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Comparative Smooth Muscle Relaxant Activity Of Dihydropyrimidine Derivatives 5-Acyl-6-Methyl-4-Phenyl-2-S-Ethyl-1,4-Dihydropyrimidine (BK VI) , 5-Acyl-6-Methyl-4(2,3 Methylenedioxy) Phenyl 2-S-Benzyl-1,4-Dihydropyrimidine (BK VII) and Nifedipine on Isolated Rat Uterus.

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ABSTRACT

Ca2+ channel antagonists find their uses as anti-arrhythmic agents and in the treatment of hypertension and CHF.Besides a wide variety of uses in angina,migraine,raynaud's disease,clinical trials are underway to evaluate their role to slow the progression of renal failure.In view of the above, efforts are on to develop new compounds having Ca2+ channel blocking activity. To compare the smooth muscle relaxant activity of dihydropyrimidine derivatives 5-acyl-6-methyl-4-phenyl-2-S-ethyl-1,4-dihydropyrimidine(BK VI) and 5-acyl-6-methyl-4(2,3 methylenedioxy) Phenyl 2-S-benzyl-1,4-dihydropyrimidine (BK VII) on isolated rat uterus. Effect of the test compounds BK-VI, BK-VII and nifedipine on the smooth muscles of isolated rat uterus was observed. Observations were made with increasing bath concentrations of each compound. Six preparations were used for each dose of BK-VI, BK-VII and nifedipine. Mean effect of increasing doses of BK-VI,BK-VII and nifedipine on the height of Ca2+ dependent, K+-induced contraction of isolated rat uterus were noted and IC-50 calculated. Test compounds BK-VI and BK-VII had a significant dose-dependent relaxant effect on K+-induced contractions of isolated rat uterus. For BK-VI, significant relaxation was seen at bath concentration starting from 9.34x10-4M (IC50=12.2x10-4 M).BK-VII showed significant relaxation at bath concentrations starting from 4.2X10-5M (IC50=12.2X10-5M), while nifedipine showed significant relaxation at much lower bath concentration starting from 2.8X10-7(IC50=7.5X10-7). BK-VI and BK-VII, like nifedipine, have calcium channel blocking activity and they can inhibit the Ca2+ dependent contractions of smooth muscles of uterus. On comparison,Nifedipine was found to be the the most potent compound followed by BK-VII and BK-VI.

Keywords: Calcium channel blockers, Dihydropyrimidines, Voltage dependent calcium channels.

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INTRODUCTION

In the development of new multicomponent reactions, organic chemists have been inspired to design large families of novel compounds.[1]Among them 3-multicomponent reaction, Biginelli reaction that allows preparation of DHPMs has attracted renewed interest[2] owing to their therapeutic and pharmacological properties as channel blockers, anti-hypertensive agents,α1a antagonists and neuropeptide Y antagonists[3].This explains widespread studies on these compounds in specialized literature[4].Various biological assays using K+ -depolarized rabbit aorta have shown that 2-Hetero substituted -4- aryl -1,4-dihydro-6-methyl-5-pyrimidine carboxylic acid esters, mimic dihydropyridine calcium channel blockers. The solid state structure of dihydropyrimidine analogue shows their conformation quite similar to dihydropyridines[5].It was found that dihydropyrimidines can inhibit depolarization-induced contractions of isolated smooth muscle preparations. Some showed anti-ischaemic activity in animal models[6].Various dihydropyrimidines have been evaluated for their calcium antagonistic activities by comparison with calcium antagonistic reference compound nifedipine[7].Two such compounds BK-VI and BK-VII were evaluated and compared for their calcium-channel blocking and smooth muscle relaxant activity activity.

MATERIAL AND METHODS

TEST COMPOUND BK-VI AND BK-VII

Both test compounds, 5-acyl-6-methyl-4-phenyl-2-S-ethyl-1,4-dihydropyrimidine(BK-VI),(Molecular weight-274) and 5-acyl-6-methyl-4(2,3 methylenedioxy) Phenyl 2-S-benzyl-1,4-dihydropyrimidine (BK-VII) (Molecular weight 380) were obtained from department of chemistry, Punjabi university, Patiala. For BK-VI, a mixture of benzaldehyde (0.01mole,1.06gms.), thiourea (0.01mole,0.76gms),acetyl acetone (0.015 mole,1.5 ml) and concentrated HCl(3-4 drops) in absolute alcohol was irradiated at 30%microwave power level. The tetrahydropyrimidine obtained was separated, dissolved in NaOH solution and to this mixture, diethyl sulfate was added. BK-VII was prepared using 2,3-methylene dioxy benzaldehyde (0.01 mole, 1.5 gms), thiourea (0.01 mole, 0.76 gm), acetylacetone (0.015 mole, 1.5 ml) and concentrated HCl (3-4 drops) in absolute alcohol (10 ml) taken in a borosil beaker (100ml) was irradiated at 30% microwave power level and to the tetrahydropyrimidine obtained, benzyl chloride was added. The solid product separated was confirmed by taking its IR,NMR,UV and mass spectra[8].Both BK-VI and BK-VII were found to soluble in carboxymethylcellulose.

Drugs and chemicals

1% carboxymethyl cellulose was used as a solvent for compounds BK-VI, BK-VII and nifedipine.Other chemicals and agents used were of pure analytical grade and obtained from local suppliers.

Animals

Adult healthy rabbits of either sex weighing between 1.5-2.5 Kg and female albino rats (250-350 gms) were used in this study. They were provided uniform environmental conditions and diet. The diet comprised of green leafy vegetables, grass, soaked grams and milk. The care and maintenance of the animals was as per the approved guidelines of the Committee For the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. All the animal procedures were approved by the Animal Ethical Committee of the establishment. Isolated rat uterus was used for the present study.

PROCEDURE

ISOLATED RAT UTERUS - IC_{50}

Smooth muscles can contract if they are exposed to membrane depolarizing high potassium bathing medium. This mechanical response can be abolished by removing Ca^{2+}ions from the high potassium bathing solution and reinstated by adding Ca^{2+}ions back to the solution [9]. It can also be prevented by addition of a Ca^{2+} channel blocking agent to the high potassium bathing solution[10] or aborted by adding such an agent after the contraction has been induced[11].

Studies have shown that depolarisation of rat uterus increases smooth muscle cell membrane permeability to extracellular calcium resulting in the contractile response, which is directly proportional to extracellular calcium concentration. Further Fleckenstein and Grun have demonstrated that calcium channel blockers like verapamil, gallopamil etc. suppress excitability and contractility in the rat uterus [12].

Rat uterus was used to quantify the inhibitory action of the test compounds and calculate IC50 in the present study. Priming was done 24 hours prior to every experiment, by administration of Diethylstilbesterol (DES), 0.1 mg/kg body weight, subcutaneously. Dissection was done and preparation mounted in oxygenated De Jalon solution as per the method described by Ghosh. Temperature of the bath was kept around 30°C. Bath capacity was kept constant at 25ml. Tissues were subjected to a tension of 1 g for half an hour for relaxation after which KCl was added to the bath to get a final concentration of 60 mM. K+ induced contractions were recorded using a frontal writing lever. Magnification was kept at 5-6 times. The contractions were recorded on a static smoked drum for half an hour so as to obtain the maximum response.[13] Fine suspensions of the test compounds in 1% carboxymethyl cellulose (CMC) were then added in geometric doses and waiting period of 15 minutes was given for each dose. A cumulative dose response curve was taken. Time matched controls were also recorded for each experiment. Six such experiments were done and IC_{50} calculated.

Mean value and standard error for all parameters were determined separately and put in tables as mean±SE. Statistical significance of the difference at various concentrations, before and after was analysed using Student's paired’t’ test.

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RESULTS

Effect on Isolated Rat Uterus

(For calculation of IC_{50})

Six experiments were performed using nifedipine and six using compound BK VI and BK-VII at different doses showing their relaxant effects on K+-induced and Ca2+ dependent contraction of isolated rat uterus.(Fig.1,2)

Results of each of six experiments are tabulated (Table 1, 2 and 3)

Table 1: Mean relaxing effect (Mean±SE) of increasing doses of compound BK-VI on K+-induced contraction of isolated rat uterus (n=6)

Bath conc. (µg/ml)	Height of K+-Induced contraction (mm) Before BK VI	After BK VI	Mean Chang	Mean %age	p value change
16 (5.8×10^{-5}M)	63.6±15.1	46.0±13.7	0.92±0.45	2.31	>0.05
32 (11.6×10^{-5}M)	63.6±15.1	44.5±13.3	2.0±1.06	4.92	>0.05
64 (23.3×10^{-5}M)	63.6±15.1	42.0±12.7	5.17±2.93	11.56	>0.05
128 (46.7×10^{-5}M)	63.6±15.1	36.8±11.9	8.83±4.07	18.67	>0.05
256 (93.4×10^{-5}M)	63.6±15.1	29.8±11.7	20.25±8.63	39.53	<0.05
512(186.8×10^{-5}M)	63.6±15.1	19.5±0.85	31.9±8.83	63.92	<0.01
1024(373.7×10^{-5}M)	63.6±15.	10.75±0.47	49.8±10.94	85.12	<0.01

Table 2: Mean relaxing effect (Mean±SE) of increasing doses of Nifedipine on K+-induced contraction of isolated rat uterus (n=6)

Bath conc. (µg/ml)	Height of K+-induced contraction (mm) Before Nifedipine	After Nifedipine	Mean Change	Mean %age	p value change
0.1 (2.8×10-7M)	31.7±9.96	27.8±8.58	3.83±1.49	10.66	<0.01
0.2 (5.7×10-7M)	31.7±9.96	18.83±5.05	12.83±4.95	36.88	<0.001
0.4 (11.5×10-7M)	31.7±9.96	7.66±1.58	24.0±8.91	71.13	<0.001
0.8 (23.1×10-7M)	31.7±9.96	2.16±1.07	29.5±10.21	90.17	<0.001
1.6 (46.2×10-7M)	31.7±9.96	0.5±0.34	30.66±10.10	98.2	<0.001

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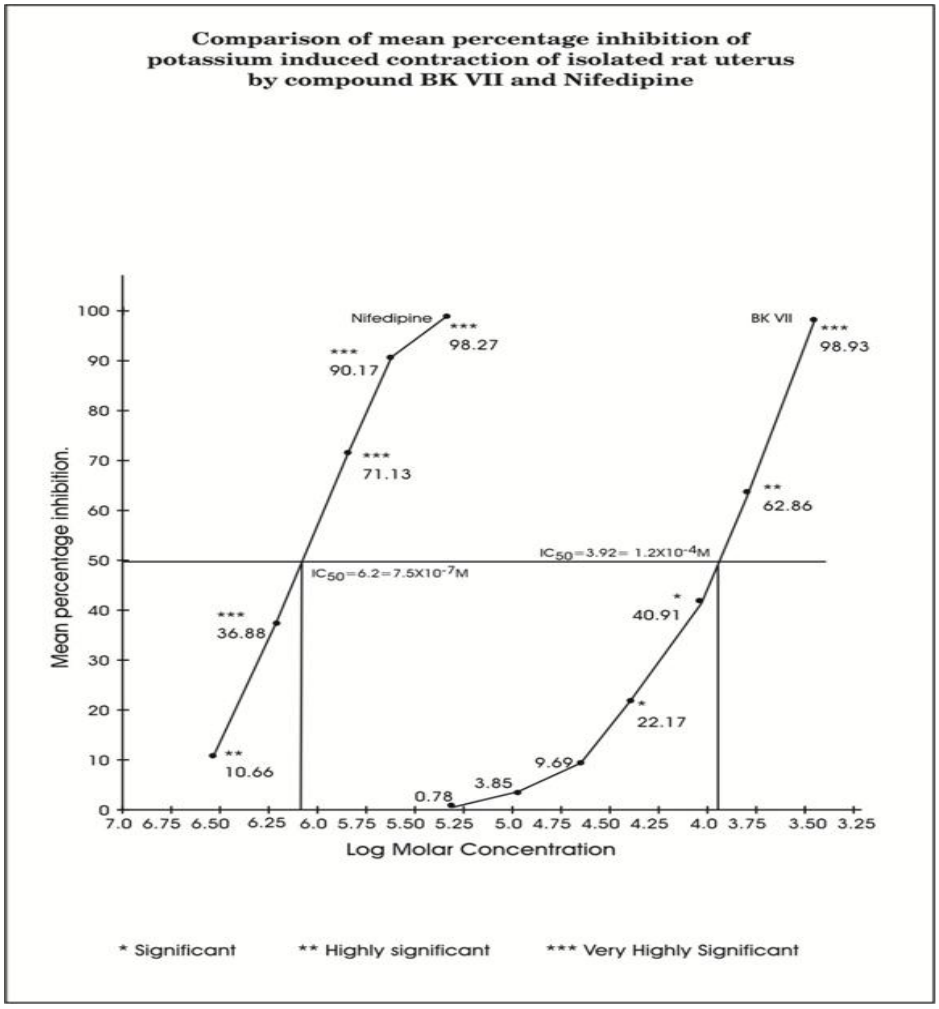
Table 3: Mean relaxing effect (Mean±SE) of increasing doses of compound BK-VII on K^+-induced contraction of isolated rat uterus (n=6)

Bath conc. (µg/ml)	Height of K^+-induced contraction (mm) Before BK VII	After BK VII	Mean Change	Mean %age change	p value
2 (0.52×10^{-5}M)	46.25±13.5	46.0±13.7	0.25±0.25	0.78	>0.05
4 (1.05×10^{-5}M)	46.25±13.5	44.5±13.3	1.75±0.62	3.85	>0.05
8 (2.1×10^{-5}M)	46.25±13.5	42.0±12.7	4.25±1.12	9.69	>0.05
16 (4.2×10^{-5}M)	46.25±13.5	36.8±11.9	9.5±3.30	22.17	<0.05
32 (8.4×10^{-5}M)	46.25±13.5	29.8±11.7	16.5±4.69	40.91	<0.05
64 (16.8×10^{-5}M)	46.25±13.5	19.5±0.85	26.79±7.42	62.86	<0.01
128 (33.6×10^{-5}M)	46.25±13.5	0.75±0.47	45.5±13.0	98.93	<0.001



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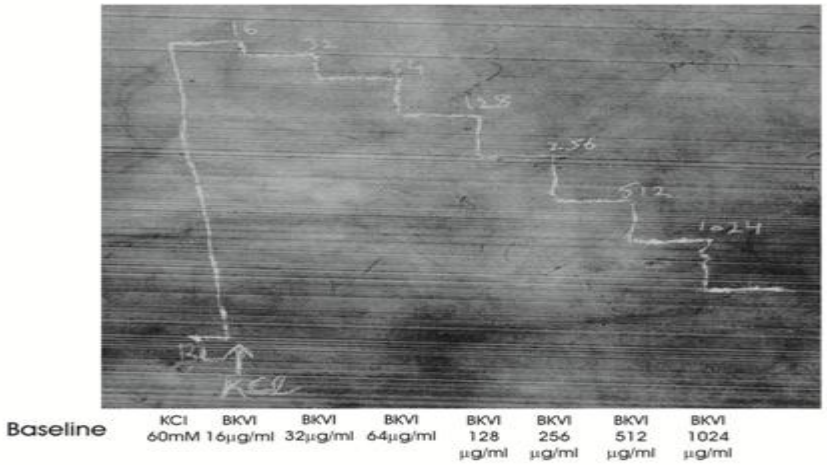
Relaxing Effect of BK-VI and Nifedipine on K^+ - induced Contraction of Isolated Rat Uterus.



Baseline KCl 60mM | BKVI 16µg/ml | BKVI 32µg/ml | BKVI 64µg/ml | BKVI 128 µg/ml | BKVI 256 µg/ml | BKVI 512 µg/ml | BKVI 1024 µg/ml

Baseline KCl 60mM | Nif. 0.1µg/ml | Nif. 0.2µg/ml | Nif. 0.4µg/ml | Nif. 0.8µg/ml | Nif. 1.6µg/ml

DISCUSION

Calcium channel blockers are the drugs having wide ranging pharmacological activity. In recent years, interest in the designing of 1, 4 - dihydropyrimidine - 5 carboxylate compounds has increased manifold. They have been presented as valuable substitutes[1] for nifedipine and other dihydropyridines[14], clinically useful in the treatment of cardiovascular disease.

In the present study, the pharmacological actions of dihydropyrimidine derivative5-acyl-6-methyl-4-phenyl-2-S-ethyl-1,4-dihydropyrimidine(BK-VI),and 5- acyl - 6- methyl -4 (2,3- methylenedioxy) phenyl 2-s-benzyl-1,4- dihydropyrimidine (BK-VII), were studied on smooth muscle. 'In vitro' preparation was used for serving that purpose viz: Isolated rat uterus. Six experiments were conducted with different concentrations of BK-VI, BK VII and nifedipine in each parameter.

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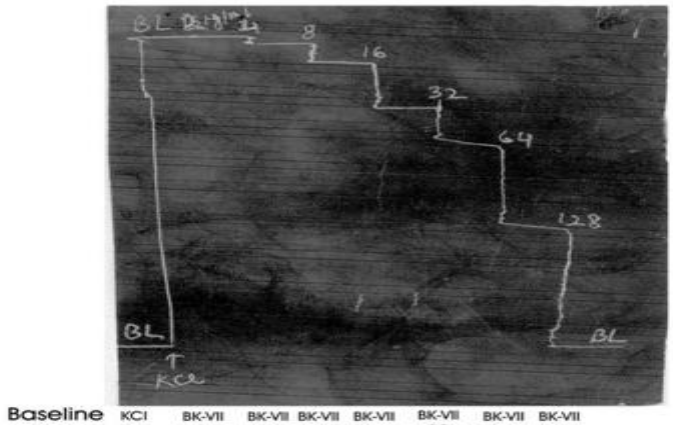
The inhibitory effect of drugs like lidoflazine, cinnarizine and chlorpromazine on the contractions of several arteries evoked by KCl-rich solutions can be reversed by increasing the concentration of calcium in the perfusate. Also depolarization in K+ containing solution does not seem to release intracellular Ca2+ unless calcium is present in the bathing medium which reinforces the idea that K+-induced contractions are dependent on entry of extracellular Ca2+ ions[12].Therefore, the drugs which inhibit such contractions may possibly do so by blocking the calcium channels present on the smooth muscles of the test preparations.

Relaxing Effect of BK-VII and Nifedipine on K^+ - induced Contraction of Isolated Rat Uterus.



Compound BK VI and BK-VII were found to be having a dose-dependent relaxant effect on the K+-induced contractions of isolated rat uterus. Significant relaxation was seen at bath concentration starting from 9.34x10-4 M (IC50=12.2x10-4 M) (Graph1) for BK-VI. Compound BK-VII showed significant relaxation at bath concentration starting from 4.2x10-5M (IC50=12.2x10-5M) (Graph2). Nifedipine in comparison shows highly significant to very highly significant dose-dependent relaxant effect at all bath concentrations starting from 2.8x10-7M (IC50=7.5x10-7M)(Graph 1,2).

From above cited experiments, conclusion can be drawn that the compounds BK VI and BK-VII do have a calcium channel blocking activity and they can inhibit the Ca2+ dependent and K+ induced contractions of the smooth muscle of uterus.A significant dose dependent relaxant effect on uterine smooth muscles was observed at doses higher than those of nifedipine.BK-VI showed the same effect at higher doses than BK-VII.Thus, it can be concluded that nifedipine is more potent thanBK-VI and BK-VII ;and BK-VII is more potent than BK-VI. In order to ascertain the status of these compounds as drugs, further studies are required not only in other animals and tissue models but also in various pathophysiological models, since some drugs show more pronounced effect in disease and in pathophysiological models than in physiological conditions[12]. The predictive value of such models can be affected by difference in various species.

REFERENCES

[1] GanemB.Acc.Chem.Res.2009,42,463.(b)Armstrong,R.W.;Combs,A.P.*etal.* Acc.Chem.Res.1996,29,123.
[2] Biginelli,P.Gazz. Chim. Ital.1893, 23,360.
[3] Atwal,K.S.;Rovnyak,G.C.;Kimball,S.Detal.J.Med.Chem.,1990,33,2629.
[4] KappeC.O.Org.React.2004,63,1.(b)C.O.Kappe,Acc.Chem.Res.2000,33,879.
[5] Atwal KS, Rovnyak GC, Kimball SD et al. J Med Chem 1990 a; 33:2629-2635.
[6] Grover GJ, Dzwonczyk S, McMullen DM et al. J Cardiovasc Pharmacol 1995; 26:289-294.
[7] Alajarin R, Vaquero JJ, Alvarez-Builla J et al. Bioorg Med Chem 1994; 2(5):323-329.
[8] Kaur J. A post-graduate thesis, Deptt. of Chemistry, Punjabi University, Patiala, 1999.
[9] Hurwitz L, McGuffee LJ, Little SA et al. J Pharmacol Exp Ther 1980;214: 574-580
[10] Godfraind T. J Pharmacol Exp Ther 1983; 224:443-450.
[11] Hurwitz L, McGuffee LJ, Smith PM et al. J Pharmacol Exp Ther 1982; 220:382-388.
[12] Godfraind T, Miller RC and Wibo M. Pharmacol Rev 1986; 38:321-416.
[13] Ghosh MN. Scientific book agency; 2005.pp3-4, 10, 22-23, 90, 92-93,130-131.
[14] Bossert F, Meyer H, Wehinger EAngen Chem Int Ed Engl 1981;20:762-769.