April 1994

Possible presence of calcium channel blocker(s) in Rubia cordifolia: An indigenous medicinal plant

A H. Gilani
Aga Khan University, anwar.gilani@aku.edu

K H. Janbaz
Aga Khan University

M Zaman
Aga Khan University

A Lateef
Aga Khan University

A Suria
Aga Khan University

See next page for additional authors

Possible Presence of Calcium Channel Blocker(s) in Rubia cordifolia: An Indigenous Medicinal Plant

Pages with reference to book, From 82 To 85

Anwar H. Gilani, K.H. Janbaz, M. Zaman, A. Lateef, A. Suria (Departments of Pharmacology, The Aga Khan University Medical College, Karachi.)
H.R Ahmed (Departments of Physiology, The Aga Khan University Medical College, Karachi.)

Abstract

Crude extract of Rubia cordifolia (PC) was tested In isolated tissue preparations for its possible calcium channel antagonislic activity. PC suppressed the spontaneous contractions of guinea- pig atria, rabbit jejunum and rat uterus in a concentration dependent manner (0.1-3 mg/ml). In rabbit aorta, It inhibited norepinephrine (10 uM) and KCl (80 mM) induced contractions. Replacement of physiological salt solution with calcium free solution abolished the spontaneous movements of rabbit jejunum. However, addition of calcium (25 pg/mi) In the tissue bath restored the spontaneous movements. When the tissues were pretreated with plant extract (1 mg/mi) or verapamil (0.5 ug/ml) addition of calcium failed to restore spontaneous contractions. These results Indicate that the plant extract exhibits spasmolytic activity similar to that of verapamil suggestive of presence of calcium channel blocker like constituent(s) in this plant (JPMA 44:82, 1994).

Introduction

Rubia cordifolia Linn. (Family: Rubiacea; Synonmy: R Purpuria DC) locally known as “Manjit” is a perennial climbing herb growing wild in northern hilly areas of Pakistan[1]. The plant has been employed to dye cotton, silk and wooly garments since ancient time[2,3]. Dried roots are considered to be useful in alleviating dropsy, paralysis, jaundice, amenorrhoea and visceral obstructions[4]. Plant is also known to exhibit antineoplastic[5], antibacterial[6] and antiviral[7] properties Moreover, it exerts uricolytic and nephrolytic[8] activities and thus promotes disintegration as well as elimination of urinary stone[9]. Furthennore, itis used as an ingredient of a native recipe "Qurse-rewand" prescribed by traditional healers to cure chronic diseases and trauma of liver[10]. In an attempt to validate this traditional use of the plant, we tested crude extract of this plant against acetaminophen and CCI4-induced liver damage in animal model and found it hepatoprotective[11] with diuretic activity (unpublished data). On the other hand, Ca^{++} + channel blocking drugs have recently been reported to exhibit hepatoprotective, as well as antilithiatic and diuretic properties[12,13] in addition to their well established uses in cardiovascular disorders. It has recently been reported that Ca^{++} channel blockers are present in plants[14,15]. The aim of this study was therefore, to see if this plant also contains Ca^{++} channel blocker like activity which could explain in part some of the folldoric uses of the plant.

Materials and Methods

Preparation of crude extract

Rubia cordifolia roots were purchased from local herbal store and authenticated with the help of a botanist at The University of 2Xarachi. The plant material was powdered and macerated in 80% aqueous-methanol (BDH Ltd., Poole, England) for one week with occasional shaking. The extract was filtered and concentrated to dark reddish brown residue under reduced pressure on a rotary evaporator,

with approximate 11% yield.

Drugs and animals

The following reference materials were obtained from the sources specified; norepinephrine hydrochloride, potassium chloride (Sigma Chem. Co., St. Louis, MO, USA) and calcium chloride (E. Merck, Darmstadt, P.R Germany). All drugs were dissolved in distilled water and dilutions were made fresh in normal saline (0.9% NaCl) on the day of experiment. Animals used in this study were housed in the Animal House of the Aga Khan University at 23-25°C and were given a standard diet and tap water ad libitum.

Pharmacodynainic studies

These experiments were carried out by the method previously used in our laboratory[16,17].

Guinea-pig atria

Gunea-pigs of either sex (400-600g) were killed by cervical dislocation. Paired atria were removed carefully and mounted in a 20 ml tissue bath filled with Krebs-Henseleit solution maintained at 32°C and aerated with 5% carbon dioxide in oxygen. The composition of physiological salt solution was (mM): NaCl, 118.2; KCl, 4.7; MgSO4, 1.2; KH2 PO4 1.2;D-glucose, 11.7; NaHCO3, 25.0 and CaCl2, 2.5 (PH, 7.4). The spontaneous atrial contractions were recorded via an isotonic transducer (T3) coupled with Bioscience (PR200) chart recorder. Each preparation was allowed to equilibrate under ig resting tension for atleast 30 min before administration of any drug.

Rabbit aorta

White rabbits (local breed) of either sex weighing 2-3 kg were killed by a blow to the back of the head. The descending thoracic aorta was quickly removed and cut into rings of 2-3mm width which were opened by cutting perpendicular to the axis of symmetry of the cylindrical vessel to make strips. Each strip preparation was mounted in a 20 ml tissue bath containing Krebs Henseleit solution, maintained at 37°C and continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide. A resting tension of2g was applied to each tissue and an equilibrium period of one hour was allowed before drug-induced changes in isometric tension of the strips were measured on Gould 3000 polygraph.

Rat uterus

Young female rats (1 70-200 g) were killeci by a blow on the head. The middle 2 cm of the uterine horns were cut longitudinally and each strip was mounted in a 10 ml tissue bath containing Tyrode's solution maintained at 37°C and gassed with a mixture of 5% of CO2 in oxygen. The composition of the Tyrode\'s solution was (mM):NaCl, 136.9; KCI, 2.7; MgCl2, 1.1; NaH2PO4, 0.4; NaHCO3,11.9; D-glucose,5.6; CaCI2,1.8(pH 7.4). The tissue was allowed to equilibrate under 0.5g basal tension for 20 min before isotonic contractions were recorded as described for atria.

Rabbit jejunum

White rabbits (2-3 kg) of either sex starved for 24 hours were killed by cervical dislocation and exsanguina tion. Segments of jejunum about 2 cm length were mounted in 20 ml tissue bath containing Krebs-Henseleit solution, maintained at 37°C and bubbled with a gas mixture of 95% 02 and 5% CO2. A preload of 1.Og was applied and spontaneous contractions were recorded isotonically via T-3 isotonic transducer on Bioscience MD4 recorder. The tissue were allowed to equilibrate for one hour before addition of any drug. The concentrations mentioned in the text or in the figures represent the final bath concentrations.

Results

Plant extract at the concentration of 0.1-3 mg/nil, caused a concentration-dependent inhibition of spontaneous contractions of guinea-pig paired atria, rabbit jejunum and rat uterus as shown in Figure 1.

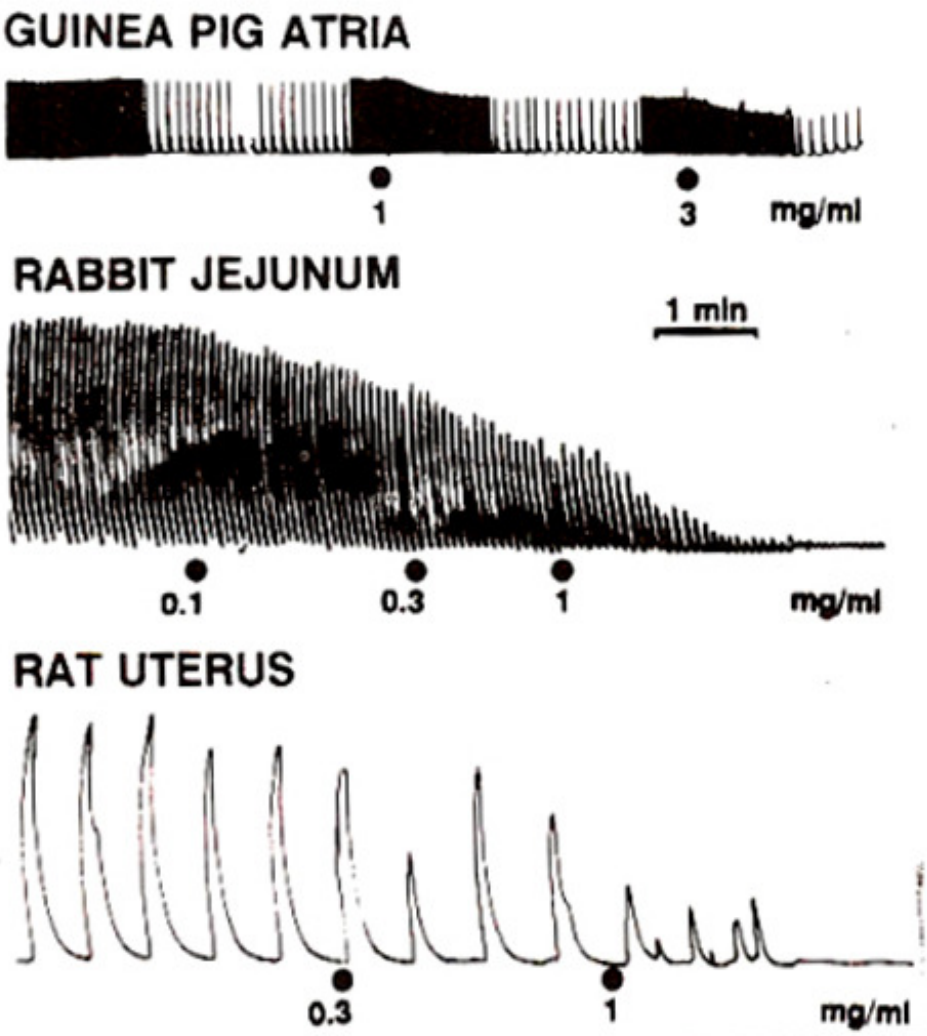

Figure 1. A representative tracing showing concentration- dependent inhibitory effects of Rubia cordifolia on spontaneous movements of different isolated tissue preparations. The time scale mentioned represents slow speed of atria and speed of the chart recorder was increased 4 times to record changes in heart rate.

In jejunum and uterus plant extract at the concentration of 1 mg/nil, completely abolished spontaneous movements whereas in atrial preparation, 3 times greater concentration produced only 50% inhibitory response indicating that the plant extract is approximately 10 times more potent in smooth muscle preparations. This inhibitory effect on all free preparations was reversible as each tissue regained its spontaneous activity after washing the tissue a couple of times with the fresh bathing fluid. In rabbit aorta, both norepinephrine (10MM) and K^+ (80 mM) caused maximal reproducible contractions and the tissue attained its resting state 20-30 minutes after wash out. Then tissue were pretreated with plant extract at different concentration (0.1- 1mg/kg) 30 minutes before redetermination of norepinephrlne or K^+ responses.

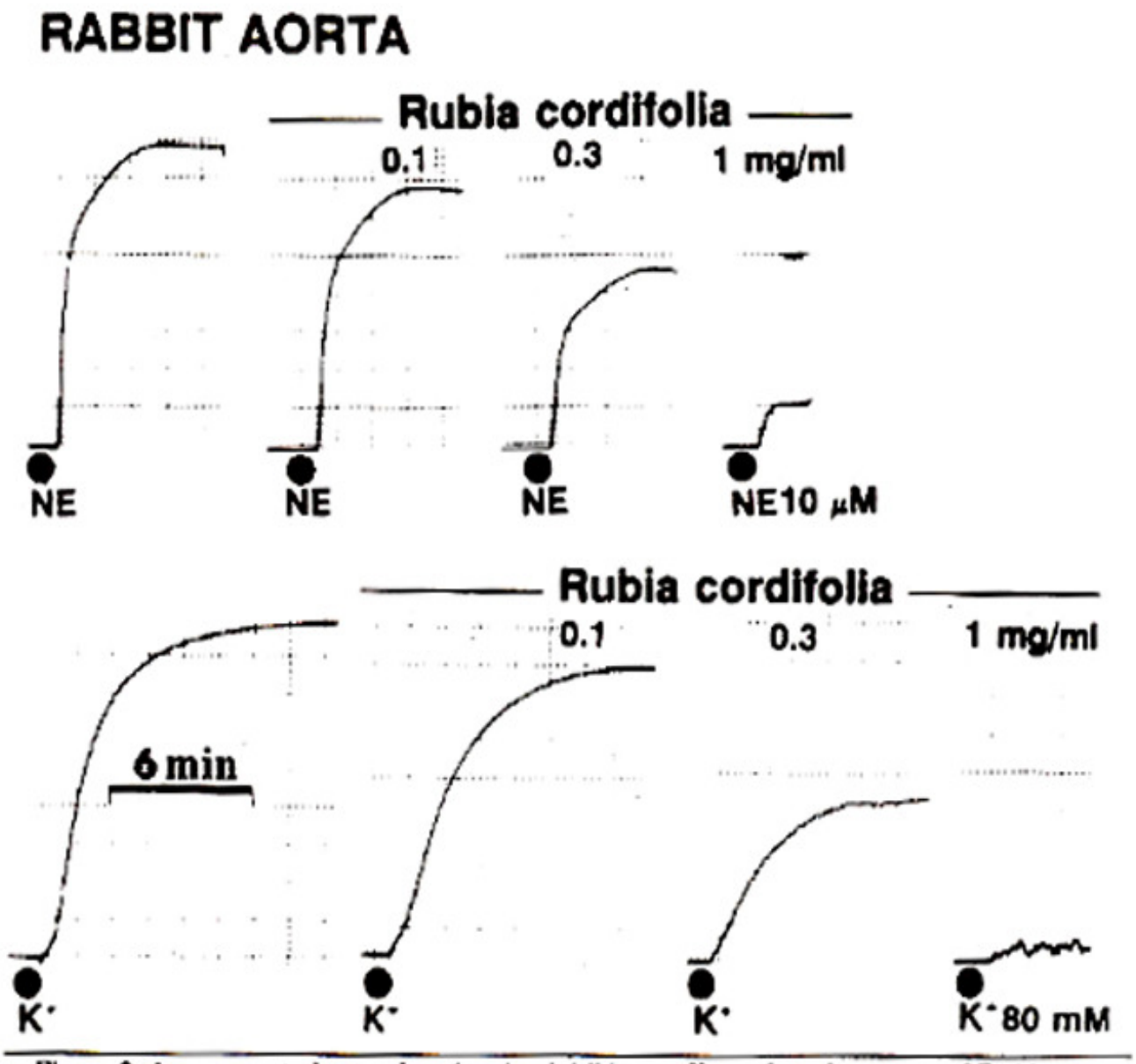

Figure 2. A representative tracing showing inhibitory effect of crude extract of Rubia cordifolia against norepinephrine (NE) and K^+-induced contractions in rabbit aorta.

As shown in Figure 2, plant extract inhibited norepinephrine or C-induced contractions in a concentration- dependent manner. The inhibitory effect of plant extract was similar against both spasmogens and was reversible after frequent washings. Plant extract was also tested in calcium free bathing fluid for its inhibitory effect on calcium supplementation in rabbit jejunum and the results were compared with that of verapamil (Figure 3).

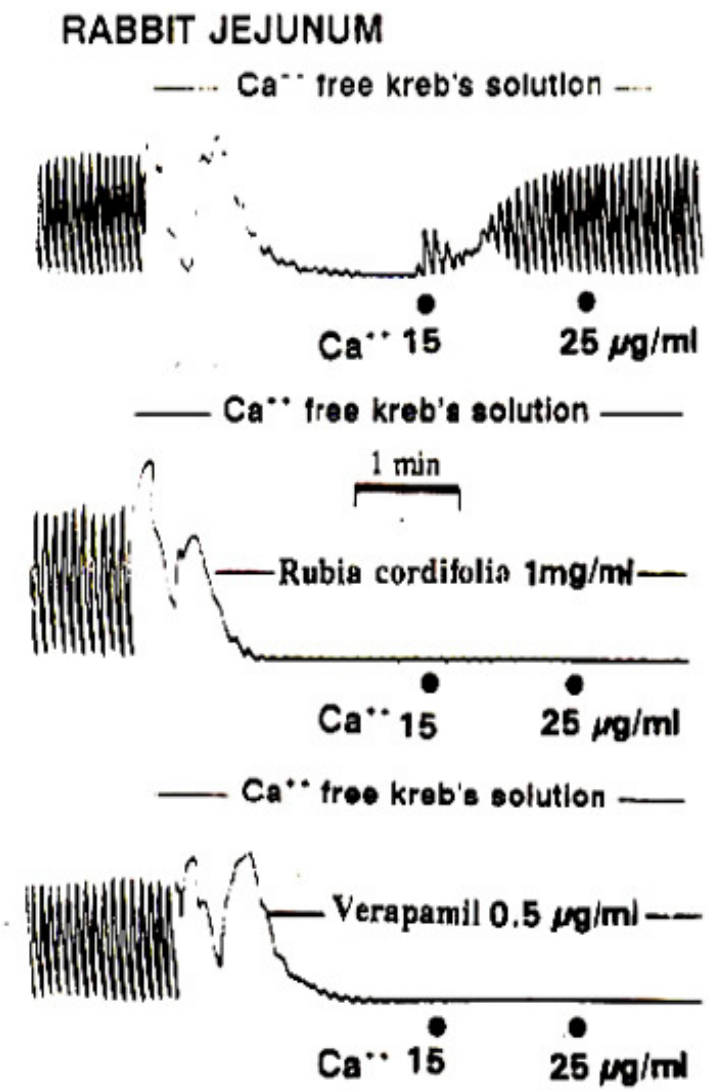

Figure 3. Comparison of plant extract and verapamil for their inhibitory effects against calcium supplementation in Ca^{++} free Kreb's solution.

When bathing fluid was replaced with the calcium free krebs solutions the spontaneous movements were abolished within one minute. Addition of calcium to the tissue bath restored spontaneous activity of the tissue in a concentration-dependent manner (15-25 ug/ml). However, addition of calcium failed to restore spontaneous movements of tissues which were treated with either plant extract (1 mg/mi) or verapamil (0.5 ,ug/ml) indicating blockade against calcium effect.

Discussion

The plant extract suppressed the spontaneous movements of guinea- pig atria, rabbit jejunum and rat uterus thus showing its spasmolytic activity with approximately 10 times more potent on smooth muscle preparations than in cardiae tissue. The rabbit aorta preparation was used for the study of mechanism of spasmolytic action. Pretreatment of tissue with the plant extract inhibited aorta contractions induced by norepinephrine (10 uM) or K^+ (8OumM). It has been shown that high K^+ - concentrations cause marked contractions in blood vessels by depolarizing smooth muscle cells and increasing the influx of calcium through L-type voltage operated channels (VOC) [18]. In contrast,

contractions induced by norepinephrine and by other neurotransmitters is due partly to calcium release by intracellular stores and partly to influx of extraceilular calcium via receptor operated channels (ROC)[18-20]. In this study, plant extract inhibited contractions induced by 80 mM Kt This vasorelaxant effect is not due to opening of K^+ channels because cromokalin and other C channel openers do not inhibit contractions induced by K^+ concentrations greater than about 30 mM[21,22]. The fact that the inhibitory effect of plant extract against K^+ induced and norepinephrine-induced contractions was observed at similar concentrations, suggests that plant extract acts intracellularly and/or on the cell membrane blocking calcium influx through voltage dependent and receptor operated channels in aortic tissue. The calcium blocking activity of plant extract was further confirmed when tested on spontaneous movements of rabbit jejunum suspended in calcium free solution. The spontaneous movements of the intestine is regulated by periodic depolarization and repolarization. At the height of depolarization, action potential appears as a rapid influx of Ca ++ + via VDCs[23]. As the plant extract inhibited the spontaneous movements of rabbit jejunum, it may be due to interference either with the depolarization process or with the Ca^{++} influx through VDCs. The spontaneous movements of rabbit jejunum were abolished when exposed to Ca^{++} + free solution, however, these movements were resumed on addition of Ca^{++} to the bathing fluid. Whereas, the same dose of calcium failed to restore such phasic movements when the tissue was pretreated with plant extract or verapamil rendering the added Ca^{++} unavailable to contractile elements in cytoplasm. Thus, hindrance to Ca^{++} influx via VDCs may be assigned to the chemical constituent(s) of Rubia cordifolia in a manner similar to verapamil, a standard Ca++ channel blocker[24]. The results of this study indicate that the crude extract of Rubia cordifolia produces direct depressant effects possibly through Ca^{++} channel blocking action. Since calcium channel blocking drugs are known to exhibit antilithiastic effect[12,13] the presence of calcium channel blocker constituent(s) in Rubia cordifolia may be responsible for the folkloric use of this plant for disintegration of urinary stones[9], though direct antilithiastic effects remain to be elucided.

Acknowledgements

This study was supported by funds provided by The National Scientific Research Development Board and The Aga Khan University.

References

1. Baquar, S.R. Medicinal and poisonous plants of Pakistan, Karachi. Printas, 1989, p. 390.
2. Reinking. K. Dyeing with madder from ancient to modem times. Melliand Textilber., 1939;20:445-48.
3. Farnsworth, M. Second century B.C. roee madder from Comith and Athena. Anti Archaeol., 1951;55:236-39.
4. Nadksrini, 1CM. Indian Materis Medics Bombay, Popular Parkshan, 1976, pp. 1075-77.
S. Itogawa, S. Search for anti-tumour agents in nature. New Neoplasm inhibitor from Rubia plants. Kagsku to Ysgaku no Kyoshitsu, 1985;90:15-23.
6. Gsw, HZ. and Wang. H.P. Survey of Chinese drugs for presence of anti-bacterial substances. Sciences, 1949;110:11-12.
7. Jin. Y.H., Wang, Y. and Cu, B.L. The study cf anti-viral effect of methanol extract of Rubia cordifolis against herpes aimplexvirua2. In vitro. ViroL Sin., 1989;4:345-49.
8. Nikonov, G.K., Rozanova, V.D. and Kraevskil, Nephrolytic preparations. USSR, 1960;134:388.
9. Keller, J. and Gorlich, B. The action of madder on bladder stones. Disintegration and elimination of

bladderstones. Z. UroL, 1944;38:I-1&
10. Said, H.M. Diseasesof the liver: Greco-Arabic concepts, Karachi Hsmdsrd Foundation Press, 1982, pp. 107-a
11. Gilani, A.H. and Jaobaz, K.H. Effect ofRubia cordifolia extract on acetaminophen and CCl4-induced hepatotoxicity. Phytotherapy Research, 1994 (in press).
12. Gambaro, 0., Cicevello, E., Msrchini, F., et sL Are calcium antagonists potential antilithissticdrugs? In: Pathogenesis and treatmentof nephrolithiasis (Eds. F. Linari, M. Marangella and M. Bruno) Basel, Karger, 1987, pp. 181-83.
13. Larochelle, P. Renal tubulareffects of calcium antagonists. Kidney lot., 1992;41(suppl. 36):49-53.
14. Rauwald, MW. and Brehm, 0. Screening of some medicinal plants for their possible calcium-antagonistic activity. Planta Med., 1991;57(suppl), A59-60.
15. Gilani, A.M. Search foroew calcium channel blockiogdrugs from Pakistani plants. 2nd International Congress on Ethnopharmacology. Sweden, Upssla, July 2-4, 1992, p.33.
16. Gilani, A.H. Antihypertensive sction of himbacine in anesthetized cats. DrugDev.Res., 1991;24:127-33.
17. Gilani, A.H. and Cobbin, LR Csrdioselectivity of himbscine: a musesrine receptor antagonist Naunyn Schmiedeberga Arch.PhsrmacoL, 1986;332:16-20.
18. Bolton, T.B. Mechsnisms of action of transmitters sod other substances on smooth muscle Physiol.Rev., 1979;59:606-718.
19. Godfraind, T., Miller, Rand Wibo, M. Calcium antagonism and calciumentty blockade. Pharmacol.Rev., 1986;38:321-416.
20. Ksraki, H. and Weias, 0. Mini-review: calcium release in smooth muscles. Life Sci., 1988;42:111-15.
2L Hamilton, T.C., Weir, S.W. and Weston, A.H. Comparison of the effect of BRL 34915 and verapamil on electrical and mechanical activity on rat portalvein. Sri. Pharmacol. 1986;88:103-11.
22. Deitmer, P., Oolenhofen, K. and Noack, T. Comparison of the relaxing effect of cicletanine and cromakalin on vascular smooth muscle. J.Cardiovasc. Pharmacol., 1992;20:35-42.
23. Brading, AF. How do drugs initiate contraction in smooth mucles. Trends Pharmacol. Sci., 1981;2:261-65.