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THE POSSIBLE ROLE OF 3':5'- CYCLIC GUANOSINE MONOPHOSPHATE IN ACETYLCHOLINE-MEDIATED CONCRACTION OF ISOLATED RAT DUODENUM

Short Communication

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Key Words:Acetylcholine, 3':5'-cyclic guanosine monophosphate, sodium nitroprusside, methylene blue
Anahtar Terimler:Asetilkolin, 3':5'-siklik guanosin monofosfat, sodyum nitroprussid, metilen mavisi.

SUMMARY

This study was planned to determine whether the possible role of 3':5'-cyclic guanosine monophosphate(cylic GMP) on acetylcholine(ACh) action in isolated rat duodenum. Therefore, sodium nitroprusside(SNP) and methylene blue(MB) were used as a guanylate cyclase activator and inhibitor respectively. MB caused significant fall in ACh-induced contraction peaks, whereas SNP did not alter them. These results suggested that cyclic GMP may play a role for ACh action in isolated duodenum smooth muscle. However further experiments are necessary to determine whether a causal relationship exists between cyclic GMP and ACh action.

OZET

3':5'-siklik Guanosin Monofosfatın Izole Sıçan Duodenumunda Asetilkoline Bağlı Kontraksiyonlar Üzerindeki Muhtemel Rolü

Sunulan çalışmada 3':5'-siklik guanosin monofosfatın(siklik GMP) izole sıçan duodenumunda asetilkoline bağlı(Ach) kontraksiyonlar üzerinde rolü olup olmadığını araştırmak için planlanmıştır. Bu amaçla bir guanilat siklaz aktivatörü olan sodyum nitroprussid(SNP) ve bir guanilat siklaz inhibitörü olan metilen mavisinden(MB) yararlanılmıştır. MB duodenumun Ach-bağımlı kontraksiyonlarını anlamlı ölçüde azaltmış, ancak SNP Ach-bağımlı kontraksiyonlar üzerinde anlamlı bir etki meydana getirmemiştir. Bu sonuçlar indirekt olarak siklik GMP'ın izole sıçan duodenumunda Ach aksiyonunu etkileyebileceğini göstermektedir. Ancak siklik GMP ve Ach aksiyonu arasındaki ilişkinin aydınlatılabilmesi için daha kapsamlı araştırmalara gereksinim vardır.

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As it was known that, acetylcholine(ACh) relaxes intact vascular smooth muscle(1) whereas it causes contraction in nonvascular smooth muscle(2) via muscarinic receptors. A hypothetical mechanism by which ACh causes relaxation in vascular smooth muscle has been offered:ACh generates an endothelium-derived relaxing factor(EDRF), since relaxation of isolated arterial smooth muscle by muscarinic receptor agonists is dependent on the presence of undamaged endothelium. EDRF causes an elevation of cyclic GMP level(3). On the other hand, the cyclic GMP content in the aorta has decreased significantly after removing the endothelium(4). However, it is not clear whether cyclic GMP involves in ACh action in nonvascular smooth muscle, especially gastrointestinal tract. Insofar as we are aware, there is no study on this topic in rat duodenum. The purpose of this study was to investigate the effectiveness of cyclic GMP dependent mechanism for ACh action in duodenum.

In this study, sodium nitroprusside(SNP) and methylene blue(MB) were used as a guanylate cyclase activator(5) and inhibitor(6) respectively to investigate the possible role of cyclic GMP on ACh action in duodenium.

Studies were perfomed in 24 adult rats of both sexes, weighing between 150-200 g. The abdomen was opened immediately after decapitation, duodenum removed and placed quickly in oxygenated tyrode solution. Strips were prepared approximately 2 mm wide and 20 mm length from the distal part of the duodenum. Each strip was placed in 10 ml isolated organ bath containing physiological solution maintained at 37°C and bubbled with air. An isometric transducer(Harvard 50-7905) and a Universal Oscillograph(Harvard 50-8648) were used for recording. The preparation was equilibrated under 1 g.tension for 60 min before drug applications. Acetylcholine chloride, methylene blue and soduim nitroprusside were obtained from Sigma Chemical Co. and drugs were dissolved in physiological solution.

Dose-response curves of ACh were determined in each strip by adding several doses of ACh into physiological solution. After a 15 min resting period, the same procedure was achieved with physiological solution containing MB(12 strips) or SNP(12 strips). Strips were incubated with MB(10^{-5} M, n=6; 10^{-6} M, n=6) or SNP(10^{-5} M, n=6; 10^{-6} M, n=6) for 3 min before ACh application.

The contraction and relaxation of strip by drugs were evaluated as the percentage of maximal response to ACh. Data were analyzed statistically by comparing responses of control and MB or SNP groups to the same dose of ACh using t test for paired observations.

When comparison of different responses was made with or without MB(10^{-5} M) it was observed that MB caused a significantly fall in ACh-induced contraction peaks, except 10^{-7} M ACh concentration (Fig.1).

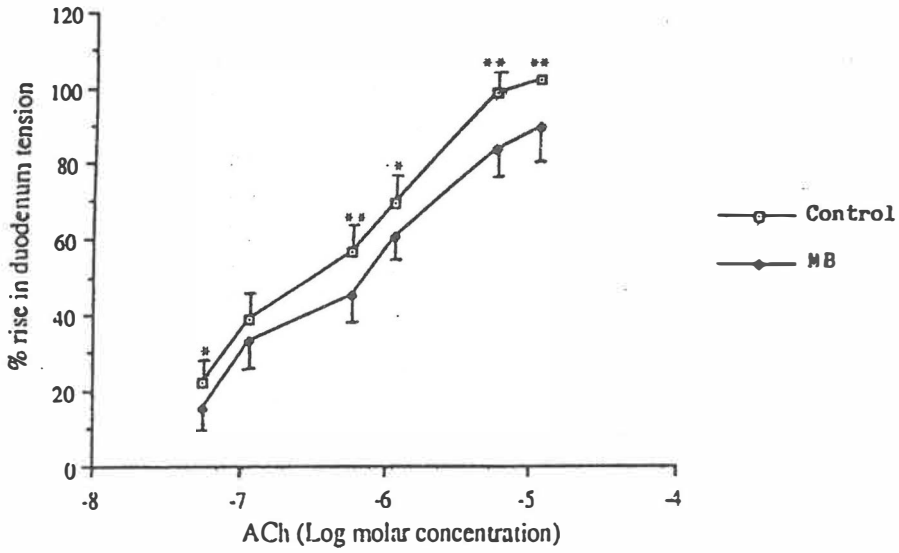

Figure 1:Effect of methylene blue(MB) on duodenum tension to acetylcholine(ACh).*, **,: Difference in responses between ACh-induced peaks in absence and presence of MB(10^{-5} M) $p<0.05$; $p<0.01$, respectively, n=6. Bars represent SD

On the other hand, 10^{-6} M MB application caused a significant fall on ACh-induced contraction peaks in only two concentrations of ACh(10^{-7}, 10^{-5}, M). It was not shown any significantly changes on duodenum contractions by other concentrations of Ach (fig.2).

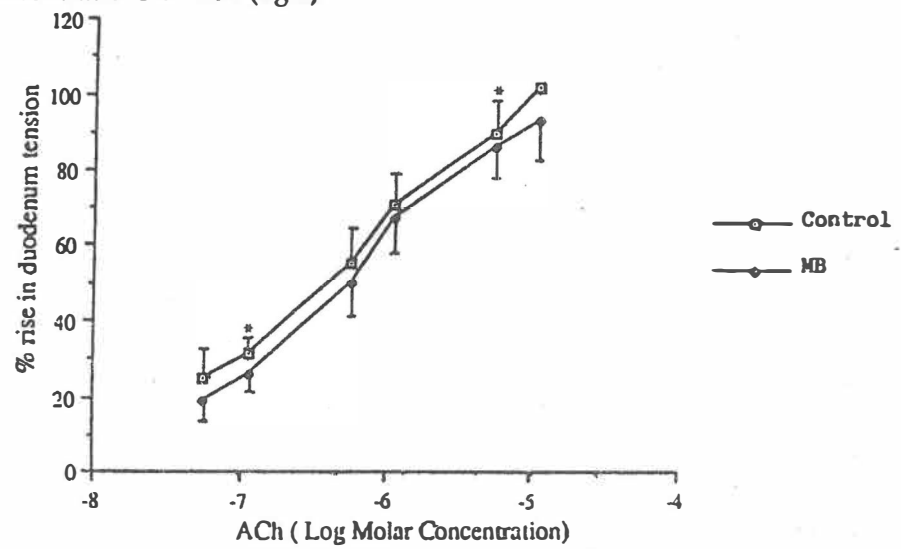

Figure 2:Effect of methylene blue(BM) on duodenum tension to acetylcholine(ACh).*: Difference in responses between ACh-induced peaks in absence and presence of MB (10^{-5} M) $p<0.05$, n=6. Bars represent SD.

SNP was applied at 10^{-5} M and 10^{-6} M concentrations and did not significantly change on contraction peaks in response to ACh.

Muscarinic receptors on nonvascular smooth muscle link to the Ca Channels by G protein and the role of cyclic AMP was known in detail on this topic(7). The occuping of muscarinic receptor results in Ca influx from exracellular sources and, the increased intracellular Ca Level brings about the increase of tension in smooth muscle(7). However, ACh causes contraction even though when tissue is bathed in Ca-free solution(6). Therefore, it is more reasonable to suspect that only mechanism by which ACh causes contraction is not Ca influx from extracellular sources. Besides, it was known that the cytoplasmic concentration of Ca^{+2}, another ubiquitous second messenger, is controlled either by regulation of several different Ca^{+2} specific channels in the plasma membrane or by its release from intracellular organellar storage depots(7). Since incubation of duodenum with MB, which is known to decrease intracellular cyclic GMP accumulation,attenuated the increase of tension induced by ACh(see "results"), it is also possible that ACh elevates intracellular Ca level by occuping muscarinic receptors linked to guanylate cyclase. Confirming with our finding, MB has been reported to inhibit relaxation response to ACh in vascular smooth muscle(1). Besides, a rise in cyclic GMP level has been shown by stimulation of muscarinic receptors in several smooth muscle types(1-8). In the present experiment, SNP did not alter ACh-induced contraction peaks. If cyclic GMP plays a role of duodenum smooth muscle why did not SNP change ACh-induced contractions? It may be argued that cyclic GMP elevation in duodenum caused by SNP occurs in a pool which is not involved in regulation of contractile activity. But, it was shown clearly that MB attenuated significantly ACh-induced contractions.

According to these results, cyclic GMP may contribute ACh action in rat duodenum. However further detail experiments are necessary to determine whether a causal relationship exists between cyclic GMP and ACh action.

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