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Research Article

Paraoxon Attenuates Vascular Smooth Muscle Contraction through Inhibiting Ca^{2+} Influx in the Rabbit Thoracic Aorta

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We investigated the effect of paraoxon on vascular contractility using organ baths in thoracic aortic rings of rabbits and examined the effect of paraoxon on calcium homeostasis using a whole-cell patch-clamp technique in isolated aortic smooth muscle cells of rabbits. The findings show that administration of paraoxon (30 μM) attenuated thoracic aorta contraction induced by phenylephrine (1 μM) and/or a high K^+ environment (80 mM) in both the presence and absence of thoracic aortic endothelium. This inhibitory effect of paraoxon on vasoconstrictor-induced contraction was abolished in the absence of extracellular Ca^{2+}, or in the presence of the Ca^{2+} channel inhibitor, verapamil. But atropine had little effect on the inhibitory effect of paraoxon on phenylephrine-induced contraction. Paraoxon also attenuated vascular smooth muscle contraction induced by the cumulative addition of $CaCl_2$ and attenuated an increase of intracellular Ca^{2+} concentration induced by K^+ in vascular smooth muscle cells. Moreover, paraoxon (30 μM) inhibited significantly L-type calcium current in isolated aortic smooth muscle cells of rabbits. In conclusion, our results demonstrate that paraoxon attenuates vasoconstrictor-induced contraction through inhibiting Ca^{2+} influx in the rabbits thoracic aorta.

1. Introduction

Organophosphorus ester pesticides (OPs) are mainly used as agricultural and domestic insecticides, leading to increasing numbers of cases of toxic effects on humans and livestock. OPs poisoning can result in neurotoxicity, myocardial damage, microvascular dysfunction, cytotoxicity, respiratory failure, and functional disorder of immune system et al. in human [1, 2]. A key mechanism of intoxication of OPs is the irreversible inhibition of acetylcholinesterase (AChE) and resulting in the accumulation of acetylcholine (ACh) and overstimulation of its receptors [3]. However, cholinergic hyperexcitability cannot account for all of the neurological, behavioral, and cardiovascular system manifestations arising from exposure to OPs. There is evidence for a wide range of novel targets that are more sensitive than AChE to exposure to OPs [2, 4].

It has been reported that some OPs altered vascular contractility and exacerbated OPs-induced hypertension in acetylcholinesterase-independent manner [5, 6]. Paraoxon is the active metabolite of parathion. The response to vasoactive agents is altered by treatment with paraoxon in hens ischiadic arteries [7, 8]. However, whether paraoxon directly modulates the function of vascular endothelium and/or smooth muscle cells in rabbits has not been examined. It has been demonstrated that paraoxon blocked GABA, NMDA, glycine, nicotinic and muscarinic receptor, and chloride channels in neurons [4, 9]. It has been also reported that paraoxon inhibited Ca^{2+} inflow during the action potential and could downregulate Ca^{2+}-activated K^+ channels leading to a reduction of the afterhyperpolarization and an increase in snail neurons firing [10, 11]. However, there are some inconsistent results between calcium homeostasis and paraoxon in nonneuronal cells [12, 13]. Thus in this study, we investigated the effect of paraoxon on vascular contractility in rabbits thoracic aortic rings and calcium homeostasis in aortic smooth muscle cells.

2. Materials and Methods

2.1. Drugs and Chemicals. Acetylcholine (ACh), sodium nitroprusside (SNP), phenylephrine (Phe), potassium chloride (KCl), N-Nitro-L-arginine (L-NNA), atropine, paraoxon (PXN), verapamil hydrochloride, nicotinamide, ryanodine, tetraethylammonium (TEA-CI), HEPES, egtazic acid, C_SOH, C_SCI, MgATP, and tetrodotoxin (TTX) were purchased from Sigma-Aldrich (St. Louis, MO,USA), Paraoxon was dissolved in ethanol in organ baths experiment and in DMSO in measurement of Ca^{2+} current experiment.

2.2. Aortic Ring Preparation and Tension Measurements. The thoracic aortas were dissected from male New Zealand rabbits (2.0 ± 0.3 Kg, supplied by the animal Center of South China University) killed by exsanguination after anesthesia with pentobarbital sodium (80 mg/kg, IV). The adhering perivascular tissue was removed carefully. The thoracic aortas were cut into ring segments, 4 to 5 mm in length. The aortic endothelium was removed by inserting a plastic club into the lumen followed by gentle rubbing in some experiments. Each ring was then placed in a 10 mL organ bath and mounted on two stainless steel hooks, one of which was fastened to the bath and the other connected to a strain gauges for measurement of isometric force. The organ bath was filled with Krebs solution at 37°C and bubbled with a mixture of 95% O_2 + 5% CO_2. The rings were equilibrated for 60 minutes under a resting tension of 2.0 g and the solution was changed at 20-minute intervals. The integrity of the endothelium was affirmed by the addition of the endothelium-dependent vasodilator, ACh (1 μM), after contractions had been induced by 1 μM Phe. This was similarly made with the denuded vessels in order to exclude any endothelial function. After Phe and ACh were washed out with Krebs solution, the aortic rings were contracted by 50 mM KCl. After recording the contractile tension of each ring following stimulation with 50 mM KCl, the baths were washed out with Krebs solution. The contractile responses were expressed relative to those measured for the 50 mM KCl-induced contraction in the test experiments [14, 15].

2.3. Treatment of the Thoracic Aortic Rings with Paraoxon. The concentration of paraoxon was 0.3, 3, or 30 μM. Paraoxon was added to the incubation system 30 minutes prior to or during the contraction induced by Phe or KCl. Vehicle-treated rings were used as controls. In some experiments, endothelium-denuded thoracic aortic rings were contracted by Phe in the presence of SNP (1 nM) without paraoxon administration. In some other experiments, thoracic aortic contractions were performed in the absence of extracellular Ca^{2+} using Ca^{2+}-free Krebs solution containing 1 mM EDTA.

2.4. Studies on the Effect of L-NNA, Verapamil, Atropine, Ryanodine, and Nicotinamide. Several inhibitors and antagonists were used in the test experiments. The concentrations of inhibitors or antagonists used in our study were as follows: L-NNA (NO synthase inhibitor; 30 μM), verapamil (L-type voltage-dependent Ca^{2+} channels blocker; 55 μM), atropine (a specific muscarinic receptors antagonist; 5 μM), ryanodine (ryanodine receptors blocker; 30 μM), and nicotinamide (ADP-ribosyl cyclase inhibitor; 6 mM). The thoraotic aortic rings were pretreated with each inhibitor for 20 minutes and then contractions were induced by 1 μM Phe or 80 mM KCl.

2.5. Exogenous Ca^{2+}-Dependent Contraction. The thoracic aortic rings without endothelium were washed three times with Ca^{2+}-free Krebs solution containing 1 mM EDTA and then exposed to the same buffer for 15 minutes. The thoracic aortic rings were pretreated with 30 μM paraoxon for 30 minutes and then stimulated with KCl (80 mM). After 30 minutes incubation, $CaCl_2$-(10^{-5} M to 10^{-2} M) induced contractions were measured for each aortic ring [14].

2.6. Measurement of Vascular Smooth Muscle Calcium Levels. The procedure was mainly the same as that described by F.P. Leung et al. with minor modifications [16]. The aortic rings were loaded with fura-2 acetoxymethyl ester (fura-2 AM) through incubation in Krebs solution at pH 7.4, containing 10 μM fura-2 AM and 0.025% Pluronic F-127 at room temperature for 2 hours. After fura-2 AM loading, extracellular fura-2 AM was washed off in Krebs solution. Then, the aortic rings were longitudinally cut open and pinned onto a block of silicone elastomer, which was fixed onto a base plate of the flow chamber. After vessel fixing, the flow chamber was placed on an inverted microscope equipped with a spectrophotometer and perfused with Krebs solution. Then, the fura-2 AM-loaded vessels were visualized through an inverted fluorescence microscope to perform measurements of fura-2 AM microfluorometry. The ratio of the fluorescence intensities at alternating 340 nm (F340) and 380 nm (F380) excitation wavelengths was monitored to measure intracellular calcium concentration ($[Ca^{2+}]_i$). Fluorescence intensities were recorded as a function of time. After being fixed, the aortic tissues were allowed to recover for 30 minutes and then exposed for 30 minutes to Ca^{2+}-free 80 mM K^+ solution containing 1 mM Na_2-EDTA. Subsequently, they were perfused with the same high-K^+ solution supplemented with 0.1, 0.3, 1, and 3 mM $CaCl_2$. Tissue was then washed several times in Ca^{2+}-free 80 mM K^+ solution until baseline was recovered. After incubating with paraoxon (30 μM) for 30 minutes, cumulative perfusion of $CaCl_2$ induced a second concentration-dependent increase in $[Ca^{2+}]_i$.

2.7. Thoracic Aortas Smooth Muscle Cell Isolation. Male New Zealand rabbits weighing approximately 2.0 Kg were stunned by heavy blow on the head. The thoracic aortas were surgically excised. Five to 10 segments (6–10 mm in length) of thoracic aortas were quickly dissected free of connective tissue in ice-cold solution containing 4.7 mM KCl, 145 mM NaCl, 1.2 mM NaH_2PO_4, 2 mM pyruvic acid, 1.17 mM $MgSO_4$, 0.02 mM EDTA, 0.1 mM $CaCl_2$, 5 mM D-glucose, and 10 mg/mL bovine serum albumin (BSA). Thoracic aortas were placed in an isolation solution: 4.2 mM KCl, 120 mM NaCl, 25 mM $NaHCO_3$, 1.2 mM $MgCl_2$, 0.6 mM

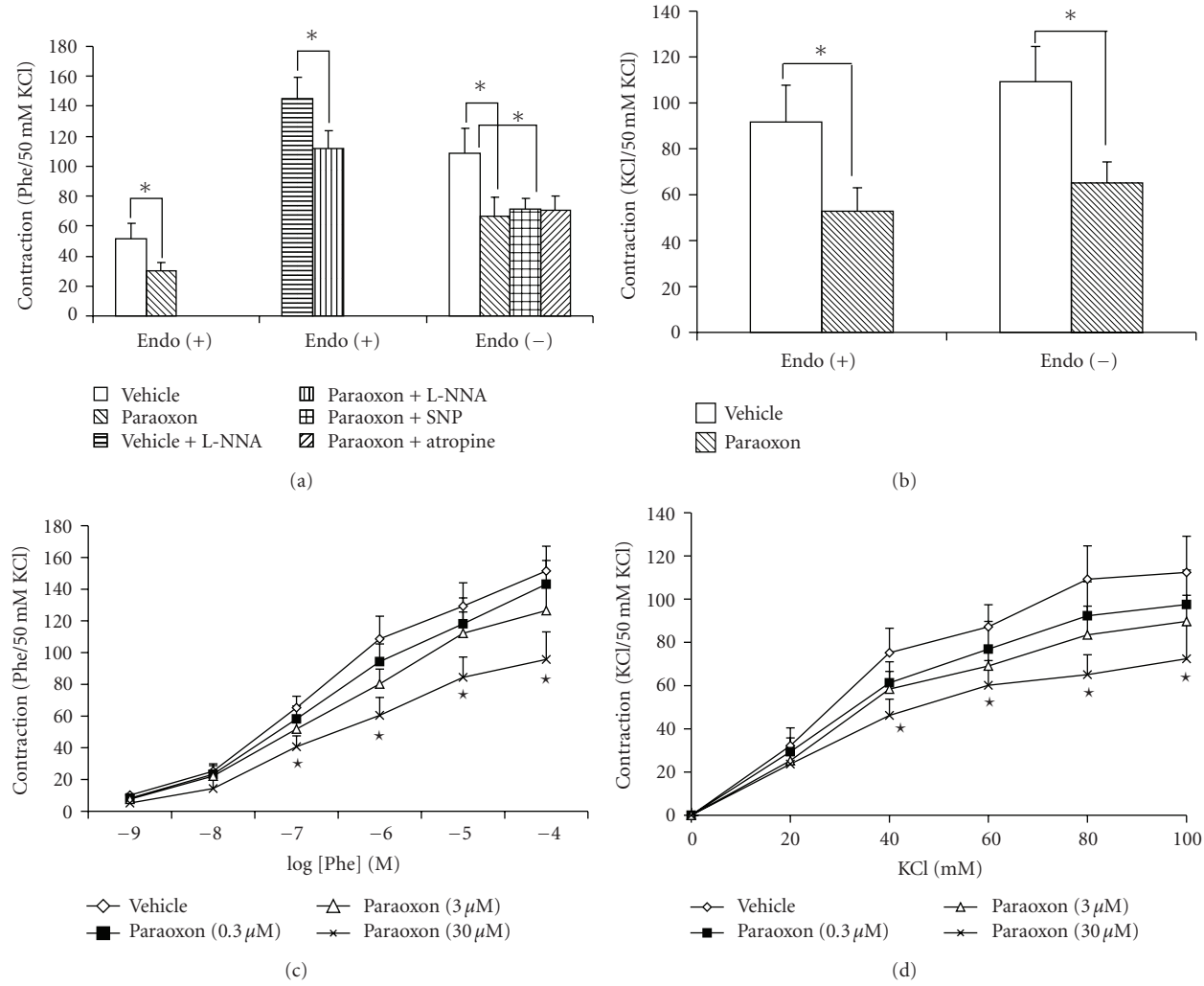

Figure 1: Paraoxon attenuated phenylephrine- (Phe-) or KCl-induced vasoconstriction in rabbit thoracic aorta. The effect of pretreatment with paraoxon on phenylephrine- (a) or KCl- (b) induced contraction in rabbits thoracic aorta in the presence or absence of aortic endothelium. The aortic ring preparations were preincubated with (30 μM) or without (vehicle) paraoxon for 30 minutes followed by stimulation with Phe (1 μM) or KCl (80 mM). In some ring preparations with aortic endothelium (Endo), L-NNA (30 μM) was incubated during Phe-induced contraction. In some other ring preparations without aortic endothelium, sodium nitroprusside (SNP: 1 nM) and atropine (5 μM) were incubated during Phe-induced contraction in the absence of paraoxon. (c) Concentration-response curves for Phe-induced vasoconstriction of endothelium-denuded aortic rings in the presence (0.3 μM–30 μM) or absence (vehicle) of pretreatment with paraoxon (30 μM) for 30 minutes. (d) Concentration-response curves for KCl-induced vasoconstriction of endothelium-denuded aortic rings in the presence (0.3 μM–30 μM) or absence (vehicle) of paraoxon (pre-treatment for 30 min). Each contractile value was standardized against the contraction level induced by 50 mM KCl in the absence of pretreatment. Data are expressed as means ± SEM ($n = 8$). $^*P < .05$; $^*P < .05$ compared with the vehicle group.

KH_2PO_4, 1 mg/mL BSA, 0.1 mM $CaCl_2$, and 11 mM D-glucose, pH 7.4, when bubbled with 5% CO_2 and 21% O_2 (at room temperature). The arteries were cut into 4 to 5 mm segments, which were then digested in isolation solution containing papain (1.0 mg/mL), BSA (1 mg/mL), and dithiothreitol (1 mg/mL) for 10 minutes at 37°C. The artery segments were then transferred to isolation solution containing collagenaseII (0.36 mg/mL), 0.1 mM $CaCl_2$, dithiothreitol (1 mg/mL), and BSA (1 mg/mL) for 10 minutes at 37°C. The tissue was then washed 10 times in ice-cold isolation solution without collagenase and triturated gently with polished Pasteur pipettes to separate the individual cells. Freshly isolated cells with characteristic elongated morphology of vascular myocytes were added directly to the chamber and allowed to adhere for 10 to 15 minutes for whole-cell patch-clamp electrophysiology.

2.8. Measurement of Ca^{2+} Current in Isolated Aortic Smooth Muscle Cells of Rabbits. Isolated aortic smooth muscle cells were placed in the experimental chamber (0.4 mL) mounted on the stage of an inverted microscope (IX-70 Olympus, Tokyo, Japan). After setting to the bottom of chamber, the cells were superfused with external solution for 15 minutes at a rate of 2∼2.5 mL/min at 25°C. Transmembrane currents

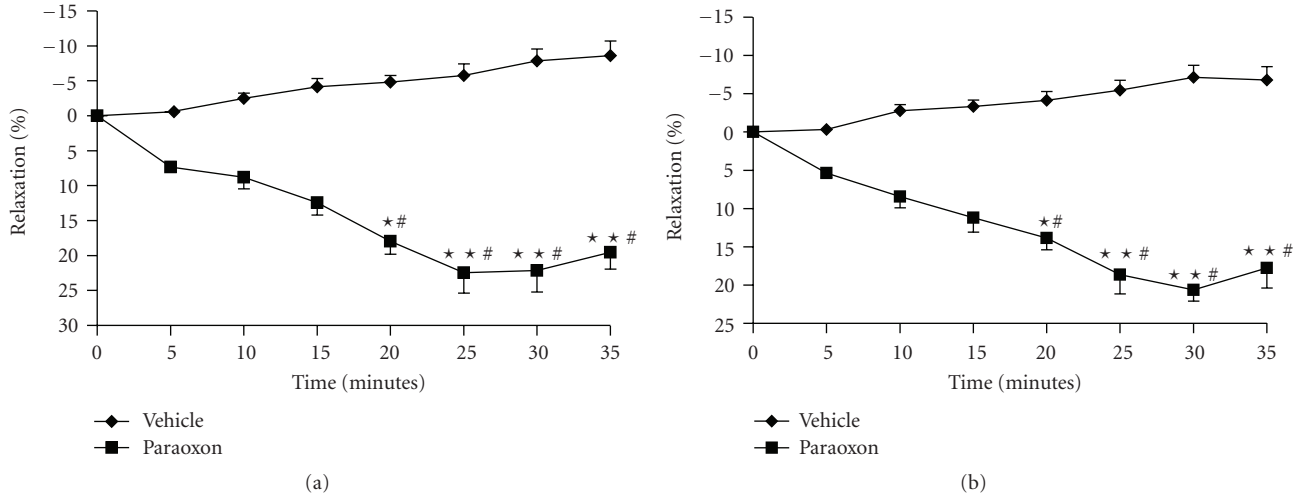

FIGURE 2: Time course of vasodilation induced by 30 μM paraoxon in thoracic aortic rings precontracted by 1 μM Phe (a) or 80 mM KCl (b). Data are expressed as means ± SEM ($n = 8$). $^{\star}P < .05$ and $^{\star\star}P < .01$, compared with vehicle group. $^{\#}P < .05$, compared with time of zero.

were recorded using an Axopatch amplifier (200B, Axon Instruments, USA). Patch-clamp pipettes were manufactured from borosilicate glass capillaries (GC 150T-7.5, Clark Electromedical Instruments, London, UK). The resistance of the patch pipette was 2–4 MΩ, when filled with electrode internal solution. Liquid junction potential between the pipette solution and external solution was corrected after the pipette tipped into the external solution. After gigaseal formation, the membrane was ruptured with a gentle suction to obtain the whole-cell voltage-clamp configuration. To minimize the duration of capacitive current, membrane capacitance and series resistance were compensated after membrane rupture. The external solution was changed to Na^+-free solution in which Na^+ was replaced by equimolar tetraethylammonium chloride (TEA-CI). Na^+ current was also inactivated at the holding potential of –40 mV and blocked by tetrodotoxin (TTX, 2×10^{-6} mol/L). K^+ current was suppressed by substituting intracellular K^+ by Cs^+. Computer-generated voltage or current pulses were programmed using the pCLAMP 8.0 software (Axon Instruments, USA). Online acquired data were stored on a hard disk of the microcomputer. All experiments were carried out at room temperature (22∼ 24°C) [17, 18].

The electrode internal solution for whole-cell recording contained (in mM) 3 MgATP, 140 CsCl, and 10 HEPES, and 10 egtazic acid, and pH was adjusted to 7.2 with CsOH. The external solution was composed of (in mM) 140 TEA-Cl, 2.0 $MgCl_2$, 1.5 $CaCl_2$, 10 glucose, 10 HEPES, and 0.002 TTX, gassed with 100% O_2, and the pH was adjusted with NaOH to 7.3∼7.4 [17, 18]. Paraoxon was dissolved in DMSO and diluted in external solution at the concentrations of 0.3, 3, and 30 μmol/L.

2.9. Statistical Analysis. All data were expressed as means ± SEM. Statistical comparisons were made using one-way ANOVA plus Bonferroni multiple comparison tests. $^{*}P < .05$ was regarded as significant.

3. Results

3.1. Effect of PreTreatment with Paraoxon on Vasoconstriction Induced by Phe and KCl. The effect of pre-treatment with paraoxon on contraction induced by Phe or high concentration of K^+ was investigated in rabbits thoracic aorta. The maximum contractions of aorta rings induced by 1 μM Phe were decreased by pretreatment with paraoxon (30 μM), regardless of the presence (41.20% ± 11.05%) or absence (38.87% ± 8.74%) of endothelium (Figure 1(a)). The vasoconstriction of aortic rings induced by 80 mM KCl was also attenuated by pretreatment with paraoxon (30 μM) regardless of the presence (42.42% ± 8.57%) or absence (40.09% ± 6.18%) of endothelium (Figure 1(b)). Meanwhile, L-NNA (30 μM), an inhibitor of NO synthesis, had little effect on the inhibitive effect of paraoxon on 1 μM Phe-induced vasoconstriction, but sodium nitroprusside (1 nM), a donor of NO, significantly attenuated 1 μM Phe-induced vasoconstriction in endothelium-denuded aortic rings (Figure 1(a)). So endothelium-denuded thoracic aortic rings were used in the succeeding experiments. Furthermore, atropine (5 μM), a specific muscarinic receptors antagonists had also little effect on the inhibitory effect of paraoxon on 1 μM Phe-induced vasoconstriction in endothelium-denuded thoracic aortic rings (Figure 1(a)). As shown in Figure 1(c), paraoxon at a concentration of 30 μM significantly inhibited 10^{-7}–10^{-4} M of concentration Phe-induced constriction in thoracic aortic rings without endothelium. Likewise, pretreatment with 30 μM paraoxon also significantly attenuated 40–100 mM KCl-induced vasoconstriction in endothelium-denuded thoracic aortic rings (Figure 1(d)).

3.2. Paraoxon Directly Relaxed Phe- and KCl-Induced Vasoconstriction in Aorta without Endothelium. As shown in Figure 2(a), treatment of paraoxon (30 μM) resulted in vasodilation in the thoracic aortic rings without endothelium that had been precontracted by Phe (1 μM). The relaxant

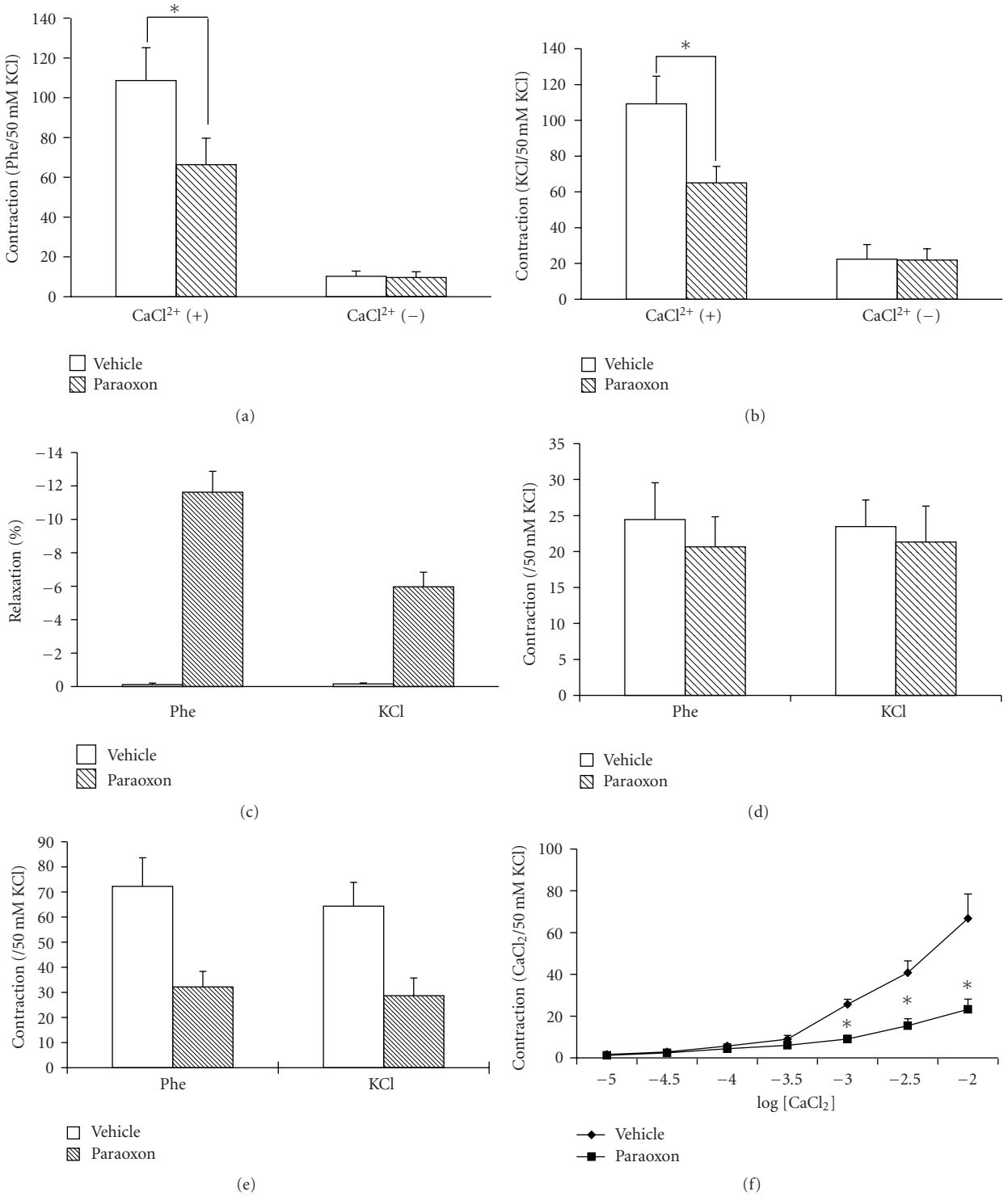

FIGURE 3: Effects of pretreatment with 30 μM paraoxon for 30 minutes on Phe- (a) or KCl- (b) induced contraction in endothelium-denuded aortic rings in the presence [Ca^{2+} (+)] or absence [Ca^{2+} (−)] of extracellular Ca^{2+}. (c) Percentage of vasodilation induced by 30 μM paraoxon or vehicle during Phe- (1 μM) or KCl- (80 mM) induced contraction in endothelium-denuded aortic rings with verapamil pre-treatment for 20 minutes. (d) Effect of pre-treatment with 30 μM paraoxon for 30 minutes on Phe- or KCl-induced contraction in endothelium-denuded aortic rings with verapamil (55 μM) pre-treatment (20 minutes). (e) Effect of pre-treatment with 30 μM paraoxon for 30 minutes on Phe-or KCl-induced contraction in endothelium-denuded aortic rings with ryanodine (30 μM) plus nicotinamide (6 mM) pretreatment (20 minutes). (f) Effect of paraoxon on $CaCl_2$-induced contraction in endothelium-denuded aortic rings prestimulated by 80 mM KCl. Each contractile value was standardized against the contraction level induced by 50 mM KCl in the presence of Ca^{2+} and in the absence of treatment. Data are expressed as means ± SEM ($n = 8$). $^*P < .05$, compared with the vehicle group.

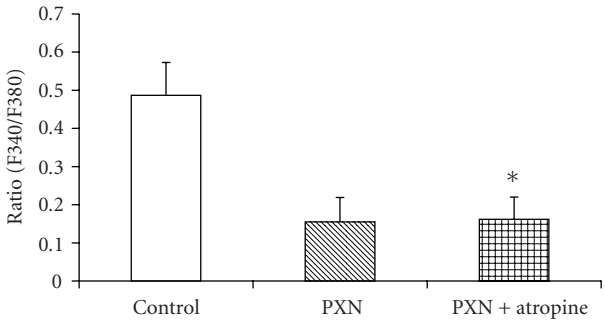

Figure 4: Effect of pre-treatment with 30 μM paraoxon (PXN) for 30 minutes on $CaCl_2$ stimulated $[Ca^{2+}]_i$ in arterial tissues without endothelia. Control refers to vehicle control. Data are expressed as means ± SEM ($n = 8$). $^*P < .05$, compared with control group.

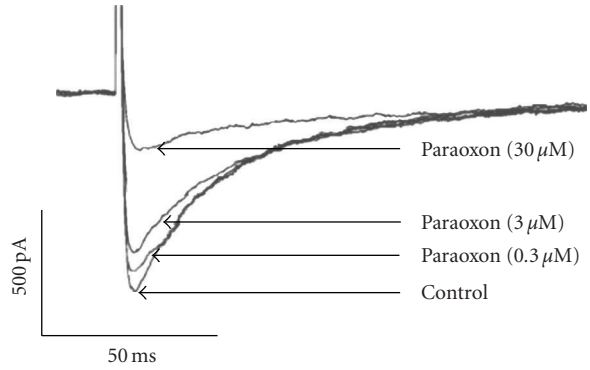

Figure 5: Effect of paraoxon on L-type calcium current (Ica,L) in isolated thoracic aortas smooth muscle cells of rabbits. Currents were recorded during 350 ms depolarization from a holding potential of –40 mV to 0 mV.

effect lasted for 35 minutes after treatment with paraoxon (30 μM). As well, paraoxon induced vasodilation in thoracic aortic rings without endothelium contracted previously by 80 mM KCl (Figure 2(b)).

3.3. Effect of Lack of Extracellular Ca^{2+} or Verapamil on the Inhibitory Effect of Paraoxon on Vasoconstriction. To investigate the involvement of extracellular Ca^{2+} in the inhibitory effect of paraoxon on vasoconstriction, thoracic aortic rings were washed and incubated with Ca^{2+}-free Krebs solution. As shown in Figures 3(a) and 3(b), a lack of extracellular Ca^{2+} almost completely inhibited Phe (94.1% ± 1.6%) and KCl- (78.3% ± 2.2%) induced vasoconstriction. Under Ca^{2+}-free conditions, paraoxon failed to inhibit vasoconstriction (Figures 3(a) and 3(b)). These results showed that the effect of paraoxon on Phe- or KCl-induced contraction depended on extracellular Ca^{2+}. Paraoxon did not relax either Phe- or KCl-induced vasoconstriction in the presence of 55 μM verapamil (Figure 3(c)). The decrease of vasoconstriction induced by paraoxon was also abolished by verapamil (Figure 3(d)). Ryanodine and nicotinamide, Ca^{2+}-induced Ca^{2+} release pathway blocker, also had no influence on the effect of paraoxon on Phe- or KCl-induced contraction (Figure 3(e)). As shown in Figure 3(f), to investigate the effect of paraoxon on Ca^{2+} influx-induced vasoconstriction, $CaCl_2$ was administrated cumulatively [10^{-5} M–10^{-2} M] to the aortic rings which had been incubated in Ca^{2+}-free Krebs solution. To activate Ca^{2+} influx into the smooth muscle cells, the aortic rings were stimulated with KCl (80 mM) prior to the cumulative addition of $CaCl_2$. Addition of $CaCl_2$ increased the contractile tension of the endothelium-denuded aortic rings in concentration-dependent manner. The contraction induced by the cumulative addition of $CaCl_2$ was attenuated by pre-treatment with paraoxon in endothelium-denuded aortic rings.

3.4. Effect of Paraoxon on $[Ca^{2+}]_i$ in Vascular Smooth Muscle Cells of Rabbit Thoracic Aorta. To investigate whether paraoxon attenuated Phe- and KCl-induced vasoconstriction in the thoracic aorta mainly through inhibiting Ca^{2+} influx, the effect of paraoxon on vascular smooth muscle $[Ca^{2+}]_i$ was measured in thoracic aorta without endothelia. $CaCl_2$ induced a rise in VSM $[Ca^{2+}]_i$ in Ca^{2+}-free 80 mM K^+ solution, and the first and second concentration-dependent responses were similar. As shown in Figure 4, changes in VSM $[Ca^{2+}]_i$ measured as the fluorescence ratio in thoracic aorta in response to $CaCl_2$ in 80 mM K^+ solution after treatment with 30 μM paraoxon for 30 minutes. $[Ca^{2+}]_i$ were significantly attenuated by treatment with paraoxon (30 μM) for 30 minutes. Moreover, treatment with 1 μM atropine did not influence the paraoxon- (30 μM) mediated inhibition of the $CaCl_2$-stimulated $[Ca^{2+}]_i$ rise (Figure 4).

3.5. Effect of Paraoxon on L-Type Calcium Current in Isolated Thoracic Aortas Smooth Muscle Cells. L-type calcium current (Ica,L) in thoracic aortas smooth muscle cells was evoked by a depolarizing step pulse from the holding potential of –40 mV to 0 mV at the frequency of 0.1 Hz. The step pulse duration was 350 ms. Paraoxon (0.3, 3, and 30 μM) inhibited the peak amplitude of Ica,L (Figure 5). Current-voltage (I-V) curve of L-type calcium current was obtained by a number of depolarizing step pulses (350 ms) from the holding potential of –40 mV to test potentials between –50 mV and 50 mV. The test step pulses were delivered in 10 mV increments. Ica,L was activated at –30 mV and the peak amplitude occurred at the potential of 0 mV. Paraoxon (0.3, 3, and 30 μM) upshifted the I-V curve (Figure 6(a)), but the current density at test potential of 0 mV was significantly decreased only in paraoxon (30 μM) group compared with the control group (Figure 6(b)).

4. Discussion

Organophosphorus ester pesticides (OPs) are well acknowledged to have the potential to result in severe, acute toxicity through the phosphorylation of serine residues of acetylcholinesterase (AChE) and the subsequent accumulation of ACh. However, many researchers have reported additional noncholinesterase actions for OPs [19]. It has been shown that OPs could interact directly with targets other than acetylcholinesterase [20, 21]. At the cellular level, the main

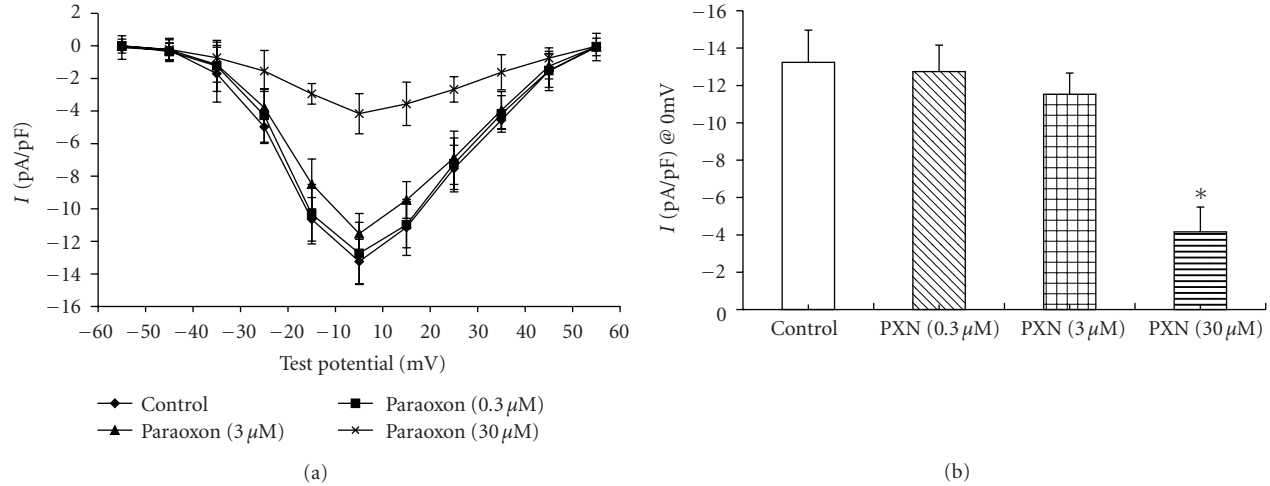

Figure 6: Effect of paraoxon (PXN) on L-type calcium current (Ica,L) in isolated thoracic aortas smooth muscle cells of rabbits. (a) Effect of paraoxon on current-voltage (I-V) curve of Ica,L in isolated thoracic aortas smooth muscle cells of rabbits. (b) Effect of paraoxon on current densities at test potential of 0 mV in isolated thoracic aortas smooth muscle cells of rabbits. Control refers to vehicle-treated cells. Data are expressed as means ± SEM ($n = 6$). $^*P < .05$, compared with control group.

targets of OPs include receptors, enzymes, ion channels, cell signaling molecules, and cytoskeletal elements et al. [22–24]. Likewise, there is multiple evidence that OPs can interact with targets other than AChE in the cardiovascular system [25, 26].

Paraoxon is the active metabolite of parathion which is one of the most acutely toxic organophosphorus ester pesticides [27]. We examined the effect of paraoxon on the vasoconstrictor-induced contraction in rabbits thoracic aortic rings. The results showed that paraoxon (30 μM) attenuated significantly both Phe- and KCl-induced aortic contraction regardless of the presence or absence of aortic endothelium. Our findings also showed that L-NNA had little effect on the inhibitory effect of paraoxon on Phe-induced contraction, while SNP inhibited Phe-induced contraction in endothelium-denuded aortic rings. These results suggested that paraoxon attenuated vascular contraction independent of generation of NO by the endothelia. But L-NNA markedly increased the response to Phe in the presence of aortic endothelium. This may be explained by the fact that L-NNA decreases the production of endothelium-derived relaxing factor, which results in an increase of vascular sensitivity to vasoconstrictor. Meanwhile, atropine had also little effect on the inhibitory effect of paraoxon on Phe-induced contraction in endothelium-denuded aortic rings. This demonstrated that paraoxon attenuated vascular contractility independent of muscarinic receptors activation.

Phe can bind to α-adrenoceptor on the plasma membrane, leading to the activation of phospholipase C, which produces inositol 1-, 4-, 5-triphosphate (IP_3) and diacylglycerol (DG) [28]. Vascular smooth muscle contraction is then triggered by an increase in $[Ca^{2+}]_i$ induced by Ca^{2+} mobilization from the sarcoplasmic reticulum and a membrane depolarization-stimulated Ca^{2+} influx from the extracellular spaces [29]. This vasoconstrictor- induced Ca^{2+} inflow from the extracellular spaces is principally mediated by L-type Ca^{2+} channels [30]. Release of Ca^{2+} from sarcoplasmic reticulum is mainly mediated by IP_3 receptors and ryanodine receptors, both of which contribute to the transient increase in $[Ca^{2+}]_i$ [31]. Ryanodine receptor is activated by influx of Ca^{2+} from the extracellular spaces, usually called Ca^{2+}-induced Ca^{2+} release. In addition, various types of K^+ channels exist in vascular smooth muscle cell. The K^+ current hyperpolarizes the vascular smooth muscle cell membrane and prohibits the entry of Ca^{2+} through closing the L-type Ca^{2+} channels, resulting in vasorelaxation [32]. So high environmental K^+ level leads to membrane depolarization and increases the entry of Ca^{2+} from extracellular spaces [33]. Our results showed that the degree of aortic contraction was significantly decreased in Ca^{2+}-free medium and/ or the presence of the L-type Ca^{2+} channel inhibitor, verapamil. Our findings also showed that ryanodine and nicotinamide, Ca^{2+}-induced Ca^{2+} release pathway blockers, had little effect on the action of paraoxon, but blockade of extracellular Ca^{2+} entry in the absence of extracellular Ca^{2+} or use of verapamil abolished the effect of paraoxon on contraction in rabbits thoracic aorta. Moreover, pre-treatment with paraoxon (30 μM) decreased contractions induced by the cumulative addition of $CaCl_2$. These data suggested that the suppressive effect of paraoxon on vascular contraction was involved in the extracellular Ca^{2+} influx, but not the release of Ca^{2+} from the sarcoplasmic reticulum.

Subsequently, we examined the effect of paraoxon on $[Ca^{2+}]_i$. The results suggested that pre-treatment with paraoxon attenuated significantly the transient increase in $[Ca^{2+}]_i$ in vascular smooth muscle cells of the rabbits thoracic aorta. Ca^{2+} inflow from the extracellular spaces is mainly mediated by L-type calcium channels. So we investigated the effect of paraoxon on L-type calcium current (Ica,L) in isolated thoracic aortas smooth muscle cells of rabbits. The results showed that paraoxon (30 μM) decreased significantly L-type calcium current. Therefore we

inferred that paraoxon attenuated vasoconstrictor-induced contraction through inhibiting Ca^{2+} influx via blocking L-type calcium channels in vascular smooth muscle cells of the rabbits.

Calcium homeostasis is a highly controlled process, essential for various cellular functions including the contraction of smooth muscle and skeletal muscle, transmitter release, the cell excitability, and cell death [34, 35]. The interaction of some OPs with membrane Ca^{2+} channels has been reported [36, 37]. Soman has been shown to decrease calcium entry through voltage-dependent calcium channels in bullfrog peripheral sympathetic neurons [38]. Methyl parathion and malathion inhibit the $[Ca^{2+}]_i$ rise induced by nicotinic agonist in bovine adrenal chromaffin cells [39]. Exposure of SN56 cells to 10 μM paraoxon for 18 hours significantly decreases the Ca^{2+} mobilization elicited by carbachol [40]. However, there are some different results about the effects of organophosphates on Ca^{2+} homeostasis. Chávez et al. reported that paraoxon did not have any influence on the $[Ca^{2+}]_i$ in tracheal smooth muscle cells of guinea pigs [12], whereas Sun et al. found an enhanced Ca^{2+} release and influx mechanisms in presence of paraoxon in the human parotid cell-line HSY [13]. So further studies are needed to examine potential mechanism.

In conclusion, we have demonstrated that paraoxon attenuates vasoconstrictor-induced contraction and induces vasodilation through inhibiting Ca^{2+} influx in the rabbits thoracic aorta.

Acknowledgment

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