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Differential contractile response of normal vas deferens of rodents in correlation to their calcium and electrolyte levels*

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Abstract. The contractile pattern of the vas deferens in three different rodents, rat, guinea pig and mouse was studied in response to adrenaline and noradrenaline. The left vas deferens of rat was more responsive to the graded doses of adrenaline and noradrenaline than the right. The same was also true for guinea pig and mouse vas deferens. This differential response has been correlated with the greater concentrations of calcium and sodium in the right vas deferens in rats and guinea pigs and it might also be related to the levels of membrane-bound and intracellular calmodulin-bound calcium. It is suggested that the left vas deferens might possess more calmodulin-bound calcium than the right, which might have instead, more membrane-bound calcium.

Keywords. Vas deferens; calcium; contractile pattern.

Introduction

The interplay of metal ions on cellular functions and membrane structure are of importance in the reproductive system. The importance of calcium in the contractility of guinea pig vas deferens has been reported (Momose and Gomi, 1978; Sugimoto *et al.*, 1978). Moreover, the response of vas deferens to KCl also involves the participation of readily available calcium ions contained in the tissue (Sugimoto and Nagata, 1973; Sugimoto and Furumichi, 1975; Sugimoto *et al.*, 1977; 1978). Westfall *et al.* (1975) reported that the decrease in membrane-bound calcium ions leads to depolarization of the cell membrane and renders the cells more excitable. Recent studies by Slater (1981) have revealed that calcium is essential for norepinephrine release, but calcium alone cannot induce the release of neurotransmitters.

Although, it is known that continuous transport of the spermatozoa takes place due to regular spontaneous contractions (Hamilton, 1972), not only during ejaculation but also at rest, the mechanism of sperm transport through the ductus deferens is still not fully understood.

A study in three different rodents, rat, guinea pig and mouse, was carried out to investigate the effects of different doses of epinephrine and norepinephrine on the contractile pattern of their left and right vas deferens as well as the spontaneous contractions. The concentrations of Ca^{2+} and electrolytes Na^{+}, K^{+} were especially

Abbreviation used: NA, noradrenaline

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determined in order to correlate the changes, if any, in the contractile pattern of vas deferens, with the electrolyte profile, in the light of recent data on the role of calcium in smooth muscle contraction.

Materials and methods

Normal, mature, male, albino rats *(Rattus norvegicus)* or Holtzman strain (200-250 g body weight) adult healthy guinea pigs *(Cavia porcellus)* weighing between 400-450 g and mature albino mouse *(Mus musculus)* (40-50 *g* weight) were used for the study. All the animals used were of proven fertility. The animals were housed in a controlled environment at a constant temperature of 26 ± 2°C and were subjected to 12-14 h daylight. They were maintained on standard diets (Hindustan Lever Ltd., Bombay) and water was provided *ad libitum.*

Recordings of the isolated vas deferens

The recordings of the contractile pattern of the isolated vas deferens *in toto,* from normal adult rats, guinea pigs and mice were carried out using a standard technique as described earlier (Chinoy and Chinoy, 1979; 1981). These recordings were carried out simultaneously on the vas deferens from left and right sides of these rodents using a double organ bath and under identical conditions. The frontal lever was adjusted at 500 mg tension for rat vas deferens, 1000 mg for guinea pig and 75 mg for mouse vas deferens. The response to different doses of adrenaline from 1 to 500 µg was recorded in all rodents, whereas, the response to noradrenaline was recorded in the rat only.

The mean amplitude (in mm) of the individual response to each dose of adrenaline or noradrenaline in normal animals were calculated for the left and right vas deferens.

Atomic absorption spectrophotometry

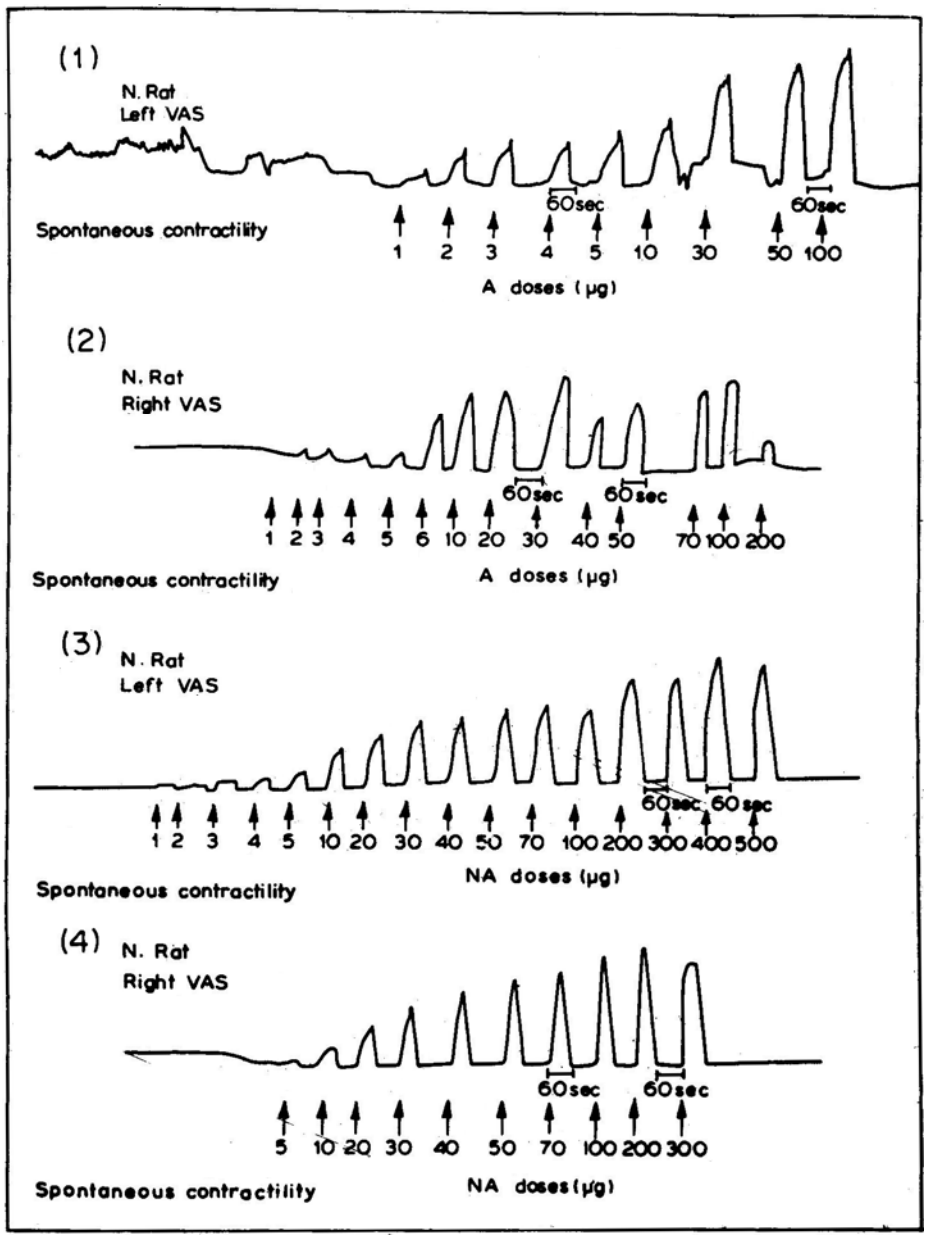
For the analysis of the different electrolytes in the vas deferens *in toto,* the study was carried out using the standard acid digestion method. Vas deferens from the left and right sides of the animals were processed separately. The concentrations of sodium, potassium and calcium were estimated separately using a Perkin Elmer 305 A atomic absorption spectrophotometer by the standard method.

For all analysis, a minimum of six replicates were done and the results were analyzed statistically by using the student's 't' test.

Results

Contractile pattern of the isolated vas deferens

Normal rat: The normal rat vas deferens from the left and right sides of the animal showed differences in the contractile pattern. Although, spontaneous contractility was obtained for the left, it was absent in the right vas deferens (figures 1,2). It was also observed that the left vas deferens on the whole was more responsive to different doses of adrenaline than the right vas deferens. Moreover, the latter responded poorly to 200 µg of adrenaline, whereas, the left side did not respond at all. On both the sides, maximum amplitude was obtained with 100 µg adrenaline.



Figures 1-4. Recordings of the isolated vas deferens (left and right sides) of normal rat showing adrenaline (A) and noradrenaline (NA) induced contractions. **1.** Note the characteristic spontaneous contractile pattern of the left vas deferens as well as its response to different doses of A. **2.** Dose dependent contractile response of the isolated right vas deferens to A. Note absence of spontaneous contractions. **3.** No spontaneous contractility of the left vas and the response to different doses of NA was more uniform and higher than that by A. **4.** No spontaneous contractility of the right vas, It did not respond to 1 to 4 µg doses of NA. At higher doses it was less responsive as compared to the left vas.

The left vas deferens responded more to the different doses of noradrenaline (NA) than the right side. This holds good only for doses upto 100 µg NA, since beyond 100 µg dose, an irregularity in the contractile response was noted (figures 3,4). The right vas deferens did not respond at all to lower doses of NA (1 to 4 µg). With 100 µg NA both the sides responded almost equally. In left vas deferens the highest amplitude was noted by 400 µg NA, whereas, in the right, the greatest response was with 200 µg dose of NA.

On the whole, a better response to exogenously applied NA at higher doses was elicited, whereas, at lower doses, adrenaline proved to be a more potent stimulant for vas deferens.

Normal guinea pig: The guinea pig ductus deferens from both the sides did not show spontaneous contractility. The left and right vas deferens were not responsive to 1 to 5 µg adrenaline, but a graded response was obtained with increase in dose of adrenaline and the amplitude was maximum with 400 µg adrenaline in the left as well as right. On the whole, left vas deferens was more responsive to all doses of adrenaline as compared to the right one, similar to the case in rat (figures 5, 6).

Normal mouse: The mouse also responded in a similar manner as the previous two rodents. No spontaneous contractions were observed in the vas deferens from left or right sides. Left vas deferens showed higher amplitude all throughout as compared to the right one (figures 7, 8).

On the whole, rat vas deferens from both the sides showed greater dose dependent response to 1 to 100 µg adrenaline, followed by mouse, and least by guinea pig. But guinea pig ductus deferens responded to the highest dose of adrenaline (400 µg) followed by rat (100 µg), and least by mouse (70 µg).

See, figure 9 (a, b, c, d) for comparative study of the contractile pattern of vas deferens in these rodents.

Metal ions

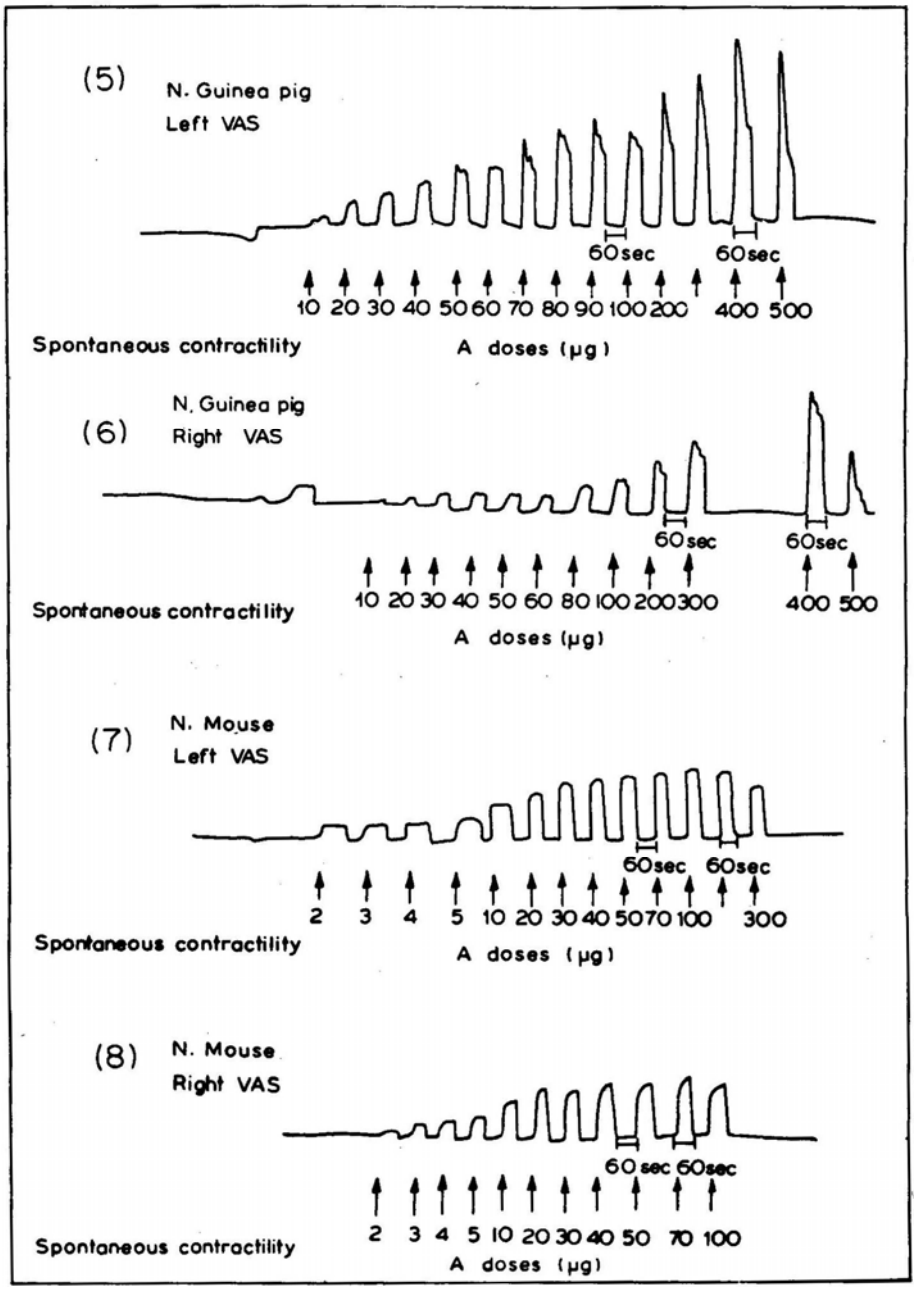
Sodium: In all the three animals, the Na^+ levels were found to be higher in the right vas deferens, than in the left one. This difference was significant in the mouse ($P<0.01$) and the rat ($P<0.02$), whereas in the guinea pig ductus deferens the difference was not significant ($P< 0.1$) (table 1).

Potassium: Guinea pig left and right vas deferens possessed the highest concentrations of K^+ and both sides had more or less same levels. The mouse had lesser levels of K^+ than the guinea pig; the rat had the least especially in the right side ($P<0.05$) (table 1).

Calcium: Ca^{2+} concentrations were not detectable in left vas deferens of the rat and the guinea pig but were detectable in the right vas deferens. The levels were insignificantly higher in the guinea pig than in the rat In the mouse vas deferens, calcium was not detectable at all (table 1).

Discussion

The vas deferens is not spontaneously active, and normally contracts only in response to neural stimulation. In the present study, spontaneous contractions



Figures 5-8. Recordings of the isolated vas deferens (left and right sides) of normal guinea pig and mouse, respectively, showing adrenaline (A) induced contractions. **5.** Note the absence of spontaneous contractility of the left vas in guinea pig. No response to lower doses of A (1 to 5 µg), but a graded response to the. higher doses was noted. **6.** The right vas showed similar pattern of response in guinea pig, which was of significantly low amplitude. **7.** No spontaneous contractions and no response of the mouse left vas to 1 µg A, but a dose-dependent response to all higher doses was noted. **8.** The contractility of the right vas was similar to that of the left one, but response were of markedly low amplitude.

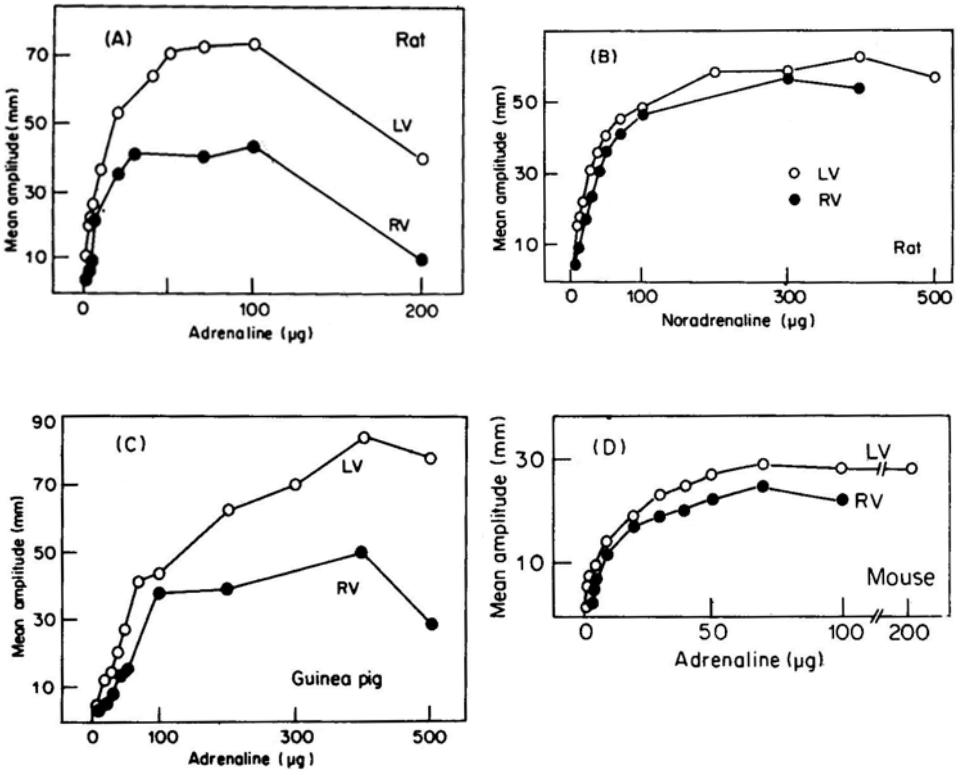


Figure 9. The graphs representing the mean amplitude of the induced response of the left and right vas deferens of rat, guinea pig and mouse plotted against the graded doses of adrenaline/ noradrenaline.

LV = Left vas deferens; RV = Right vas deferens.

Table 1. Comparative data on the concentrations of different electrolytes and calcium (Na^+, K^+, Ca^{2+}) in the vas deferens from left and right sides of normal adult rat, guinea pig and mouse.

Experimental animal	Sodium*		Potassium*		Calcium*	
	L.V.	R.V.	L.V.	R.V.	L.V.	R.V.
Rat	1193.3 ±141.6	2576.7 ±587.8	3383.3 ±310.2	2655.0 ±282.9	N.D.	1404.4 ±370.5
Guinea pig	3595.7 ±794.7	4204.2 ±754.9	5104.2 ±247.5	5287.4 ±321.3	N.D.	1539.7 ±877.9
Mouse	2693.7 ±851.5	5152.03 ±1123.3	4366.2 ±1121.6	3523.3 ±858.5	N.D.	N.D.

Values are mean ± S.E. L.V. = Left vas deferens R.V.= Right vas deferens. ★ µg/gm fresh tissue weights. N.D.= Not detectable.

were observed only in the left vas deferens of the rats. The nervous stimulation gives rise to excitatory junction potential which add to each other and when a critical value is reached, a spike is initiated. In the guinea pig, the spikes are all-or-none with overshoots of 20 mV. In the mouse, however, the spikes are graded in amplitude, varying from "humps" to overshoots of 5 mV (Bülbring and Bolton, 1979).

It is known that the density of innervation, and hence the effect of nerve activity shows much variation in different animals (Bülbring and Bolton, 1979), and that norepinephrine is the major neurotransmitter in rat, guinea pig and human vas deferens (Hafez, 1980); which also showed variations in its content in the human proximal and distal vas deferens (Baumgarten *et al.,* 1971). The density of innervation can therefore complement the physiological properties. In the present study too, the left vas deferens of rat was more responsive to different doses of adrenaline and noradrenaline (except at lower doses) than the right one. The same trend was observed for guinea pig and mouse left and right vas deferens.

Smooth muscle contraction is generally associated with alpha receptors and relaxation with β-receptors. The alpha receptor activation involves the increase in membrane permeability and calcium, whereas, the activation of β-receptor promotes the decrease of calcium and hence relaxation (Bülbring, 1979). This calcium which activates norepinephrine-induced contraction, originates from the loosely-bound calcium pool in extracellular fluid as well as from the pool of tightly-bound Ca^{2+} found intracellularly in the muscle fibre.

In the present study, the differential response of the vas deferens of the two sides from the same animal to adrenaline has been correlated with the greater concentrations of calcium and sodium in the right vas deferens of rats and guinea pigs, since it is known that the high levels of membrane-bound calcium renders the cells less excitable (Westfall *et al.,* 1975). The differential response of the left and right vas deferens might also be related to the levels of intracellular calcium, which is bound to calmodulin, a polypeptide (Cheung, 1960; Slater, 1981), which acts as an activator for norepinephrine release and muscle contraction as well as other cellular processes, only when bound to calcium. It is therefore probable that the left vas deferens might possess more calmodulin-bound calcium than the right, which has instead, more membrane-bound calcium. Hence, it is necessary to investigate further, the levels of bound and free forms of calcium in the vas deferens from the two sides, since it plays a key role in the release of neurotransmitters and muscle contraction via activation of calmodulin–the calcium binding protein (Lenz and Cormier, 1982). This calmodulin-bound calcium acting synergistically or antagonistically with c-AMP (Means *et al.,* 1982) is involved in discrete cellular activities as muscle contraction, neurotransmitter release, sperm motility etc.

The alpha-adrenergic nerves of the vas deferens help in transport of sperms and ejaculation (Bruschini *et al.,* 1977; Anton *et al.,* 1977). The exact mechanism of sperm transport needs further elucidation. Understanding of fine structure of smooth muscle of vas deferens as well as determination of levels of free and bound calcium and calmodulin would help to understand the mechanism whereby contractile pattern is modulated. The use of specific Ca^{2+} inhibitors or calmodulin binding drugs among them might be advantageous in the development of male contraceptives.

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