Indian J Physiol Pharmacol 1992; 36(3) : 181-184

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

CEM SÜER*, AYDIN ERENMEMISOGLU**†, SADUN TEMOCIN*, SEDAT AKAR*, HÜSEYIN BEYDAGI* AND IBRAHIM TASPOLAT**

*Departments of *Physiology and **Pharmacology, Erciyes University, Medical Faculty, 38039 Kayseri/TURKEY*

(Received on February 2, 1992)

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 agonosts mobilize Ca^{++} by different mechanisms in rat duodenum.

METHODS

Isolation of smooth muscle preparations : 10 Swiss Albino rats (180-350 g) were sacrified 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% O_2-5% CO_2) (4). When Ca^{++} free solution was used, 2 mM ethylene glycol bis (beta-aminoethyl eter)-N, N'-tetraacetic acid (EGTA) and equilomar Na Cl, instead of Ca Cl_2, 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 Oscilograph, 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.

† Corresponding Author

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 tachyphlaxis 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.

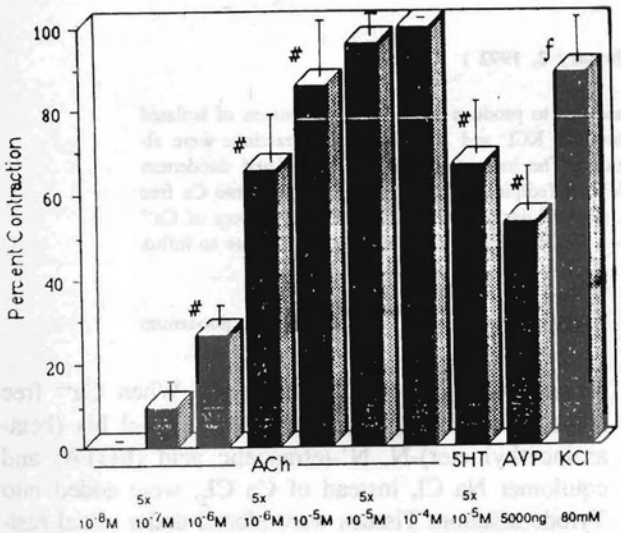

Fig. 1 : Concentration-dependent contractions to Acetylcholine (ACh), and contractions to single dose of Serotonin (5-HT), argynin vasopressin (AVP) and KCl in rat duodenum bathed in Tyrode solution. Values represent the mean±SD using 7-13 deuodenum isolated from 10 animals. Contraction is expressed as the percentage of increase in $1x10^{-4}$ M ACh-induced tone. Comparison for statistical evaluation : f P<0.01; #: P<0.001 when the means of contraction were compared with next one for ACh and with $1x10^{-4}$ M ACh for other substance, respectively.

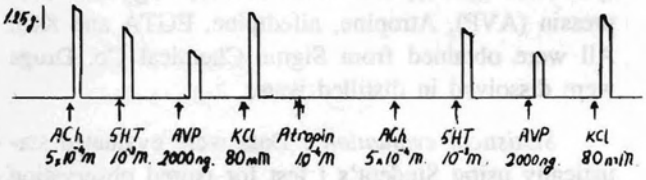

Fig. 2 : A schematic trace belongs to duodenum in which examined whether contractions to acetylcholine (ACh), serotonin (5-HT), argynin vasopressin (AVP) and KCl are mediated via muscarinic receptor.

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 Ca^{++} 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 Ca^{++} channel blocker into Ca^{++} free bath solution (Fig. 4). Extracellular Ca^{++} removal prevented KCI-or AVP evoked contraction (Fig. 3) ($P < 0.001$). After replacement of Ca^{++} free solutioon with Tyrode solution, agonists evoked contractions appeared within one hour.

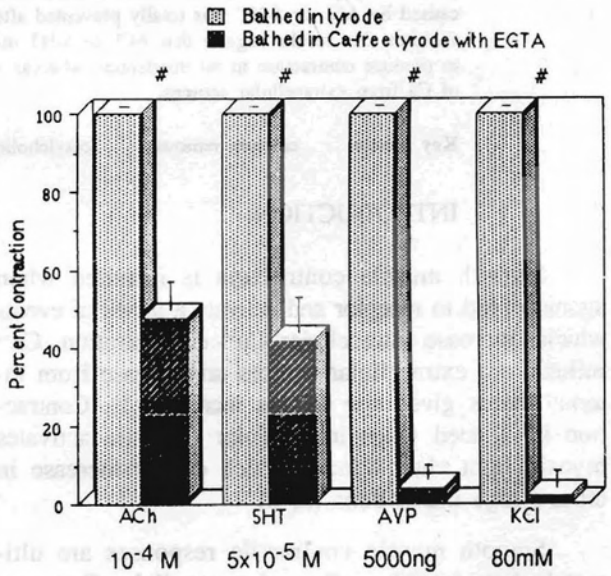

Fig. 3 : Comparative figure of the tension recorded from deudenum exposed acetylcholine (ACh), serotonin (5-HT), argynin vasopressin (AVP) and KCl in Ca-containing tyrode with that recorded from duodenum exposed to same agents in Ca-free tyrode with EGTA. Contraction is expressed as the percentage of increase in each agonist-induced tone. Values persent the mean±SD using 7-13 duodenum isolated from 10 animals. Comparison for statistical evaluation **#: P<0.001 when the means of contraction to agonist were compared with each other.**

In three duodenums bathed in Ca^{++} free solution with EGTA, 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.

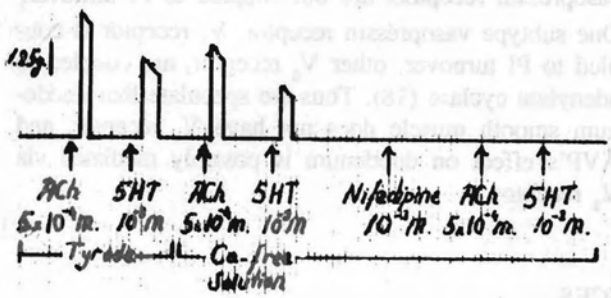

Fig. 4 : A schematic trace belongs to duodenum in which examined the effect of nifedipine on contraction to acetylcholine (ACh), serotonin (5-HT), attenuated by Ca removal.

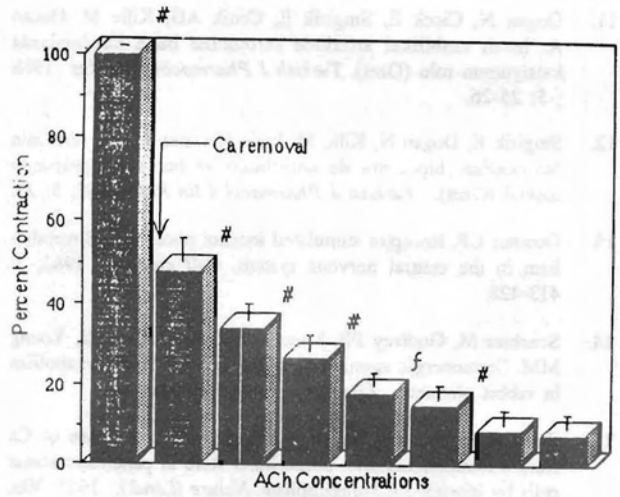

Fig. 5 : The effect of Ca removal from solution on acetylcholine-evoked duodenum contraction. Comparison for statistical evaluation; f: P<0.01; #: P<0.001 when the means of contraction were compared with next one.

DISCUSSION

ACh and 5-HT caused an increase in tension of duodenum, suggesting the existence of a binding site for these agonists in duodenum. ACh and 5-HT produced a stronger contraction in a Ca^{++} containing medium than in a Ca^{++}-free medium. Such a response is similar to those of bovine coronary artery (6), swine trachealis (7), the circular muscle layer of guinea pig and human stomach (8), the circular muscle layer of guinae pig ileum (9) to ACh, and rat stomach fundus (10), human umbilical artery (11) and calf coronary artery (12) to 5-HT. The observation that ACh or 5-HT was able to elicit a small but significant, residual contractile response even in the absence of extracellular Ca^{++} suggests that these agonists utilize another source of Ca^{++}, probably intracellular. The results of the present investigation do not provide direct evidence for ACh or 5-HT-induced intracellular Ca^{++} release. However, several experimental observations support this hypothesis :

1. Contractions 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 Ca^{++} - free solution (present study), indicating the exhaustion of intracellular Ca^{++} 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 Ca^{++} release in pancreatic cells (15).

6. Nifedipine prevented ACh-and 5-HT-evoked contraction in duodenum (present study) and in human umblical artery (11), indicating that nifedipine blocks Ca^{++} 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 Ca^{++} 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 Ca^{++} and Ca^{++} enters from extracellular source (1). In the present study, supporting this classic knowledge, KCl produced an increase in tension of duodenum. Ca^{++} 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 **vasopressin receptors are not coupled to PI turnover.** One subtype vasopressin receptor, V_1 receptor is coupled to PI turnover, other V_2 receptor, are coupled to adenylate cyclase (18). Thus we speculate that duodenum smooth muscle does not have V_1 receptor, and AVP's effect on duodenum is possibly mediated via V_2 receptor.

REFERENCES

1. Bolton TB. Mechanisms of action of transmitter and other substances on smooth muscle. *Physiol Rev* 1979 ; 59 : 606-718.
2. Kamm KE, Stull JT. The function of myosin and myosin light chain kinase phosphorilation in smooth muscle. *Annu Rev Pharmacol Toxicol* 1985; 25:593-620.
3. Taylor DA, Bowman BF, Stull JT. Cytoplasmic Ca^{++} is a primary determinant of myosin phosphorilation in smooth muscle cells. *J Biol Chem* 1989; 264: 6207-6213.
4. Porry WLW (Ed) : *Pharmacological experiments on isolated preparations,* Edinburgh, Livingstone, 1970, p2.
5. Kayaalp O (Ed) : *Rasyonel tedavi yonunden tibbi farmakoloji,* Ankara, Ankara, Toroman ve Ulucan Matbaasi, 1987, p 237.
6. Ratz PH, Flaim SF. Acetylcholine-and 5-hydroxytryptamine-stimulated contraction and Calcium uptake in bovine coronary arteries. Evidence for two populations of receptor operated calcium channels. *J Pharmacol Exp Ther* 1985; 234:641-647.
7. Shieh CC, Petrinic MF, Dwyer TM, Farley JM. Concentrations-dependence of ACh-induced changes in calcium and tension in Swine trachealis. *J Pharmacol Exp Ther* 1991; 256: 141-148.
8. Bitar KN, Burgess GM, Putney JV, Makhlouf GM. Source of activator Ca in isolated guinae pig and human gastric muscle cells. *Am J Physiol* 1986; 250:G280-G286.
9. Grider JG, Makhlouf GM. Contraction mediated by Ca release in circular and Ca influx in longitudinal intestinal muscle cells. *J Pharmacol Exp Ther* 1988; 244: 432-437.
10. Secrest RJ, Schoepp DD, Cohen ML. Comparision of contractions to serotonin, carbamylcholine and prostaglandin $F_{2\alpha}$ in rat stomach fundus. *J Pharmacol Exp Ther* 1989; 250: 971-978.
11. Dogan N, Cicek E, Singirik E, Cenik AG, Kilic M, Ozcan A. Insan umbilikal arterinde serotonine bagh kasilmalarda kalsiyumun rolu (Ozet). *Turkish J Pharmacol Clin Res* 1988 ; 5: 25-26.
12. Singirik E, Dogan N, Kilic M. Izole Koroner arterde serotonin cevaplarimn hipotermi ile arttirilmasi ve bunlarin kalsiyumla iliskisi (Ozet). *Turkish J Pharmacol Clin Res* 1988; 5: 25.
13. Downes CP. Receptor-stimulated inositol phospholipid metabolism in the central nervous system. *Cell calcium* 1982; 3: 413-428.
14. Scachter M, Godfrey PP, Minchin MCW, McClue SJ, Young MM. Serotonergic agonists stimulate inositol lipid metabolism in rabbit platelets. *Life Sci* 1985; 37:1641-1647.
15. Streb H, Irvine RF, Berridge MJ, Schultz I. Release of Ca from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-triphosphate. *Nature (Lond.),* 1983; 306: 67-69.
16. Ratz PH, Flaim SF. Mechanism of 5HT contraction in isolated bovine ventricular coronary arteries : Evidence for transient receptor-operated calcium influx channels. *Cir Res* 1984; 54:135-143.
17. Hays RM. Agents affecting the renal conservation of water. The Pharmacological Basis of Therapeutics (Eds: AG Gilman, TW Rall, A Nies, P Taylor), New York, Pergamon Press, 1990, P 739.
18. Kinter LP, Hoffman WF, Strassen FL. Antagonists of antidiuretic activity of vasopressin. *Am J Physiol* 1988; 254:F 165-F171.