Indian J Physiol Pharmacol 2008; 52 (2) : 157–163

# *PTYCHODISCUS BREVIS* TOXIN DECREASES THE SPONTANEOUS ACTIVITY OF RAT RIGHT ATRIA INVOLVING MUSCARINIC RECEPTORS AND POTASSIUM CHANNELS

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**( Received on July 15, 2007 )**

**Abstract :** Marine dinoflagellate *Ptychodiscus brevis* toxin (PbTx), is known to produce toxic effects on cardiovascular system. The present experiments were conducted to evaluate the effect of synthetic phosphorus containing *Ptychodiscus brevis* toxin on spontaneously beating right atrium *in vitro*. The PbTx (0.84-84 µM) decreased the rate and force of right atrial contractions in a concentration-dependent manner. Ethanol, a vehicle present in highest concentration of PbTx, had no effect on atrial rate or force of contraction. Pretreatment with atropine blocked the PbTx-induced decrease in atrial rate and force of contraction. The tetraethylammonium, a potassium channel blocker, blocked the PbTx-induced decrease in atrial rate and force, where as, L-type of calcium channel blocker, nifedipine blocked the PbTx-induced force of contraction but not the rate changes. The results indicate that the PbTx decreased the atrial rate and force of contraction via cholinergic receptors involving $K^+$ channel.

**Key words :** brevetoxin PbTx right atrial potential nifedipine TEA

## INTRODUCTION

Brevetoxins are lipid soluble cyclic polyether neurotoxins produced by marine dinoflagellate (*Ptychodiscus brevis* toxin; PbTx), an organism linked to periodic “red tide” blooms in the Gulf of Mexico, Florida and Indian seacoast (1, 2). These toxins are liberated into water column during blooms conditions (1, 3) and pose serious health problems to man or livestock (1). PbTx produce deleterious effects on various systems such as respiratory, gastro-intestinal, nervous and cardiovascular systems (4–11). The cardiovascular abnormalities such as bradycardia, hypotension, conduction blockade have been reported along with respiratory abnormalities (apnea followed by hyperapnea) in cats *in vivo* (6). The *in vivo* bradycardia can be due

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to the direct effect of the toxin on the pacemaker cells or indirectly by altering the cardio-respiratory reflexes as shown for scorpion venom (12, 13). In our earlier report, we have shown that PbTx did not involve Bezold–Jarisch reflex/J-reflexes (12). Thus, the effect could be due to the direct action of the toxin on the atria. It is known that PbTx alters the $Na^+$ channel activity in variety of tissue including the neuronal one (14). In this context it may be mentioned that the pacemaker cells minimally involve $Na^+$ channels either for the prepotential or for the spike potential. Thus, the effects of PbTx unlikely to be due to the alteration of $Na^+$ channel activity of cardiac cells. The pre-potential determines the cardiac rate and is produced by $I_K$ decay followed by the opening of transient $Ca^{2+}$ channels. The direct effect of PbTx on cardiac pacemaker cells is not known. Therefore, this study was undertaken to examine the effect of PbTx induced changes on spontaneously beating right atrium and to delineate the underlying mechanisms.

## MATERIAL AND METHODS

All the experiments were performed according to the guidelines of the Institute of Medical Sciences, Banaras Hindu University, Varanasi for conducting the animal experiments. Care was taken to restrict the number of animals to the minimum possible. The detailed description of the methods for recording atrial contractions *in vitro* from albino rat has been described earlier (15, 16).

### *Isolation of atrial preparation and recording of the contractions*

Adult male albino rats (Charles Foster strain) were sacrificed by cervical dislocation and exsanguination. The thorax was opened; heart was carefully dissected out and placed in a petridish containing chilled Krebs Ringer solution bubbled with 100% $O_2$. The right atrium was carefully separated from the rest of the heart and mounted vertically by securing one end to a glass tube placed in an organ bath (volume = 10 ml) containing Krebs Ringer kept at 28±1°C and bubbled continuously with 100% $O_2$. The other end of the atrium was fastened firmly to a force displacement transducer by a fine thread. The atrial preparation was then given resting tension of 0.1 g and was allowed to equilibrate for 30 min before making the control recordings. The isometric contractions were recorded on a chart recorder after stabilization. The Krebs Ringer solution was changed at every 15-min intervals unless mentioned otherwise.

### *Experimental Protocol*

After recording the initial contractions the tissue was exposed to cumulative concentrations of PbTx (0.84–84 µM) allowing 20 min at each concentration. At the end the effect of PbTx was washed with normal physiological solution for 30 min. In case of antagonists, the tissue was pretreated with atropine (0.3 µM) or nifedipine (1.0 µM) or tetraethylammonium (10 mM) for 20 min and the recording was made, subsequently exposed to different concentrations of PbTx as above.

### *Drugs and solutions*

Krebs Ringer solution had the following composition (mM): NaCl, 137; KCl, 2.68; $CaCl_2$, $2H_2O$, 1.8; $MgCl_2$, $6H_2O$, 0.88;

$NaH_2PO_4$, $2H_2O$, 0.36; $NaHCO_3$, 7 and glucose 11. Synthetic PbTx (O, O-Diphenyl-N-Cyclooctylphosphoramldate, Mol. Wt. 359) was obtained from Defence Research and Development Establishment, Gwalior, India. Atropine sulfate, tetraethylammonium chloride, and nifedipine were obtained from Sigma chemical company, St. Louis, MO, USA. The stock solution of PbTx was prepared in absolute ethanol and other drugs were prepared in distilled water. The final dilutions of all the drugs were made in Krebs Ringer. All the stock solutions ($10^{-2}$ M) were stored in a freezer and thawed just before use.

*Statistical analysis*

The force of atrial contractions was expressed as percentage of initial response and was pooled to obtain mean±S.E.M. values. Differences between various groups were compared by using one-way or two-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test. Student's *t*-test was also performed as required. A $P<0.05$ was considered significant.

## RESULTS

*PbTx produced a concentration-dependent decrease of rate and force*

PbTx (0.84–84 µM) decreased the rate and force of spontaneously beating rat right atrium in a concentration-dependent manner (Fig. 1, $P<0.05$, one way ANOVA). At 0.84 µM of PbTx, there was no decrease in rate and force of contraction. At 2.8 µM concentration of PbTx rate and force were decreased by 17%. At 84 µM, the rate and force were decreased by 43%. The inhibitory concentration to produce 50% of maximal depression ($IC_{50}$) for the PbTx was around 4.0 µM. Ethanol (vehicle used for PbTx; 10 µL/ml) had no effect on the rate or force (97.8±8.3 and 95±9.4% of initial) of atrial contractions.

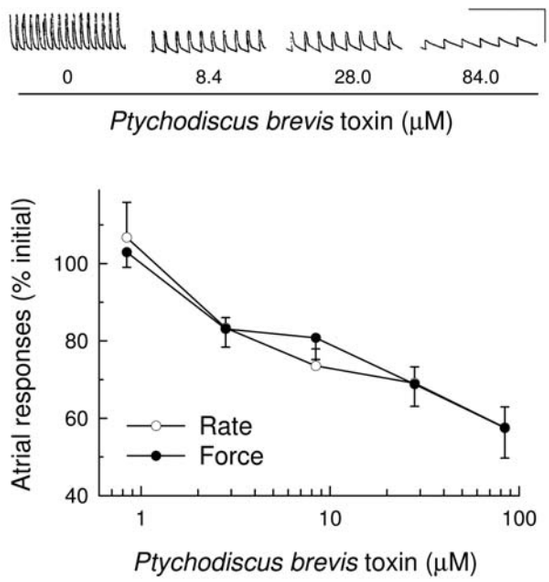


Fig. 1: *Ptychodiscus brevis* (PbTx; 0.84–84 µM) decreased atrial rate and force in a concentration-dependent manner. Cumulative concentration-response of PbTx was obtained after exposing the atria to a concentration for 20 min. In the upper panel, the original tracings of an experiment are shown to depict the PbTx-induced decrease in rate and force at different concentrations. Horizontal line = 5 sec and vertical line = 0.1 g. In the lower graph, the mean±S.E.M values of pooled observations obtained from different experiments (n = 9). The decreases at various concentrations were significantly different ($P<0.05$; one way ANOVA).

*Atropine blocked the PbTx induced depression*

Atropine (0.3 µM) pre-treatment *per se* decreased the rate by 15±2.5% and force by 31±15.2% of the initial, respectively. In the

presence of the atropine, the PbTx (2.8–84 μM)-induced decrease of atrial rate and force of contraction were significantly blocked ($P<0.05$, Two way ANOVA followed by Student-Newman-Keuls test, Fig. 2). The blockade of force changes was complete (Fig. 2).

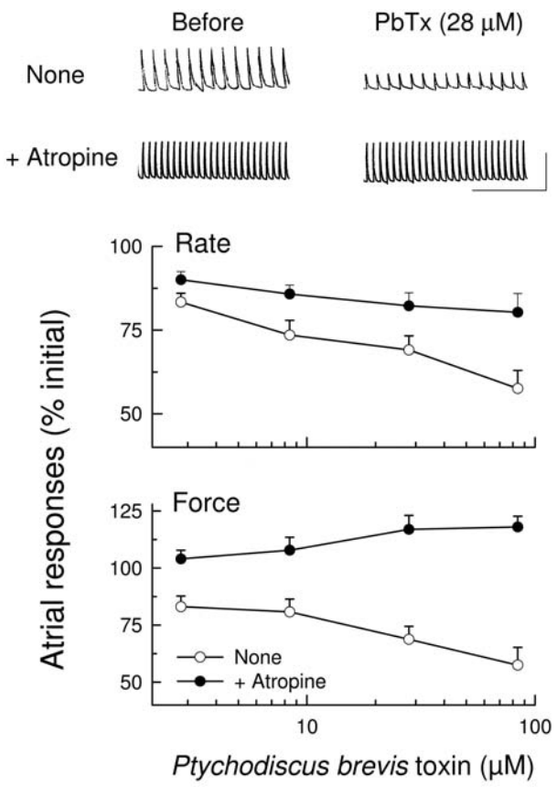


Fig. 2 : Atropine (0.3 μM) blocked the PbTx-induced changes in rate as well as force. The original tracings of atrial contractions before (20 min after exposure to atropine/saline) and after 28 μM of PbTx are presented in the upper panel. Horizontal line = 5 sec and vertical line = 0.1 g. The mean±S.E.M values as percentage of initial were obtained from 6 different experiments. The values are significantly different from one another ($P<0.05$; two way ANOVA followed by Student-Newman-Keuls test).

*Tetraethyl ammonium antagonized the PbTx-induced changes*

TEA (10 mM), a potassium channel blocker, itself did not alter either atrial rate (97.8±5.7% of initial) or force of contraction (109±4.8% of the initial). However, in the presence of TEA, the PbTx-induced decrease in atrial rate and the force were attenuated significantly (Fig. 3).

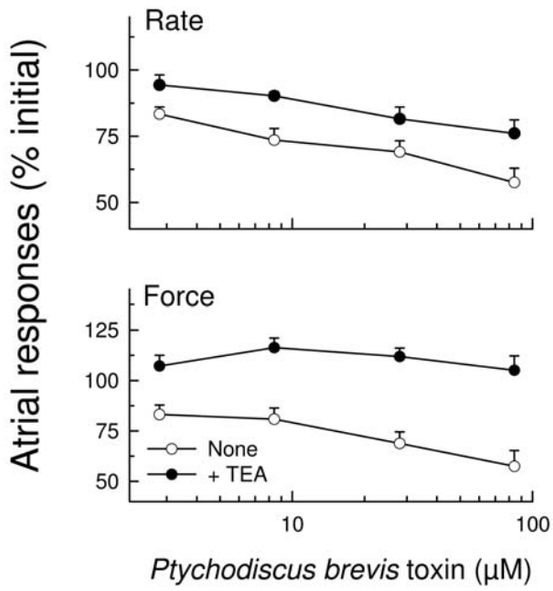


Fig. 3 : Tetraethylammonium, (TEA, 10 mM) blocked the PbTx-induced decrease in atrial rate and force of contractions. The mean±S.E.M were obtained from 7 different experiments. The values after TEA are significantly different from the control ($P<0.05$; two way ANOVA followed by Student-Newman-Keuls test).

*Nifedipine antagonized the PbTx-induced changes*

Nifedipine (1.0 μM) by itself decreased the atrial rate and force of contraction by 17% and 13%, respectively. Nifedipine did

not alter the PbTx-induced decrease in atrial rate but blocked the force of contraction produced by the toxin significantly (Fig. 4).

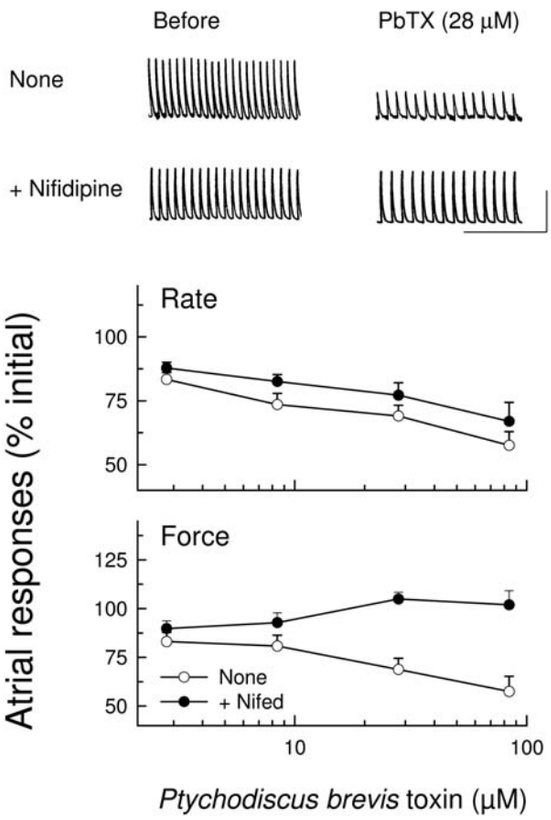


Fig. 4 : Nifedipine (Nifed, 1.0 µM) blocked the force changes but not the atrial rate changes induced by PbTx. The original tracings of atrial contractions before (20 min after exposure to nifedipine/saline) and after 28 µM of PbTx are presented in the upper panels. Horizontal line = 5 sec and vertical line = 0.1 g. In the lower graph, the mean±S.E.M values from 7 different experiments are depicted. The force changes induced by PbTx are significantly different from the control ($P<0.05$; two way ANOVA followed by Student-Newman-Keuls test).

## DISCUSSION

Our results demonstrate that PbTx induced decrease in rate and force of contraction involves muscarinic receptors, as evidenced by the blockade with atropine. The atrial rate is determined by the pace maker potential of SA node, which is generated by the decrease in $K^+$ conductance followed by increase in $Ca^{2+}$ conductance. SA node has very rich cholinergic nerve plexuses. Binding of ACh to muscarinic receptors (G-protein coupled receptors) modulates inwardly rectifying potassium channels ($GIRK/K_{Ach}$). This in turn increases the $K^+$ conductance and bring about hyperpolarization of atrial pace maker cells (17, 18). This decreases the slope of prepotential or diastolic depolarisation to produce negative chronotropic effect (17, 19, 20). The $Na^+$ channels minimally participate in the generation of pace maker potential or in the action potentials of cardiac pace maker cells. On the other hand, PbTx is known to activate voltage gated $Na^+$ channels in neuronal tissue and induce $Ca^{2+}$ influx (21). Therefore, PbTx is likely to produce its action on cholinergic nerve plexuses present in the atrial tissue. Thus, it appears that PbTx stimulates the cholinergic nerve plexus by activating $Na^+$ channels to release ACh through $Ca^{2+}$ influx as suggested before (21). ACh thus released decreases rate and force through its action on muscarinic receptors. Since there is no increase in rate or force of atrial contractions after PbTx, it is presumable that the contribution from adrenergic nerves is minimal.

Further, our findings with TEA indicates the involvement of $K^+$ channel in mediating the effect. TEA is not specific $K_{Ach}$ channel blocker but blocks majority of $K^+$ channels including $K_{Ach}$ channel (22, 23). Therefore, it is clearly evident that the effect of PbTx is mediated through the release of ACh from cholinergic nerve plexuses of atria involving $K^+$ channel.

The ability of atropine and TEA to block the force changes indicates the involvement of muscarinic receptor-dependent actions. The activation of Gi by muscarinic receptor agonists has been demonstrated in rat atria (16). The Gi in turn activate G-cyclase and enhance the cGMP production. The cGMP is known to suppress cAMP activity. The cAMP modulates $Ca^{2+}$ channel activity and brings about force related changes (24, 25). Thus in our study, suppression of cAMP activity is a possibility. This is supported by our observation with nifedipine. Further, it is to be noted that L-type of calcium channels are not involved in rate changes.

In conclusion, PbTx by activating cholinergic plexuses mediate the negative inotropic and chronotropic actions involving muscarinic receptor-dependant $K^+$ channels. However, further studies are required to explore the detailed mechanisms underlying the calcium channels in mediating PbTx induced force changes.

## ACKNOWLEDGMENTS

The authors wish to thank Director, Defence Research and Development Establishment, Gwalior, India for providing PbTx.

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