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## **Comparison of Smooth Muscle Contractility in Rat Vas Deferens (Tube) and Rat Stomach Strip (Sheet) in Various Physiological Salt Solutions**

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**Abstract:** Prototype agents such as Acetylcholine ( $1.0 \times 10^{-9}$  to  $5.0 \times 10^{-9}$  M), Nor-adrenalin ( $2.5 \times 10^{-8}$  to  $4.2 \times 10^{-7}$  M) and Potassium (50 mM) which stimulate different receptor populations in smooth muscle contraction have been examined on the contractility in Rat Vas Deferens (RVD) and Rat Stomach Strip (RSS) in various Physiological Salt Solution (PSS), varying the extra-cellular calcium in the medium shows a reversed sigmoidal curve in both tissues. In  $\text{Ca}^{2+}$ -free medium, the rat vas deferens contractility diminished rapidly <10 min, but the rat stomach strip, maintained successively diminished and remained stable after 1 h of this treatment. The contractile heights diminished as extracellular  $\text{Ca}^{2+}$  decreases in the bathing PSS from 1.8 mM  $\text{Ca}^{2+}$  to 0.9 mM  $\text{Ca}^{2+}$  and 0.45 mM  $\text{Ca}^{2+}$  and  $\text{Ca}^{2+}$ -free medium, (64, 81 and 98.2% in RVD and 31, 68 and 87% in RSS. In Depolarising medium, both tissues loses contractility rapidly less than five minutes in RVS and RSS maintained diminished contractions for over 1 h. The results in this study suggest that the rat stomach strip under the experimental conditions has more intra cellular calcium storage when compared to contractility in rat vas deferens.

**Key words:** Comparison, smooth muscle, contractility, physiological salt solution

### **INTRODUCTION**

Smooth muscles forms a very heterogenous group of tissues and the properties of vascular muscle differ greatly from those of the airways, gut or reproductive tracts. Many of these differences arise from tissue specific expression of cells surface receptors. The variations between different smooth muscles is at least greater as that between different types of striated muscle (Illingworth <http://www.bmb/eeds.ac.uk/Illingworth/muscle>). This reference support this study which was conducted in May 2007, in the University of Swaziland). The present study aimed at comparing contractility in two different smooth muscles (RVD) and (RSS) from different anatomical sites in order to examine the pattern of contractility under different experimental conditions and assess the properties of intra cellular calcium in these tissues.

### **MATERIALS AND METHODS**

The adult albino rats 8 weeks old; Sprague Dawley Strain used in these experiments were supplied from the Swaziland Institute for research, animal house. The animals were maintained in well ventilated conditions, under constant temperature (30°C) and humidity (50%) and exposed to light dark cycle for 3 weeks before use. The animals were fed on standard livestock pellets with free access to water and were treated ethically according to the guidelines for the treatment of experimental animal as determined in Southern Africa. Animal right council. This study was conducted in the University of Swaziland in May 2007. The rats were killed by cutting one of the common carotid arteries. The RSS was prepared according to the method of Vane (1957) and Vas Deferens were excised in different experiment and placed in a Petri dish containing physiological salt solutions at room temperature. The epididymal end of the Rat Vas Deferens was carefully cleansed of adhering connective tissue. Both

ends of the tissue were tied with fine cotton threads and mounted in 15 mL organ bath filled with physiological salt solution maintained at 37°C and aerated with a gas mixture containing 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The preparations were equilibrated for 1 h in the bath in an unstretched condition and the bathing solution renewed every 20 min. After equilibration, the contractile responses were recorded isometrically using Ugo Basile displacement transducer and signal amplified with a dynamometer (Gemini).

### Physiological Salt Solutions

The physiological salt solution used in these experiments were modified Tyrode solution with the following ionic composition in mM L<sup>-1</sup>. NaCl 118.0, NaHCO<sub>3</sub> 25.0, KCl 1.2, CaCl<sub>2</sub> 1.8, KH<sub>2</sub>PO<sub>4</sub> 1.2, MgCl<sub>2</sub> and glucose 25.0. The solution was bubbled with a 95% O<sub>2</sub> and 5% CO<sub>2</sub> gas mixture which maintained the solution at pH between 7.2-7.4. The depolarising Tyrode was prepared by replacing NaCl with KCl.

In other preparations the calcium ions were varied in the PSS by reducing Ca<sup>2+</sup> in the physiological solution from 1.8 to 0.9 mM (half), 0.45 mM (quarter) and Ca<sup>2+</sup>-free medium, (Ca<sup>2+</sup> was completely omitted in the PSS). This treatment was examined on sub-maximal contractions in both tissues in this study.

### Drugs

Drugs used: were acetylcholine bromide (Sigma, St. Louis, MD, USA). Noradrenalin hydrochloride (Sigma, St. Louis MO, USA). Potassium (Ubi Laboratory). The Stock solution of the drugs were prepared by dissolving in a 0.1 M HCl, while all other solutions were prepared by dissolving in distilled water. The stock solutions were stored at -20°C until use. The data obtained from these experiments were expressed as the mean ± Standard Error of the Mean (SEM). The values obtained in the different groups were compared using the student's t-test.

## RESULTS

### Effect of ACH (2.0×10<sup>-9</sup> M-5.6×10<sup>-7</sup> M) and Noradrenalin (NA) (2.0×10<sup>-8</sup> M to 4.2×10<sup>-7</sup> M) on Rat Stomach Strip and Rat Vas Deferens Respectively

Dose effect relationship was established using Acetylcholine concentration (ACH) ranging from (1.0<sup>-9</sup> to 5.6×10<sup>-9</sup> M) in the rat stomach strip, similarly Noradrenalin (NA), (2.0×10<sup>-8</sup> to 4.2×10<sup>-7</sup> M) was used in Rat Vas deferens. A sub maximal dose of ACH (2.0×10<sup>-9</sup> M) and Noradrenalin (2×10<sup>-7</sup> M) was chosen and examined in varying extra-cellular Ca<sup>2+</sup> from 0.9 mM to 0.45 mM and Ca<sup>2+</sup>-free media in different experiments. Contractile heights in both muscles diminished as shown in Fig. 1 and 2.

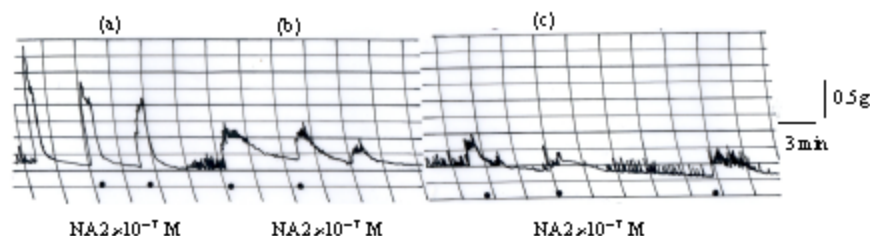


Fig. 1: Representative trace recording of effects of Extra cellular variations PSS on Noradrenaline (2×10<sup>-7</sup> M) in, RVD (a) 1.8 mM, (b) 0.9 mM and (c) 0.45 mM Ca<sup>2+</sup> Ca<sup>2+</sup>-free medium horizontal bar tension(g) Vertical bar (time interval). All contractions were recorded from the same experiments

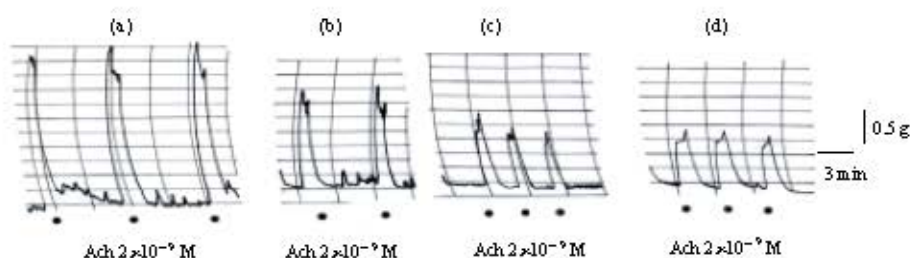


Fig. 2: Representative trace recording of effects of Extra cellular  $\text{Ca}^{2+}$  variations PSS on Ach-induced Sub maximal contractions ( $2 \times 10^{-7} \text{ M}$ ) in Rat stomach strip preparations, (a)  $1.8 \text{ mM Ca}^{2+}$ , (b)  $0.9 \text{ mM Ca}^{2+}$ , (c)  $0.45 \text{ mM Ca}^{2+}$  and (d)  $\text{Ca}^{2+}$ -free medium horizontal bars T (g) Vertical bar time interval, All contractions were recorded from the same experiments

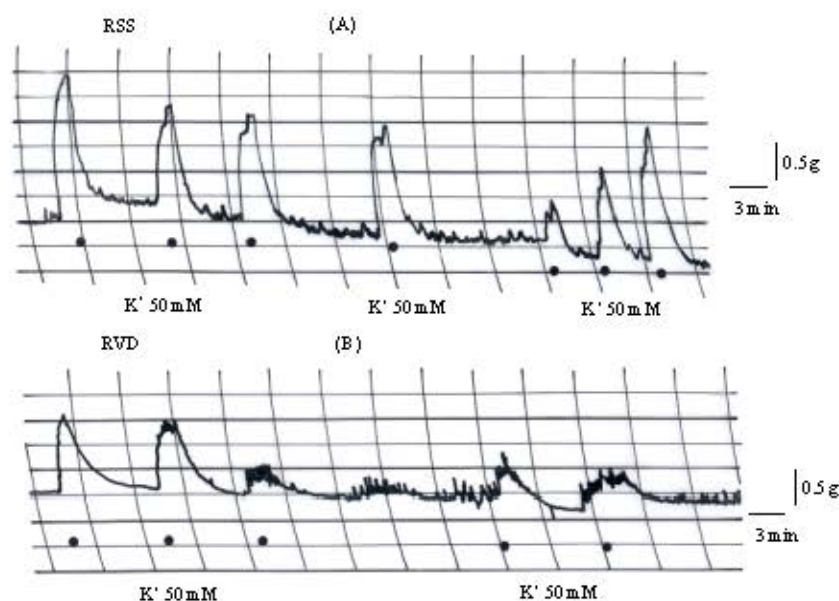


Fig. 3: Effects of  $\text{K}^+$   $50 \text{ mM}$  induced contractions in (A) RSS and (B) in RVD. All preparations were recorded from same experiments

#### **Effect of $\text{Ca}^{2+}$ -Free Pss on Sub-Maximal Doses of ACH and NA**

The effect of this treatment was examined on contractility in RSS and RVS in different experiments. Contractile height in both muscle decline exponentially (Fig. 1, 2). The percentage decline in heights of contractions is shown in the Table 1.

#### **Effects of $\text{K}^+$ (50 mM) on Contractile Action of RSS and RVD**

The result of the contractile action of both RSS and RVD in this medium the Rat stomach strip maintained higher contractions than the rat vas deferens (Fig. 3).

#### **Time Effect of $\text{Ca}^{2+}$ -Free Medium on Sub Maximal Contractions in RSS and RVD**

Contractions in RVD decline rapidly  $< 10 \text{ min}$  up to (98.2%) of its contractile heights, but the RSS diminished contractions 87% remained stable for over 1 h.

Table 1: Showing contractile heights (%) decline in extra cellular  $Ca^{2+}$  variations from 1.8 to 0.9 mM. 0.45 mM  $Ca^{2+}$   $Ca^{2+}$ -free PSS

Tissue	0.9 mM $Ca^{2+}$	0.45 mM $Ca^{2+}$	$Ca^{2+}$ -free
RSS	31.0±1.5	68.0±2.0	87.5±2.5 (n = 10)
RVS	64.0±2.5	81.5±1.5	98.2±1.5 (n = 10)

Level of significance values are mean±SEM (n = 10), p<0.05 student t-test

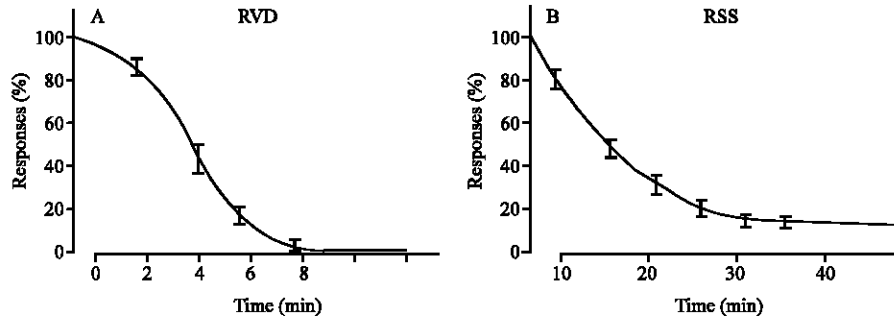


Fig. 4: Time related decline in responses of (A) RVD and (B) RSS in  $Ca^{2+}$  free PSS, each point is a mean of 10 experiment, responses elicited every 5 min after  $Ca^{2+}$  withdrawal from PSS. Each response was expressed as % of the mean of 10 responses from the same experiment

#### Effects of Depolarising PSS on Submaximal Doses of ACH and NA in RSS and RVD

Both tissues did not respond to contraction in this medium (Fig. 4).

### DISCUSSION

Smooth muscles differ in their contractions, that it would be difficult to propose a preparation representative of all (Gillespie, 1972), Nonetheless, there would be a general agreement that for experimental purposes certain properties are desirable. The preparation should consist of cells which are arranged in parallel bundles to form a thin sheet, such an arrangement could minimise the problem of diffusion for drug access and ion exchange. The muscle should be bilateral so that the control and test preparation can be taken from the same animal. No existing preparation fulfils all of these properties since most smooth muscle are found in the walls of hollow viscera (Gillespie, 1972). From this background the contractile agents that increase free intracellular calcium initiate muscle contraction (Hurwitz and Suria, 1971).

The isolated RSS and the RVD are known to possess most of, if not all these desirable features. The results obtained with Acetylcholine and Noradrenalin, both drugs induce-contractions in smooth membranes by mobilising  $Ca^{2+}$  from intra cellular stores by opening  $Ca^{2+}$  Channels in the plasma membranes thus permitting sustained influx of extracellular  $Ca^{2+}$  (Chiu *et al.*, 1986; Minnermann, 1988; Koch *et al.*, 1990; Nishimura, 1991) the increased permeability of the cell membrane for  $Ca^{2+}$  entry is common to different agonists and different preparation (Putney, 1970). The cellular mechanism of contraction involved in the response of smooth muscle to Nor-adrenalin or Acetylcholine and high potassium solution is different, the former is produced by receptor mediated supply of  $Ca^{2+}$  and the latter by influx of  $Ca^{2+}$  through voltage gated  $Ca^{2+}$  channel (Karaki *et al.*, 1997). The results in this study shows higher muscles contractions in receptor mediated responses when compared to  $K^+$ -mediated responses in both tissues (Fig. 3). The findings in varying, extra cellular calcium from 1.8 to 0.9 mM  $Ca^{2+}$ , 0.45 mM  $Ca^{2+}$  and  $Ca^{2+}$ -free medium in these muscles showed a reversed sigmoidal curve... which indicated the importance of both extra cellular  $Ca^{2+}$  and intra cellular  $Ca^{2+}$  are needed

for muscle contraction. However, in this study, the rat stomach strip contractions diminished and remained stable after 1 h, while in the rat vas deferens, contractility diminished rapidly under 10 min and this treatment; confirms our earlier study (Aziba and Okunola, 1999). The decreased in heights of muscle contractions to 77 and 98% in RSS and RVD, respectively in  $\text{Ca}^{2+}$ -free medium showed that intra-cellular  $\text{Ca}^{2+}$  store is more in RSS than in RVD (Fig. 3). The reason for this may be due to poor endoplasmic or Mitochondria development in this muscle.

Contractions induced by high  $\text{K}^+$  is a direct effect which results in cell depolarisation which account for the non response to, Noradrenalin nor Acetylcholine in depolarise medium in this study and consequently activation of voltage-dependent calcium channels and  $\text{Ca}^{2+}$  influx into smooth muscle cell is inhibited.

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