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Original Article

EFFECTS OF DRUGS AND IONIC VARIATIONS ON CONTRACTIONS OF RAT SMOOTH MUSCLES

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Chemical agents, Acetycholine (5.0 x 10^{-9}M - 4.0 x 10^{-8}M), Noradrenaline (6.6 x 10^{-5}M - 5.2 x 10^{-4}M) and Potassium (25mM, 50mM) which stimulate different receptor populations in smooth muscle contractions and their ionic channel properties have been examined in different isolated preparations of Rat ileum (RI), Rat Stomach Strip (RSS), and Rat Vas Deferens (RVD) using known Ca^{2+} channel and specific receptor blockers. Atropine and Phentolamine respectively blocked Ach and NA competitively. While effect on K^+- induced contraction was unaffected. The Rat ileum and Rat Stomach Strip has more pool of intracellular Ca^{2+} store than the RVD because in Ca^{2+}-free medium, contraction declined markedly. The Vas Deferens showed unstable contraction in depolarised medium. The result of this suggests that as membrane depolarisation increases contraction in Rat Vas Deferens smooth muscle declined, while Verapamil and Nifedipine were more sensitive in blocking K^+ than Ach and NA induced contractions.

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INTRODUCTION

Smooth muscles differ in their properties that it would be difficult to propose preparation representative of all (Gilespie, 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 drugs access and in ion exchange studies. The muscle should be bilateral so that the control and test preparation can be taken from the same animal. Both motor and inhibitory in autonomic studies. No existing preparation fulfils all of these properties since most smooth muscles are found in the walls of hollow viscera, the interpretation of mechanical responses.

From this background, the contraction agents that increase free intracellular Ca^{2+} levels initiate the contraction of smooth muscles (Hurwtts and Suria, 1971). The release of Ca^{2+} may be triggered from intracellular Ca^{2+} through membrane ionic channels (Bolton, 1979).

The isolated rat stomach strip, rat ileum, and the rat vas deferens are known to possess most if not all these desirable features, the aim of the present investigation is to examine the interactions of drugs and ionic variation on different rat smooth muscles.

MATERIAL AND METHODS

Animals:

The animals used for these studies are male albino rats of the Wistar strain Animals were fed with adequate rat cubes and had access to water. The weight of the rat used ranged between 150-250g.

Physiological Salt Solution:

The physiological salt solution was in millimolar concentration (mM) NaCl 136,

KCl 26.85, NaHCO3 11.90, NaH_2 PO_4 8.33, $CaCl_2$ 16.21, $MgSO_4.7H_2O$ 10.56, Glucose 5.55.

Depolarizing Tyrode Solution (mM): KCl 24.96, $MgSO_4$. $7H_2O$ 8.33, $NaH_2PO4_2H_20$ 10.56, Glucose 5.53.

Preparation of Tissue:
Male albino rats of Wistar of strain 150-250g-body weight were stunned, decapitated and the vas deferentia was dissected free from the surrounding mesenteries. Each *Vas deferens* was removed from its urethral and epididymal connections, placed in a petri-dish containing tyrode solution bubbled with air. A length from the epididymal end of the *vas deferens* approximately 2cm long was cut and carefully suspended in a 15ml organ bath filled with normal tyrode. The Rat Stomach Strip was isolated and prepared according to the method of Vane (1957). The rat ileum was isolated and prepared according to the method of Rang and Ritter (1970).

RESULT

Effect of Specific Antagonists on Muscle Contraction :
Ach ($5.0x10^{-9}$M-$4.0x10^{-8}$M) Na ($6.6x10^{-5}$ M-$5.2x10^{-4}$M) caused concentration dependent contractions of the isolated Rat Stomach Strip and Rat Vas Deferens respectively. Ach concentration - response curves were shifted to the right with maximum unchanged, indicative of competitive antagonism (Fig.1). At 10^{-9}M-10^{-7}M of Nifedipine Ach, NA contractions were unaffected, but blocked contractions evoked by K^+

Effect of Ca^{2+} variation in RSS
After obtaining a dose response effect of Ach in rat stomach strip, Noradrenaline in rat vas deferens and k^+ in rat ileum, a dose causing 75% of the response was selected, after which it was subjected to Ca^{2+} variations in physiological salt solution from 1.8mM, 0.9mM and 0.45mM Ca^{2+}.

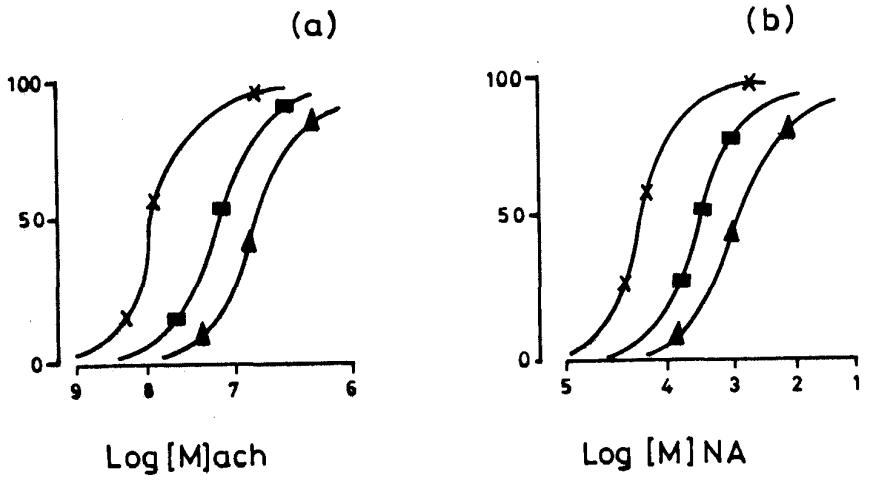

FIG. 1.
Dose-response to Ach and Noradrenaline on rat stomach strip. Rat Vas Deferens in the absence (X) and in the presence of Atropine 10^{-9}M (□-□) , 10^{-8}M (Δ-Δ) in A and Phentolamine 10^{-5}M (□-□), 10^{-4}M (Δ-Δ), in B concentrations are expressed as percentage of the maximum control values. Each point represents the mean of 6 experiments.

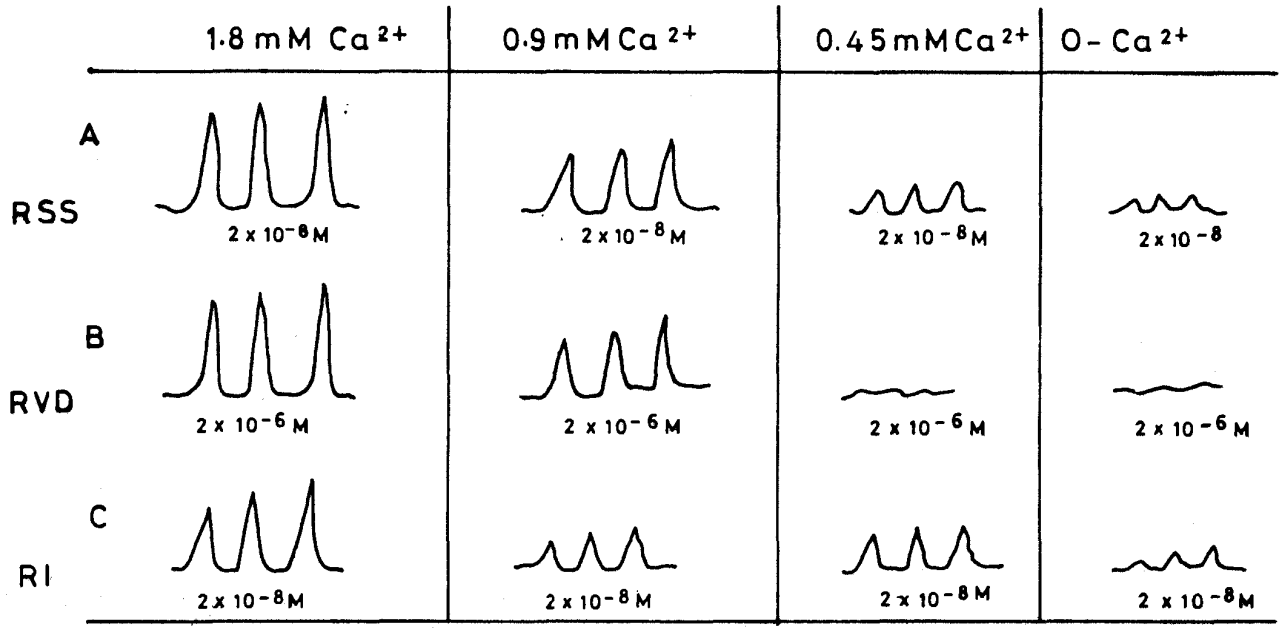

FIG. 2.
Effects of extracellular Ca^{2+} variation in Physiological salt solution on Contrations induced by submaximal dose of acetylcholine (2 X 10^{-8}M):, Noradrenaline (2 X 10^{-6}M) in Rat stomach Strip (A), Rat Vas Deferens (B) and Rat ileum (C) in 1.8, 0.9 and 0.5 mM and 0 – Ca^{2+} Medium.

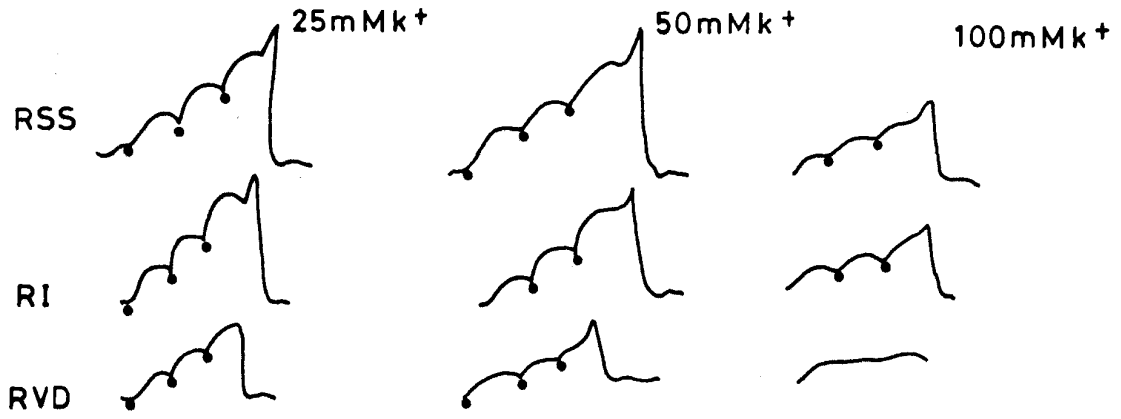

FIG 3.
Effects of Depolarisation (25, 50, 100mM K^+ on cumulative contractions in Rat Stomach Strip. The vertical bar indicates time interval in minutes and the horizontal bar is the tension in the muscle strip. The dots indicate points of drug addition.

As the extracellular Ca^{2+} varies in response declined markedly, while the rat stomach the medium, the vas deferens strip and rat ileum responses declined and stabilized contractions after 30 minutes of this treatment (Fig 2).

Effect of Depolarisation on Muscle Contractions and Time Course Study

In these studies a dose effect response was established cumulatively according of Van Rossum (1963) in normal physiological solution: thereafter were incubated in various depolarising solutions in different experiments (k^+ 25mM, 50mM 100mMk+). The mechanical responses of tissues in this medium differ greatly. Muscle contractions in RSS did not change from control at 50mM K^+, while the Vas deferens declined completely in 100mM K^+; the rat ileum contractions faded in less that 5 minutes under this treatment (Fig 3)

Effect of Organic Ca^{2+} Blockers on Agonist-Induced Contractions

Since channels are opened during depolarisation in muscle contractions this study investigated the ionic channels interaction employed by these agents in these muscles. After obtaining a dose-effect curve, the muscles in different experiment were incubated in various concentrations of Nifedipine and Verapamil. The blocking effect was non-competitive because there was no shift to the right and the maximum was suppressed Nifedipine 10^{-9}M effectively blocked k^+ -induced contractions.

DISCUSSION

The result presented in this study generally showed that the various muscles used possess similar ionic properties with the different chemical agents used and their Ca^{2+} ionic properties vary with the agents stimulating different receptor populations. Ach in RSS and NA-induced responses in RVD and Ach in RI were competitively blocked by atropine and phentolamine. These findings confirmed that the RSS and RI are well endowed with muscarinic receptors. Similarly phentolamine blocked competitively the effect of NA-induced responses in the RVD which again suggests the presence of alpha-receptors in the muscle. In Ca^{2+} variation studies on the three tissues, the rat stomach strip and ileum could still elicit responses when stimulated with Ach, while the Vas deferens faded out in less than 5 minutes of this treatment, this showed that the Ca^{2+} stores of the muscles differ in their ability to hold Ca^{2+} under the experimental condition. Their Ca^{2+} holding capacities in the tissues as follows RSS>RI>RVD. This finding could be attributed to the development and functioning of the sarcoplasmic reticulum the storage site of intracellular Ca^{2+} in mammals.

The result on ion channel interactions with agonist was envisaged because opening of Ca^{2+} channel requires membrane depolarisation. Agents that produce hyperpolarisation will cause Ca^{2+} channel to close which in turn will reduce Ca^{2+} (Gurney, 1994) influx and promotes muscle relaxation or inhibition. Our result in these studies, showed that the RVD lost contractility in high K^+ medium, while RSS and RI still showed contractions in 100mM which faded out in less than 2 minutes.

This finding showed that the rat smooth muscle in high K^+ medium loses contractility. It is therefore proposed that these tissues can respond to contraction in 50mM K^+ medium which is appropriate for GIT smooth depolarisation.

The organic Ca^{2+} channel blockers are more effective in inhibiting K^+-induced contractions that receptor mediated contractions by Ach and NA in these smooth muscles.

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