The Effects of Manganese Chloride on Acetylcholine-Induced Contractions of Isolated Rat Duodenal Smooth Muscle

Nezahat ZALOĞLU, MD., Emine KOÇ, MD., Gülseli YILDIRIM, MD.,

Department of Physiology, Faculty of Medicine, Ankara University, ANKARA

✔ Mn^{2+} is an essential trace element, known as Ca^{2+} antagonist and suppress the action potential and contraction of various types of smooth muscle cells. It exerts its effects by entering the cells through L type Voltage-dependent Ca^{2+} channels, or receptor-operated Ca^{2+} channels and/or perhaps by other non selective cation channels. It may produce contraction by directly activating contractile proteins.

In the present study, we measured the Acetylcholine (ACh)-Induced ($2.7x10^{-7}$M) duodenal smooth muscle contractions containing standard and modified Tyrode (Ca^{2+}-free, and 2 fold Ca^{2+}) bath solutions with adding Atropine ($1.4x10^{-7}$ M), Diltiazem ($5.5x10^{-7}$ M) and $MnCl_2$ (5 mM). In the experiments, rats (n=10) were used. Animals were killed by decapitation. Abdomen opened and duodenal pieces (1 cm) were taken. Their contents were flushed with ice-cold Tyrode solution. The duodenum pieces were suspended in an organ bath containing 10 ml of Tyrode solution and aerated with 95% O_2 and 5% CO_2, at 37 °C. After equilibration period of 30 minutes, contractile force was recorded isometrically with a force displacement transducer (Ugo-Basile 7003) connected to a microdynograph recorder (Ugo-Basile 7050). A resting tension of 0.3 g were used. At the three bath solutions while atropine and diltiazem inhibited the ACh-induced contractions, $MnCl_2$ (5 mM) induced a rapid and transient suppression of contractions. The average amplitudes of contractions with $MnCl_2$ were significantly lower than those of diltiazem and atropine.

The results suggested that the suppression of contractions with Mn((influx may due to other factors such as cAMP content and its effects on contractile elements.

Key words: *Isolated duodenal smooth muscle contractility, manganese chloride*

✔ **Manganklorürün İzole Sıçan Duodenum Düz Kasında Asetilkolinle İndüklenmiş Kontraksiyonlar Üzerine Etkisi**

Manganez, iki değerlikli formu ile Ca^{+2} antagonistidir. Düz kasta aksiyon potansiyeli ve kontraksiyonu inhibe edebilir. Hücre içine girişi voltaj bağımlı L tipi Ca kanalı, reseptör bağımlı Ca kanalı ve/veya non selektif iyon kanalları aracılığı ile olmaktadır. Bu nedenle çeşitli organ düz kaslarında kontraktil proteinlere direkt etki ile kontraksiyon oluşturabileceği bildirilmektedir. Sunulan çalışmada standart, $2xCa^{+2}$ ve Ca^{2+}'suz ortamlarda $2.7x10^{-7}$ M ACh ile oluşturulan duodenum kontraktilitesi üzerine $MnCl_2$ (5 mM)'ün etkisi araştırıldı. Diltiazem, Atropin ile oluşan kontraksiyon yükseklikleri ile karşılaştırıldı. Çalışmada 10 adet rat kullanıldı. Uygun şekilde izole edilen duodenal düz kas örneklerinde kontraksiyon yükseklikleri Ugo Basile izometrik force transdüseri yardımı ile yazdırıldı. Kontraksiyonlar atropin ve diltiazemle inhibe olurken, $MnCl_2$ ile ani olarak kayboldu. Bu sonuç manganez etkisi ile oluşacağı bildirilen düz kas kontraktilitesinde hücre içi diğer faktörlerin de, örneğin; cAMP düzeyinin de rol oynayabileceğini düşündürdü.

Anahtar kelimeler: *İzole duodenum düz kas kontraksiyonu, manganklorür*

INTRODUCTION

Manganese (Mn^{2+}) is considered as a Ca^{2+} antagonist[1,2]. It suppresses the action potential and the contractions of various types of smooth muscle cells. It is also known that Mn^{2+} enters the cell from L-type Ca^{2+} channels[1], and blocks Ca^{2+} channels of T and R- type[3,4]. Mn^{2+} and some other organic cations such as Ni^{2+}, Co^{2+} and La^{3+} are necessary in very high concentrations to block receptor-mediated Ca^{2+} influx. Moreover in some but not all cell types, some divalent cations can pass through the receptor-mediated Ca^{2+} influx pathway[5]. Several reports have been published on the effects of Mn^{2+} on different smooth muscle mechanical activity especially in gastrointestinal tract, airway smooth muscle and uterus smooth muscle[1-3,9]. Studies of different isolated segments from the gastrointestinal tract, it has been shown that Mn^{2+} may have dual effects on the contractility as an inhibitory or excitatory agent[2,9].

In this study we aimed to investigate the effects of $MnCl_2$ on the amplitudes of ACh-induced contractions of isolated rat duodenal smooth muscle.

MATERIALS AND METHODS

10 wistar rats, average weighting of 150 g were used. Animals were taken from animal, care and research center of medical faculty where they were housed. Rats were sacrified by decapitation, then abdomen was opened and duodenal pieces of 1 cm were taken and their contents flushed by ice-cold Tyrode solution. Tyrode solution contains (in mM): NaCl 137, KCl 2.7, $MgCl_2$ 1.05, $CaCl_2$ 1.8, NaH_2PO_4 0.42, Glucose 5.5 and PH adjusted to 7.4. In the experiment we investigated the contractions with standard and modified Tyrode solutions which were Ca^{2+}-free (omitting $CaCl_2$ and adding equimolar NaCl) and 2xCa^{2+} (omitting NaCl adding equimolar 2 fold Ca^{2+} into the standard Tyrode) than we investigated the contractions with atropine ($1.4x10^{-7}$ M), a muscarinic receptor antagonist, diltiazem ($5.5x10^{-7}$ M), an L-type Ca^{2+} channel blocker and $MnCl_2$ (5 mM) containing standard, Ca^{2+}-free and 2xCa^{2+} organ bath mediums. The duodenal pieces were suspended in organ bath containing 10 ml of Tyrode solution kept at 37 °C and aerated with 95% O_2 and 5% CO_2 continuously. The contractile force was recorded isometrically with a force displacement transducer (Ugo Basile 7003) connected to a microdynograph recorder (Ugo-Basile 7050). Changes in isometric tension were recorded. After the equilibrium period of 30 minutes the duodenal pieces were contracted with ACh. In order to find maximal contractions to ACh, different doses of ACh added into organ bath (data not shown) and we continued the experiments with the selected dose of ACh. Tissue were pre-treated with atropine ($1.4x10^{-7}$ M) for 3 min, than ACh ($2.7x10^{-6}$ M) added into the bath solution and contractions were recorded. After the tissue were washed for three times with standard Tyrode solution and waited for equilibrium, the next agent were applied. The same procedure were done at the three bath medium with diltiazem ($5.5x10^{-7}$ M) and $MnCl_2$ (5 mM) respectively. Before testing the contractions in Ca^{2+}-free Tyrode medium, the duodenal pieces were pre-incubated in Ca^{2+}-free Tyrode solutions for 30 minutes. Mn^{2+} was applied as $MnCl_2$. The amplitudes were measured as mm from recorded traces and the calibration was made as 1g per 10 mm with a resting tension of 0.3 g. Atropine and diltiazem were obtained from Sigma Chemical Company, $MnCl_2$. $4H_2O$ were obtained from E. Merck Darmstadt Co.

The statistical analysis were done by student's t test $p<0.05$ was considered as significant.

RESULTS

The amplitudes of ACh-induced isolated duodenal contractions, at the standard, $2xCa^{2+}$, and Ca^{2+}-free Tyrode containing mediums, were given in (Table) and (Figure). As shown in Table the average peak tension of ACh-induced contraction recorded in standard Tyrode was 28±4.24 mm and significantly decreased to 11.3±2.19 in Ca^{2+}-free medium ($p<0.05$). The amplitudes of ACh-induced contractions were significantly decreased with atropine and diltiazem ($p<0.05$). As shown in Table and also in Figure addition of $MnCl_2$ to the bath solution, the ACh-induced contractions suddenly suppressed at all three investigated bath solutions. The ACh-induced contractions were recovered after the $MnCl_2$ removed from the solution by washing out the medium by standard Tyrode.

Table. The Effects of Atropine, Diltiazem, $MnCl_2$ on The Amplitudes of ACh-induced Contractions Mean±SE (in mm) in Standard and Modified ($2xCa^{2+}$ and Ca^{2+}-Free)Tyrode.

Tyrode	ACh	Atropine+ACh	Diltiazem+ACh	Mn+ACh
Standard	28+1.34	0.1±0.03*	11.4±0.82*	-0.4±0.39*
2x Calcium	26.4±1.07	0±0*	13.8±0.82*	0.5±0.12*
Calcium-free	11.3±0.69*	0	6.7±0.56	-0.5±0*

*$p<0.05$

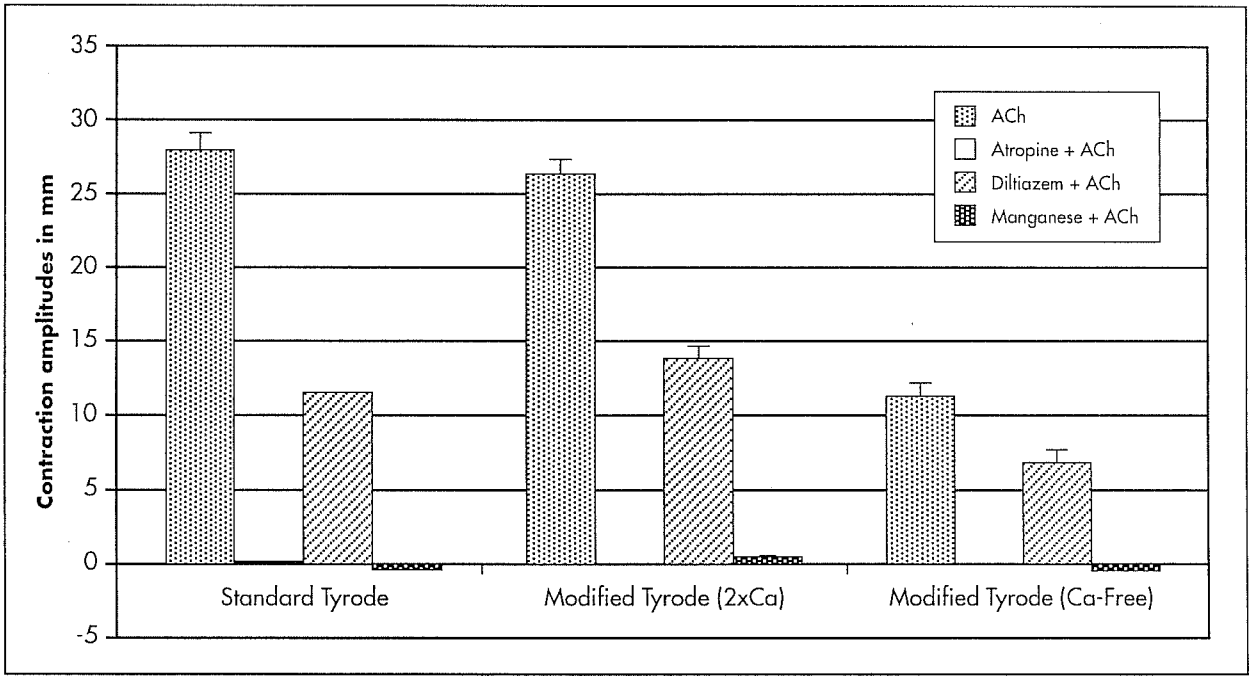

Figure. The effects of Atropine, Diltiazem and Manganese on the ACh-induced contraction amplitudes of isolated rat duodenum in standard and modified ($2xCa^{2+}$ and Ca^{2+}-free) Tyrode.

DISCUSSION

In the present study we investigated the effects of $MnCl_2$ on the ACh-induced isolated rat duodenal contractions. As seen in Figure the ACh-induced contractions were abolished with $MnCl_2$ addition to the mediums. The mechanism of the contractions induced by ACh is due to the increase in internal Ca^{2+} concentration. ACh increases the internal Ca^{2+} through both release of intracellular store and Ca^{2+} entry from extracellular space[10,11]. As internal Ca^{2+} near the inner surface of the cell membrane increases, Ca^{2+} sensitive K^+ channels activated and leads to membrane hyperpolarized. This hyperpolarization increases the driving force for Ca^{2+} influx. It was previously demonstrated that ACh causes maintained hyperpolarization which is strictly dependent on Ca^{2+}-influx[11].

In the present study we investigated the effects of Mn^{2+} on the amplitudes of ACh-induced contractions on the isolated duodenal muscle. Wang and Bremeen had found that Mn^{2+} was able to pass through the depletion activated Ca^{2+} entry pathway in isolated endothelial cells[12]. In guinea pig isolated vas deferens Tsunobuchi and Gomi had found that the ACh-induced contractions dependent on extracellular Ca^{2+} and they showed that the contractions were abolished or markedly reduced within 20 min after removal of Ca^{2+} from the medium[13]. These results of the above study was similar to that of ours in Ca^{2+}-free solution. Tsunobuchi et al [13] loaded Mn^{2+} to the medium and stimulate the tissue and observed that the rate of decrease of these contractions in Ca^{2+}-free medium were much slower than those of normal preparations. In our study we were not able to observe these contractions evoked by Mn^{2+} in loaded preparations. Although we waited for 3 hours and added $MnCl_2$ with 30 minute intervals, in the presence of ACh in Ca^{2+}-free medium (Data not shown). It seems that the actions of Ca^{2+} and Mn^{2+} on the ACh-induced contractions may due to both external and internal concentrations of Ca^{2+} and Mn^{2+} In our study it is possible that the main cause of abolishing the contraction was the dissociation of internal and external Ca^{2+} and Mn^{2+}. concentration. On the other hand, it was also reported that $MnCl_2$ modifies the Ca^{2+} influx and penetrates the cell, thus causing either a suppression or an acceleration of the mechanical responses in various visceral muscles[2]. Itoh et al showed that in the cells of the stomach antrum, when Mn^{2+} is present in an extremely low concentration ($2x10^{-9}$ M), it may accelerate the interaction between Ca^{2+}-Ca^{2+}-receptor and suggested that, Mn^{2+} itself binds with Ca^{2+} receptor for contractile proteins. There are conflicted data on this topic, perhaps the response of tissues to $MnCl_2$ presumably depends on the permeability of membranes in various tissues. On the other hand, there may be the other causes of the suppression of the ACh-induced contractions. Mogami et al had suggested that the contractile depression was accompanied by a decrease in the duration of the action potential[14]. It was also reported that intracellular cAMP concentration, depending on the activation or inactivation of adenylate cyclase by Mn^{2+} in a concentration-dependent manner, may be the main cause of the suppression or recovery of the contractions in various smooth muscles[14-19].

In conclusion, the depression of the ACh-induced contractions by $MnCl_2$ in all three mediums may be caused by several factors, including the dissociation of the internal and external Ca^{2+} and Mn^{2+} concentrations and internal cAMP concentration and even energy metabolism of the tissue for the contraction. It needs further investigations.

Geliş tarihi : 26.02.2001
Yayına kabul tarihi : 04.06.2001
Correspondence:
Dr. Nezahat ZALOĞLU
Ankara Üniversitesi, Tıp Fakültesi,
Fizyoloji Anabilim Dalı
06100 Sıhhıye-ANKARA

REFERENCES

1. Tsien RW, Hess P, McCleskey EW, et al. Calcium channel mechanisms of selectivity, permeation and block. Ann Rew Biophys Chem 1987; 16: 265–290.
2. Osa T, InoH. And Okabe K. Effects of porcine relaxin on contraction a membrane response and cyclic AMP content in rat myometrium in comparison with the effect of isoprenalyne forskolin. Br J pharmacol 1991; 104: 950–960.
3. Hofmann F, Nastainczyk W, Rohrkasten A, et al. Regulation of the L-type calcium channel. Trends Pharmacol Sci 1987; 8: 393–398.
4. Hong SJ, Chang CC. Calcium channel subtypes for the sympathetic and parasympathetic nerves of guinea pig atria. Br J Pharmacol 1995; 116: 1577–1582.
5. Krause K. H, Demaurex N, Lew. D.P. Mechanism and pharmacology of receptor mediated Ca^{2+} influx in nonexitable tissues. In Chung KF, Barnes PJ (eds.) Pharmacology of the Respiratory Tract Experimental and Clinical Research 1993 67: Marcel Decker, New York, NY 63–102.
6. Nasu T, Baba K. Mechanics of Manganese ions-induced contraction of ileal longitudinal muscle in Ca^{2+}-free, high-K^+ medium. Gen Pharmac 1997; 28: 503–507.
7. Itoh T, Kuriyama H, Nanjo T: Effects of calcium and manganese ions on mechanical properties of intact and skinned muscles from the guinea-pig stomach. J Physiol 1982 ; 333: 555–576.
8. Warner DO, Keith AJ, Robert RL, Christina MP: Muscarinic receptor stimulation modulates the effect of halothane on Mn^{2+} influx in airway smooth muscle. Am J Physiol 1997; 273 (cell Physiol, 42): C868–C873 .
9. Magraner J, Esteban M. Austina P,et al. Effects of Mn^{2+} on the responses induced by different spasmogens in the oestrogen-primed rat uterus. Eur J of Pharm 1997; 326: 211–222.
10. Wang X, Lau F, Li I, et al. Acetylecholine-sensitive intracellular Ca^{2+} store in fresh endothelial cells and evidence for ryanodine receptors. Circ Res 1995; 77: 37–42.
11. Wang X, Chu W, Lau F, Van BC. Bradykinin potantiates acetylcholine induced responses in native endothelial cells from rabbit aorta. Biochem Biophys Res Commun 1995; 213: 1061–1067.
12. Wang X, Breemen CV. Multiple mechanisms of activating Ca^{2+} entry in freshly isolated rabbit aortic endothelial cells. J Vasc Res 1997; 34: 196–207.
13. Tsunobuchi HU, Gomi Y. The specific effect of Mn^{2+} on the tonic components of receptor-mediated contractions in isolated vas deferens of the guinea pig. Eur J of Pharm 1996; 295: 235–241.
14. Mogami K, Todoroki N. Relationship between Mn-induced contractile inhibition and cyclic AMP content in the longitudinal muscle of estrogen-treated rat uterus. Jpn J Phys 1997; 47: 143–146.
15. Osa T, Inoue H, Todoroki N, Cui D: Effects of Mg and Mn ions on the inhibitory action of cyclic nucleotides in the longitudinal myometrium of rat. Jpn J Physiol 1994; 44: 49–66.
16. Todoroki N, Mogami K, Cui D, Osa T. Influence of intracellular Mn^{2+} on the contractile inhibition caused by db cAMP, forscholin and porcine relaxin in the circular muscle of the estrogen-treated rat uterus. Jpn J Physiol 1995; 45: 1087–1091.
17. Nasu T. Actions of manganese ions in contraction of smooth muscle Gen Pharmacol 1995 26: 945-953.
18. Wiemmer G, Kaiser G, Paim D: Effects of Mg^{2+} and Mn^{2+} on adenylcyclase activity. Naunyn-Schmiedeberg's Arch Pharmacol 1978; 303: 145–152.
19. Krall JF, Leshon SC, Korenman SG. Phospholipid and guanine nucleotide sensitive properties of the smooth muscle adenylate cyclase catalytic unit. Biochemistry 1988; 27: 5323–5328.