INFLUENCE OF CALCIUM REMOVAL FROM BATH SOLUTION ON ISOLATED RAT DUODENUM CONTRACTIONS

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Abstract: In this study whether extracellular Ca** is essential to produce an increase of tension of isolated rat duodenum by ACh, 5-HT, AVP and KCl, was examined. KCl and AVP-evoked contractions were almost totally prevented by Ca** removal from bath solution. The increase of tension of isolated duodenum caused by ACh or 5-HT was totally prevented after adding nifedipine, a Ca** channel blocker, into Ca free solution. Our results suggest that ACh or 5-HT utilizes intracellular as well as extracellular sources of Ca** to produce contraction in rat duodenum, whereas AVP or KCl evoked contraction was mainly due to influx of Ca from extracellular sources.

Key words: calcium removal acetylcholine 5-hydroxytryptamine vasopressin duodenum

INTRODUCTION

Smooth muscle contraction is initiated when agonists bind to receptor and activate a series of events which increase intracellular Ca** concentration. Ca** influx from extracellular sources and release from internal stores gives rise to this increase (1). Contraction is induced when intracellular Ca** rise activates myosin light chain kinase which causes increase in cross-bridge interactions (2, 3).

Smooth muscle contractile responses are ultimately linked to the influx of extracellular Ca** and/or the mobilization of intracellular Ca**. The present study is a preliminary study, designed to examine whether contractile agonists mobilize Ca** by different mechanisms in rat duodenum.

METHODS

Isolation of smooth muscle preparations: 10 Swiss Albino rats (180-350 g) were sacrificed by cervical dislocation. After removal duodenums were cleaned of adherent connective tissue and cut into 10 mm long pieces. Duodenums were suspended in 10 ml-tissue bath filled with Tyrode solution (37°C aerated with 95% O₂-5% CO₂) (4). When Ca** free solution was used, 2 mM ethylene glycol bis (beta-aminoethyl ether)-N, N'-tetraacetic acid (EGTA) and equimolar Na Cl, instead of Ca Cl₂, were added into Tyrode solution. Tissues were placed under initial resting force of 10 g and were allowed to equilibrate for approximately 1 hr before exposure to drugs.

Tension recordings: The development of tension of duodenum was measured with a transducer (Harvard Isometric transducer, 50-7905) and recorded on an oscillograph (Harvard Universal Oscillograph, 50-8648).

Drugs: The following agents were used: Acetylcholine (ACh), Serotonin (5HT), Argynin vasopressin (AVP), Atropine, nifedipine, EGTA and KCl. All were obtained from Sigma Chemical Co. Drugs were dissolved in distilled water.

Statistical evaluation: Data were evaluated statistically using Student’s t test for paired observation and are expressed as means ±SD. When P values were less than 0.05, the difference was considered to be significant.

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RESULTS

ACh, 5-HT, AVP or KCl caused an increase in tension of duodenum bathed in Tyrode solution. Dose dependent % contractions to ACh are shown in Fig. 1. Since tachyphylaxis was reported in smooth muscle to 5-HT and AVP (5), the relation between concentration of these agonists and tension was not examined. The contractile doses of 5-HT, AVP and KCl were determined in a separate experiment and are also shown in Fig. 1.

Atropine, a muscarinic receptor antagonist, did not alter the contraction to 5-HT, AVP or KCl, whereas ACh did not cause any increase in tension in atropinized duodenum (Fig. 2).

The removal of extracellular Ca²⁺ attenuated, but did not prevent, the development of an ACh or a 5-HT contraction (P < 0.001). These attenuated contractions to ACh and 5-HT disappeared after adding nifedipine, a Ca²⁺ channel blocker into Ca²⁺ free bath solution (Fig. 4). Extracellular Ca²⁺ removal prevented KCl- or AVP evoked contraction (Fig. 3) (P < 0.001). After replacement of Ca²⁺ free solution with Tyrode solution, agonists evoked contractions appeared within one hour.

In three duodenums bathed in Ca²⁺ free solution, we also studied whether contraction to ACh (1.10⁻⁴ M) decreased by repetitive adding or not. The results of this examination are shown in Fig 5. The peak tension of ACh-stimulated duodenum gradually decreased by adding equimolar ACh doses.
Influence of Calcium Removal on Duodenum

In the absence of extracellular Ca²⁺, residual contractile response even in the absence of extracellular Ca²⁺ suggests that these agonists utilize another source of Ca²⁺, probably intracellular. The results of the present investigation do not provide direct evidence for ACh or 5-HT-induced intracellular Ca²⁺ release. However, several experimental observations support this hypothesis:

1. Contraction of rat duodenum to ACh were markedly attenuated by atropine indicating muscarinic receptor-mediated response (present study).

2. Muscarinic receptors in various tissues are coupled to phosphoinositide (PI) hydrolysis (13).

3. Equimolar ACh doses gradually decreased the peak tension of duodenum in Ca²⁺-free solution (present study), indicating the exhaustion of intracellular Ca²⁺ stores.

4. Contraction of rat duodenum induced by 5-HT is not mediated via muscarinic receptor (present study), and 5-HT has a direct effect on PI breakdown (14).

5. Inositol-1, 4, 5-triphosphate (IP3), a product of PI hydrolysis, can induce intracellular Ca²⁺ release in pancreatic cells (15).

6. Nifedipine prevented ACh-and 5-HT-evoked contraction in duodenum (present study) and in human umbilical artery (11), indicating that nifedipine blocks Ca²⁺ entering from intracellular stores as well as extracellular sources.

Thus, although the formation of IP3 in the presence of ACh or 5-HT was not measured, the current data suggest that these agonist utilizes intracellular sources of Ca²⁺ as well as extracellular ones to produce contraction in rat duodenum, an effect that may be linked to its ability to increase PI hydrolysis.

It seems that high-K solution exerts its effects mainly because it reduces the K gradient across the membrane, and the reduction in membrane potential increases permeability of Ca²⁺ and Ca²⁺ enters from extracellular source (1). In the present study, supporting this classic knowledge, KCl produced an increase in tension of duodenum. Ca²⁺ removal prevented high-K-induced contractions. Similar results was reported in calf coronary artery (12) and in bovine ventricular coronary artery (16).
AVP is a potent vasoconstrictor in the systemic circulation, but its effects on the nonvascular smooth muscle are less clear. AVP has been reported to contract the intestinal smooth muscle, but only at high doses (17). We also indicated AVP-induced contraction in duodenum at 5000 ng dose. This contraction was totally prevented by Ca++ removal, indicating

**REFERENCES**