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Involvement of protein kinase C and IP_3-mediated Ca^{2+} release in activity modulation by paraoxon in snail neurons

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Abstract

We have previously reported that paraoxon, an organophosphate compound, at submicromolar concentrations effectively suppresses Ca^{2+} action potentials and modulates the activity of snail neurons. This effect was unrelated to acetylcholinesterase inhibition but was found to involve the direct or indirect modulation of ion channels [Vatanparast, J., Janahmadi, M., Asgari, A.R., Sepehri, H., Haeri-Rohani, A., 2006a. Paraoxon suppresses Ca^{2+} action potential and afterhyperpolarization in snail neurons: Relevance to the hyperexcitability induction. Brain Res. 1083 (1), 110–117.]. In the present work, the interaction of paraoxon with protein kinase C (PKC) and inositol 1,4,5-trisphosphate (IP_3)-mediated Ca^{2+} release, on the modulation of Ca^{2+} action potentials and neuronal activity was investigated. Phorbol 12, 13 dibutyrate (PdBu), the activator of PKC, suppressed afterhyperpolarization and increased the activity of snail neurons without any significant effect on the Ca^{2+} action potential duration. Pretreatment with PKC activator attenuated the suppressing effect of paraoxon on the duration of Ca^{2+} action potentials. Staurosporine, a selective blocker of PKC, did not block the effect of paraoxon on Ca^{2+} action potential suppression and hyperexcitability induction. Our findings did not support the involvement PKC in the paraoxon induced Ca^{2+} action potential suppression and neuronal activity modulation, although activation of this protein kinase could attenuate some effects of paraoxon. Pretreatment with 8-(*N*,*N*-diethylamino)octyl-3,4,5-trimethoxybenzoate hydrochloride (TMB-8), an antagonist of IP_3-mediated Ca^{2+} release, abolished the secondary silencing effect of paraoxon, which is observed after primary paraoxon-induced hyperexcitability. It was concluded that slow activation of intracellular cascades by paraoxon could induce an IP_3 mediated Ca^{2+} release from intracellular stores and participate to its secondary silencing effect by mechanisms dependent on intracellular calcium homeostasis.

Keywords: Paraoxon; Firing frequency; Protein kinase C; IP_3-induced Ca^{2+} release; Snail neuron

1. Introduction

Apart from the ubiquitous actions in different cells, Ca^{2+} has a critical function in the regulation of neuronal firing behavior (Sah, 1996; Berridge, 1998; Hallworth et al., 2003). The K^+ channels that contribute to the afterhyperpolarization generation are mainly Ca^{2+} dependent and this allows the electrical activity of a neuron to be coupled to the changes in intracellular Ca^{2+}. The intracellular concentration of Ca^{2+} in neurons can be modulated both by Ca^{2+} influx through voltage dependent Ca^{2+} channels and/or Ca^{2+} release from internal stores (Witt et al., 1994; Parekh and Penner, 1997; Schousboe et al., 1997; Pivovarov et al., 2000; Louvet and Collin, 2005). Any changes in the function of membrane Ca^{2+} channels or Ca^{2+} release from internal stores could potentially affect the state of neuronal activity (Conn et al., 1989a,b; Frandsen and Schousboe, 1993; Catterall, 2000; Yamamoto et al., 2002). The interaction of some organophosphates with membrane Ca^{2+} channels has been reported (Heppner and Fiekers, 1991; Vatanparast et al., 2006a,b). Biochemical and electrophysiological approaches have also addressed organophosphate modulation of some metabotropic receptors (in particular muscarinic type) and downstream elements (Bakry et al., 1988; Katz and Marquis, 1989; Ward and Mundy, 1995; Sun et al., 2000).

Signal transduction cascades activated by metabotropic receptors can underlie transient modulation of intracellular Ca^{2+} concentration via phosphorylation or dephosphorylation

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of Ca^{2+} channels (Swartz et al., 1993; Zhou and Ikeda, 1994; Catterall, 1997, 2000; Strock and Diversé-Pierluissi, 2004), and/or by activating intracellular messenger cascades that lead to Ca^{2+} mobilization from intracellular stores. Phosphoinositol system is well known as a pathway which is activated by muscarinic receptors. A receptor bound G protein (G_q) activates the primary effecter, the enzyme phospholypase C (PLC). This enzyme yields a pair of second messengers, diacyleglycerol (DAG) and inositol 1, 4, 5-triphosphate (IP_3). In turn, IP_3 mobilizes Ca^{2+} from internal stores and DAG activates PKC (Kandle et al., 2000). Activation of muscarinic receptors modulates variety of L-, N- and P-type Ca^{2+} channels that involves PKC (Howe and Surmeier, 1995; Wicher, 2001). It also mediates an initial small release of Ca^{2+} from IP_3-sensitive Ca^{2+} stores, which triggers the major component of the Ca^{2+} response through ryanodine-sensitive Ca^{2+} stores by a Ca^{2+}-induced Ca^{2+} release process (CICR) (Berridge, 1998; Pivovarov et al., 2000).

Under physiological conditions, intracellular Ca^{2+} is tightly regulated in accord with the physiological demands (Louvet and Collin, 2005). However, in pathophysiological conditions, as after exposure to organophosphates, Ca^{2+} homeostasis can be disrupted and result in neural dysfunction (Olney et al., 1983; Savolainen and Hirvonen, 1992).

We have recently demonstrated that low concentration of paraoxon (0.3 μM), within a few minutes of application, can significantly suppress Ca^{2+} action potentials and following afterhyperpolarizations in snail neurons and increase their firing rate. It was concluded that direct inhibition of Ca^{2+} channels increases the neuronal excitability by downregulating small conductance Ca^{2+}-dependent K^+ channels (SK) that underlie afterhyperpolarizations. Interestingly we found that a secondary increase in the amplitude and duration of afterhyperpolarizations, associated with a decrease in the neural firing develop by time that could not be explained by simply blockade of Ca^{2+} channel and points out toward other interaction(s) of paraoxon with subcellular element(s) (Vatanparast et al., 2006a). Considering the importance of metabotropic receptors in organophosphate induced consequences (Olney et al., 1983; Savolainen and Hirvonen, 1992), we hypothesized that the interaction of paraoxon with these receptors (especially muscarinic receptors) could contribute to some effects of paraoxon. An alteration in the activity of PKC might be involved in the suppression of Ca^{2+} action potentials by paraoxon. On the other hand, Ca^{2+} release from IP_3-sensitive Ca^{2+} stores could serve as a cellular basis for the secondary silencing effect of paraoxon. To pursue such mechanisms, the effect of submicromolar concentrations of paraoxon and its interactions with PKC and IP_3-induced Ca^{2+} release were studied on electrical activity of snail neurons.

2. Materials and methods

2.1. Preparation

Experiments were performed on central neurons in the subesophageal ganglia of land snail *Caucasotachea atrolabiata*. The circum-oesophageal ganglia were dissected out and were fixed dorsal side up on a sylgard coated recording chamber (Dow Corning Midland, MI, U.S.A) in normal snail Ringer. The composition of normal snail Ringer was (in mM): NaCl, 84; $CaCl_2$, 10; KCl, 4; $MgCl_2$, 5; glucose, 10; HEPES, 5; (pH 7.4). To expose neurons, the connective sheathes were mechanically torn using fine forcipes without any pretreatment with proteolytic enzymes. These procedures were in accordance with the guidelines of the Institutional Animal Ethics Committee at Shaheed Beheshti Medical Sciences University. All experiments were performed at room temperature (21–24 °C).

2.2. Data acquisition and analysis

To elicit Ca^{2+} action potentials the extracellular medium was changed by a circulating Ringer in which the NaCl content of normal snail Ringer was replaced by TEA, and to which 4-aminopyridine (4AP, 5 mM) was added (Ca^{2+} Ringer). Microelectrodes (Clark instrument, UK) were filled with 3 M KCl and those with a resistance of 2–5 MΩ were used for recording. The reference electrode was an agar bridge containing normal Ringer and 3 M KCl in series, which was connected to earth via an Ag–AgCl wire. Intracellular recordings were obtained in current clamp mode using an Axoclamp 2B amplifier. The recorded data were digitized using an A/D converter (AD instrument, Australia) and saved on computer for offline analysis. The parameters of spontaneously recorded Ca^{2+}action potentials and following afterhyperpolarizations, duration and amplitude, were measured using Chart5 software as described before (Vatanparast et al., 2006a). Data were presented as means ± S.E.M. with n being the number of neurons on which the measurements were done. The statistical differences were determined by the Student's t-test and ANOVA followed by Tukey's multiple comparison test with a significance level of 0.05.

2.3. Drugs

Paraoxon, O, O-diethyl p-nitrophenyl phosphate (Sigma, USA) was prepared as 1 M stock in absolute ethanol and diluted (0.3-0.6 μM) daily with Ca^{2+} Ringer. In some experiments ganglia were pretreated in a Ca^{2+} Ringer containing atropine sulphate (Sigma, 5 μM) to block the muscarinic receptors. Phorbol 12, 13 dibutyrate (PdBu), staurosporine and 8-(*N*,*N*-diethylamino)octyl-3,4,5-trimethoxybenzoate hydrochloride (TMB-8) were added to bath medium from stock solutions in dimethyl sulphoxide (DMSO). Final concentration of ethanol and DMSO in superfusing solutions was less than 0.01%.

3. Results

Experiments were made on 50 neurons that showed spontaneous firing in calcium Ringer containing voltage dependent potassium channel blockers (4AP and TEA). In control conditions, the recorded spontaneous Ca^{2+} action potentials had a mean duration of 0.28 ± 0.06 s, afterhyperpolarization of 4.43 ± 0.22 s and a frequency of 0.143 ± 0.02 Hz (n = 21).

3.1. Paraoxon suppressed Ca^{2+} action potentials and had a dual effect on the firing rate

Paraoxon decreased the duration of Ca^{2+} action potentials in a time dependent manner. Application of paraoxon (0.3 μM) within 10 min induced a reduction of 34.7±7.2% in the duration of action potentials. Paraoxon also induced a reduction of 28.6±8.1% and 21.4±9.4% in the duration of afterhyperpolarization within 5 min and 10 min of application, respectively, which was along with an increase in the frequency of firing (Fig. 1). The maximum frequency was observed 5 min after paraoxon application by 52.5±16.4% increase compared to control condition ($P<0.05$). In the previous study we showed that a direct blockade of Ca^{2+} channels participates as a mechanism of paraoxon induced hyperexcitability via downregulation of Ca^{2+} dependent K^+ channels that underlies afterhyperpolarization. We also found that within 10–15 min of exposure to 0.3 μM the duration of afterhyperpolarization showed a secondary increase associated with a decrease in firing frequency and this was despite of ongoing suppression of Ca^{2+} action potentials (Vatanparast et al., 2006a). Application of higher concentration of paraoxon (0.6 μM) within 10 min caused a reduction of 65.72±6.71% in the duration of action potentials (Fig. 1A). Although increasing the concentration of paraoxon to 0.6 μM produced further decrease in the duration of action potentials, it led to an increase in the duration of afterhyperpolarization along with more suppression in the firing rate (Fig. 1B).

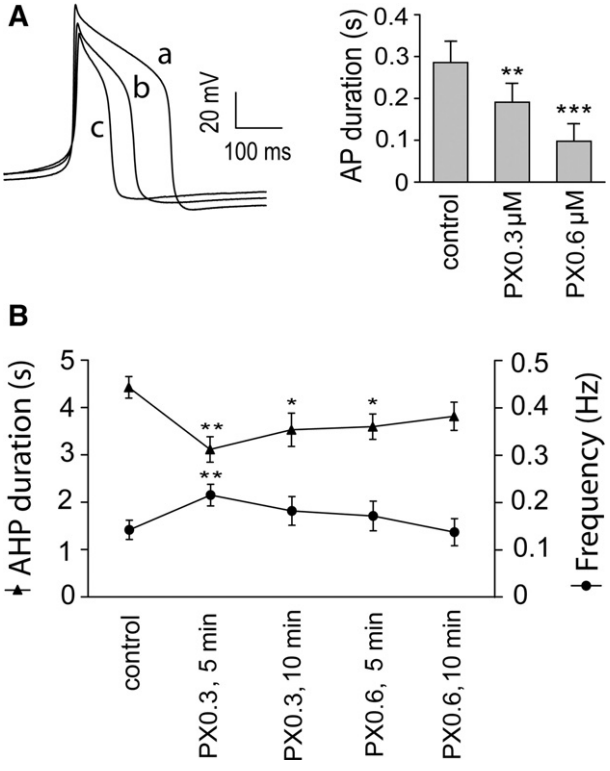

Fig. 1. Paraoxon (PX) decreased the duration of Ca^{2+} actionpotentials (AP) and had a dual effect on the duration of afterhyperpolarization (AHP) and firing rate. A: superimposed Ca^{2+} action potentials recorded from a neuron in normal Ca^{2+} Ringer (a), 10 min after exposure to 0.3 μM paraoxon (b) and 10 min after application of higher concentration of paraoxon (0.6 μM) (c). Bar histogram shows the effects of 10 min of exposure to the 0.3 μM and 0.6 μM paraoxon on the mean duration of Ca^{2+} action potentials. B: A graph representing the mean frequency of Ca^{2+} action potentials and mean duration of afterhyperpolarization in control condition, 5 and 10 min after application of 0.3 μM paraoxon and also 5 and 10 min after increasing the concentration of paraoxon to 0.6 μM. ($n=21$) *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. control.

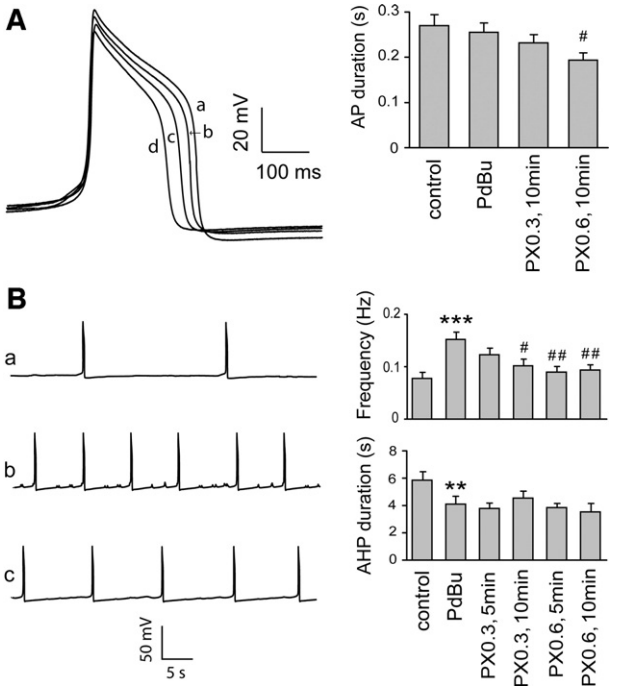

Fig. 2. PdBu attenuated the suppressing effect of paraoxon on the duration of Ca^{2+} action potentials and eliminated paraoxon induced hyperexcitability. A: superimposed Ca^{2+} action potentials recorded from a neuron in control condition (a), 10 min after exposure to 5 μM PdBu and 10 min after application of 0.3 μM paraoxon (c) and 0.6 μM paraoxon (d) in the presence of PdBu. B: Trains of Ca^{2+} action potentials recorded from a neuron in normal Ca^{2+} Ringer (a), 10 min after exposure to PdBu containing Ca^{2+} Ringer (b) and 10 min after application of paraoxon to PdBu containing Ringer (c). Histograms represent quantitative comparisons on the mean duration of Ca^{2+} action potentials, frequency of action potentials and duration of afterhyperpolarization between control condition, 10 min after application of PdBu and in different time point of application of 0.3 μM paraoxon and 0.6 μM paraoxon in the presence of PdBu ($n=11$). **$P<0.01$, ***$P<0.001$ vs. control and #$P<0.05$, ##$P<0.01$ vs. PdBu treated condition.

3.2. Modulations of PKC activity modify the Ca^{2+} action potentials and neuronal activity but it does not contribute to the actions of paraoxon

Involvement of PKC in paraoxon-induced Ca^{2+} action potential suppression and frequency modulation was investigated using activator and inhibitor of PKC and following application of paraoxon. Exposure of neurons to PdBu (5 μM), a potent PKC activator within 10 min of application did not considerably change the duration of Ca^{2+} action potentials. The suppression of Ca^{2+} action potential duration by 0.3 μM paraoxon was not significant in the presence of PdBu, but this effect was significant within 10 min of exposure to 0.6 μM paraoxon by a decrease of 23.92% compared to PdBu treated condition (Fig. 2A). Exposure of neurons to PdBu containing Ringer for 10 min induced 89.2±11.7% increases in the firing

rate. The increase in the frequency in the presence of PdBu was associated with 29.3±9% decrease in the duration of afterhyperpolarization. In the presence of PdBu, paraoxon application did not induce the expected primary hyperexcitability in the neuronal activity but within 10 min of paraoxon application a decrease in the frequency of firing was observed. The silencing effect of paraoxon was intensified by increasing its concentration to 0.6 μM (Fig. 2B).

The finding that PKC activator can induce neuronal hyperactivity and paraoxon is not able to increase the firing rate in the presence of PKC activator suggests that PKC activation might act as mediator for paraoxon induced primary hyperexcitability. According to this hypothesis, paraoxon is not able to increase the firing rate in the presence of PdBu because PKC has been already activated to a point of saturation by PdBu. Other parallel mechanism(s), which are not affected in the presence of PKC activator, could induce the secondary silencing effect. To further examine this hypothesis, in some experiments the neurons were exposed to Ca^{2+} Ringer containing staurosporine, a PKC inhibitor, before paraoxon application. Staurosporine (5 μM) suppressed Ca^{2+} action potentials. The decrease of Ca^{2+} action potentials duration within 15 min of staurosporine application was 32.7±8.6% ($P<0.01$). Paraoxon application at concentration of 0.3 μM and 0.6 μM, respectively, within 10 min induced 41.3±11.3% ($P<0.01$) and 57.4±9.5% ($P<0.001$) decrease in the duration of Ca^{2+} action potentials compared to staurosporine treated condition (Fig. 3A). Staurosporine did not change the firing rate and afterhyperpolarization duration, but paraoxon (0.3 μM) within 5 min caused 36.1±7.8% increase in the frequency of spikes ($P<0.01$), which was associated with 12.7±4% decrease in duration of afterhyperpolarization ($P<0.05$). With longer exposure of neurons to paraoxon (10 min) the firing rate slightly decreased but it was still significantly higher than staurosporine treated condition. This was associated with an increase in the duration of afterhyperpolarization, so that within 10 min of exposure to paraoxon the afterhyperpolarization duration was not significantly different from control condition. Further application of paraoxon (0.6 μM) did not induce more changes in the firing rate and duration of afterhyperpolarization (Fig. 3B).

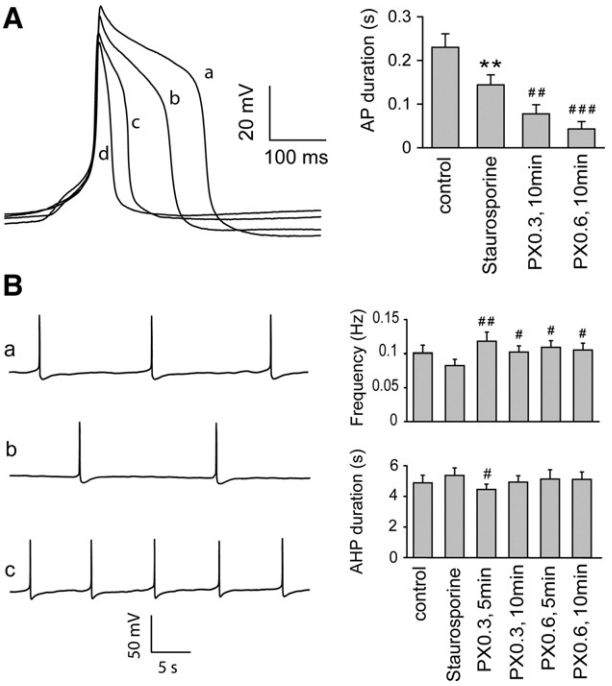

Fig. 3. Staurosporine did not block the suppressing effect of paraoxon on Ca^{2+} duration and paraoxon induced hyperexcitability. A: Staurosporine and paraoxon additively decreased the duration of Ca^{2+} action potentials. Ca^{2+} action potentials recorded in control condition (a), 15 min after exposure to 5 μM staurosporine (b) and 10 min after application of 0.3 μM paraoxon (c) and 0.6 μM paraoxon (d). B: Trains of Ca^{2+} action potentials recorded in control condition (a), 15 min after exposure to staurosporine (b) and 10 min after application of 0.3 μM paraoxon (c). Bar histograms show the mean duration of Ca^{2+} action potentials, frequency of firing and duration of afterhyperpolarization in control condition, 15 min after application of staurosporine and in different time points of application of 0.3 μM paraoxon and 0.6 μM paraoxon in the presence of staurosporine ($n=8$). #$P<0.05$, ##$P<0.01$ vs. staurosporine treated condition.

3.3. Antagonist of IP_3 receptor-mediated intracellular calcium release, TMB-8, eliminates the paraoxon induced secondary increase in afterhyperpolarization duration and neuronal silencing

The secondary increase in the afterhyperpolarization duration represents an increase in the activity of Ca^{2+} dependent K^+ channels and this is not expected by the inhibitory action of paraoxon on Ca^{2+} channels. To examine the possible involvement of Ca^{2+} mobilization from intracellular stores in paraoxon-induced secondary augmentation of afterhyperpolarization, some experiments were carried out in the presence of TMB-8, an inhibitor of IP_3 –assisted Ca^{2+}-induced Ca^{2+} release (IP_3 –assisted CICR). Application of TMB-8 (100 μM) for 20 min did not change the duration of Ca^{2+} action potentials but paraoxon significantly suppressed them in the presence of TMB-8. The decrease in the duration of Ca^{2+} action potentials within 10 min of exposure to 0.3 μM and 0.6 μM paraoxon was 40.9±11.7% ($P<0.01$) and 58.3±7.2% ($P<0.001$), respectively (Fig. 4A). Paraoxon also increased the frequency of firing in the presence of TMB-8, while TMB-8 did not change the firing rate per se. In the presence of TMB-8, the paraoxon induced increase in the firing rate was growing both by time and dose (Fig. 4B). Under this condition, the secondary neural silencing and increase in afterhyperpolarization duration were not observed. Within 10 min of paraoxon application at 0.3 μM and 0.6 μM concentration, the frequency of firing showed 98.3±16.4% and 123.6±13.8%($P<0.001$) increase compared to TMB-8 treated condition. The paraoxon induced hyperexcitability in the presence of TMB-8 was along with an ongoing decrease in the duration afterhyperpolarization. In the presence of TMB-8, the decrease in the duration of afterhyperpolarization within 10 min of exposure of the neurons to 0.3 μM and 0.6 μM paraoxon was 38.1±10.8% ($P<0.01$) and 60.7±8.4% ($P<0.001$), respectively (Fig. 4B). In a separate group of experiments, contribution of cholinergic receptors to the secondary increase in afterhyperpolarization duration was examined. Pretreatment of the ganglia for at least 30 min with atropine (5 μM), a muscarinic antagonist, did not prevent the excitatory effect of paraoxon. Paraoxon (0.3 μM)

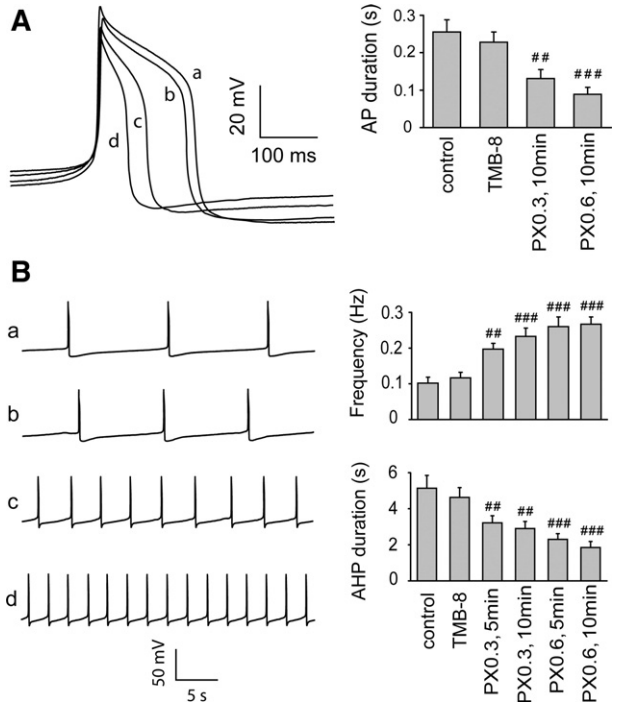

Fig. 4. TMB-8 did not change the effect of paraoxon on the duration of Ca^{2+} action potentials but eliminated the secondary silencing effect of paraoxon and associated increase in the duration of afterhyperpolarization. A: Superimposed Ca^{2+} action potentials from a neuron in control condition (a), 20 min after incubation in TMB-8 containing Ringer (b) and 10 min after application of 0.3 μM paraoxon (c) and 0.6 μM paraoxon (d) to the TMB-8 containing Ringer. Trains of Ca^{2+} action potentials recorded in control condition (a), 20 min after exposure to TMB-8 (b) and 10 min after application of 0.3 μM paraoxon (c) and 0.6 μM paraoxon (d) in the presence of TMB-8. Histograms show the mean duration of Ca^{2+} action potentials, frequency of spikes and duration of afterhyperpolarization in control condition, 20 min after application of TMB-8 and in different time points of application of 0.3 μM paraoxon and 0.6 μM paraoxon in the presence of TMB-8 ($n=10$). ##$P<0.01$, ###$P<0.001$ vs. TMB-8 treated condition.

in the presence of cholinergic antagonist induced 41.7±11.4% increase in the firing rate within 5 min of application. Following to this excitation there was a decrease in the frequency of spikes that often led to cessation of firing within 10–15 min but this effect was not associated with an increase in the duration of afterhyperpolarization (data not shown).

4. Discussion

The present study demonstrated that the modulation of neuronal activity by paraoxon is not mediated by PKC. On the other hand, a slow activating IP_3-dependent Ca^{2+} release seems intimately correlated with the secondary neural silencing effect that is observed after primary paraoxon induced hyperexcitability. The relevance of the interplay of the PKC and IP_3-induced Ca^{2+} release and low concentration of paraoxon for the modulation of Ca^{2+} action potentials and spontaneous activity of snail neurons is discussed.

PdBu did not significantly change the duration of Ca^{2+} action potentials. In the presence of PKC activator, application of paraoxon caused a reduction in the duration of Ca^{2+} action potentials, but in a lesser extent than with paraoxon alone. The reduction in the duration of Ca^{2+} action potentials within 10 min of exposure to 0.3 and 0.6 μM paraoxon alone was 35% and 65%, respectively. However in the presence of PdBu the decrease in the duration of Ca^{2+} action potential by these concentrations of paraoxon was 9% and 26%, respectively. This finding shows that the activation of PKC attenuates the suppressing effect of paraoxon on the duration of Ca^{2+} action potentials. The effect of PKC on Ca^{2+} currents has been reported in different neurons (Conn et al., 1989a,b; Catterall, 2000). In F_1 neurons of *Helix aspersa* has been shown that catalytic subunit of PKC reverse the rundown of Ca^{2+} current that happens in the dialyzed neuron under whole cell clamp, while the blockade of protein phosphates increase the Ca^{2+} current (Golowasch et al., 1995). The increment in the Ca^{2+} current in the presence of activators of PKC has been attributed to the modulatory effect of PKC on Ca^{2+} channel by phosphorylation that increases their conductance (Hill, 2001). Since the suppressing effect of paraoxon on the duration of Ca^{2+} action potentials happened in the presence of PKC inhibitor, staurosporine, it does not necessarily involve the modulation of PKC activity. The effect of PKC on the attenuation of paraoxon induced Ca^{2+} action potential suppression seems to happen as a physiological antagonism.

Staurosporine increased the frequency of Ca^{2+} action potentials along with a decrease in the duration of afterhyperpolarization. The excitatory effect of PKC activators on activity of different neurons, including snail neurons, have already been reported (Hill, 2001; Conn et al., 1989a,b; Zhang et al., 2001). Some neurotransmitters (e.g. acetylcholine) decrease the action potential frequency adaptation via metabotropic receptors in hippocampal neurons. This process involves attenuation of the SK channel mediated current, which in turn suppresses sI_{AHP}. As a result, afterhyperpolarization is sharply reduced and causes the cell to fire repetitively. The cholinergic suppression of sI_{AHP} involves a G-protein-mediated protein phosphatase (Krause and Pedarzani, 2000; Hill, 2001). Previous studies have reported the mechanisms by which the activation of PKC can facilitate membrane excitability. Exposure of hippocampal neurons to phorbol 12,13-diacetate increases the number of action potentials evoked by a constant depolarizing current and also suppresses the slow afterhyperpolarization, suggesting that this phorbol ester inhibited potassium currents in CA_1 neurons (Baraban et al., 1985). The inhibition of K^+ currents by PdBu has been reported in rat sensory neurons and snail photoreceptors (Etcheberrigaray et al., 1992; Zhang et al., 2001). Our findings of the increase in afterhyperpolarization duration and decrease in firing rate after exposure to staurosporine could be resulted from suppression in the basal level of PKC activation. We found that paraoxon can not increase the frequency of action potentials in the presence of PdBu. The inhibitory effect of PdBu on paraoxon induced neuronal excitability raise the question that whether PKC could be a mediator whereby paraoxon induces the neural excitation, so that a saturate activation of PKC by PdBu masks the effect of paraoxon. However, our finding of excitability induction by paraoxon in the presence of staurosporine, refuted such a mechanism.

The lack of secondary increase in the duration of afterhyperpolarization and decrease in firing rate in the presence of TMB-8 indicated that IP_3-induced Ca^{2+} release contributes to the

secondary silencing effect of paraoxon. Ca^{2+} mobilization from intracellular Ca^{2+} stores can serve as a negative feedback on the neural firing through activation of Ca^{2+}-dependent K^+ currents (Yamamoto et al., 2002). It has been reported that paraoxon increases the intracellular free Ca^{2+} in human parotid cell-line (Sun et al., 2000) and neuroblastoma cell-line (Hong et al., 2003). Katz and Marquis (1992) reported that exposure of neuroblastoma cell line to ultra low concentration (0.1 nM) of paraoxon increases IP_3 in a time dependent manner. This effect was partially antagonized by muscarinic antagonists but completely blocked by neomycin, a phospholipase inhibitor, suggesting a direct interaction between paraoxon and phospholipase (Katz and Marquis, 1992 Sun et al. (2000) showed that elevated basal cytosolic free Ca^{2+} in parotid cells following to chronic exposure to paraoxon (0.1–1 nM) is derived from the interaction with IP_3 receptor rather than with muscarinic receptors (Sun et al., 2000). Here, the secondary afterhyperpolarization augmentation was eliminated after pretreatment with atropine. This finding suggests that the interaction of paraoxon with muscarinic receptors and Ca^{2+} mobilization from IP_3-sensitive Ca^{2+} stores via activation of PLC could be the cellular basis for secondary increase in duration of afterhyperpolarization. Involvement of phosphatidyl inositol metabolism has been reported in organophosphate-induced convulsions. It is thought that the enhancement of IP_3 by some organophosphates could be partially responsible for organophosphate-induced neural damages (Bodjarian et al., 1993, 1995; Mobley, 1990). Soman intoxication is associated with a biphasic enhancement of IP_3 level in rat hippocampal neurons. The primary build-up of IP_3 happens within 10 min of the onset of soman induced seizures, which is mainly coupled to activation of muscarinic receptors. However, histamine H_1 subtype and glutamate metabotropic receptors are also involved in the later second step of IP_3 accumulation (Bodjarian et al., 1993, 1995).

The IP_3 induced Ca^{2+} release has been reported in snail neurons (Belan et al., 1990). Our study supports the assumption that muscarinic receptor system linked to inositol phosphate system signaling pathway is most likely to be involved in the secondary silencing effect of paraoxon in snail neurons. The participation of phosphoinositide hydrolysis system seems to be rather common in organophosphate intoxication (Savolainen and Hirvonen, 1992; Bodjarian et al., 1993, 1995). The precise pathway and mechanism that underlies contribution of this system to neural activity modulation by paraoxon, remains to be elucidated. This system might be particularly important in clinical consequences that develop following to the long time exposure to low doses of organophosphates, e.g. Persian Golf war syndrome.

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