

ORIGINAL ARTICLE

EFFECTS OF VARIOUS PHARMACOLOGICAL AGENTS ON EXPOSED HEART OF *UROMASTIX HARDWICKII*

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Background: The pharmacological and physiological studies on cardiac activity of reptiles specifically of *Uromastix hardwickii* are scarcely available in literature, as well as the effects of parasympathetic and sympathetic agonists together are also not available. Therefore, the mechanical and electrophysiological effects of pharmacological agents, like Physostigmine and its effects before and after Adrenaline administration were observed on the exposed and intact heart of a reptile, *Uromastix hardwickii*. **Method:** To work on exposed heart of *Uromastix hardwickii*, Physostigmine and Adrenaline were prepared by dissolving 0.01 gm in 10 ml of distilled water. Oscillograph was used to record the mechanical and electrical activity of intact heart through isotonic transducer. **Result:** Physostigmine was found to produce significant effect on Systolic Force (SF), Duration of cardiac cycle (DCC) and Duration of Phase 4 (DP4). Significant effect of Physostigmine was also observed on heart rate (HR) before Adrenaline administration and on DP4 after Adrenaline administration. **Conclusion:** It was confirmed that Physostigmine does not exhibit its normal effect on Amplitude of Action Potential, cardiac cycle (CC), Duration of action potential (DAP), Plateau Duration and DP4. Physostigmine is affecting the cardiac activity of this reptile without inhibiting the cholinesterase and not accumulating the Acetylcholine. It modulates the effects of Adrenaline when used before the administration of Adrenaline.

Keywords: Physostigmine, Adrenaline, Oscillograph, Systolic Force, Duration of cardiac cycle, Duration of Phase 4, Heart rate, Amplitude of Action potential, plateau duration, Acetylcholine

INTRODUCTION

Parasympathomimetic drugs can influence cholinergic transmission either by acting on acetylcholine (ACh) receptor or by affecting the release or destruction of ACh. Therefore drugs that act on ACh receptor may:

- 1) Mimic the action of ACh, e.g., cholinergic agonist such as muscarine and nicotine¹
- 2) Block the action of ACh, e.g., cholinergic antagonist such as atropine²

Physostigmine (Phy) is an anticholinesterase³ which increases the activation of both sympathetic and parasympathetic ganglia supplying the heart and at the ACh receptor on the neuro-effector cells. It mimics the action of the vagal nerve activation, negative chronotropic, dromotropic and inotropic effects are produced and a decrease in the cardiac output is observed.⁴

Adrenaline (Adr) acts affectively on both alpha and beta receptors, indicating it has a strong inotropic and chronotropic effect, but also has a mixed peripheral action.⁵ It is a potent inhibitor for the effect of endogenous ACh. The present study has been conducted to investigate the physiological effects of certain Parasympathomimetic and Sympathomimetic agonists on cardiac parameters which are still unexplainable for their normal and reverse effect on lacertilian specie, *Uromastix hardwickii*. Literature is almost silent regarding pharmacological effect on various cardiac parameters. Hence, effect of specific concentration of

Physostigmine before and after administration of adrenaline was used on exposed heart of the lizard.

MATERIAL AND METHODS

Spiny-tailed lizards *Uromastix hardwickii* were used for experiments. The animals were pithed, dissected and thoracic cavity was exposed. The mechanical and electrical activity of intact heart was recorded on oscillograph (Harvard apparatus Ltd. UK, Cat. No. 60-9315). Normal activity of heart was maintained by 'Reptilian buffer' containing 100 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 5 mM Na₂HPO₄, 1.2 mM KH₂PO₄. Dilutions of drugs were prepared by dissolving 0.01 g in 10 ml of distilled water to get particular concentration.

The mechanical activity including Heart Rate (HR), Duration of Cardiac Cycle (DCC) and Systolic Force (SF) of exposed heart of the animal were recorded using isotonic transducer (Harvard apparatus Ltd. UK, Cat. No. 50-6360), while electrical activities like Amplitude of Action Potential (AAP), Duration of Action Potential (DAP), Plateau Duration (PD) and Duration of Phase 4 (DP4) were recorded by fine electrodes before and after infusion of 1 ml of drug.

SPSS-16 was used for the statistical analysis. Mean and standard deviation of all parameters were calculated, $p < 0.05$ was taken as significant.

RESULTS

Physostigmine was found to increase HR, AAP, PD and decrease SF, DP4 and DCC when induced without Adrenaline administration. However, its significant

effects were found only on DCC ($p < 0.025$), SF ($p > 0.05$) and highly significant on DP4 ($p < 0.0005$). No statistical significant effect was observed on HR ($p > 0.05$), AAP ($p > 0.05$), and PD ($p > 0.05$) (Table-1). After Physostigmine administration, adrenaline produced no significant stimulatory effect on HR, PD, and AAP ($p > 0.05$) and highly significant stimulatory effect only on DP4 ($p < 0.005$) however, a non-significant decrease was observed in all other parameters (Table-2).

Physostigmine produced significant increase in the heart rate ($p < 0.005$) when introduced after adrenaline administration (Table-3). Decrease in the heart rate was observed that was non-significant on DCC SF, DAP, AAP ($p > 0.05$) and a non-significant increased effect was observed on DP4 (Figure-3).

Table-1: Comparison of mechanical parameters between control and Physostigmine (1.5 mM) treated exposed heart of *Uromastix hardwickii*

Parameters	Control	Physostigmine	p-value
HR	11.73±1.59 (16)	7.76±0.98 (16)	<0.025
DCC	3.88±0.52 (16)	2.46±0.34 (16)	<0.025
SF	8.69±2.71 (16)	7.06±2.23 (16)	<0.005

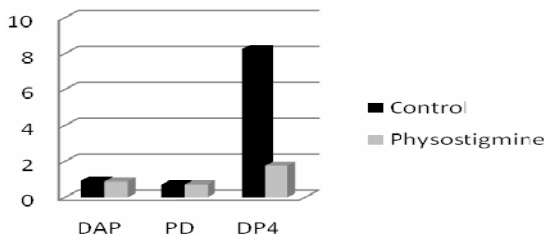


Figure-1: Comparison of electrical parameters between Control and Physostigmine (1.5 mM) treated exposed heart of *Uromastix hardwickii* (sec, mV)

Table-2: Comparison of mechanical parameters between control and Physostigmine (1.5 mM) in presence of adrenaline (3 mM) treated exposed heart of *Uromastix hardwickii*

Parameters	Control	Adrenaline+ Physostigmine	p-value
HR	23.4±2.29 (10)	23.87±2.9 (10)	<0.005
DCC	2.86±0.29 (10)	2.76±0.54 (10)	>0.05
SF	4.2±0.29 (10)	4.15±0.28 (10)	<0.005

Table-3: Comparison of mechanical parameters between control and adrenaline (3 mM) in the presence of Physostigmine (1.54 mM) treated exposed heart of *Uromastix hardwickii*

Parameters	Control	Adrenaline+ Physostigmine	p-value
HR	19.81±2.54 (20)	25.56±2.14 (20)	>0.05
DCC	3.88±0.52 (20)	2.63±0.18 (20)	>0.05
SF	8.68±2.71 (20)	8.115±2.57 (20)	>0.05

All values are Mean±Standard error. Figure in parenthesis represents the number of observations. Adr=Adrenaline, Phy=Physostigmine

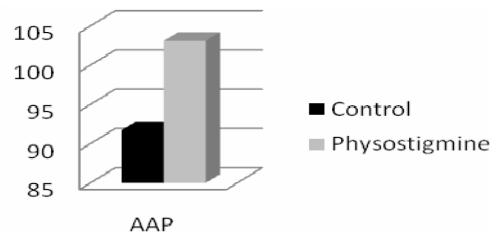


Figure-2: Comparison of electrical parameters between Control and Physostigmine (1.5 mM) in the presence of Adrenaline treated exposed heart of *Uromastix hardwickii* (Sec, mV)

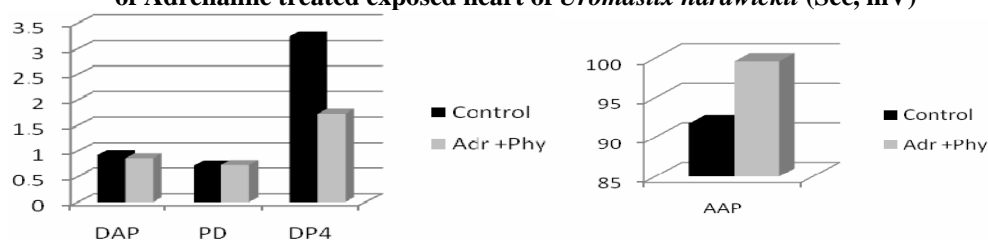


Figure-3: Comparison of electrical parameters between Control and Adrenaline (3 mM) in the presence of Physostigmine (1.5 mM) treated exposed heart of *Uromastix hardwickii*

DISCUSSION

In this study Physostigmine was found to produce negative chronotropic as well as negative inotropic

effect on *Uromastix* heart. Previously, Physostigmine was shown to decrease the rate of degradation of ACh and amplify the effects of parasympathetic nervous activity to reduce heart rate in human⁶ and

mimics the effect of vagal nerve activation and produced negative inotropic effect⁷. Decreased HR has been associated with a simultaneous increase in the duration of cardiac cycle.⁸ But in this study reverse effect has been observed and statistically significant decreased DCC was observed than that of control on Uromastix heart. The cholinergic agonist has been identified earlier to prolong the DAP⁹ and Plateau duration in human.¹⁰⁻¹² In the present study, lower values of DAP and PD than control shows the reverse effect on Uromastix heart. It may be due to the elevation of Ca⁺⁺ as reported by King *et al*¹³ that Physostigmine increases the action potential stimulation and decreases the plateau duration in Retzius cells of leech. Thus, the membrane effects of Physostigmine appear to be independent of any inhibition of cholinesterase or accumulation of ACh on Uromastix heart as in CNS.

Although not statistically significant, Physostigmine modulation of Uromastix's heart produced three well-recognised effects of catecholamine.

1. Negative inotropic effect
2. Prolong Plateau duration of action Potential
3. Increased Amplitude of Action Potential

No studies have been reported yet that assessed directly the interaction between Physostigmine and Adrenaline on other animals. However, according to Watanabe and Besch¹⁴, ACh as cholinergic agonist produces antagonising effect on catecholamine on depolarized fibres. It was further demonstrated that when adrenergic stimulation was applied during simultaneous vagal stimulation, the negative inotropic effects were produced.¹⁵⁻¹⁸ It appears that cholinergic effects on ventricular contractile function are dicholinergic antagonism of adrenergic effect by modulation of sympathetic effects on the ventricles.¹⁸ Among the possible mechanisms for this interaction, ACh released from vagal nerve endings can interact with muscarinic cholinergic receptors on sympathetic nerve endings and thereby inhibit the release of nor-epinephrine from these nerve terminals.¹⁹ According to Watanabe *et al*¹⁴ and Gardner²⁰ muscarinic cholinergic agonists inhibited catecholamine-induced activation of glycogen phosphorylase in isolated heart. However, based on results of previously published studies, several possibilities seem worthy of consideration, suggests that cyclic adenosine 3':5'-monophosphate (cAMP) mediates the effects of catecholamine on the slow inward current.¹⁴ It is likely that the cholinergic modulation of sympathetic effects involves cAMP. It has been shown by several groups of investigators that ACh can attenuate the amount of cAMP generated in response to catecholamines in the heart.^{21,22} Thus, one possible explanation for the observed electrophysiological

interaction is that, in the presence of Physostigmine, less cAMP was generated in the myocardial cells, in response to adrenergic receptor stimulation that is responsible to produce the reverse effect on these three parameters of Uromastix heart. However administration of Physostigmine + Adrenaline on HR, CC, DAP and DP4 produce same effect as produced by Adrenaline. It shows that Physostigmine does not antagonising the effects of Adrenaline on these parameters, may be higher concentration is required to produce antagonising effect on these parameters on Uromastix heart.

Sympathetic stimulation inhibits the release of ACh by acting on β_1 -adrenalineenergetic receptors on vagal terminals.²³ The same antagonistic effects have been observed by sympathetic activity or cholinergic activity on some parameters of Uromastix heart, i.e., modulation by adrenaline of two effects of Physostigmine on Uromastix heart; (1) positive chronotropic effect (2) decreased Amplitude of Action Potential. But these reverse effects were not statistically significant. Possible mechanism for the observed electro physiological interaction may be that, on the administration of Adrenaline, more cAMP was generated in the myocardial cells, that is involved to produce positive chronotropic effect, as reported earlier that simultaneous application of ACh and nor-epinephrine produced a similar biphasic chronotropic effect.²⁴ These procedures produced an initial increase in cyclic guanosine 3':5'-monophosphate (cGMP) and a delayed elevation in cAMP in isolated SA-node of rabbit heart. However, decreased AAP may produced by decreased Na⁺ current inhibited by Adrenaline on Uromastix. While the effect of Adrenaline + Physostigmine on other parameters, i.e., CC, SF, DAP, PD and DP4 were same as observed in the effect of Physostigmine, it shows that Adrenaline is unable to block or antagonizing the action of Physostigmine on these parameters.

CONCLUSION

Physostigmine does not exhibit its normal effect on amplitude of action potential, cardiac cycle, duration of action potential, plateau duration, and duration of Phase 4. Physostigmine affects the cardiac activity of this reptile without inhibiting the cholinesterase and not accumulating acetylcholine, and it modulates the effects of Adrenaline when used before the administration of Adrenaline.

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