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Comparative Analysis of Physiological and Pharmacological Parameters of Vas deferens of Uromastix and Rabbit

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ABSTRACT:

Background: The male reproductive aspect of mammals and lizards is essentially different on the bases of the morphology of their reproductive tract. The lizards are devoid of secretary glands. Even then they have the seminal plasma for the suspension of their sperms. However, in addition to semen plasma, smooth musculature of vas deferens (VD) also helps in providing the driving force for the propulsion of sperm along with their own motility. Apart from differences in reproductive cycle and seasonal effects on lizard's reproduction in comparison to mammals, it seems that there is a probable difference in the physiological and pharmacological properties of the smooth muscles of their VD as well.

Objectives: Therefore, the present study was designed to compare the physiological and pharmacological responses on receptors of VD against acetylcholine (ACh), adrenaline (Adr) and their antagonists in Uromastix (lizard), and Rabbit (mammal).

Methodology: Freshly isolated muscle strips of Uromastix *hardwickii* & Rabbit *Oryctologous cunniculus* were mounted on organ bath assembly and their physiological (mechanical) activity was continuously monitored by adding different pharmacological agonists and antagonists on data acquisition system, Power-lab.

Results: Results demonstrated that in Uromastix *hardwickii* the basal tone of VD was decreased by the administration of both the acetylcholine (Ach)&adrenaline (Adr) without the appearance of rhythmicity and their actions were significantly antagonized by Atropine (Atr) and Atenolol (Ate), respectively. While, the basal tone in Rabbit VD was increased by both the ACh & Adr, which later followed by the appearance of spontaneous rhythmic contraction. This preliminary study on the smooth muscles of VD in Uromastix highlights the differences in the physiological activity and the presence of adrenergic and cholinergic receptors.

Key words: Vas deferens, Acetylcholine, Adrenaline. Atropine, Uromastix, rabbit

INTRODUCTION:

Uromastix *hardwickii* reptile, found in deserted area of Sind. Like other species they

live in groups of several individuals have over 20 years of wild, reach sexual maturity around four years old and lay eggs between 10 to 40 eggs per year depending on the individual size and species (Zug, 1993; Highfield and Slimani, 1998; Bouskila and Amitai, 2001, Nemtoz, 2008). Regarding ecology, behavior, physiology and pharmacology little is known about the genus Uromastix *hardwickii*. The present study is on pharmacology of male organ, vas defrentia of Uromastix. Vas deferens is a simple convoluted tubule, one of the accessory male genital organs that develop from Wolfian duct (Shanbhag 2002). There is great variability seen in sexual maturity of several species of Uromastix that too depend on seasonal pattern (González-Espinoza, *et al*, 2017; Fox 1958). Studies on VD are generally made because of an interest in the diversity of the neurotransmitters and co-transmitter involved that mediate contractions (Kuriyama *et al*., 1998; Koslov and Andersson, 2013).The existence of VD receptors have been investigated in many species (Guinea pig, Rat, Mouse, Rabbit, Dog and Human) using histo-chemical, electron microscopic, and pharmacological procedures, leading to prediction about the distribution of adrenergic and cholinergic terminals and receptors. (Burnstock, and Verkhrastsky, 2010; Klingeand and Sjostrand,1994; Steinmeyer*et al*., 1991).

VD, first describe by Hukovic in 1961,is non-rhythmically active smooth muscle, innervated by extrinsic nerve supply from central nervous system (Burnstock and Verkhrastsky, 2010). The nerve ending secrete ACh or Noradrenaline (NA) to cause depolarization of the smooth muscle membrane & elicit the contraction. However, action potential often does not develop because the fibers are too small to generate action potential (Guyton and Hall, 2015). The general structure and function of the VD show several differences among different animals. In reptile *C.versicolor* the outer layer of smooth muscle has luminar trabeculae with psedostratified epithelium and during breeding season the terminal part of VD become swollen (Shanbhag 2002). Rodent VD muscles are arranged in three layers, outer & inner longitudinal layers with circular muscle layer in between. The relative thickness of these layers varies from species to species and from one end to the other. The muscle cells are electrically coupled wave of depolarization to travel from one cell to other (Dixon *et al.*, 1998; Kuriyama*et al*., 1998; Kaleczyc, 1998; Westfall and Westfall, 2001; Burnstock and Verkhratsky, 2010). It is generally accepted that in the mammalian VD, adrenergic innervations are the most common among the nerve fiber groups supplying the tissues. Connaughton and Docherty (1990) reported dense innervations of adrenergic nerves with α_1-adrenoceptors located postjunctional & α_2 adrenoceptors present predominantly on pre junctional nerve terminal in mammals . Similarly Stjarne *et al.* (1995) and Knight *et al.* (2001) revealed that NA produces slow & small depolarization via the α_2-adrenoceptor. It is

also evident from different studies that adrenergic neuron supplying the mammalian VD contains ATP and NPYas a co-transmitter with NA and they act cooperatively during excitatory transmission in sympathetic nerves (Koslov and Andersson, 2013). Studies on VD also suggested that the excitatory responses in human and dog VD were blocked by α-adrenergic blocking agents (Wagner and Sjostrand, 1988; Koslov and Andersson, 2013). However contradictory to α–adrenoceptors, activation of β-adrenoceptors in the Guinea pig VD was found to hyperpolarized smooth muscle cell membranes, via the synthesis of cAMP (Bulbring and Tomita, 1987), β-adrenergic stimulation can also be evoked by ATP (Todorov *et al*., 2001). In a electrophysiolgical study Harhum, *et al* (2003) characterize two types of voltage gated K currents in epididymal part of rat VD.

Cholinergic innervations have also been identified in the VD of various species.High concentrations of nicotinic agonists produced depolarization on guinea pig VD, by activating the post junctional M_2 receptors (Fukushi and Wakui., 1986; 1987). While the M_1 receptor are distributed on adrenergic pre junctional nerve terminals of human where they suggested to be responsible for the negatively control of release of NA and thus mediate contractile responses (Fong and Jentsch, 1995; Miranda *et al.,* 1992). However, the action of exogenous ACh on smooth muscle cells of VD was weaker than NA and ATP (Wakui and Inomata, 1985a & b; Arver and Sjostrand, 1982) but ACh release from cholinergic nerve terminals can be enhanced when prejunctional nicotinic receptors were activated (Alina*et al.,* 2005). Thus it seems that cholinergic nerve fibers supplying the mammalian vas deferens are fewer than the adrenergic ones (Kaleczyc,1998; Burnstock and Verkhratsky, 2010; Koslov and Andersson, 2013).

In view of such a complex musculature our present study was design to identify the presence of cholinoceptors & adrenoceptors of VD of Uromastix *hardwickii*. Further, the pharmacological studies on Uromastixhardwickii are yet not available. The attempt has been made to identify the responses of Acetylcholine (ACh) & Adrenaline (Adr) with their antagonist on VD of Uromastix and compare the effect with VD of Rabbit.

MATERIALS & METHOD

Animals: Freshly obtained adult male *Uromastix hardwickii* & Rabbits *Oryctologus Cunniculus* were used in all the experiments. Their handling, captivity & dissection were according to the international ethics for laboratory animals.

Tissue Preparation: The procedure for handling, dissection and isolation of smooth muscle tissue strips was approved by institutional ethics committee and describe earlier (Arifa et al, 2014; 2004; 2000; 1997 a & b; 1995; Azeem, *et al* 1999) with slight modification for VD

strips. Briefly after decapitation, the abdomen was opened immediately by making a longitudinal cut from abdominal to pelvic cavity. Gut was lifted on one side so that VD was easily seen as it was a narrow convoluted duct lies posterior from the epididymis close to the testes. It was then separated out just below the epididymis up to the urethra, emptied out from any semen by gently pressing it with cotton swab or finger without stretching. Strips from Uromastix and rabbits were placed in reptilian buffer (**Azeem & Shaikh (2006), Farooq *et al.* (2007)** and Kreb's Buffer (Azeem *et al* 1999; Arifa et al., 2014; 2000) respectively. However, at room temperature VD of Uromastix & Rabbit survives well without oxygen & there was no rush to get the tissues to an oxygenated bath. A perfectly isolated VD measures about 1 to 1.5 inches. Strips were mounted in gut tube associated with organ bath assembly (Bioscience; 61300) which was also equipped with thermostat, glass coil, and oxygen tube. The distal part of preparation was fixed between oxygen tube & force transducer. Transducer was connected with data acquisition system, Powerlab (AD instruments ML825) and its associated software Chart.

Mechanical recording: For mechanical recording the preparation was allowed to stabilize for 30 minutes in the gut tube. There was continuous oxygen bubbling & 37°C temperature was maintained for Rabbit VD, while Uromastix recording were made at room temperature without oxygen bubbling because of cold blooded animal. After stabilization a record was obtained before and after drug administration at slow speed.

Data analysis:

The graphical records obtained from Powerlab were used for the calculations of graded contraction & time of graded response to peak. For statistical analysis average & standard error were calculated and comparison of data was done by using student's t-test.

Drugs:

Following drugs were used in these experiments; Acetylcholine (Riedel-de Haen AG D-3016), Adrenaline (BDH Ltd. England, 27053), Atropine (E-Merck-Darmstadt) and Atenolol (ZAFA Ltd. Regn. No.009727,Mfg Lic. No 000513).

RESULTS:

Effects of Acetylcholine & Adrenaline and their antagonist on Uromastix *hardwickii:*

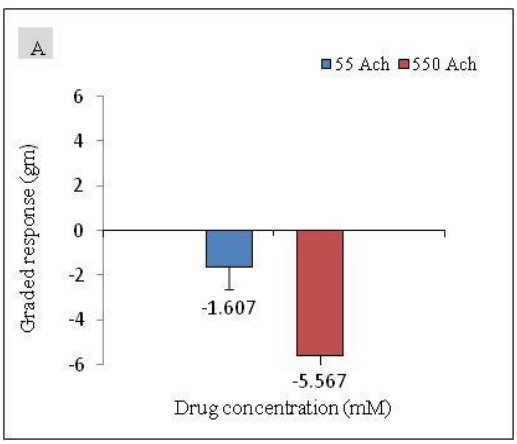
In order to establish resting length all slacks were removed from the preparation and set at zero tension. The preparation of Uromastix had produced no change/response on resting length even when used ACh in concentration below 55mM. However, it does exhibited decrease in basal tone on administration of high concentration of ACh i.e., 55mM &550mM (Fig. 1A). The comparison between the two concentration i.e., 55 & 550 mM demonstrated

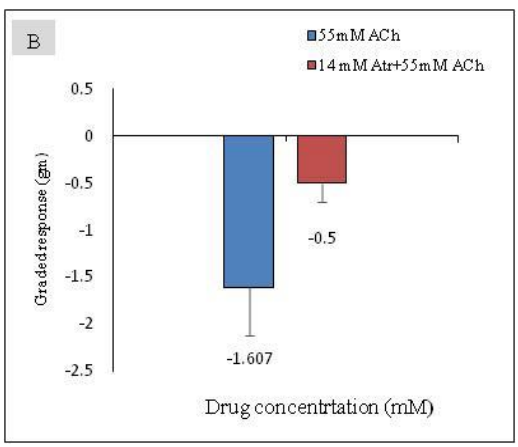
highly significant differences (P<0.0005). Moreover, the duration to achieve the peak response was increased significantly (P<0.025) by 91% by administration of 550mM of ACh when compared to 55mM of ACh (Fig. 1B).When the effects of cholinergic antagonist atropine was tested and applied before the administration of 55mM ACh, it showed 69% lesser response as compared to ACh alone (Fig. 1B, Fig. 1E). Statistically this difference was also significant (P<0.05). While the duration of peak response was about 76% lesser when obtained in the presence of Atr than observed with ACh alone & that gave significant (P<0.025) difference on statistical comparison (Fig.1B, Fig.1E).

The result regarding the administration of 30mM Adr also showed decrease in graded response similar to ACh response obtained by higher concentration (55 & 55mM) (Fig 1C; Fig 1F). However this response was non-significantly antagonized by Atenolol (Ate) when administered before the administration of Adr (Fig. 1C). However, the time to reach peak response showed significant difference (P<0.05) when compared with Adr and Adr in the presence of Ate (Fig.1F).

Effects of acetylcholine & Adrenaline and their antagonist on Rabbit:

Contradictory to Uromastix VD, Rabbit VD showed increase in basal tone by only 5.5mM of ACh followed by the appearance of rhythmic contractions (Fig. 2A). However this graded response or tone reduced significantly (P<0.0005) by 92% when ACh administered in the presence of atropine (Fig. 2A; Fig 2C). Time to peak response was also found to be significantly reduced by 79% (P < 0.005) when ACh administrated in the presence of Atr compared with ACh alone (Fig. 2C). Similarly, by administration of Adr on rabbit VD was again found to increase the basal tone and this response was significantly (P<0.0005) reduced or antagonized by 86% when Adr administered in the presence of Ate (Fig 2B). The duration of response to peak was about 63% lesser in the presence of Ate than observed with Adr alone (Fig 2D).While statistically this difference was a significant (P<0.025) between them.





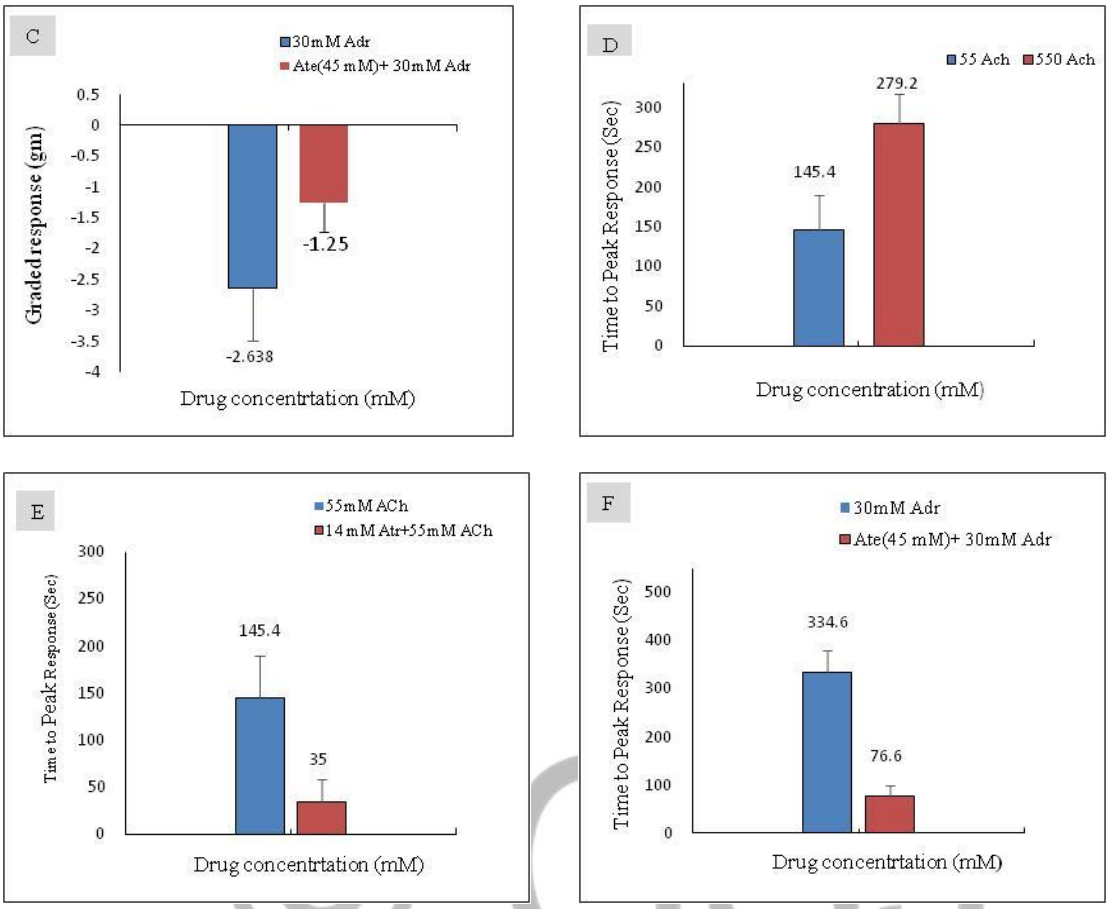
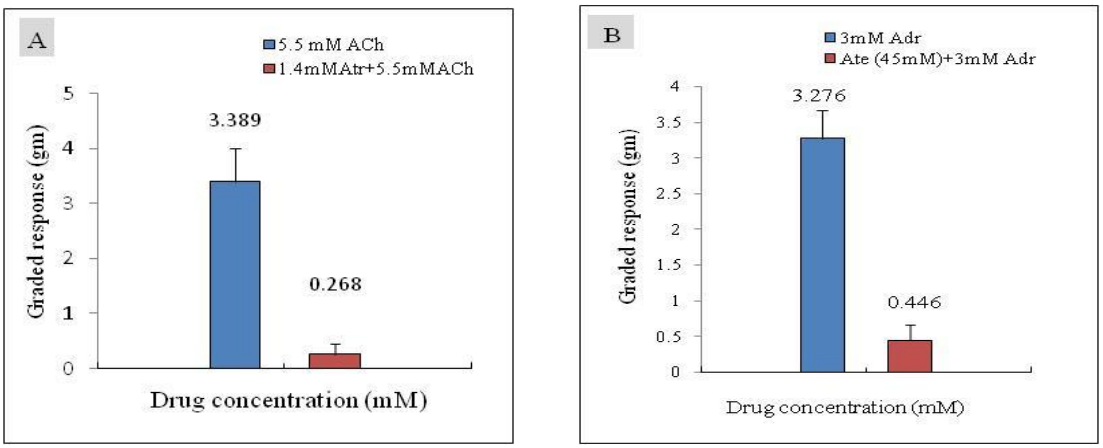


Fig 1: Effect on Graded Mechanical Response of VD of Uromastix *hardwickii*.

(A): ACh at 55 & 550 mM (B): ACh alone and ACh with Atr

(C): Adr alone and Adr with Ate. (D): Time to Peak ACh 55 & 550 mM.

(E): ACh alone and ACh with Atr. (F): Adr alone and Adr with Ate.



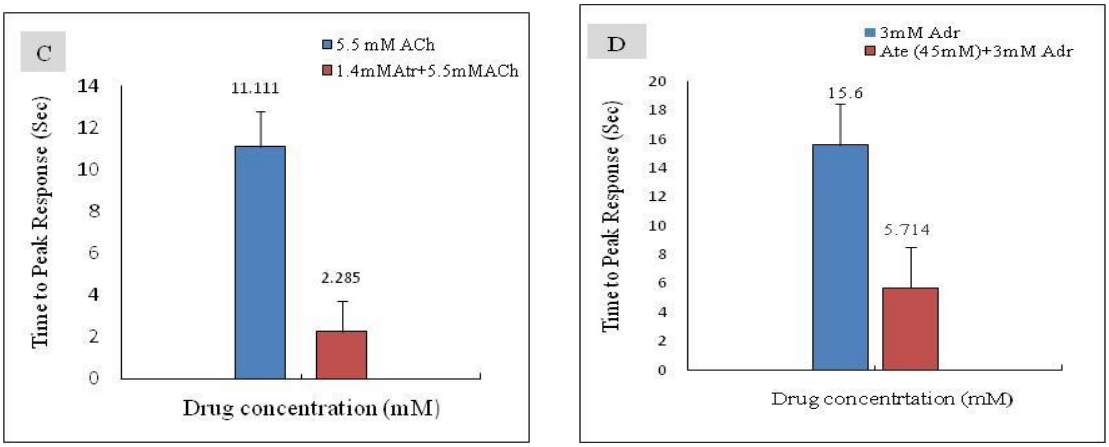


Fig 2: Mechanical Response of VD of Rabbit.
(A): ACh alone and ACh with Atr. (B): Adr alone and Adr with Ate.
(C): Time to Peak; ACh alone and Ach with Atr. (D): Adr alone and Adr with Ate.

DISUSSION:

VD is the universal sperm storage organ across the reptile species, providing de-synchronization of the timing of spermiogenesis, ovulation, and mating (Gang et al., 2011Gist & Congdon, 1998, Almeida-santos *et al*, 2004). The smooth muscle in the wall of vas propels spermatozoa toward urethra (Reynolds 1943). During breeding season of reptile the terminal part of the vas swollen (Shanbhag 2002). This preparation has proven to be useful for studying the physiological mechanism and the drug effect that modifying neurotransmission (Westfall and Wesfall, 2001; Knight, *et al* 2001; Koslov and Anderssson, 2013). The physio-pharmacological study of the reptile are not well understood and are less studied because its significance is not well appreciated and partly because it most difficult to study. Although around 456 species of reptile are inhabit of subcontinent. In male reptile Wolffian duct give rise to vas deferens (Shanbhag 2002; Fox 1958;). In this study pharmacological aspect of Uromastix VD was focused along with Rabbit. Our results revealed that when Uromastix VD exposed to exogenous ACh and Adr its basal tone was reduced. While this effect was significantly antagonize by antagonist Atr, and insignificantly antagonize by Ate respectively. The interpretation of the results regarding VD is not simple because of its complex structure and varicose nerve terminals. However, it do indicate that ACh does not showed excitatory response, rather it relaxes the tissues and probably responsible to activate voltage gated K channel, or release NO which induced relaxation (Murphy and Rembold, 2005). Harhun, (2003; 2005) identified outward K current in different portion of rat VD that relaxes the smooth muscle. Further Andrianpsitohaina, (1992) revealed that the mechanisms under lying cholinergic relaxation either by neural release or by the exogenous application of ACh is actually the release of nitric oxide. According to him nitric oxide is primarily responsible for relaxation. It has also revealed that nitric oxide released

from noradrenergic noncholinergic (NANC) nerve terminals of smooth muscles cells mediate slow inhibitory junction potential via activation of cGMP that leads to muscle relaxation (Sanders and Ozaki, *et al*., 1994; Jury et al., 1992; Crist et al., 1991). Thus, it might be possible that in Uromasitx VD large number of NANC neurons are present that activate the voltage gated K^+ channels or release NO from their nerve terminals that affect the basal tone and relaxes the smooth muscle both by the ACh and Adr.

Contradictory to the Uromastix, in Rabbit VD the basal tone was enhanced after the exogenous application of ACh and Adr. This increase was followed by irregular rhythmic contractions probably indicates the excitatory effect of ACh or Adr that is responsible for increase in intracellular calcium being the basis of muscle contraction on this tissue. Bolton et al (1999) reveled that receptors for excitatory signals molecules generally depolarizes the cell & then Ca^{++} release from intracellular stores by the action of inositol tri phosphate (IP3) or by Ca^{++} induced Ca^{++} release phenomena. This Ca^{++} waves then spread throughout the cell and cause muscle contraction.

Our results regarding rabbit VD clearly indicates the presence of cholinergic and adrenergic innervations that also confirms from the earlier findings (Fukushi andWakui.,1987; Fukushi and Wakui.,1986; Wakui, and Inomata., 1985a&b). However the functionally dominant role of adrenergic innervations (Banks *et al.,* 2006) and presence of pace maker cells that initiate the contraction (Metzgeret al, 2008; Burnstock and Lavin 2002) can also be observed in present study where Adr was required in less amount than the ACh which required high concentration to produce its response.

CONCLUSION:

It is concluded from above discussion that unlike rabbit VD where ACh and Adr stimulation has excitatory effects, in uromastix, both agents decreases its basal tone and thus relaxes VD and it might be due to activation of voltage gated K channels or release of NO. However, responses of VD against ACh in uromastix were observed on high doses indicating less number of cholinoceptors in this tissue. Advanced histological and biochemical studies are required to know the details about the distribution of cholinergic and adrenergic receptors in VD.

ETHICAL APPROVAL:

Ethical approval was obtained from the DRC Department of Physiology, Animal Ethics Committee, University of Karachi and all experiments involving animals were conducted under the strict guidelines of said committee.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interests among them.

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