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MECHANISM OF ACTION OF 6, 7, 8, 9, 10, 12-HEXAHYDRO-AZEPINO-[2, 1-B] QUINAZOLIN-12-ONE-(RLX) – A NOVEL BRONCHODILATOR

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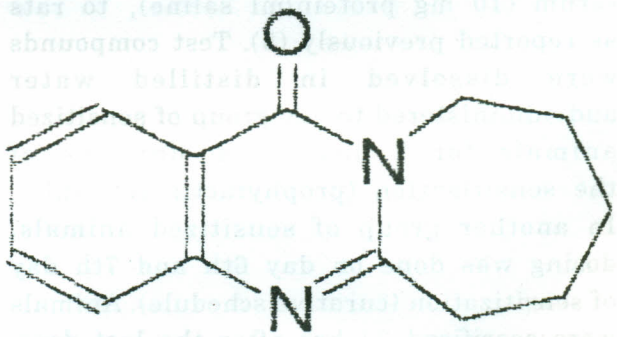
Abstract : The present study presents a mode of action profile of RLX (6, 7, 8, 9, 10, 12-hexahydro-azepino-[2, 1-b]-quinazoline-12-one) a bronchodilator obtained by the chemical modification in the molecule of alkaloid vasicine (Ex: Adhatoda vesica). The effect of RLX (p.o.) was observed on: (a) mast cell degranulation, (b) release of histamine and prostaglandin E (PGE), (c) ^{45}Ca uptake and (d) activities of cAMP phosphodiesterase (PDEase) and lipoxygenase enyzmes in mesenteries/peritoneal mast cells/ lung tissue homogenates in rats under systemic anaphylaxis. RLX (10 and 20 mg/kg) inhibited antigen-induced mast cell degranulation and released of histamine from target tissues. An increased outflow of PGE (lungs) and an inhibited ^{45}Ca uptake (peritoneal mast cells) were noted. Lung PDEase and lipoxygenase activities were decreased. These results suggested that RLX could be acting like disodium cromoglycate and aminophyline with additional attributes its oral efficacy and long duration of action.

Key words : bronchodilator, histamine, calcium uptake, anaphylaxis

INTRODUCTION

Adhatoda vasica Nees, is a highly reputed Ayurvedic medicinal plant, the leaves of which have been used in Indian medicine for more than 2000 years. Vasicine, a major alkaloid from this plant has been chemically modified to yeild highly active compounds of which 6,7,8,9,10,12-hexahydro-azepino-[2,1-b]quinozolin-12-one (RLX) (Fig. 1) is reported as potent bronchodilator (1). It also possesses anti-inflammatory and anti-arthritic activities (2, 3) and its involvement in cell-mediated and humoral components of the immune system is also documented (4). The present inestigation was carried out to evaluate the mechanism of action of RLX as bronchodilator in rats.



6, 7, 8, 9, 10, 12-hexahydro-azepino-[2, 1-b] quinazolin-12-one

Fig. 1 : Structure of RLX.

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METHODS

Healthy adult male albino rats (150-175 g) kept under uniform husbandry conditions were obtained from the animal house of the laboratory. They were maintained on pelleted food and water *ad libitum*. A light period of 12 hours was allowed.

The drugs and other chemicals were obtained from following sources: Chromatographically pure RLX (Department of Natural Products Chemistry, RRL, Jammu), aminophylline (Burroughs Wellcome & Co., Bombay), disodium cromogylcate (DSCG, Unique Chemicals, Bombay), horse serum (King's Institute, Madras), arachidonic acid 5'-nucleotidase, histamine hydrochloride and components of the scintillation fluid (Sigma Chemicals Co., USA); ^{3}HC-AMP and ^{45}Ca (BARC, Trombay); PGE_2 RIA kit (NEN, Amersham, England). All other chemicals were of analytical grade.

Active and passive sensitization was produced by injecting (sc) 0.5 ml of horse serum (10 mg protein/ml saline), to rats as reported previously (5). Test compounds were dissolved in distilled water and administered to one group of sensitized animals for 7 days from day one of the sensitization (prophytactic schedule). In another group of sensitized animals, dosing was done on day 6th and 7th day of sensitization (curative schedule). Animals were sacrificed 24 hrs after the last dose. Mesenteries were dissected away from the small intestines, cut into several small pieces and challenged with horse serum (10%) for 30 minutes at 37°C. Mesenteric bits were fixed, stained (toluidine blue 1.0% in 70% alcohol followed by light green 0.1% in water) and examined as a whole mount preparation. Peritoneal fluid was recovered by direct lavage, centrifuged for 5 min at 350 g and the cell pellet (10^6 cells/0.5 ml) was challenged with horse serum as described. The degranulated mast cells were scored and results represented as percentage of the total (5). The supernatant was assayed for histamine released and expressed as % of the total histamine content of the cells i.e. released plus residual histamine and corrected for non-anaphylactic histamine release by substraction (6). For *in vitro* Ca^{++} uptake studies, mast cell suspension (500 ul) was preincubated for 30 min 37°C in presence of ^{45}Ca (10,000 DPM/tube) with different concentrations of the test compounds. Cells were washed thrice with 5 ml of cold physiological saline. The residual free radioctive ^{45}Ca was removed through glass fibre filter (Whatman pore size 0.2–10 um). The DPM count of ^{45}Ca in each cell suspension was determined using a LKB 1214 Rack Beta Scintillation Counter. Lung tissue (1.0 g) was cut into small pieces, challenged with horse serum, as described abve. Tissue was homogenised in Tris-HCL buffer (0.01 M; pH 7.5) and centrifuged for 20 min at 750 g and the supernatants (2 mg protein/ml) used for assay of released histamine (6), Prostaglandin E (by RIA), and cAMP phosphodiesterase activity (as nmole of ^{3}H cAMP hydrolysed/mg protein) (7, 8). Lipoxygenase activity was determined using arachidonic acid as substrate (9). Protein was measured by the method of Lowry et al (10).

RESULTS

Protective effect of test compounds on mast cells against anaphylaxis:

The results on the effect of different doses of RLX and DSCG on degranulation of peritoneal and mesenteric mast cells under active [Gr I] and passive [Gr II] anaphylaxis are summerised in Table I. In mesenteric mast cells, the degranulation was 80% under anaphylactic shock. Prophylactically RLX (10 mg/kg) has shown 57% and 66% protection in Gr I and Gr II (Figs. 2-4), was enhanced to 65% and 76% at 20 mg/kg respectively. In the curative treatment schedule, RLX (10

TABLE I: Effect of RLX on mast cell degranulation in rats under anaphylaxis.

Treatment	*Dose (mg/kg)*	*Masenteric mast cells*		*Peritoneal mast cells*	
		Gr. I	Gr. II	Gr. I	Gr. II
Sensitized (control)		80±1.7	80±1.7	82±4.0	86±3.0
RLX[P] (po)	10	35±1.7* (57)	28±1.7* (66)	39±4.0* (53)	29±1.7* (66)
	20	28±1.4* (65)	20±2.0* (76)	24±3.0* (70)	24±1.4* (72)
RLX[C] (po)	10	34±1.7* (58)	32±1.4* (60)	38±2.0* (53)	32±1.4* (63)
	20	21±1.4* (75)	25±1.4* (69)	21±1.0* (75)	19±1.0* (78)
DSCG[P] (ip)	25	28±1.7* (65)	21±1.4* (74)	21±1.4* (75)	21±1.7* (76)
DSCG[C] (ip)	25	32±1.4* (60)	32±1.4* (60)	30±1.5* (60)	35±1.4* (60)

P = Prophylactic treatment; the drug was administered for 7 days of sensitization. C = Curative treatment; the drug was administered on 6th & 7th day of sensitization. Gr I, Active anaphylaxis; Gr II, Passive anaphylaxis. Results are expressed as Mean ± SE (n = 10). Figures in parenthesis represent protection.* = P<0.001 vs sensitized control (Student's t-test).

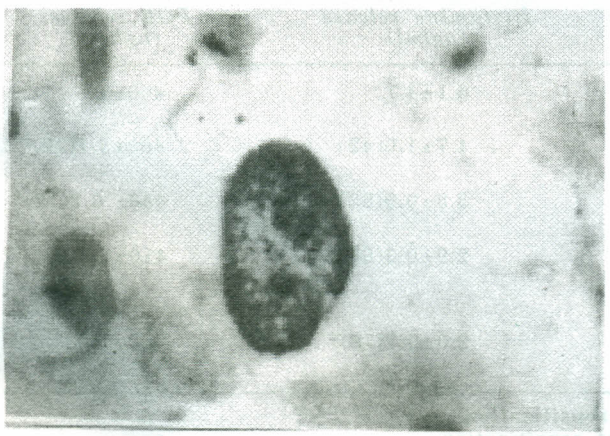

Fig. 2: A normal mast cell (x100).

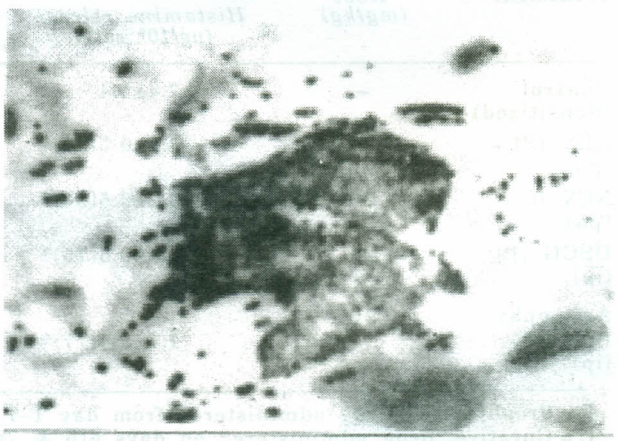

Fig. 3: A mesenteric mast cell (x100) under anaphylaxis.

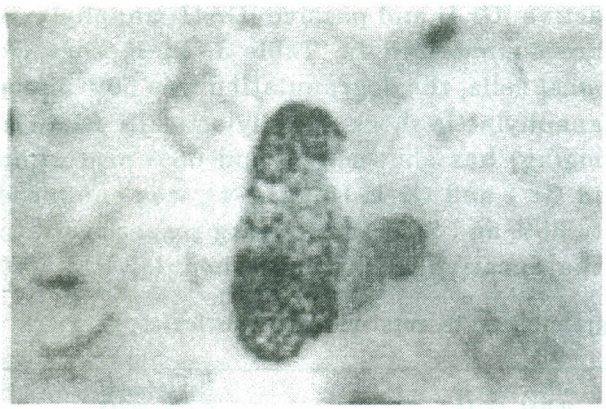

Fig. 4 : A mast cell (x100) from RLX (20 mg/kg, po)-treated group.

mg/kg) prevented mast cell disruption by 58% and 60% which increased to 75% and 69% in active and passive anaphylaxix, respectively at 20 mg/kg dose. DSCG (25 mg/kg) produced 65% protective effect in Gr I and 74% in Gr II. In peritoneal mast cells 82±4% and 86±3% cells were found to be disrupted following active [Gr I] and passive [Gr II] anaphylactic shock. Prophylatic treatment with RLX (10 mg/kg) produced 53% and 66% protection in Gr I and Gr II, was increased to 70% and 72% at (20 mg/kg) respectively. RLX (10 mg/kg) in curative schedule showed 53% and 63% protection in Gr I and Gr II, at higher dose (20 mg/kg). It is increased to 75% and 78% respectively. DSCG (25 mg/kg) showed 75-76% protection when administered prophylactically. The anti-anaphylactic of DSCG in curative treatment was 60% in Gr I and Gr II.

Effect of test compounds on histamine release from peritoneal mast cells and lung tissue:

The effect of the test compound on release of histamine from peritoneal mast cells and lung tissue under passive anaphylaxis is summerised in Table II. Anaphylactically induced histamine release in mast cells was 7.4 ± 1.4 µg/10^6 cells. RLX (20 mg/kg, po) caused 56% inhibition. The inhibitory effect in DSCG and aminophylline treatment groups was 75% and 77% respectively. In lung tissue, a maxium release of 8.1 ± 1.7 µg/ml was observed due to

TABLE II : Effect of RLX on release of histamine and PGE from rat tissues under passive peritoneal analyaxis.

Treatment	*Dose (mg/kg)*	*Peritoneal mast cells*	*Lung tissue*	
		Histamine release (µg/10^6 cells)	*Histamine release (µg/ml)*	*PGE release (ng/ml)*
Control (Sensitized)	–	7.4±1.4	8.1±1.7	400±4.7
RLX [P] (po)	20	3.2±0.5(56)*	4.7±1.0(42)	450±5.7(12)*
RLX [C] (po)	20	3.2±0.5(56)*	3.8±0.9(53)*	440±`6.2(12)*
DSCG [P] (ip)	25	1.8±0.5(75)*	2.9±0.3(64)*	410±6.9
Aminophylline [C] (ip)	25	1.7±0.4(77)*	2.0±0.7(75)*	380±5.83*

P = Prophylactic; drug administered from day 1–7 of sensitization.
C = Curative; drug administered on days 6th & 7th of sensitization.
Data represented as Mean ± SE [n = 6]; Figures in the parenthesis represent % change. *P <0.01 (t-test).

anaphylaxis, which was reduced in RLX treated groups by 42% (prophylactic) and 53% in curative treatments. The inhibitory effect due to DSCG (25 mg/kg) and aminophyllin (25 mg/kg) was 64% and 75% respectively.

Effect of test compounds on PGE release from lung tissue:

Effect of RLX on PGE release is depicted in Table II. After anaphylactic shock, the amount of PGE released was found to be 400 ± 4.7 ng/ml, which was enhanced by 12% due to treatment with RLX given in either prophylactic or curative mode. A marginal effect on PGE release was noted in DSCG (25 mg/kg) and aminophylline (25 mg/kg) treated groups.

Effect of test compounds on enzyme activities:

The results are shown in Fig. 5. RLX inhibited the lung enzyme activities in passive peritoneal anaphylaxis. Prophylactically or curatively administered RLX (20 mg/kg, po) inhibited the activity of PDEase in the range of 22-24%. DSCG (25 mg/kg, ip) produced 49% inhibition whereas 66% inhibition was observed with aminophylline (25 mg/kg, ip). RLX (20 mg/kg) inhibited 5'-lipoxygenase activity by 20-24% whereas DSCG and aminophylline did not produce significant effect.

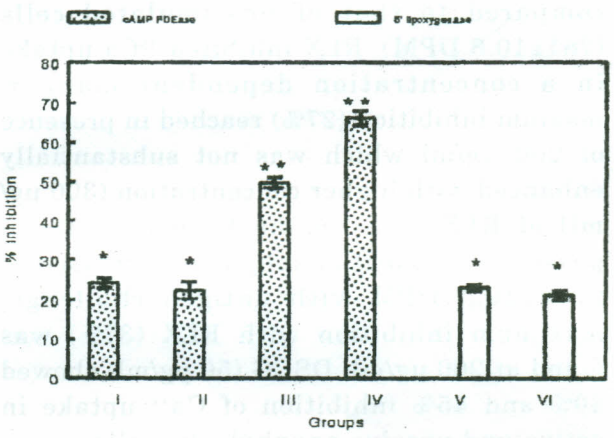

Fig. 5 : Percent inhibition of enzyme activity in rat lung tissue under passive anaphylaxis.

(N = 6); P = prophylactic; C = curative; RLX (20 mg/kg, po); DSCG (25 mg/kg, ip); Aminophylline (25 mg/kg, ip). **P<.001; *P < .05

Gr	I	RLX[p]	cAMP PDEase
Gr	II	RLX[C]	cAMP PDEase
Gr	III	DSCG[C]	cAMP PDEase
Gr	IV	Aminophylline (C)	cAMP PDEase
Gr	V	RLX [P]	5' -Lipoxygenase
Gr	VI	RLX [C]	5' -Lipoxygenase

TABLE III : Effect of RLX on ^{45}Ca uptake (DPM/10^6 cells) by mast cells stimulated by antigen (*in vitro* study).

Treatment	*Active anaphylaxis*	*Passive anaphylaxis*
Sensitized (control)	781±10.6	
None	2085±10.1	2707±10.33
RLX [µg/ml of Tyrode solution]		
100	1574±9.5 (24)*	1786±9.1 (34)*
200	1509±8.7 (27)*	1707±8.9 (37)*
300	1500±9.7 (28)*	1701±8.6 (37)*
DSCG		
50	1260±5.9 (40)*	1525±7.5 (45)*

Values are Mean ± SEM. Figures in parenthesis represent % inhibition of uptake; * = P<.001.[N=5]

Effect of test compounds on Ca^{++} uptake by peritoneal mast cells:

The effect of different concentration of RLX (0-300 µg/ml) and DSCG (50 µg/ml) on Ca^{++} uptake in mast cells is shown in Table III. The ^{45}Ca uptake in antigen-induced cells was 2085±10.1 DPM in active anaphylaxis, compared to that of unstimulated cells (781±10.6 DPM). RLX inhibited ^{45}Ca uptake in a concentration dependent manner, maxium inhibition (27%) reached in presence of 200 µg/ml which was not substantially enhanced with higher concentration (300 µg/ml) of RLX. ^{45}Ca uptake by mast cells in passive peritoneal anaphylaxis increased to 2707±10.3 DPM with antigen challenge, maximum inhibition with RLX (37%) was found at 200 µg/ml. DSCG (50 µg/ml) showed 40% and 45% inhibition of Ca^{++} uptake in active and passive anaphylactic cells.

DISCUSSION

Earlier investigation on pharmacological effect of RLX showed that it attenuates the histamine induced bronchoconstriction and as compared to aminophylline, it was found to be 5-10 times more potent with a high therapeutic index (1). In this paper, we have evaluated the mode of action of RLX vis-a-vis the activity profile of two antiasthmatic drugs (DSCG and aminophylline). The results show that like DSCG (11) RLX has direct protective effect on mast cells in rats under anaphylaxis. Further, it also inhibited histamine-release from sensitized mast cells and lung tisue subsequent to antigen-antibody combination.

In the sequence of the events leading to the release of histamines, cAMP has been suggested to play a central role by acting as a controlling factor (12). There exists an inverse relationship between cAMP concentration and antigen activated release of histamine (13) and therefore PDEase inhibition has been suggested as a mechanism of relaxant action (14). Our results show that RLX could be effective through a cAMP dependent mechanism, by inhibiting PDEase, which could alleviate the deficiency of intracellular cAMP. Earlier pharmacological studies have shown that RLX did not cause increase in heart rate and force of cardiac contraction (1), and it may be expected that elevated cAMP levels in presence of RLX remain within physiolgical lilmit.

However, histamine alone cannot satisfactorily account for all the effects on smopoth muscles observed during anaphylactic reaction (15), since antigen-antibody reaction results in the formation of other smooth muscle stimulating substances, leucotrienes (LTC4, LTD4) which in turn regulate release of prostaglandins (13). A key enzyme, in the biosynthesis of prostaglandins and leucotrienes is 5'-lipoxygenase and one of the eventuality of possible inhibition of this enzyme, is the shunting of arachidonate to synthesis of prostaglandins (13). Prostaglandins of E series have been shown to possess bronchodilator activity (16). The present results show that RLX, which inhibits 5'-lipoxygenase and increase PGE outflow from anaphylactically treated rat lungs, could play a pathophysiological modulator role.

Further experiments were directed to reveal a ppssible role of Ca^{++} ions in the mode of action of RLX. It has been established that after the attachment of the releasing stimulus (antigen-antibody reaction) to the cell membrane a calcium

gating mechanism is activated, since a close relation between the rate of histamine release and amount of Ca^{++} uptake in the cells has been observed (17). The antiallergic drugs, DSCG and aminophylline are documented to block Ca^{++} influx into the mast cells (13). A similar finding with respect to RLX suggests that this compound is capable of altering cell permeability so that calcium uptake in mast cells is significantly inhibited.

In conclusion, the studies presented here suggested that RLX is effective against both active and passive anaphylaxis, which imparts it a superior efficacy against antigen challenge, irrespective of the mode of immunisation. The mode of action profile of RLX seems to be similar, if not identical to DSCG and aminophylline (31) with additional attributes of its oral efficacy, long duration of action and high therapeutic index.

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