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EFFECT OF STREPTOMYCIN ON THE RELAXATIONS OF ISOLATED RAT DUODENUM INDUCED BY BRADYKININ, ADRENALINE AND PAPAVERINE: ITS MECHANISM OF ACTION ON SMOOTH MUSCLES

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SUMMARY:

The present study was designed to examine the effects of streptomycin on the bradykinin-, adrenaline- and papaverine-induced relaxations of the isolated rat duodenum. The main purpose of this study was to approach the mechanism of action of streptomycin on smooth muscles using rat duodenum as assay organ. It was observed that streptomycin inhibits the relaxations of rat duodenum elicited by bradykinin, adrenaline and papaverine. This inhibitions were non-competitive in nature. Furthermore, it was noted that this antibiotic do not cause a contraction or relaxation, even its high doses. The results obtained in this study was discussed on the basis of the data obtained in previous investigations.

STREPTOMİSİN'İN BRADİKİNİN, ADRENALİN ve PAPAVERİN İLE OLUŞTURULAN İZOLE SIÇAN DUODENUMU GEVŞEMELERİ ÜZERİNE ETKİSİ: DÜZ KASLAR ÜZERİNDEKİ ETKİSİNİN MEKANİZMASI

ÖZET:

Bu çalışma, izole sıçan duodenumunda bradikinin, adrenalin ve papaverin ile oluşturulan gevşemeler üzerine streptomisin'in etkilerini incelemek için tasarlandı. Çalışmanın ana amacı, deney organı olarak sıçan duodenumu kullanıp streptomisin'in düz kaslar üzerindeki etkilerine yaklaşım sağlamaktı. Streptomisin'in, bradikinin, adrenalin ve papaverin ile ortaya çıkarılan sıçan duodenumu gevşemelerini inhibe ettiği gözlendi. Bu inhibisyonlar, nonkompetitif nitelikte idi. Ayrıca, bu antibiyotiğin yüksek derişimlerde bile sıçan duodenumunda gevşeme ya da kasılmaya neden olmaması da dikkat çekiciydi. Bu çalışmada elde edilen sonuçlar, önceki araştırmalarda elde edilen veriler temel alınarak tartışıldı.

INTRODUCTION

Streptomycin, an aminoglycoside antibiotic, is produced by the growth of soil microorganism, streptomyces griseus. It was discovered in 1944 and was the next important antibiotic to be isolated after

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penicillin. It is used in therapy of tuberculosis, tularaemia and plague, but it can also be useful against E.coli, Proteus vulgaris, Ps.aeruginosa, H.influenzae, Br. abortus and Kl.pneumonie. It is well-known that serious side effects may occur during the treatment with streptomycin. For example, streptomycin causes autotoxicity, hypersensitivity reactions, hepatotoxicity and nephrotoxicity in high doses(1,2). One of most important side effect of streptomycin is neuromuscular blocking action(3). It has been shown that streptidin is responsible for the action on the striated muscle(4). It is known that streptomycin also exerts some effects on the smooth muscle. It has been reported that peristaltic reflex of the guinea-pig ileum was abolished in the presence of streptomycin(5). On the other hand, streptomycin has been found to be a non-competitive inhibitor of acetylcholine, histamine, bradykinin(6), angiotensin II, PGE_2(7), scorpion venom from androctonus crassicauda(8) on the guinae-pig ileum, 5-hydroxytryptamine on the rat gastric fundus(9) and noradrenaline on the rat vas deferens(10). Furthermore, it has been reported that the in vivo bronchoconstrictor responses to histamine, bradykinin(11) and 5-hydroxytryptamine(12) in the guinea-pig are inhibited by streptomycin. Exact mechanism of these actions is still unestablished. In this paper, it was aimed to approach to the mechanism of the inhibitory effects of streptomycin using isolated rat duodenum.

MATERIALS AND METHODS

Isolated Rat Duodenum

The isolated rat duodenum was prepared according to the method described by Horton(13). Adult albino rats (150-250 g) were killed by cervical dislocation and duodena were removed immediately thereafter. Then, the tissues were kept in atropinized Krebs' solution (0.143 mM) at 6°C for two hours. In this way, the isolated duodena behaved well and no tachphlaxis was observed. After this period, the duodena were suspended in a 10-ml organ bath containing the atropinized Krebs' solution at 31°C and gassed with a mixture of 95 % O_2 and 5 % CO_2. To record the relaxations, the tissues were connected to an isotonic transducer (Ugo Basile, No: 7006) coupled to a recording microdynamometer (Ugo Basile, No: 7050). The tissues were allowed to equilibrate with a 1.00 g basal tension for approximately 60 minutes prior to drug testing. During this incubation period, the isolated rat duodena were rinsed every 15 minutes with 20 ml of the atropinized Krebs' solution. After this initial incubation, non-cumulative dose-response curves obtained for a relaxant agent using two individual dose-response procedure in all experiments. The relaxant agents used in this study were bradykinin, adrenaline and papaverine. In each experiment, merely one concentration of streptomycin was tested. The isolated rat duodenum was incubated with these drugs for 10 minutes as described before(6,7) and the dose-response procedure was repeated in the presence of streptomycin. The relaxations of the rat duodenum were magnified 8-folds.

Analysis of Data

Non-competitive antagonist affinity constants (pD'_2 values) as elaborated Ariens and Van Rossum(14) were used to quantify the action of streptomycine on the isolated rat duodenum. All values reported show the results of individual experiments in which the dose-response curves obtained in the presence and in the absence of streptomycin were evaluated by means of linear regression analysis. When indicated, significance of differences between the mean values were determined by the Student's t-test(15).

Drugs used

Adrenaline bitartarate (Sigma), atropine sulphate (Merck), bradykinin, triacetate

(Sigma), papaverine hydrochloride (Sigma), streptomycine sulphate (Pfizer). All dilutions, except of papaverine were prepared with fresh Krebs' solution. Papaverin was dissolved in saline.

RESULTS

Even at the high doses (up to $2x10^{-3}$ g/ml), streptomycin did not cause a relaxation and a contraction on the rat duodenum. However, bradykinin-, adrenaline- and papaverine-induced relaxations were inhibited by streptomycin (in a dose range of $2.7x10^{-4}$ — $1.1x10^{-3}$M). According to regression analysis, this inhibitions were non-competitive in nature (Figure 1, 2 and 3). The pD'_2 values calculated were summarized in Table I.

Table I pD'_2 values concerning the inhibitory action of streptomycine on the rat duodenum relaxations elicited by bradykinin, adrenaline and papaverine in vitro.

Relaxan Agent	pD'_2	± SE
Bradykinin	3.044	± 0.026
Adrenaline	2.767	± 0.207
Papaverine	3.123	± 0.007

pD'_2 : Non-competitive antagonist affinity constant

SE : Standard error of mean (n = 6 for each set of experiment)

Table II Inhibitory effects of streptomycin against certain agonists which had been previously shown on some smooth muscle preparations(6,7,9,10). Abbreviations used: pD'_2 and pA_2 non-competitive and competitive antagonist affinity constants respectively. GPI: Guinea-pig ileum, RFS: Rat fundus strips, RVD: Rat vas deferens, DRD: K^+-depolarized rat duodenum, n.a: non-applicable.

Agonists	pD'_2	pA_2	Preparations
Acetycholine	3.96	n.a	GPI
Histamine	3.32	n.a	GPI
Bradykinin	3.35	n.a	GPI
Angiontensin II	3.09	n.a	GPI
Prostaglandin E_2	3.69	n.a	GPI
Serotonin	2.64	n.a	RFS
Noradrenali	3.42	n.a	RVD
Calcium	n.a	3.40	DRD

DISCUSSION

The main purpose of this study was to make a convincing approach for the mechanism of streptomycin actions on smooth muscles. Seeing that the pD'_2 values which had been obtained in the previous studies (6,7,9,10) are closely resembling (Table II), it has been proposed that streptomycin exerts its effects on smooth muscles through a common mechanism(7,9). Recently, it has been demonstrated that streptomycin inhibits Ca^{2+}—induced contractions of K^+— depolarized rat duodenum in a competitive manner(16). This demonstration is comfirming the previous

Figure 1

Effect of streptomycine on the bradykinin-induced relaxations of isolated rat duodenum. Vertical bars show standard error of mean.
Dose-response curves of bradykinin: ● in the absence of streptomycin (Control), in the presence of ◒ $2.7x10^{-4}$M, ○ $5.4x10^{-4}$M and ⊕ $1.1x10^{-3}$M streptomycine (n = 6 in all cases).

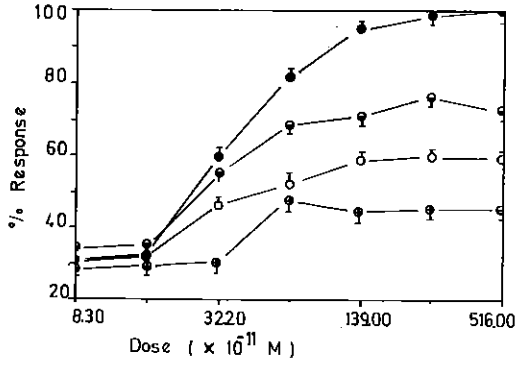


Figure 2

Effect of streptomycine on the adrenaline-induced relaxations of isolated rat duodenum. Vertical bars show standard error of mean.
Dose-response curves of adrenaline: ● in the absence of streptomycin (Control), in the presence of ◒ $5.4x10^{-4}$M and ○ $1.1x10^{-3}$M streptomycin (n = 6 in all cases).

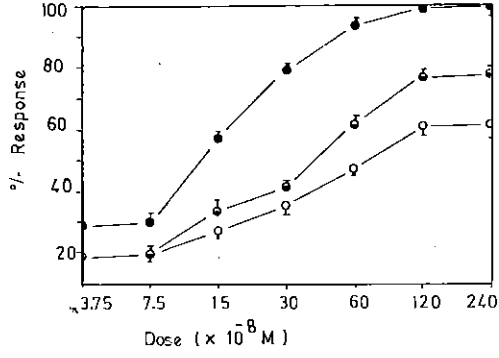
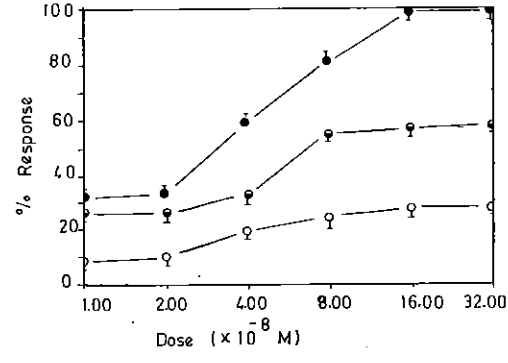


Figure 3

Effect of streytomycine on the papaverine-induced relaxations of isolated rat duodenum. Vertical bars show standard error of mean.
Dose response curves of papaverine: ● in the absence of streptomycin (Control), in the presence of ◒ $5.4x10^{-4}$M and ○ $1.1x10^{-3}$M streptomycin (n = 6 in all cases).



studies. Therefore, it would appear likely that Ca^{2+} ions take a role in the streptomycin-induced inhibitory responses of smooth muscles. In fact, some evidences for the Ca^{2+}-antagonistic action of streptomycin have been obtained in several electrophysiological studies(17,18).

The calcium-antagonistic action of streptomycin might be a responsible in its antagonistic effects againstbradykinin, adrenaline and papaverine. Confirmatively; it has been shown that calcium and calmodulin antagonists such as verapamil and trifluoperazine are able to inhibit the effects of the above-mentioned relaxant agents on the isolated rat duodenum(19). It is known that the relaxant effects of bradykinin(20), adrenaline(21) and papaverine(22) are more or less related with extracellular Ca^{2+} concentrations.

On the other hand, some other aminoglycoside antibiotics such as neomycine and gentamycine are believed to inhibit the calcium channels named as "superficial Ca^{2+} channel"(23,24). Therefore, it would be of interest to perform a comparative study in terms of the Ca^{2+}-antagonistic actions of aminoglycosides.

REFERENCES

1. Laurence, D.R., Bennett, P.N. (ed.), "Chemotherapy and chemotherapeutic agents", in: Clinical Pharmacology, London, Churchil Livingstone, p. 191, 1986.
2. Crossland, J. (ed.), "The chemotherapy of bacterial infections", in: Lewi's Pharmacology, London, Churchill Livingstone, p. 815, 1980.
3. Brazil, O.V., Corrado, A.P., "The curariform action of streptomycin.", J. Pharmacol. Exp. Ther. 120, 452-459, 1957.
4. Altınkurt, O., "Streptidin'in çizgili adale üzerindeki asetilkolinokompetitif kürarizan etkisi.", Türk Hij. Tec. Biol. Derg. 20, 245-249, 1970.
5. Dzoljic, M., Atanackovic, D., "Action of streptomycin on guinea-pig ileum peristalsis.", Arch. int. Pharmacodyn. 156, 279-284, 1965.
6. Altınkurt, O., Kanzık, I., "Non-competitive inhibition of bradykinin, acetylcholine and histamine on guinea-pig ileum with streptomycin and vitamin K_3.", Arzneim. Forsch. 30, 41-44, 1980.
7. Altınkurt, O., Abacıoğlu, N., "Non-competitive inhibition of angiotensin II and PGE_2 on guinea-pig ileum with streptomycin, vitamin K_3 and rutin.", Arzneim. Forsch. 30, 610-613, 1980.
8. Altınkurt, O., Altan, M., "Urfa yöresi akrep (Androctonus crassicauda) zehirinin deney hayvanlarındaki farmakolojik etkileri ile bu etkilerden bir çoğuna streptomisin'in antagonistik cevapları.", Ank. Ecz. Fak. Mec. 10, 41-60, 1980.
9. Altınkurt, O., Yıldızoğlu, N., "Non-competitive inhibition of serotonin on rat stomach fundus with vitamin K_3 and streptomycin.", Turkish J. Pharmacol. Clin. Res. 2, 89-93, 1984.
10. Altınkurt, O., Öztürk, Y., Unpublished observations on the isolated rat vas deferens, 1984.
11. Altınkurt, O., Kanzık, I., Inhibitory effects of streptomycin on bronchoconstrictor, hypotensive and inflammatory responses to bradykinin and histamine.", Arch. int. Pharmacodyn. 246, 277-285, 1980.
12. Yıldızoğlu, N. "İlaçların deney hayvanlarında serotonin (5-HT) in kan düzeyleri ile bazı farmakolojik yanıtlarına etkileri.", Doktora Tezi, Ankara, 1984.
13. Horton, E.W., "Human urinary kinin excretion.", Brit. J. Pharmacol. 14, 125-132, 1959.
14. Ariens, E.J., Van Rossum, J.M., "pD_x, pA_x and pD'_x values in analysis of pharmacodynamics.", Arch. int. Pharmacodyn. 110, 275-299, 1957.
15. Goldstein, A. (ed.), Biostatistics, The Mac Millan Co., New York, 1964.
16. Altınkurt, O., Öztürk, Y., "K^+-depolarize sıçan duodenumunda streptomisin'in kalsiyum antagonistik etkisinde olası mekanizma", Turkish J. Pharmacol. Clin. Res. 2, 122-123, 1984.

17. Farley, J.M., Wu, C.H., Narahashi, T., "Mechanism of neuromuscular block by streptomycin: A voltage clamp analysis", J. Pharmacol. Exp. Ther. 222, 488-493, 1982.
18. Maeno, T., Enomoto, K., "Reversal of streptomycin-induced blockade of neuromuscular transmission by 4-aminopyridine. Proc. Jpn. Acad. 56B, 486-491, 1980.
19. Öztürk, Y., "Sıçan duodenumundaki bradikinin reseptörleri ve bunlara etkiyen faktörler ile streptomisin ve K_3 vitamininin etki özellikleri.", Doktora Tezi, Ankara, 1985.
20. Boschkov, P., Paiva, A.C.M., Paiva, T.B., Shimuta, S.I., "Further evidence for the existence of two receptor sites for bradykinin resposible for the diphasic effect in rat isolated duodenum.", Brit. J. Pharmacol. 83, 591-600, 1984.
21. Anderson, R., "Role of the cyclic AMP and Ca^{2+} in the metabolic and relaxing effects of catecholamines in intestinal smooth muscle.", Acta Physiol. Scand. 85, 312-322, 1972.
22. Sunagane, N., Uruno, T., Kubota, K., "Mechanism of relaxant action of papaverine II. Differences between modes of relaxant effects of papaverine and amytal in guinea-pig taenia coli", J. Pharm. Dyn. 6, 209-216, 1983.
23. Goodman, F.R., Weis, G.B., Adams, H.R., "Alterations by neomycin of ^{45}Ca movements and contractile responses in vascular smooth muscle", J. Pharmacol. Exp. Ther. 188, 472-480, 1974.
24. Weiss, G.B., "Delineation of actions of calcium antagonists in vascular smooth muscle", in: Ca^{2+} Entry Blockers, Adenosine and Neurohumors., Ed. Merrill, G.F., Weiss, H. R., Baltimore, Urband and Schwarzenberg, p. , 1983.