Serotonin-Induced Contraction in Isolated Rat Duodenal Muscle*

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✔ The majority of the serotonin stored in the body is located in gastrointestinal enterochromaffin cells.
We investigated the effects of serotonin (5-HT) on the isolated rat duodenal muscle contraction. Acetylecholine (ACh) produced a greater contraction than 5-HT did. Selective antagonist at L-type Ca^{2+} channels, diltiazem inhibited more significantly the contractile response to ACh than to 5-HT. Pretreatment with ketanserin inhibited the contractile response to 5-HT but did not inhibit the contractile response to ACh. 5-HT and ACh evoked contractions were decreased in Ca^{2+} free medium.
These results indicate that, in the rat duodenal muscle 5-HT and ACh do not utilize the same calcium channels, and both ACh and 5-HT utilizes intracellular as well as extracellular sources of Ca^{2+} to produce contractions. 5-HT acts via its specific receptors on rat duodenal smooth muscle.
Key words: *Isolated smooth muscle contractility, serotonin*

✔ **Sıçanda İzole Duodenum Kontraktilitesi Üzerine Serotoninin Etkisi**
Organizmadaki serotoninin en büyük kısmı sindirim kanalı enterokromafin hücrelerinde bulunur.
Sunulan çalışmada serotoninin izole duodenal düz kas kontraktilitesi üzerine etkisi araştırıldı. Asetilkolin (ACh) ile stimule edilen kontraksiyon cevapları serotonin ile oluşturulandan daha yüksekti.
L tipi Ca^{++} kanal blokörü olan diltiazemin ACh'ın oluşturduğu kontraksiyon cevaplarını inhibe edici etkisi serotonin üzerine olan etkisinden daha fazla idi. Ketanserin uygulaması ise serotonine cevabı inhibe ederken ACh'a karşı cevabı etkilemedi. Kalsiyumsuz ortamda Serotonin ve ACh ile stimule edilen kontraksiyonlar azalmış olarak bulundu.
Sonuçta sıçan duodenum düz kasında serotonin ve ACh'ın aynı Kalsiyum kanallarını kullanmadığı ve her iki agonistin de kontraksiyon oluşturmak için ekstraselüler ve intraselüler kalsiyum kaynaklarını kullandığı ve serotoninin spesifik reseptörleri üzerinden etki gösterdiği öne sürülmektedir.
Anahtar kelimeler: *İzole düz kas kontraksiyonu, serotonin*

INTRODUCTION

Diseases characterised by inflammation are important causes of mobidity and mortality in humans. During the inflammatory response, inflamed tissues release histamine, serotonin, prostaglandins and kinins locally; these agents mediate manifestations of inflammation such as pain, vasodilatation, increased capillary permeability and smooth muscle contraction[1]. The

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majority of the serotonin (5-HT) from these mediators, stored in the body is located in gastrointestinal enterochromaffin cells[2], approximately 95% of the intestinal content of 5-HT being localised to these cells[3]. However little is known about the control of 5-HT release from such cells or the physiological functions of 5-HT upon this release. Although 5-HT is predominantly synthesised and stored in enterochromaffin cells of the gut mucosa[4] a small amount is located in the myenteric plexus[5]. Histological and physiological evidences suggest that enterochromaffin cells are closely associated with nerve endings. Stimulation of the splancnic nerves or vagal adrenergic fibers decreases the 5-HT content of these cells and increases the concentration of 5-HT in the intestinal lumen or portal circulation[6-8]. In enteric neurons, 5-HT functions as a neurotransmitter[9-13]. Cell bodies of serotonergic neurons are found only in the myenteric ganglia[5,14].The principle projection of these neurons is directed to other myenteric ganglia and to the submucosal plexus[14]. Since cell bodies of serotonergic neurons are not found in the submucosal plexus, it seems unlikely that 5-HT is released by enteric motor neurons that innervate enterocytes[15]. It is also well established that 5-HT is involved in the stimulation of intestinal secretion and has been implicated in the pathogenesis of several diseases[16].

On the other hand, basal serotonin release is slightly higher in the jejenum and ileum than in the duodenum[17]. One can say that 5-HT acts partly non neurally and partly neurally.

In the present study, we investigated the effect of serotonin on thesmooth muscle contraction in the isolated rat duodenum.

MATERIALS AND METHODS

Wistar rats, irrespective of sex, (250-300 g) (n=20) were allowed food and water ad libitum prior to experiments. The animals were sacrified by servical dislocation. The duodenum was quickly removed and cleaned off adherent connective tissue and flushed with ice-cold Tyrode solution. 10 mm duodenum pieces mounted in a 40 ml isolated organ bath containing Tyrode solution which was (mM): NaCl 137, KCl 2.7, $MgCl_2$,$CaCl_2$ 1.8, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.5 and bubbled with 95% O_2/ 5% CO_2 mixture at 37 °C, PH=7.4. For tension recordings, samples were mounted vertically between a fixed holder, and a force transducer (Ugo Basile isometric transducer No 7003) and resting tension on each sample was adjusted to 0.3 g. The tissue preparations were allowed to maintain equilibrium for 30-40 minutes before the experiments. The isometric tensions were recorded by Ugo Basile No 7050 recorder.

The first experimental protocol (n=10) was designed to determine the effective contractile doses of serotonin. For this purpose, we induced contractions at four different concentrations of 5-HT ($6.4X10^{-9}$ M, $6.4X10^{-8}$ M, $6.4X10^{-7}$ M, $6.4X10^{-6}$ M) and acethylcholine (ACh) ($6.8x10^{-8}$ M, $6.8x10^{-7}$ M, $6.8x10^{-6}$ M, $6.8x10^{-5}$ M) in standard Tyrode and Ca^{2+}-free medium. Ca^{2+}-free solution was prepared by equimolar substitution of $CaCl_2$ with NaCl in Tyrode solution. By using Wilcoxon Matched-Pairs Signed Ranks test, we decided to continue the experiments at the concentrations of $6.4x10^{-7}$ M for 5-HT and $6.8x10^{-7}$ M for ACh which stimulated the contractions effectively. In the second experimental protocol (n=10), contractions were induced by 5-HT and ACh both in standard and Ca^{2+}-free Tyrode solutions. We compared the effects of serotonin to those of

other contracting agent ACh, which is widely studied in this muscle type.

In order to investigate the involvement of L-type Ca^{2+} channels in the contractile responses elicited by each agonist, we used the selective antagonist of L-type Ca^{2+} channels, diltiazem ($5.6x10^{-7}$ M). In addition also, muscle segments were also pre-treated with ketanserin ($5.6x10^{-7}$ M) which is a specific 5-HT_2 receptor blocker. The samples were pre-incubated with these blockers for 3 minutes prior to testing.

After each test, the samples were washed for three times by Tyrode. After the equilibrium is maintained the next agent is applied and the same procedure is repeated for all agents.

Serotonin, ACh and diltiazem were obtained from Sigma Chemical Company, ketanserin was supplied by Fluca Biochemica. Agents were dissolved in distilled water.

Statistical analses were evaluated by using Student's t test. Data is expressed as means±SD. A p value of 0.05 or less was considered statistically significant.

RESULTS

As the aim of this study was to investigate the effect of serotonin on isolated duodenum muscle, to start with we compared the already known contractile effects of ACh with those of serotonin at four different concentrations in standard Tyrode and Ca^{2+}-free medium. The appropriate concentrations of ACh and 5-HT were determined for the succeeding stages of the experiment. In the second experimental protocol, we measured the contractions induced by 5-HT and ACh both in standard and Ca^{2+}-free mediums. Contractions induced by ACh were more effective than that induced by 5-HT in both standard Tyrode ($p<0.05$) and Ca^{2+}-free Tyrode ($p<0.05$) (Fig 1).

Results of the experiments related to the mechanism of 5-HT induced contractility, pre-incubation with ketanserin did not alter the contractile response to ACh ($p>0.05$)

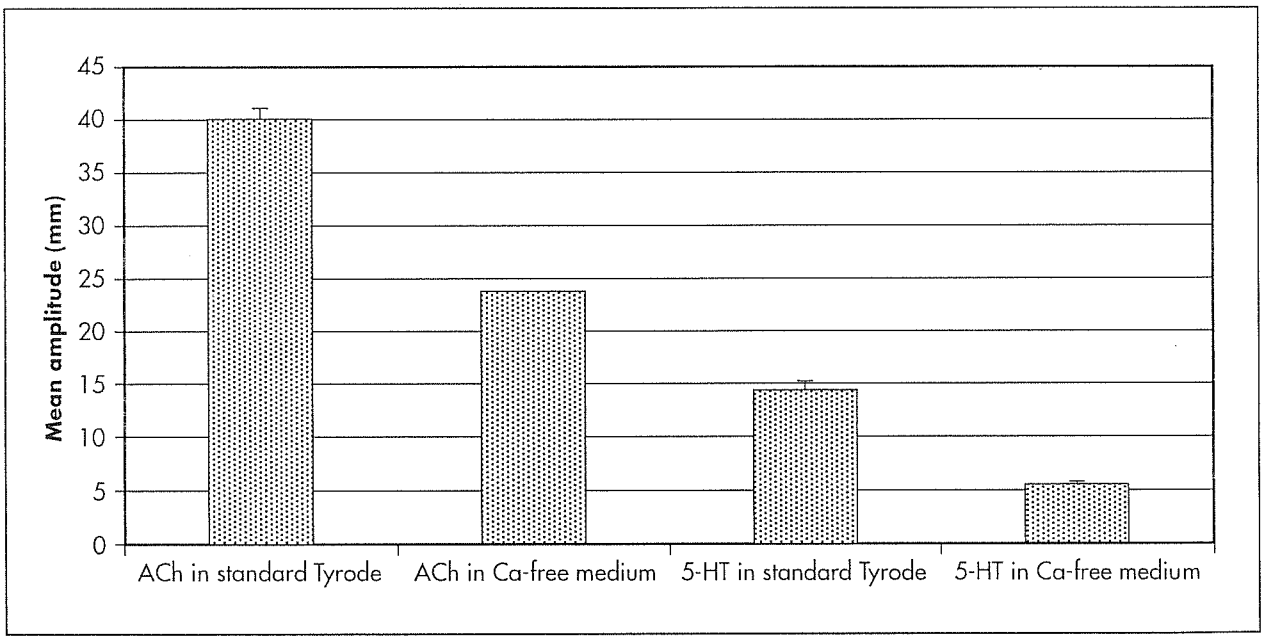

Figure 1. ACh- and 5-HT-induced contractions(mean amplitude) both in standard tyrode and Ca^{2+}-free mediums.

(Fig 2) on isolated duodenal smooth muscle but this process completely blocked the contractility produced by 5-HT in standard Tyrode solution ($p<0.05$) (Fig 3). In Ca^{2+}-free medium the results were similar to those in standard Tyrode solution ($p<0.05$ for 5-HT) ($p>0.05$ for ACh) (Fig 2, 3). On the contrary, pre-incubation with diltiazem significantly inhibited the ACh-induced contractility just as expected ($p<0.05$) (Fig 2) and

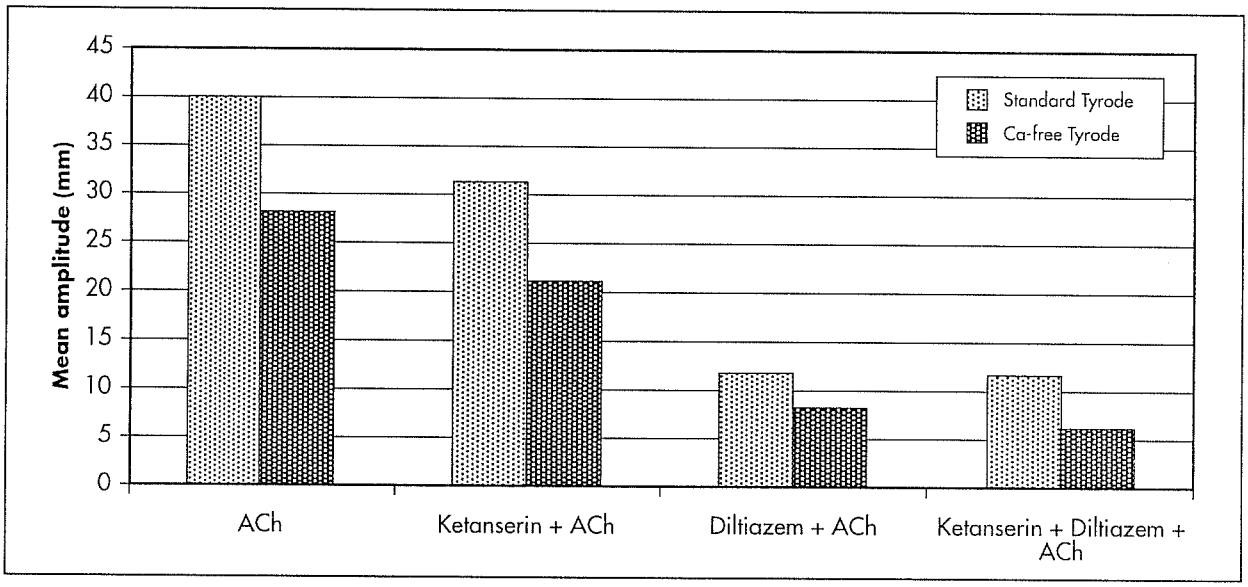

Figure 2. Effects of ketanserin, diltiazem and ketanserin+diltiazem pre-treatment on ACh-induced duodenal contractions (mean amplitude) both in standard tyrode and Ca^{2+}-free mediums.

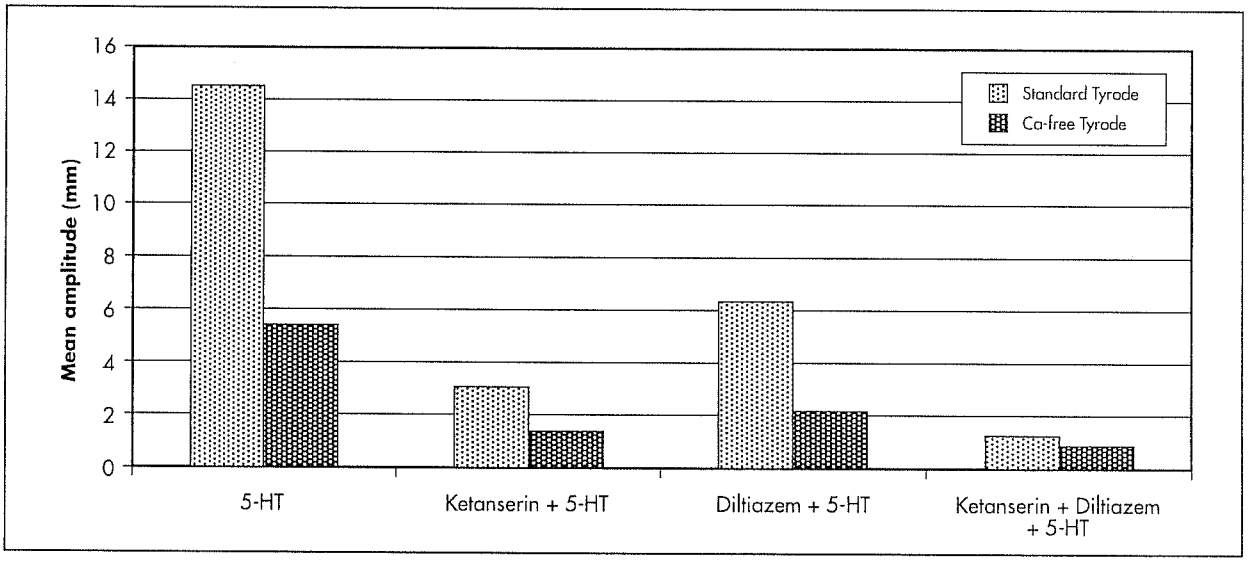

Figure 3. Effects of ketanserin, diltiazem and ketanserin+diltiazem pre-treatment on 5-HT-induced contractions(mean amplitude) both in standard tyrode and Ca^{2+}-free mediums.

5-HT-induced contractions also decreased by diltiazem pretreatment ($p<0.05$) (Fig 3). However, diltiazem inhibited the contractile response induced by ACh more significantly than that by 5-HT (Fig 2, 3). On the other hand, inhibition of 5-HT induced contractions by ketanserin were more evident than those produced by diltiazem (Fig 3).

When diltiazem and ketanserin were used together, the net inhibitor effect of these two antagonist was more effective for both agonists (Fig 2, 3). By removal of extracellular Ca^{2+}, contractile responses to both ACh and 5-HT were decreased ($p<0.05$ for ACh and $p<0.05$ for 5-HT).

But in Ca^{2+}-free medium 5-HT-induced contractions were not completely blocked, therefore, pretreatment with diltiazem ($p<0.05$) and ketanserin ($p<0.05$) enhanced the inhibition (Fig 3). When they were used together the inhibitory effect observed was greater than their individual effects ($p<0.05$) (Fig 3). However in Ca^{2+}-free solution, ACh induced contractions were almost totally blocked, that's why, diltiazem ($p>0.05$) and ketanserin ($p>0.05$) did not alter the results (Fig 2).

DISCUSSION

ACh and 5-HT caused an increase in tension of duodenum, thus indicating the existence of the binding sites for these agonists in duodenum. Previous investigators suggested that muscarinic agonists release 5-HT from the enterochromaffin cells of the rabbit mucosa[17], and they suggested that ACh was likely act on enterochromaffin cells to release 5-HT which eventually mediated the response[15,17]. On the other hand, many investigators suggest that 5-HT acts on submucosal plexus and enteric cholinergic neurones[18–21]. That's why we thought that ACh might have exaggerated its effect by stimulating the release of 5-HT. As it is known ACh produces a sustained increase in calcium influx and relatively sustained contractions. Contractions produced by 5-HT have been previously shown to be more transient than those produced by ACh and 5-HT increases Ca^{2+} influx only transiently[22]. This may be considered as one of the reasons which tell why, ACh produced more strong contractions than 5-HT did. ACh and 5-HT produced stronger contractions in Ca^{2+} containing medium than in Ca^{2+}-free medium. This result is similar to those of coronary artery, tracheal, stomach, ileum tissues in various species[22–26]. The observation that, 5-HT but not ACh was able to induce small but significant contraction even in the absence of extracellular Ca^{2+} indicates that 5-HT utilises somehow another source of Ca^{2+}. In other words, removal of extracellular Ca^{2+} attenuated but did not prevent the development of 5-HT induced contractions. It has been convincingly demonstrated that the outflow of 5-HT is dependent on the presence of Ca^{2+}. For example, the release of 5-HT is reduced by 70% after omission of extracellular calcium; similarly blockage of plasma membrane calcium channels significantly inhibits the 5-HT release[1,17,27]. In the present study, we suggest that in Ca^{2+}-free medium, releasing of 5-HT under the stimulant effect of ACh was not possible and additional contractile effect of 5-HT was not observed. But exogenously applied 5-HT exerted a small contractile response on isolated duodenum in Ca^{2+}-free medium. This result indicates that 5-HT induces a rise in cytosolic free calcium concentration and this increase in calcium is the cause of contraction. This suggestion totally coincides with the findings of some investigators[1].

In the present study, we used ketanserin

which is a specific 5-HT_2 receptor antagonist and observed that ketanserin inhibited the contractile effect of 5-HT but did not alter the response to ACh. The contractile effect of 5-HT is a compound response resulting from activation of at least two pathways which converge to regulate the cytosolic level of Ca^{2+} the main determinant of mechanical activity in muscle cell[28].

Previous studies using intact vascular and visceral (e.g., tracheal, intestinal) smooth muscle tissue have showed that 5-HT caused contraction by releasing contractile neurotransmitters as well as by interacting with 5-HT2 receptors on smooth muscle cells [29-31]. Kummerle et al. proposed that ketanserin inhibited the increase in $[Ca^{2+}]i$ by 90%[28], and ketanserin-sensitive 5-HT_2 receptor, probably mediated the contractile effect of 5-HT. Since ketanserin did not alter the response to ACh; ACh and 5-HT acted via separate receptors. Although the formation of intracellular signal molecules in the presence of ACh or 5-HT were not determined in this study, the current data suggest that, 5-HT utilizes intracellular sources as well as extracellular ones to produce contraction in rat duodenum. We thought that 5-HT might elicit an increase in inositol 1,4,5-three phosphate (Ins P3) levels, this is indeed in agreement with some previous investigations [25,30,33]. Ketanserin inhibited the 5-HT induced contractions in duodenal segments probably by the same manner as that of calcium channel blockers or by inhibiting the formation of second messengers such as Ins P3, diacylglycerol and protein kinase C which are involved in the release of Ca^{2+} from the sarcoplasmic reticulum as well as in the opening of the channels.

When we used a L-type calcium channel blocker, diltiazem, the contractile responses produced by ACh and 5-HT were both decreased. But ACh-induced contractions were more strongly inhibited than those induced by 5-HT did. It is known that, diltiazem prevents the entrance of Ca^{2+} from intracellular stores as well extracellular sources. On the basis of all these observations, we suggested that both agonists use the extracellular and intracellular Ca^{2+} sources together.

As a result, our observations indicate that in smooth muscle of rat duodenum both ACh and 5-HT utilizes intracellular as well as extracellular sources of Ca^{2+} to produce contractions. We strongly suggest that 5-HT acts via its specific receptor on rat duodenal smooth muscle. Muscarinic and serotonergic receptors do not stimulate calcium influx through "shared" channels and ACh and 5-HT receptor operator calcium channels may be totally different.

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