerted by $H_2O_2$ and CPA, since CPA, a chemical of large molecule, may need more time than $H_2O_2$ to diffuse and target the SR $Ca^{2+}$-pump. However, at least two lines of evidence do not support this reasoning. First, it was demonstrated that a successful detection of intracellular $H_2O_2$ in rat aortic smooth muscle cells requires the exogenous added $H_2O_2$ at least 100 μmol/L[4], indicating that if $H_2O_2$-induced contraction is truly due to its direct inhibition of intracellular SR $Ca^{2+}$-pump, then a threshold concentration of 100 μmol/L must be achieved for $H_2O_2$-induced contraction. This is contrasted with our observation that as low as 3 μmol/L $H_2O_2$ can elicit a detectable threshold contraction in rat aorta with an $EC_{50}$ of 30 μmol/L. Second, if the fast termination of $H_2O_2$-induced contraction is the result of scavenging by intracellular catalase and/or other enzymes, then a second challenge of $H_2O_2$ will be expected to re-contract aortic smooth muscles. The fact that $H_2O_2$ failed to contract aorta in the presence or absence of the first-added $H_2O_2$ apparently does not support this postulation. On the other hand, it may be also argued that the functional tachyphylaxis exerted by $H_2O_2$ might be the result of damage of vascular smooth muscles due to $H_2O_2$ challenge. Indeed, our previous result that a brief pretreatment (15 min) of aortic rings with $H_2O_2$ 30 μmol/L did not impair caffeine- and KCl-induced contractions[9], combined with the present observation that $H_2O_2$ treatment did not affect CPA-induced contraction would also argue against a non-selective impairment of vascular smooth muscles and their SR $Ca^{2+}$-pumps.

It is well established that both intracellular $Ca^{2+}$ release and extracellular $Ca^{2+}$ influx all participate in PE-induced contraction of vascular smooth muscles. Our finding that PE only evoked a phasic contraction in $Ca^{2+}$-

free medium is consistent with this notion. In addition, the fact that CPA pretreatment in $Ca^{2+}$-free medium nearly abolished PE-induced phasic contraction, well supports the previous view that CPA could deplete $IP_3$-related $Ca^{2+}$ stores through specific inhibition of SR $Ca^{2+}$-pump[13]. Under the same experimental conditions, $H_2O_2$ exposure failed to affect subsequent PE-induced contraction, suggesting no involvement of SR $Ca^{2+}$-pump inhibition exerted by $H_2O_2$, at least at the testing concentration of 30 μmol/L. Such a view is further supported by our another important finding that $H_2O_2$ but not CPA could further contract rat aortic rings after the maximal contraction evoked upon the full inhibition of SR $Ca^{2+}$-pump by thapsigargin.

Recently, we proposed a model that $H_2O_2$ might activate $P_2$-purinoceptors, thereby eliciting the vasoconstrictile response[8,9]. The present data that suramin and RB-2, two widely used $P_2$-purinoceptor antagonists, all nearly abolished $H_2O_2$-induced contraction of rat aorta are consistent with this hypothesis. Again, the failure of suramin and RB-2 on CPA-induced contraction lends additional support to our contention that $H_2O_2$ and CPA may use different signaling pathways in mediating their contractile responses in rat aorta. This view is further supported by the finding that only $H_2O_2$- but not CPA-induced contraction could be attenuated, to different degree, by various inhibitors of receptor-coupled or downstream signaling enzymes, including NCDC (PLC), H7 (PKC), genistein (protein tyrosine kinases), mepacrine ($PLA_2$), and indomethacin (COX).

The unexpected and interesting finding of the present study is that 2-APB, a membrane-permeable $IP_3$ receptor blocker[15], not only inhibited $H_2O_2$- but also CPA-induced contractions. This result is inconsistent with the data that NCDC, a PLC inhibitor, failed to influence CPA-induced contraction, thereby indicating no involvement of $IP_3$. The nature of this discrepancy is unclear. However, a recent study has shown that $IP_3$ receptor itself rather than $IP_3$ molecule is required for CPA-evoked extracellular $Ca^{2+}$ influx[15]. On the other hand, the overall finding of the present study is contrasted with that reported by Grover *et al*, who showed ROS including $H_2O_2$ inhibited SR $Ca^{2+}$-pump in coronary smooth muscles[11,12]. The reasons for this apparent contradiction are unknown. However, it should be noted that Grover *et al* challenged coronary arteries with ROS generated from xanthine oxidase/xanthine system which indeed produced not only $H_2O_2$ but also $O_2^-$ and ·OH. An early study in isolated cardiac and skeletal muscle SR vesicles had demonstrated that it was the ·OH rather than $H_2O_2$ itself that competitively inhibited the activity of SR $Ca^{2+}$-pump[16]. And our previous study had excluded the involvement of $O_2^-$ and ·OH in $H_2O_2$-induced contraction of rat aorta[8]. Nevertheless, despite all of these arguments, we still could not fully rule out the possibility that SR $Ca^{2+}$-pump in vascular smooth muscles might be somewhat impaired if the challenge time and concentration of $H_2O_2$ were raised to 45 min and 1 mmol/L respectively as employed by one study[12].

In summary, we have provided functional evidence against the involvement of SR $Ca^{2+}$-pump inhibition in the $H_2O_2$-induced contraction of rat aorta in the absence of endothelium. It seems that $H_2O_2$ contracts rat aorta through receptor (presumably $P_2$-purinoceptors)-sensitive signaling pathways, which are not shared by that of CPA, a specific inhibitor of SR $Ca^{2+}$-pump. This finding would lead to better understanding of the nature of the oxidative signaling exerted by $H_2O_2$.

**ACKNOWLEDGMENT** This work was supported by grants in part from the Heart and Stroke Foundation of Ontario (CYK). Mr SHEN Jian-Zhong was a visiting scholar of McMaster University (Canada).

## REFERENCES

1 Suzuki YJ, Forman HJ, Sevanian A. Oxidant as stimulators of signal transduction. Free Radic Biol Med 1997; 22: 269-85.
2 Nose K. Role of reactive oxygen species in the regulation of physiological functions. Biol Pharm Bull 2000; 23: 897-903.
3 Katusic ZS, Vanhoutte PM. Superoxide anion is an endothelium-derived contracting factor. Am J Physiol 1989; 257 (1 Pt 2): H33-H37.
4 Sundaresan M, Yu ZX, Ferrans VJ, Irani K, Finkel T. Requirement for generation of $H_2O_2$ for platelet-derived growth factor signal transductions. Science 1995; 270: 296-9.
5 Finkel T. Oxygen radicals and signaling. Curr Opin Cell Biol 1998; 10: 248-53.
6 Rhee SG. Redox signaling: hydrogen peroxide as intracellular messenger. Exp Mol Med 1999; 31: 53-9.
7 Suh YA, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D, *et al*. Cell transformation by the superoxide-generating oxidase Mox1. Nature 1999; 401: 79-82.
8 Shen JZ, Zheng XF, Kwan CY. Differential contractile actions of reactive oxygen species on rat aorta: selective activation of ATP receptor by $H_2O_2$. Life Sci 2000; 66: PL291-PL296.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jun; 22 (6)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

9 Shen JZ, Zheng XF, Kwan CY. Evidence for $P_2$-purinoceptors contribution in $H_2O_2$-induced contraction of rat aorta in the absence of endothelium. Cardiovasc Res 2000; 47: 574-85.
10 Ralevic V, Burnstock G. Receptors for purines and pyrimidines. Pharmacol Rev 1998; 50: 413-92.
11 Grover AK, Samson SE. Effect of superoxide radical on $Ca^{2+}$ pumps of coronary artery. Am J Physiol 1988; 255: C297-C303.
12 Grover AK, Samson SE, Fomin VP. Peroxide inactivates calcium pumps in pig coronary artery. Am J Physiol 1992; 263: H537-H543.
13 Deng HW, Kwan CY. Cyclopiazonic acid is a sarcoplasmic reticulum $Ca^{2+}$-pump inhibitor of rat aortic smooth muscle. Acta Pharmacol Sin 1991; 12: 53-8.
14 Treiman M, Casperson C, Christensen SB. A tool coming of age: thapsigargin as an inhibitor of sarco-endoplasmic reticulum $Ca^{2+}$-ATPase. Trends Pharmacol Sci 1998; 19: 131-5.
15 Ma HT, Patterson RL, van Possum DB, Birnbaumer L, Mikoshiba K, Gill DC. Requirement of the inositol trisphosphate receptor for activation of store-operated $Ca^{2+}$ channels. Science 2000; 287: 1647-51.
16 Xu KY, Zweier JL, Becker LC. Hydroxyl radical inhibits sarcoplasmic reticulum $Ca^{2+}$-ATPase function by direct attack on the ATP binding site. Circ Res 1997; 80: 76-81.

## 肌浆网钙泵的抑制不参与过氧化氢诱导的大鼠主动脉收缩

沈建中，郑秀凤[1]，魏尔清，關超然[2]

（浙江大学医学院药理教研室，杭州 310031，中国；[2] McMaster大学医学院平滑肌研究组，安大略省 L8N 3Z5，加拿大）

**关键词** 过氧化氢；环匹阿尼酸；胸主动脉；平滑肌；嘌呤能 $P_2$ 受体；舒拉明；肌浆网；钙镁 ATP 酶；2-氨乙氧基二苯酯硼酸

**目的**：研究肌浆网钙泵抑制是否参与 $H_2O_2$ 诱导的大鼠主动脉收缩反应. **方法**：离体主动脉环张力实验比较 $H_2O_2$ 及钙泵特异性抑制剂环匹阿尼酸(CPA)缩血管效应及其信号机制的差异. **结果**：$H_2O_2$ 和 CPA 均收缩去内皮主动脉环，但 $H_2O_2$ 触发快速短暂相位相收缩，而 CPA 诱导缓慢持续的张力相收缩. 在无钙液中，仅 CPA 30 μmol/L 而非 $H_2O_2$ 30 μmol/L 预处理取消苯肾上腺素 10 μmol/L 缩血管效应. Thapsigargin 30 μmol/L 诱导最大收缩反应时，仅 $H_2O_2$ 能使血管环进一步收缩. 另外，$P_2$ 受体拮抗剂 suramin、RB-2 (各 100 μmol/L)以及多种酶抑制剂包括 PLC、PKC、$PLA_2$、COX 和蛋白质酪氨酸激酶均能抑制 $H_2O_2$ 而非 CPA 诱导的缩血管效应，但 2-APB 50 μmol/L 对两者都有抑制作用. **结论**：肌浆网钙泵抑制不是 $H_2O_2$ 收缩大鼠去内皮主动脉的机制.

（责任编辑 吴民淑）