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THE INHERENT RHYTHMIC CONTRACTION PROPERTY OF PENILE TISSUE IN SEXUALLY ACTIVE ADULT MALE RATS AND THE EFFECT OF SILDENAFIL CITRATE (VIAGRA): AN *IN VITRO* PILOT STUDY

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ABSTRACT:

Isolated human cavernosal muscles and those of various animals have extensively been studied for understanding the causes and treatment of erectile dysfunctions. Usually these tissues are first pretreated with suitable drugs, i.e. Noradrenaline (N-Adr), phenylephrine, carbacol, KCl and nicorandil etc. to induce resting tone or contractions in them, followed by the application of relaxing drugs like sildenafil. We undertook this project to show first of all that these penile tissues were capable of generating rhythmic contractions if properly treated with suitable drugs, either alone or in combination, and then to study the properties of these induced tensions and/or contractions. Attempt was also made to study the effects of sildenafil on various parameters of these contractions.

Pretreatment of the tissues with atropine and N-Adr both initiated rhythmic contractions, the atropine effect being more potent (2½ times larger) on the contraction rate while N-Adr effect being significantly higher on the resting tone. Atropine also increased the contraction rate and strength of contractions in the washed tissues or when it was applied in combination with N-Adr. Ach had no effect on the rate and strength of contractions in atropine treated tissues but it decreased contraction rat in washed tissues or when applied in combination with sildenafil. Sildenafil decreased the rate and strength of contractions in all the tissues pretreated with atropine or N-Adr or in the washed tissues. The effect of sildenafil was more potent on atropine treated tissues than those on N-Adr treated ones. Atropine, Acetylcholine (Ach) and sildenafil had no effect on the resting tone of the tissues while N-Adr significantly increased the resting tone in both the atropine treated and washed tissues.

INTRODUCTION

For the last two decades, erectile dysfunction (ED) has become a common and most severe problem in men all over the world. In the mean time, scientists have also worked extensively on both the human and animal models, using different types of muscle tissues, i.e. corpus cavernosum, various blood vessels, heart, intestine etc. and have worked out several etiologies for ED. These include vasculogenic, neurogenic, hormonal and/or psychogenic factors (Thomas, 2002). Simultaneously, there have also been several significant advances in the pharmacological treatment of this disease. The treatment usually includes agents that are not only orally effective but possess either local or central acting mechanisms of action. One of these agents is sildenafil citrate (Viagra) that is being used extensively for the successful treatment of ED.

ED is the consistent inability to obtain or maintain an erection for satisfactory sexual relation. It is predominantly a disease of vascular origin where a large number of biochemical factors and intracellular mechanisms affect the corpus cavernosal smooth muscle contraction and relaxation (Bivalacqua *et al.,* 2000). Usually penile erection follows relaxation of the corpus cavernosum in

which nitric oxide (NO) released during sexual stimulation from non-adrenergic, noncholinergic nerve endings and from endothelial cells of the corpus cavernosum itself increases the second messenger system cGMP levels. This in turn modulates intracellular Ca^{++}, which in turn regulates the smooth muscle contractility (Moreland *et al.,* 1998). Sildenafil shows an intense and prolonged inhibitory effect on the smooth muscle cells of human corpus cavernosum by selectively blocking phosphodiesterase type 5 (PDE-5) activity that destroys NO-stimulated cGMP. This blockade of PDE-5 increases cGMP concentrations in the muscles in the presence of nitric oxide (Moreland *et al.,* 1998), the increase being twice in rabbit corpus cavernosum (McAuley *et al.,* 2001). Thus sildenafil enhances the NO-dependent relaxation of the isolated human corpus cavernosum, the relaxation also being dose dependent (Seidler *et al.,* 2002). Intracavernosal sildenafil at millimole tissue levels can cause relaxation of vascular smooth muscle and penile erection by a novel mechanism independent of the classical NO/cGMP pathway (McAuley *et al.,* 2001). It has further been reported that sildenafil enhances the relaxing effects of Na-nitroprusside and acetylcholine (Aydin *et al.,* 2001) and also of NO and Na-nitroprusside (Frith and Gibson, 2000) in amplitude and duration. Evidence has also been provided that sildenafil causes concentration dependent relaxation and amplifies the relaxation induced by Na-nitroprusside in human penile arteries and veins probably by inhibiting noradrenergic contractions, enhancement of neurogenic NO-mediated relaxation and inhibition of smooth muscle contraction (Medina *et al.,* 2000).

Several scientists, working on isolated corpus cavernosum smooth muscle strips from various sources, have induced resting tone or contractions in these muscles by pretreatment with noradrenaline (Taher *et al.,* 2000; Seidler *et al.,* 2002), phenylephrine (McAuley *et al.,* 2001; Palea and Barras, 2003; Aydin *et al.,* 2001; Kim *et al.,* 2000), carbacol (Frith and Gibson, 2000), KCl (Palea and Barras, 2003) and nicorandil (Ishizuka *et al.,* 2000). This pretreatment of various tissues, particularly of corpus cavernosal muscle, either increased the resting tone of the muscle or enhanced mechanical contractions in them. There is however, no evidence in literature regarding the characteristics of these contractions when the tissues were pretreated with noradrenaline, phenylephrine, carbacol, KCl or nicorandil.

The visceral smooth muscle fibers generally produce two types of tensions during their rhythmic contractions, the active and passive tensions, and both of these are affected by drugs (Burnstock *et al.,* 1963; Nasreen *et al.,* 1983a, 1983b). In most of the pharmacological studies, the investigators generally make use of passive tension or resting tone of the smooth muscle where drug induced changes are thought to occur more prominently while the use of active rhythmic contractions is limited to the measurements of the rate of contractions. It is however, of utmost importance to use the time dependent active contraction parameters while studying the pharmacological effect of drugs, as these are equally well sensitive to demonstrate drug induced effects and also help visualize some of the basic mechanisms involved.

We therefore, undertook this project to study the properties of induced contractions in the rat cavernosal smooth muscle pretreated with noradrenaline, acetylcholine and atropine. The drugs were used either alone or in combination. Attempt was also made to study the effects of sildenafil on various parameters of these contractions.

MATERIALS AND METHODS

Sexually active adult male rats of Wister strain, weighing from 380 to 475 gm were used throughout the experiments reported here. These animals were obtained from our own breeding house. For recording the contractile activity, a slightly modified Krebs-Henseliet solution (Winegrade and Shanes, 1962) was used which had the following composition: NaCl, 118mM;

KCl, 4.8mM; $CaCl_2$, 1mM; $MgSO_4$, 1.2mM; KH_2PO_4, 0.8mM; $NaHCO_3$, 25mM; Glucose, 5.55mM with pH 7.4. The chemicals used were obtained either from Merck, Germany or from BDH, England and were of analytical grade. In general, this solution was made in a 10 times concentrated stock which had all the ingredients except $CaCl_2$ and glucose. Whenever needed, 300ml of this stock solution were diluted to 3 liters and $CaCl_2$ and glucose were added in amounts mentioned above.

Similarly, all the drugs used in these experiments were obtained from Sigma, Germany and the solutions were made in concentrations of 10^{-3}. Noradrenaline and sildenafil citrate were always prepared fresh just before their use in each experiment. To test the effect of various drugs, 1ml (10^{-3}) of each drug was added into the gut bath containing 50m1 of Krebs solution, constituting a $10^{-4.5}$ drug solution

The animals were killed by decapitation. The abdomen was then immediately opened up to the lower pelvic region. The penile tissue was then dissected out along with the penis and testis and placed in a bulk (400m1) of Krebs-Henseliet solution through which a mixture of 95% O_2 and 5% CO_2 was bubbled. The tissue was then washed, cleared of penis, testis and the surrounding connective tissues and transferred to another 400ml of Krebs solution with bubbling O_2-CO_2 mixture. The distal end of each penile tissue was then fixed to the bottom of the gut bath of an isolated organ bath assembly by means of a stainless steel pin while the proximal end was pinned and attached to a photoelectric isometric force transducer, Model 60-2997 (0.5-5g) using a nylon string. The isometric force transducer was mounted on a macro-manipulator which could be moved in the vertical direction for adjusting the resting tension of the tissue, which was generally kept at about 2 gm.

For tension recordings, the force transducer was connected to a Polygraph curvilinear channel amplifier from Harvard Apparatus, England through a transducer interface. The Polygraph also served as a chart recorder. The isometric contractions were recorded on a slow moving paper, usually at a paper speed of 2.5mm/sec. The rate of contractions and the changes in the active and passive tensions were calculated from the records obtained. All the experiments were done at a constant temperature of 37^{o}C.

The penile tissue contractions were recorded under a constant load of about 2 gm. For the quantitative estimations of tensions, the distance between the base line and the maximum pen deflection was correlated with the pen deflection at the same sensitivity of the transducer, while a given weight hanged in its leaf spring. This correlation gave the tension produced by the tissue in milligrams.

For the initiation of rhythmic contractions in the rat penile tissue, the experiments were performed in two sets. In the first set, atropine 1 ml (10^{-3}) was applied to the fresh tissue while the same concentrations of noradrenaline were used in the second set. If the first dose of any of these drugs did not produce the desired effect, a second or third dose was used. Once the rhythmic contractions were initiated, the drugs atropine, noradrenaline, acetylcholine and sildenafil citrate were tested on it and the rate of rhythmic contractions, the strength of contractions and the resting tensions of the contracting tissues were measured. These drugs were used alternately one after the other to observe the consistency of the drug's effect.

RESULTS

1. Initiation of Rhythmic Contractions:

For the initiation of rhythmic contractions in the rat penile tissue, both atropine and

noradrenaline were used. The results showed that atropine was more potent in initiating rhythmic contractions in the penile tissue than noradrenaline. Atropine not only initiated rhythmic contractions within a smaller time period but the rate of the contractions was also about 2½ times greater than that obtained with noradrenaline (Table-1). This effect was highly significant ($P<0.001$). The strength of contractions also appeared to be greater in the case of atropine (Table-2) where it was about 12% greater, but this difference was not significant ($P>0.05$). It was further noted that atropine had no effect on the initial resting tension of the tissue (Table-3) while noradrenaline on the average increased it to about 48mg in less than a minute.

Table-1

Atropine and noradrenaline 1ml (10^{-3}) were initially applied to the fresh rat penile tissue to initiate rhythmic contractions

S.No.	Experimental Conditions	Rate of Contractions/min. Mean ± S.E.
1.	Atropine applied on fresh tissue to initiate rhythmic contractions	39 ± 8(8)
2.	Noradrenaline applied on fresh tissue to initiate rhythmic contractions	16 ± 2 (5)

Table-2

Strength of rhythmic contractions in the fresh rat penile tissues. The rhythmic contractions were initiated by atropine and noradrenaline, when used in volumes of 1ml (10^{-3})

S.No.	Experimental Conditions	Strength of Rhythmic Contractions (mg) Mean ± S.E.
1.	Atropine applied on fresh tissue to initiate rhythmic contractions	42.5 ± 13.6(8)
2.	Noradrenaline applied on fresh tissue to initiate rhythmic contractions	38.0 ± 8.2 (5)

Table-3

Development of resting tensions in the fresh rat penile tissues. The resting tension development was associated with the use of atropine and noradrenaline, when used in volumes of 1ml (10^{-3}) for initiating rhythmic contractions. The arrows (↑) and (↓) indicate increase or decrease in the strength of contractions respectively.

S.No.	Experimental Conditions	Maximum Change in Resting Tension (mg) Mean ± S.E.
1.	Atropine applied on fresh tissue to initiate rhythmic contractions	No change in resting tension
2.	Noradrenaline applied on fresh tissue to initiate rhythmic contractions	↑ 48 ± 14.9 (6) (In average 56.0 ± 10.8 sec.)

Table-4

Effect of atropine, noradrenaline, acetylcholine and sildenafil citrate (Viagra) on the rate of rhythmic contractions of rat penile tissue under various experimental conditions. The arrows (↑) and (↓) indicate increase or decrease in the rate of contraction respectively. All the drugs were used in volumes of 1ml (10^{-3}).

S.No.	Experimental Conditions	% Change Rate of Contractions/min. (Mean ± S.E.)
	ATROPINE	
1.	Atropine was applied after noradrenaline	↑ 51.2 ± 10.24 (7)
2.	Atropine was applied after acetylcholine	↑ 133.2 (2)
3.	Atropine was applied after washing the tissue	↑ 33.4 ± 7.6 (5)
	NORADRENALINE	
4.	Noradrenaline was applied after atropine	↑ 30.0 ± 6.1 (11)
5.	Noradrenaline was applied after washing the tissue	↑ 50.8 ± 8.5 (6)
	ACETYLCHOLINE	
6.	Acetylcholine was applied after atropine	No Effect (3) (Contraction rate remained same)
7.	Acetylcholine was applied after sildenafil citrate	↓ 16.2 (2)
8.	Acetylcholine was applied after washing the tissue	↓ 24.5 ± 8.7 (4)
	SILDENAFIL CITRATE (Viagra)	
9.	Sildenafil citrate (Viagra) was applied after atropine	↓ 23.4 ± 5.0 (11)
10.	Sildenafil citrate (Viagra) was applied after noradrenaline	↓ 36.7 ± 15.7 (5)
11.	Sildenafil citrate (Viagra) was applied after washing the tissue	↓ 35.1 ± 5.1 (9)

2. Effect on the Rate of Rhythmic Contraction:

When atropine was applied after noradrenaline, the contraction rate was increased by about 51%. A similar 33% increase was observed when atropine was applied on washed tissues (Table-4). Both these increases from their previous values were significant ($P<0.005$). These results were consistent in all the experiments done. A comparison of the atropine effect after noradrenaline or after washing the tissue showed them to be nonsignificant. When atropine was applied after acetylcholine, the increase in this parameter was about 1.25 fold, but these results were obtained in only 2 experiments out of 5. In the other 3 experiments, atropine had no effect at all.

The application of noradrenaline on the atropine initiated tissues caused a 30% increase in the rate of contraction. Similar applications of noradrenaline on washed tissues increased this parameter by about 51% (Table-4). Both these increases from the previous values were consistent

and significant. A comparison of the noradrenaline effect on atropine initiated and washed tissues showed the later to be significantly higher ($P<0.005$).

A comparison between atropine effect on noradrenaline initiated tissues and noradrenaline effect on atropine initiated tissues showed the former effect to be significantly higher. However, when the tissue was washed, noradrenaline produced about 17% greater effect in the rate of contraction than atropine, but this was nonsignificant.

When acetylcholine was applied on atropine initiated tissues, no effect was observed and the contraction rate remained the same in all the 3 experiments done (Table-4). However, acetylcholine decreased the contraction rate by about 25% when applied on the washed tissues. In 2 experiments, where acetylcholine was applied on sildenafil treated tissues, it further decreased the rate of contractions by about 16%.

Sildenafil was also applied on atropine initiated tissues. It decreased the rate of contractions by about 23%. This was a very consistent effect and was observed in all the 11 experiments done (Table-4). A similar application of sildenafil on noradrenaline initiated tissues caused about 37% decrease in this parameter. These two effects were however, nonsignificant. Sildenafil also decreased the rate of contractions by about 35% in the washed tissues. Quantitatively, this effect corresponded to the effect of sildenafil on noradrenaline initiated tissues. Further, the effect of sildenafil on the contraction rate of washed tissues was about 10% greater than that of acetylcholine but nonsignificant.

3. Effect on the Strength of Rhythmic Contractions:

Atropine increased the strength of rhythmic contractions in all the cases when applied on noradrenaline initiated tissues, acetylcholine treated tissues or the washed tissues, the increases being about 73%, 57% and 130% respectively (Table-5). Atropine effect on this parameter of the washed tissues was highly significant ($P<0.001$) as compared to its effect on noradrenaline initiated tissues.

Similarly, noradrenaline increased the contraction strength in both the cases when applied on atropine initiated tissues or the washed tissues, the increases being similar and about 110% (Table-5). It was also noted that atropine and noradrenaline effects on contractile strength in all the experimental conditions were more or less similar, there being nonsignificant differences in this parameter.

Acetylcholine was again found to have no effect on contraction strength of atropine initiated tissues and this parameter remained unaltered. However, acetylcholine decreased the contraction strength by about 30% and 85% when applied after sildenafil or on washed tissues. This decreasing effect of Ach was larger, consistent and significant for washed tissues ($P<0.005$).

Unlike atropine, sildenafil decreased the contraction strength in all the cases, when applied on atropine or N-Adr initiated tissues as well as on washed tissues, the decreases being about 43%, 24% and 42% respectively (Table-5). A comparison showed that sildenafil had a much potent and significant effect when applied on atropine initiated tissues than its effect on N-Adr initiated ones ($P<0.005$). Similarly, sildenafil effect on N-Adr initiated tissues was significantly greater than that on the washed tissues ($P<0.005$). When sildenafil was compared with Ach for its effect on contraction strength of washed tissues, it was found to have a significantly greater effect than Ach ($P<0.005$).

Table-5

Effect of atropine, noradrenaline, acetylcholine and sildenafil citrate (Viagra) on the strength of rhythmic contractions of rat penile tissue under various experimental conditions. The arrows (↑) and (↓) indicate increase or decrease in the strength of contraction respectively. All the drugs were used in volumes of 1ml (10^{-3}).

S.No.	Experimental Conditions	Strength of Rhythmic Contractions (mg) (Mean ± S.E.)
	ATROPINE	
1.	Atropine was applied after noradrenaline	35.0 ± 8.9 (7) (72.6% ↑)
2.	Atropine was applied after acetylcholine	50.0 (2) (57% ↑)
3.	Atropine was applied after washing the tissue	82.0 ± 10.8 (5) (130% ↑)
	NORADRENALINE	
4.	Noradrenaline was applied after atropine	40.0 ± 6.2 (11) (110% ↑)
5.	Noradrenaline was applied after washing the tissue	61.7 ± 15.6 (6) (110% ↑)
	ACETYLCHOLINE	
6.	Acetylcholine was applied after atropine	No Effect (3) (Contraction strength remained same)
7.	Acetylcholine was applied after sildenafil citrate	15.0 (2) (30.4% ↓)
8.	Acetylcholine was applied after washing the tissue	23.1 ± 4.4 (4) (85% ↓)
	SILDENAFIL CITRATE (Viagra)	
9.	Sildenafil citrate (Viagra) was applied after atropine	28.6 ± 1.9 (11) (42.9% ↓)
10.	Sildenafil citrate (Viagra) was applied after noradrenaline	37.0 ± 13.8 (5) (24.5% ↓)
11.	Sildenafil citrate (Viagra) was applied after washing the tissue	41.7 ± 3.1 (9) (25.2% ↓)

4. Effect on the Resting Tensions:

Atropine had no effect on the resting tensions of tissues previously treated with Ach and this parameter remained unchanged. It was a consistent finding and observed in all the 4 experiments done. Atropine however, increased the resting tension to about 85mg in N-Adr initiated tissues in an average time period of 49 seconds (Table 6). It was further noted that this resting tension, although seemed to be larger in intensity and time duration in the N-Adr initiated tissues, was actually nonsignificant from that of washed tissues.

Table-6

Effect of atropine, noradrenaline, acetylcholine and sildenafil citrate (Viagra) on the resting tensions of rat penile tissue under various experimental conditions. The arrows (↑) and (↓) indicate increase or decrease in the strength of contraction respectively. All the drugs were used in volumes of 1ml (10^{-3}).

S.No.	Experimental Conditions	Maximum Change in Resting Tensions (mg) (Mean ± S.E.)
	ATROPINE	
1.	Atropine was applied after noradrenaline	↑ 84.8 ± 30.7 (4)* (In average 87.5 ± 25.6 sec.)
2.	Atropine was applied after acetylcholine	No change in resting tension
3.	Atropine was applied after washing the tissue	↑ 48.8 ± 11.9 (4) (In average 48.5 ± 11.8 sec.)
	NORADRENALINE	
4.	Noradrenaline was applied after atropine	↑ 111.0 ± 39.5 (5)* (In average 22.0 ± 2.0 sec.)
5.	Noradrenaline was applied after washing the tissue	↑ 90.0 (1) (In 22 sec.) This effect was produced in 1 experiment out of 4
	ACETYLCHOLINE	
6.	Acetylcholine was applied after atropine	↓ 75.0 (2) (In average 135 sec.) This effect was produced in 2 experiments out of 4
7.	Acetylcholine was applied after sildenafil citrate	No change in resting tension
8.	Acetylcholine was applied after washing the tissue	No change in resting tension
	SILDENAFIL CITRATE (Viagra)	
9.	Sildenafil citrate (Viagra) was applied after atropine	↑ 25.0 (1) (In 12 sec.) This effect was produced in 1 experiment out of 9
10.	Sildenafil citrate (Viagra) was applied after noradrenaline	↑ 33.0 (2) (In average 101 sec.) This effect was produced in 2 experiments out of 5
11.	Sildenafil citrate (Viagra) was applied after washing the tissue	No change in resting tension

N-Adr increased the resting tension in both the atropine initiated and washed tissues, the observations being consistent in the former case (Table-6). In the later case, the increased resting tension was observed in only 1 experiment out of 4. These results resembled those of atropine effect on N-Adr initiated tissues and were nonsignificant to each other. The time period for N-Adr effect on washed tissues was however, smaller and highly significant ($P<0.0005$).

Ach had no effect on the resting tensions of washed tissues or on tissues previously treated with sildenafil. However, Ach decreased the resting tensions in atropine initiated tissues in two experiments out of 4.

Sildenafil had no effect on the resting tensions of the washed tissues. It however, increased this parameter in 1 or 2 experiments when applied on atropine or N-Adr initiated tissues, the effect being larger but occurring at a slower rate.

DISCUSSION

The smooth muscles are generally classified into single-unit, rhythmically contracting and multi-unit, non-rhythmically contracting types. As the cavernosal smooth muscles normally do not show spontaneous rhythmic contractions, they fall into the later type. For our mechanical contraction study, the whole of the rat cavernosal tissue was fixed with a slight stretch so that some initial passive tension or tone is produced. This slight stretching produces what is known as 'passive stretch resistance' which is essential for stretch activation. Most of the smooth muscles are stimulated by moderate stretch which causes membrane depolarization and initiation of spikes or an increase in spike frequency (Bulbring, 1955). It has further been demonstrated (Bozler, 1969) that a release of Ca^{++} is also associated with the initiation of contraction without the continuous or transient depolarization of the cell membrane, a state of stretch resistance, in which the stretched muscle cell is not excited as demonstrated from the electrical state of the membrane. This condition is regarded as non-electrical tone. In our experiments, the initial resting tension or passive stretch resistance was fixed at 2gm for about 1.5 inch long cavernosal tissue. This 2 gm resting tension gave maximum contraction amplitude. This made possible for us to record and analyze both the active tension and resting tone from the rat cavernosal muscle for the study of relaxing effects of various drugs.

Rat cavernosal muscle is a smooth muscle. It possesses a well defined structure like any other visceral muscle, along with a contractile and non-contractile component. It is also innervated by both the sympathetic and parasympathetic nerves, with the plexus of the two nervous systems being located in the muscle itself. Generally, the release of noradrenaline (N-Adr) at the nerve endings is followed by a blockade of nitric oxide (NO) release and this causes a sustained tension in the muscle and an inhibition of penile erection (Omote, 1999). However, parasympathetic stimulation releases acetylcholine (Ach) causing the release of NO from the nonadrenergic, noncholenergic nerve endings and from the endothelial cells of cavernosal muscle itself (Moreland *et al.,* 1998). NO in turn increases the second messenger cGMP levels provided that PDE5 activity is lower or blocked. Increased cGMP modulates the intracellular Ca^{++} levels and the muscle relaxes (Moreland *et al.,* 1998).

We used isolated cavernosal muscles which were devoid of any sexual stimulation. Under such conditions, the sympathetic activity would be expected to dominate resulting in the release of N-Adr and a sustained tone in the muscle. This would represent an *in vivo* non-erectile penis. When we blocked the parasympathetic activity by atropine, the whole relaxing system of the muscle was blocked and the sympathetic activity became even more prominent, leading to the generation of mechanical contractions, rhythmic in nature and superimposed on the initial resting tone. The rate and strength of contractions in such a muscle would be expected to depend upon the amount and rate of release of N-Adr from the sympathetic plexus already present in the muscle and the availability of Ca^{++}.

Our results clearly showed that direct stimulation of the tissue with N-Adr also initiated

rhythmic contractions but these were low in frequency and strength but superimposed on an elevated resting tone. These rhythmic contractions of low frequency and strength were probably due to the simultaneous relaxing activity of the parasympathetic plexus which were not blocked and were actively releasing Ach. It is further to be noted that the total tension (resting tone plus active tension) generated in the atropine treated and N-Adr stimulated tissues were almost the same with nonsignificant differences between them. We therefore, suggest that direct stimulation of the rat cavernosal muscle with N-Adr increases its resting tone and decreases the active tension in the same proportion so that the total tension generated remains the same. We further suggest that isolated rat cavernosal muscle has an inherent rhythmic contractile ability associated with sympathetic innervation but it is generally kept suppressed by the parasympathetic innervation. If however, parasympathetic activity is blocked or the sympathetic stimulation is increased, the muscle immediately develops rhythmic contractions.

Atropine is an antagonist of Ach. Thus, its application to N-Adr stimulated tissues blocked the activity of Ach in all our experiments and the muscle developed rhythmic contractions, higher in frequency and strength but without any change in the resting tone. This consistent finding seems to suggest that parasympathetic activity or the relaxing effect of endogenously released Ach is always stronger than the externally applied Ach or the sympathetic activity in these tissues. Vice versa, N-Adr application to atropine stimulated tissues again produced similar effects, i.e. contractions of increased frequency and strength, along with an increase in resting tone. It is to be noted that we have used high concentrations of N-Adr (10^{-3}) which would be expected to produce a greater stimulatory effect on contraction parameters, and this was found to be true for its effects on the washed tissues. We also observed a lesser stimulatory effect of N-Adr on atropine stimulated tissues. Since atropine used in our experiments was also high in concentration, it is probable that intermolecular actions of these two drugs might have reduced the excitatory effect of N-Adr, as has also been suggested earlier (Bivalacqua *et al.,* 2000).

It is to be noted that N-Adr produced a very marked increase in the resting tone of atropine stimulated tissues. It would mean that a larger number of muscle fibers were in a condition of sustained contraction and were not involved in rhythmic contractions generated in these muscles. This would also mean that the tension generated by the contracting fibers must also be small. This is in contradiction to the results shown in Table-5 (N-Adr column) where the increase in the developed tension was shown to be 110% in both the cases. However, it is obvious from Table 5 that N-Adr actually produced a lesser increase in the tension developed by atropine treated tissues (40 ± 6.2) mg as compared to the tension developed by the washed muscles (61.7 ± 15.6) mg, the nonsignificant difference between the two values being due to lesser number of observations and larger variations in the later case. If it is assumed that N-Adr actually produced lesser tension in atropine stimulated tissues, then we can safely say that N-Adr had a greater stimulatory effect on these tissues, the total tension generated being 151 mg against 61 mg in washed tissues.

We observed no effect of Ach on atropine initiated contractions both in rate and strength and on the resting tone. Since Ach receptors were already blocked by atropine, these results were according to our expectations. On the other hand, Ach decreased contraction rate but had no effect on the strength of contractions or on the resting tone of the washed tissues. These results were again expected since washing of the tissues removed atropine from the Ach receptors and allowed it to produce its inhibitory effects. These results are in accord with the previous findings (Aydin *et al.,* 2001) where Ach was shown to produce dose dependent gradual relaxations in rabbit cavernosal muscles. We believe that Ach can alter the rate of contractions but has no part in altering the strength of contractions, which is probably dependent on other factors like the availability of C^{++} etc.

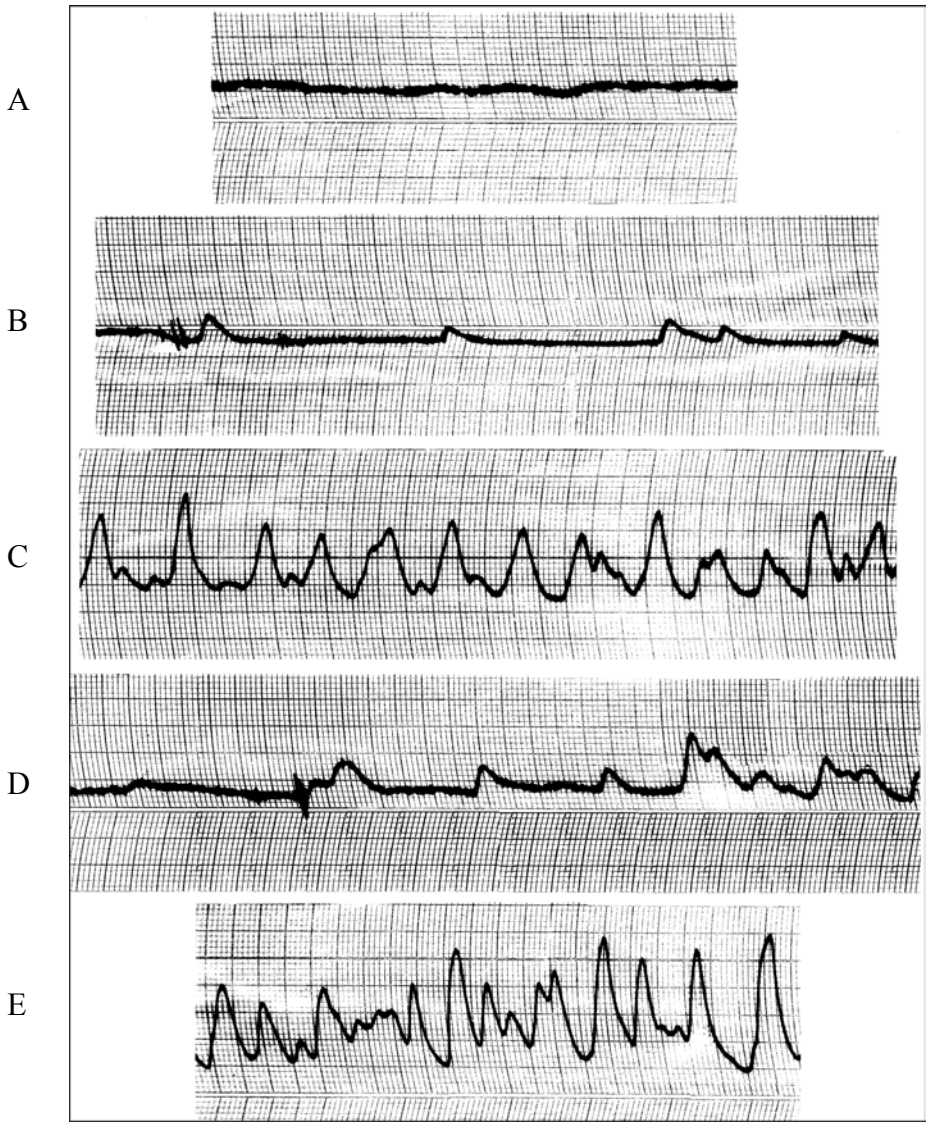

Fig. 1: Initiation of rhythmic contractions in the penile tissues of sexually active adult male rats. A) Fresh tissue showing no contractile activity. B) Addition of third dose of atropine 1ml (10^{-3}) caused an instant initiation of irregular rhythmic contractions. C) A typical record of rhythmic contractions about 2 minutes after the application of atropine. D) Fresh penile tissue before and after the application of noradrenaline 1ml (10^{-3}). Instant initiation of rhythmic contractions can be seen. E) A typical record of rhythmic contractions about 1 minute after the application of noradrenaline.

Our experiments have further shown that Ach enhanced the relaxing effect of sildenafil and further decreased the rate and strength of contractions, but had no effect on the resting tone. These results clearly demonstrated that the relaxing effect of Ach was comparatively larger and stronger than that of sildenafil when used separately.

We have further observed that sildenafil decreased both the rate and strength of contractions whether applied after atropine, N-Adr or after washing the tissues. These results are also in accordance with the previous findings where sildenafil has been shown to relax rat cavernosal muscle by selectively inhibiting PDE-5 activity that destroys NO-stimulated cGMP (Taher, *et al.,* 2000; McAuley *et al.,* 2001; Moreland *et al.,* 1998; Palea and Barras, 2003). It is again probable that sildenafil relaxed rat cavernosal muscle in our experiments using the same classical NO/cGMP pathways as described earlier by others (Moreland *et al.,* 1998; Omote, 1999). Since our experiments showed sildenafil to decrease both the rate and strength of contractions in the tissues, it is suggested that probably the Ca^{++} availability to the contractile proteins of the tissue has also been reduced directly or indirectly by this drug to produce its inhibitory effect. Further, it has been shown earlier (Medina *et al*., 2000) that the relaxation of human penile arteries and veins by sildenafil involves inhibition of nor-adrenergic contractions. It is probable that sildenafil blocked the effect N-Adr in our experiments as well and thus enhanced relaxation of the muscle.

We have further observed that sildenafil increased the resting tone in both the atropine and N-Adr treated tissues, the effect being very fast in the former case and comparatively slower in the later. However, these results were obtained in a very few experiments and need further investigation.

On the bases of our experimental results, we thus suggest that under non-sexual stimulation conditions, which prevail most of the time, the normal sympathetic stimulation of the cavernosal muscle is dominant due to the released N-Adr. This neurotransmitter blocks the release of NO in this tissue and it is followed by decreased levels of the second messenger system cGMP and increased PDE-5 activity. This phenomena leads to an increased resting tone in this muscle along with a non-erectile condition of the penis that prevails most of the time (Omote, 1999). When the animal is sexually stimulated, which is a neural and psychological phenomenon, the parasympathetic activity becomes dominant. The neurotransmitter Ach then causes increased release of NO from the nonadrenergic, noncholenergic nerve endings and from the endothelial cells of cavernosal muscle itself (Moreland *et al.,* 1998). NO lowers or blocks the activity of PDE-5 and this leads to increased cGMP levels. This also modulates the intracellular Ca^{++} levels and the muscle relaxes to allow penis to go into erection (Moreland *et al.,* 1998). When sildenafil citrate is given under sexually stimulated conditions, this drug decreases or abolishes the sustained tension in the muscle, probably by selectively inhibiting PDE-5 activity that destroys NO-stimulated cGMP (Taher *et al*., 2000; McAutey *et al.,* 2001) and a decrease in the Ca^{++} availability (Moreland *et al.,* 1998). Increased release of Ach during sexual stimulation further enhances the relaxing effect of sildenafil citrate and a very strong erection of penis in developed.

CONCLUSION

It is concluded that 1) rat cavernosal muscle has inherent rhythmic contraction ability but it is normally kept suppressed by the parasympathetic activity. 2) Ach generally relaxes the penile tissue while N-Adr stimulates it. 3) Sildenafil also relaxes the penile tissue and Ach can enhance this relaxing effect of sildenafil.

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