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THE EFFECTS OF BAY K 8644 ON AGONIST-INDUCED CONTRACTIONS IN GUINEA-PIG URINARY BLADDER

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ÖZET: Bu çalışmada Kobay Mesanesinde agonistler tarafından oluşturulan kontraksiyonlara yeni bir kalsiyum kanal aktivatörü olan Bay K 8644'ün etkileri araştırıldı. Histamin ve Serotonin (10^{-9}-10^{-4}) doza bağımlı kontraksiyonlar ortaya çıkardı. Maksimum kontraksiyon 10^{-5} M konsantrasyondaki serotonin ile elde edildi. Bu kasılma 990mg gücündeydi. Bay K 8644 histamin ve serotonin tarafından oluşturulan kontraksiyonları arttırmasına rağmen asetilkolin ile oluşturulan kontraksiyonları anlamlı olarak arttırmadı. Agonistlerin bütün cevapları Kalsiyum kanal blokörü olan nifedipin (10^{-6} M) tarafından inhibe edildi. Artan asetilkolin konsantrasyonları nifedipin etkisini azalttı. Takdim edilen bilgiler, histamin ve serotonin mesanedeki kontraksiyonlarının, büyük ölçüde membrandan geçen ekstrasellüler kalsiyumun hücre içine akışına bağımlı olduğu ve bu kontraksiyonların Bay K 8644 tarafından potansiyalize edildiği görüşünü destekler. Diğer taraftan muskarinik reseptör aracılı kontraksiyonlar Bay K 8644 tarafından potansiyalize edilmeyebilir.

ABSTRACT: Nejat GACAR, Hasan GACAR, Hilal MOCAN, Zehra ÜÇÜNCÜ, Güner K. ÖZGÜR, Nuri İ. KALYONCU, From the Departments of Pharmacology, Pediatrics and Urology, Karadeniz Technical University, Faculty of Medicine, Trabzon/TURKEY and from the Department of Pharmacology, 9 Eylül University, Faculty of Medicine, İzmir/TURKEY. The effects of Bay K 8644 on Agonist-Induced contractions in Guinea-Pig urinary bladder.

In this study the effects of Bay K 8644, a new ca^{2+} channel activator, on agonist-induced contractions in guinea-pig urinary bladders were investigated. Histamin and Serotonin (10^{-9}-10^{-4} M) produced a dose-dependent contractions. Maximum contraction was obtained by 10^{-5} M serotonin. Its value was 990.00mg. Histamin and serotonin induced contractions were enhanced by Bay K 8644 (10^{-6} M), but acetylcholine (10^{-9}-10^{-4})-induced contraction amplitudes were not increased.

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All responses of agonists were inhibited by calcium channel blocker nifedipine (10^{-6} M). This inhibition was decreased with increasing acetylcholine (Ach) concentrations. The present data support the view, that histamine and serotonin-induced contractions in urinary bladder are greatly dependent on transmembrane extracellular Ca^{2+} influx and these contractions are potentiated by Bay K 8644. On the other hand muscarinic receptor mediated contractions may not be potentiated by Bay K 8644.

Anahtar sözcükler: Mesane, Bay K-8644, Kalsiyum Kanalları
Key words: Urinary Bladder, Bay K-8644, Calcium Channels.

INTRODUCTION: There are two of ion channels in the cell membrane that allow calcium (and possibly sodium) to enter the cell. These are called potencial sensitive and receptor operated calcium channels(1).

Dihydropyridines such as Bay K 8644-CGP 28392 and YC 170 are the newer transient Ca^{2+} channel activator agents and Bay 8644 is more potent than the others(2,3,4).

It is well known that Bay K 8644 increases smooth and cardiac muscle contractions(5). The contractile activity of the smooth muscle is generally dependent on the concentration of extracellular calcium(1,6). There is, however, a marked variation in this dependence between different agents and different smooth muscles(1). In the urinary bladder both resting tone, spontaneous activity, various agonist-induced contractions and direct electrical stimulations are dependent on extracellular calcium(7). So calcium channel blockers have inhibitory effects on bladder contraction. This effect is more significant with dihydropyridines(7). On the other hand, these drugs (such as nifedipine, nimodipine etc) interacts competetively with Bay K 8644 at specific dihydropyridine site(5,8). In this view the contractions of bladder (spontaneous and agonist-induced) must be increased by Bay K 8644. However, Hertle and Herman suggested that Bay K 8644 increases the rate of spontaneous contractions of bladder but doesn't influence the amplitude of tonic contractions induced by norepinerpharine(9). Thus the present experiments were designed to study the effects of Bay K 8644 on various agonist-induced contractions in guinea-pig urinary bladder.

MATERIALS AND METHODS: Isolated bladder smooth muscle preparations were obtained from 12 adult male guinea-pigs weighing 350-700g Animals were decapitated, and urinary bladder removed. The strips of smooth muscles were cut from whole body of the bladder.

Tissue preparations (n=12) were placed in temperature regulated (37°C), 20ml organ baths containing krebs solution composition in: g/L: ($NaCl$ 6.9, KCl 0.42, $CaCl_2$ 0.37, $MgSO_4$ 7, H_2O 0.[illegible], $NaHCO_3$ 2.1, KH_2PO_4 0.3, Glucose 1.8). The solution was continuously gassed with 95% O_2 and 5% CO_2. 1g resting tension were carried out to the bladder strips. Each strip was allowed to equilibrate for 40-60 minutes. Isometric tension changes were measured with Nihon Kohden TB 612 T force-displacement transducers and recorded on a 4 channel Nihon Kohden polygraph system.

Cumulative concentration-response curves were obtained to agonits, presence or absence Bay K 8644 and nifedipine. Student's "t" test were used to determine the significance of differences. Results were considered significant at $p < 0.05$).

RESULTS: Within the concentration range 10^{-9}-10^{-4} M serotonin produced concentration-dependent contractions [illegible] bladder strips. Maximal contractil force, obtained at 10^{-5} M, was 952.08±7.61mg (n=12). These contractions were enhanced by 16.94% by Bay K 8644 10^{-6} M and contractil force was 1113.43±38.57mg (n=12).

On the other hand, nifedipine 10^{-6} M inhibited serotonin-induced contractions. At this serotonin concentration of 10^{-5} M inhibition was 77.[illegible]%.

Table 1. Values for pD_2 (the negative logarithm of the concentration of agonist producing half the maximal response)

Agonist	Control	Bay K 8644	n
Serotonin	7.056±0.10	7.804±0.081*	12
Histamin	7.304±0.16	8.094±0.088**	12
Acetylcholine	7.506±0.10	7.438±0.098	12

* Values are ± SE. Significant difference between control and Bay K 8644 + agonist.

Table 2. The effects of Bay K 8644 and nifedipine on serotonin-induced contractions in guinea-pig bladder

Serotonin Concentration	% max responses			
(-log M)	Control	Bay K 8644	Nifedipine	n
9	18.29±0.52	29.92±1.09	11.63±0.19	12
8	29.34±1.52	39.94±2.34	12.42±0.27	12
7	49.32±3.27	70.19±3.37	13.64±0.49	12
6	80.67±0.78	91.60±4.54	17.17±0.79	12
5	99.81±0.17	116.52±3.31	22.25±1.30	12
4	91.69±0.85	108.94±2.79	21.60±2.79	12

At the histamin concentration of 10^{-5} M (916.00±0.23mg) the response was enhanced by 13.56% by Bay K 8644 10^{-6} M (1040.25±24.09).

These contractions were inhibited approximately 80.86% by nifedipine 10^{-6} M. Although serotonin and histamin-induced contractions were significantly enhanced by Ca^{++} channel activator Bay K 8644 ($p < 0.001$), increasing of acetylcholine induced contractions were not sifnificant ($p < 0.50$).

Table 3. The effects of Bay K 8644 and nifedipine on histamin-induced contractions in guinea-pig bladder

Histamin Concetration (-logM)	% max responses			
	Control	Bay K 8644	Nifedipine	n
9	24.[illegible]±0.35	33.89±1.47	11.46±0.31	12
8	38.02±2.42	50.21±2.53	13.16±0.49	12
7	52.80±3.61	75.51±2.39	14.05±0.57	12
6	88.82±2.49	95.24±2.43	15.83±0.64	12
5	96.05±0.91	111.52±0.91	18.35±0.76	12
4	97.57±0.90	140.60±1.95	16.05±0.55	12

Table 4. The effects of Bay K 8644 and nifedipine on acetylcholine -induced contractions in guinea-pig bladder

Acetylcholine Concentration (-log M)	% max responses			
	Control	Bay k 8644	Nifedipine	n
9	25.01±0.52	25.66±0.41	16.40±0.79	12
8	33.30±0.83	32.19±0.88	18.05±0.86	12
7	60.18±3.18	58.66±3.09	21.27±1.07	12
6	76.40±0.96	74.58±0.85	28.43±2.80	12
5	70.00±2.13	70.77±1.78	31.68±3.04	12
4	66.38±1.58	65.65±1.65	45.31±2.96	12

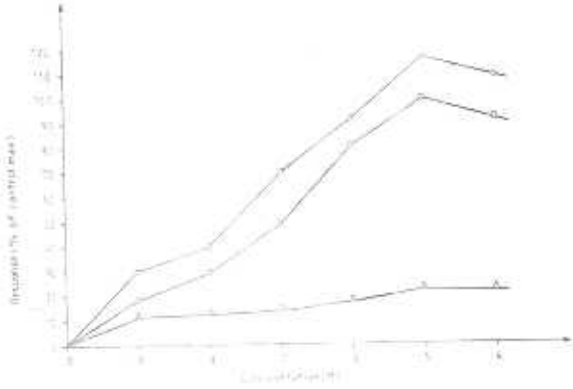

Fig.1: Concentration-response curves obtained by cumulative addition of serotonin (□) and presence of 10^{-6}M Bay K 8644 (○), nifedipine (Δ)

Figure 1. Concentration-response curves obtained by cummulative addition of serotonin(|_| and presence of 10^{-6} M Bay K 8644 (o), nifedipine ( )

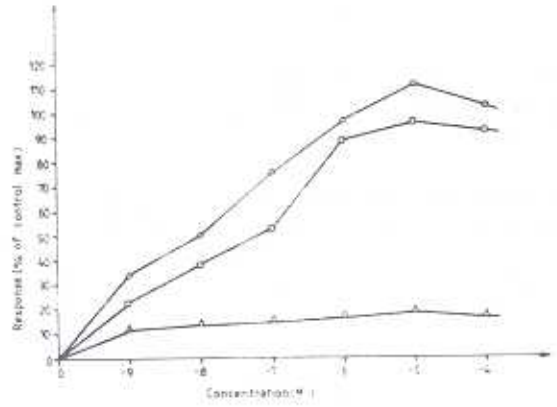

Fig.2: Concentration-response curves obtained by cumulative addition of histamin (□) and presence of 10^{-6}M BayK8644 (○), nifedipine (Δ)

Figure 2. Concentration-response curves obtained by cumulative addition of histamin (|_|, and presence of 10^{-6} M Bay K 8644 (o), nifedipine ( )

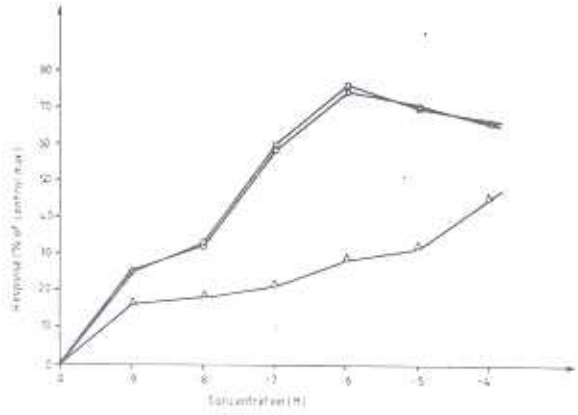

Fig.3: Concentration-response curves obtained by Cumulative addition of Acethylcholin (o) and presence of 10^{-6}M Bay K8644 (o), nifedipine (a)

Figure 3. Concentrations-response curves obtained by Cumulative addition of Acethylcholin(|_|)and presence of 10^{-6} Bay K 8644 (o), nifedipine ( )

DISCUSSION: The present data showed that serotanin and histamine but not acetylcholine-induced contractions were enhanced by Bay K 8644 in guinea-pig urinary bladder.

It is well known that calcium in necerrasy for smooth muscle contraction. These contractions require an increase in the intracellular free calcium concentration. Intracellular free calcium concentration is increased by two important sources: a) Intracellular calcium pools b) Extracellular calcium.

The contractile responses of the urinary bladder to various agonists, neurotransmitters and to potassium have been reported to be dependent upon an extracellular calcium source(6,7). Extracellular calcium can enter the cell through receptor or potantial operated calcium channels(1,6,10) and may be activated by a number of drugs and substances.

Bay K 8644 is a dihydropyridine Ca^{2+} agonist that binds to a specific dihydropyridine receptor(11,12,13) and enhances Ca^{2+} influx through potantial operated calcium channels(14,15,16,17) but not receptor operated channels(14). On the other hand, other investigators reported that Bay K 8644 potantiated contractil responses to -adrenoceptor agonist NA in rat aorta(18), dog saphenous artery(19), to anglotensin receptor agonist AII(20), to 5-TH_2 receptor agonist serotonin(21) in rabbit aorta, to H_1 receptor agonist histamine in guinea-pig intestinal smooth muscle(22).

These findings are similar to our results although pertaining to a differient smooth muscle.

The histamine H_1 receptor in quinea-pig urinary bladder is involved the histamine-induced contraction(25) and Bay K 8644 potentiated the action of histamine in quinea-pig bladder (in this study) and intestinal smooth muscle(22). The ED_{50} value of histamine in bladder was similar to that in the quinea-pig small intestine(25).

Bay K 8644 also potentiated an augmented serotonin-induced contractions, which are 5-HT_2 receptor mediated, in the rabbit isolated aorta preparation(21) and the responses of quine-apig bladder were similar.

On the other hand Bay K 8644 increased acethylcholine-induced contractions but this increasing was not significant. The values of contractions were 667.62±21.18mg and 675.50±18.74mg absence and presence of 10^{-6} M Bay K 8644 respectively.

Agonist-induced contractions were reduced by nifedipine(10^{-6}M), but the effect of nifedipine was decresed by increasing acethyl choline concentrations. This is consistent with findings of other investigators who studied carbachol(1,23,24). The effect of nifedipine was also decreased with increasing carbachol concentrations.

Carbachol-induced contractions were unaffected by Bay K 8644 and this suggests, therefore, that cholinergic contraction in human airways in independent of calcium influx through potantial dependent channels(26). In addition, Donoso et al(27) demonstrated that Bay K 8644 increases the contractile responses of neurotensin in rat stomach fundus. The neurotensin receptors in the rat fundus are coupled to calcium channels, sensitive to nifedipine and related drugs where as the muscarinic receptors are not linked to the same channels(27).

The study of Roy and Pruneau(20) shows that the receptor-operated system in the rabbit aorta, when stimulated by AII, is highly sensitive to the α-adrenoceptors. It indicates that Bay K 8644 activates calcium channels opening upon occupancy of a membrane receptor. In spite of these data, it is still not clear whether or not Bay K 8544 interacts with receptor operated calcium channels.

In conclusion the present study suggest that serotonin and histamin-induced transmembrane Ca^{2+} influx were increased by Bay K 8644 but muscarinic receptor-mediated transmembrane Ca^{2+} influx was not increased in quinea-pig urinary bladder.

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