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# Mechanisms of the Antiallergic Action of *N*-Methylmequitazine (LG 30435)

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**Abstract**—LG 30435 (*N*-methylmequitazine) was assayed in passive lung (PLA) and cutaneous (PCA) anaphylaxis in guinea-pigs and rats. At doses from 0·3 to 3 μmol $kg^{-1}$ i.v., it produced a dose-dependent inhibition of guinea-pig PLA and of rat PCA and PLA, while the parent compound was ineffective or poorly effective up to 3 μmol $kg^{-1}$. An attempt was made to elucidate the mechanism of LG 30435's action in these anaphylactic models, by means of various antagonists. It was tentatively concluded that different mechanisms are involved in the protective action of LG 30435 in each of the three models: histamine antagonism, possibly accompanied by an inhibition of the effects of peptido-leukotrienes in guinea-pig PLA; histamine antagonism in rat PCA and 5-hydroxytryptamine antagonism in rat PLA, possibly accompanied by a mast-cell stabilizing action in both cases. LG 30435 is devoid of smooth muscle relaxant effects on the airways and its demonstrated anticholinergic and anti-PAF effects do not appear to be involved in its antiallergic action.

LG 30435 (*N*-methylmequitazine) is a potent inhibitor of the guinea-pig bronchoconstriction induced by different agonists, such as acetylcholine (ACh), histamine, 5-hydroxytryptamine (5-HT), leukotriene $D_4$ ($LTD_4$) and platelet-activating factor (PAF) (Subissi et al 1986; Criscuoli et al 1986).

Asthma is a complex disease and its symptoms are the outcome of the effects of several mediators, possibly interacting with each other. No single mediator may be of overriding importance in its pathogenesis (Lessoff 1984), thus explaining the apparent lack of therapeutic success with competitive antagonists such as antihistamines (Eiser 1982) and perhaps, more recently, SRS-A antagonists (Sheard et al 1984). Therefore, a compound like LG 30435, which interferes with multiple biological functions likely to be involved in asthma, could be effective in the therapy of this disease. LG 30435 was also found to have antiallergic activity in guinea-pigs and rats, but the mechanism of this action was not elucidated (Subissi et al 1986). It is not easy to establish which are the relevant mechanisms, since LG 30435 is a multipotent antagonist and a number of different mediators are released from mast cells and basophils during anaphylaxis, including histamine, arachidonate metabolites both of the cyclo-oxygenase and of the lipoxygenase pathway and PAF (Church 1985). Therefore, the object of our work was a more extensive study of the antiallergic action of LG 30435 in three different models of anaphylaxis, namely passive lung anaphylaxis (PLA) in guinea-pigs and rats and passive cutaneous anaphylaxis (PCA) in rats. An attempt was made, by using antagonists of histamine (mepyramine), peptido-leukotrienes (FPL 55712) and PAF (CV 3988 or brotizolam) and a mast cell stabilizer (disodium cromoglycate, DSCG), to identify the mechanisms of LG 30435 which may be relevant to its antiallergic properties in each of the examined models.

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## Materials and Methods

### *Animals*

Male Dunkin-Hartley guinea-pigs (Rodentia, Torre Pallavicina, Bergamo), 300–400 g, and male Sprague-Dawley rats (Nossan, Correzzana, Milano), 200–250 g (preparation of the antiserum) or 120–150 g (PCA) or 250–300 g (PLA), were used.

### *Passive lung anaphylaxis (PLA) in the guinea-pig*

The method of Davies & Johnston (1971) was used. For production of antiserum, a group of guinea-pigs was sensitized by injecting into each hind foot 0·15 mL of an emulsion containing equal volumes of egg albumin 5 mg $mL^{-1}$ and Freund's complete adjuvant. Six weeks later further injections of 0·1 mg/animal of egg albumin were given intradermally, once weekly for 3 weeks. The animals were bled after the last injection and the pooled serum was stored at −20°C. Twenty four hours before the experiment the serum was thawed, diluted 1:25 with 0·9% NaCl (saline) and injected into an ear vein of guinea-pigs in a volume of 1·5 mL $kg^{-1}$. The animals were anaesthetized with sodium pentobarbitone 70 mg $kg^{-1}$ i.p. and prepared according to the method of Konzett & Rössler (1940). A tracheal cannula was connected to a Miniature Ideal Pump (Bioscience) calibrated at 4–5 mL and 56 rev $min^{-1}$. The ventilation of the animal was regulated to equal a pressure of 10 cm water and the volume of overflow was diverted to a piston recorder connected to a Basile 7006 isotonic transducer, and recorded on a Basile 7070 Gemini Recorder. Saline or the test drugs were injected i.v., followed after an appropriate time by a challenge dose of antigen (egg albumin 5 mg $kg^{-1}$ i.v.). The antigen-induced bronchoconstriction in treated animals was compared with that observed in control animals.

### *Bombesin-induced bronchoconstriction in the guinea-pig*

The animals were prepared essentially as described above, but intratracheal pressure instead of lung overflow was recorded through a side arm of the tracheal cannula,

connected to a Bentley Trantec 800 pressure transducer. After an equilibration period bombesin 30 $\mu g\ kg^{-1}$ i.m. was injected. When the bombesin-induced bronchoconstriction had stabilized, the test drugs were administered intravenously, each dose being injected when the effects of the preceding one had reached a steady state.

*Passive cutaneous anaphylaxis (PCA) in the rat*

The method described by Martin & Römer (1978) was followed. Briefly, for production of antiserum rats were sensitized by s.c. administration of 1 mg egg albumin and 200 mg aluminium hydroxide gel suspended in 1 mL of saline and i.p. administration of *Bordetella pertussis* $2 \times 10^{10}$ organisms (Sclavo). Fourteen days later the animals were exanguinated and the pooled serum was stored at −20°C. After thawing the serum was injected intradermally (0·1 mL of a 1:10 dilution) in one site on the shaved back of the test animals. 24 h later the rats received either the solvent (controls) or the test compound intravenously, followed immediately or 5 min later by an intradermal injection of histamine (10 $\mu g$) or 5-HT (0·3 $\mu g$) at two further sites. Immediately afterwards the animals received an i.v. injection of 20 mg $kg^{-1}$ egg albumin and 20 mg $kg^{-1}$ Evans blue in saline. 20 min later the rats were killed by cervical dislocation and the diameter of the blue spot at each antiserum, histamine and 5-HT injection site was measured. The percent inhibition of the diameters of the blue spots in treated animals vs controls were calculated.

*Passive lung anaphylaxis (PLA) in the rat*

The same antiserum prepared for PCA was used. 24 h before the experiment 10 mL $kg^{-1}$ of undiluted serum were injected i.v. to the test animals. These were anaesthetized with sodium pentobarbitone 60 mg $kg^{-1}$, respiration was abolished with succinylcholine chloride 5 mg $kg^{-1}$ i.p. and the animals were prepared according to Konzett & Rössler (1940), essentially as described for guinea-pig PLA, except that ventilation was regulated to equal a pressure of 6 cm water. Saline or the test drugs were injected i.v. followed after an appropriate time by a challenge dose of antigen (egg albumin 20 mg $kg^{-1}$ i.v.). The antigen-induced bronchoconstriction in treated animals was compared with that observed in control animals.

*Drugs and chemicals*

LG 30435 and mequitazine were prepared at Laboratori Guidotti by Prof. L. Turbanti. Mepyramine maleate, egg albumin (grade V), succinylcholine chloride, 5-hydroxytryptamine creatinine sulphate (5-HT) and bombesin (Sigma), ketotifen hydrogen fumarate and methysergide maleate (Sandoz), disodium cromoglycate (I.S.F.), pentobarbitone sodium (Abbott), Evans blue (BDH), histamine dihydrochloride and atropine sulphate (Merck, Darmstadt), ipratropium bromide (Chiesi), salbutamol sulphate (Glaxo) and aminophylline (Malesci) were used. FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4*H*-1-benzopyran-2-carboxylate), BW 755c (3-amino-1-[(*m*-trifluoromethyl)phenyl]-2-pyrazoline), CV 3988 (rac-3-(*N*-n-octadecylcarbamoyloxy)-2-methoxypropyl-2-thiazolioethyl phosphate) and brotizolam were kindly donated by Fisons, Wellcome Research Laboratories, Takeda and Boehringer Ingelheim, respectively.

*Statistics*

The statistical comparisons were made using Student's *t*-test. When possible, ED50s and 95% confidence limits were calculated by means of linear regression analysis of the response/log dose curves.

## Results

*PLA in the guinea-pig*

The effects of LG 30435 on PLA were assayed at different times from antigen challenge. Table 1 shows that the compound appeared more effective at 5 min rather than at 1 or 10 min before antigen challenge. Therefore, in a separate

Table 1. Effects on passive lung anaphylaxis in the guinea-pig.

| Substance | Dose $\mu mol\ kg^{-1}$ i.v. | Time of admin. (min) | n | Bronchoconstriction (% of the response to total occlusion of the tracheal cannula, mean values ± s.e.m.) 1 min | 3 min | 5 min | area 0–5 min |
|---|---|---|---|---|---|---|---|
| Vehicle | — | — | 15 | 100 | 96±3 | 93±5 | 89±3 |
| LG 30435 | 3 | − 1 | 5 | 59±16(c) | 61±11(c) | 44±9(c) | 48±8(c) |
| | | − 5 | 5 | 32±15(c) | 39±12(c) | 38±10(c) | 32±11(c) |
| | | −10 | 5 | 56±15(c) | 54±14(c) | 39±12(c) | 42±11(c) |
| Mepyramine | 10 | − 5 | 13 | 62±10(c) | 62±7(c) | 50±7(c) | 53±7(c) |
| Ketotifen | 3 | − 5 | 5 | 39±13(c) | 46±17(c) | 39±13(c) | 35±12(c) |
| Disodium cromoglycate | 20 | − 1 | 5 | 100 | 100 | 98±2 | 92±1 |
| BW 755 c | 50 | −10 | 6 | 100 | 88±6 | 83±8 | 82±6 |
| FPL 55712 | 20 | − 1 | 7 | 78±13(a) | 63±15(b) | 56±14(b) | 60±13(b) |
| CV 3988 | 5 | − 5 | 5 | 100 | 100 | 100 | 95±1 |
| Mepyramine+ FPL 55712 | 10<br>20 | − 5<br>− 1 | 6 | 4±0·4(d) | 13±2(d) | 13±2(d) | 10±2(d) |
| Mepyramine+ FPL 55712 | 10<br>5 | − 5<br>− 1 | 6 | 33±13 | 40±10 | 30±7 | 34±8 |
| Mepyramine+ CV 3988 | 10<br>5 | − 5<br>− 5 | 7 | 51±16 | 63±13 | 49±11 | 52±13 |

a) $P<0·05$; b) $P<0·01$; c) $P<0·001$ versus vehicle; d) $P<0·01$ versus mepyramine

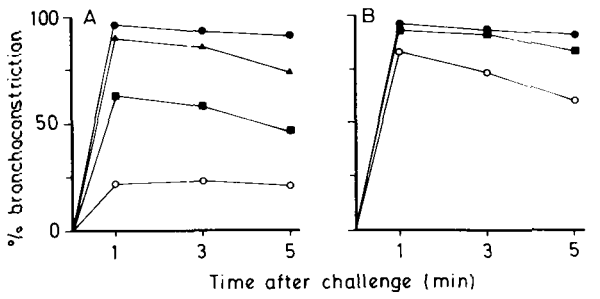


FIG. 1 Effects of (A) LG 30435 and (B) mequitazine, administered 5 min before antigen challenge, on passive lung anaphylaxis in the guinea-pig. Doses: ● 0, ▲ 0·3, ■ 1 and ○ 3 μmol kg$^{-1}$ i.v. Each point represents the mean of at least 6 animals.

experiment, a dose response study was performed, in comparison with mequitazine, with a 5 min pretreatment time (Fig. 1). The effects of LG 30435 were dose-related and its ED50, calculated at the time of maximal inhibition, was 1·6 (0·7–3·6) μmol kg$^{-1}$. Mequitazine, on the contrary, was nearly ineffective up to 3 μmol kg$^{-1}$. As shown in Table 1, a mast cell stabilizer, DSCG, a dual inhibitor of cyclo-oxygenase and lypoxygenase, BW 755c, and a PAF-antagonist, CV 3988, were ineffective in this model at relatively high doses. Also atropine sulphate and ipratropium bromide were devoid of any effect at 3 μmol kg$^{-1}$ (data not shown). An $H_1$-histamine antagonist, mepyramine, and a peptido-leukotriene antagonist, FPL 55712, showed a modest protective action (30–40%). When given in combination with a fixed dose of mepyramine, FPL 55712 potentiated dose-dependently the inhibitory effects of the antihistamine, up to an almost complete suppression of the anaphylactic response, while CV 3988 did not augment the action of mepyramine.

*Bombesin-induced bronchoconstriction in the guinea-pig*

Bombesin (30 μg kg$^{-1}$ i.m.) produced a long-lasting bronchoconstriction of 15–30 cm water. LG 30435, when injected in successive doses of 0·1, 0·3, 1 and 3 μmol kg$^{-1}$ i.v. did not affect this bronchoconstriction, while salbutamol and aminophylline produced a dose-dependent inhibition, threshold doses being 0·1 μg kg$^{-1}$ and 1 mg kg$^{-1}$, respectively.

*PCA in the rat*

Titre of the antiserum (reciprocal of final serum dilution yielding PCA reaction of 5 mm in diameter) was ~100. When the antiserum was warmed up at 56°C for 1 h, no cutaneous reaction was detected, thus providing evidence that our PCA model was essentially IgE dependent (Fügner 1985). As shown in Table 2, LG 30435 inhibited dose-dependently both PCA and the histamine-induced anaphylactoid effect with a similar potency, while showing a scant

Table 2. Effects on passive cutaneous anaphylaxis in the rat.

| Substance | Time of administration (min) | ED50 and 95% confidence limits (μmol kg$^{-1}$ i.v.; n = 20–24) antigen | histamine | 5-HT |
|---|---|---|---|---|
| LG 30435 | 0 | 2·9(1·1–8·3) | 1·5(0·93–3·7) | >3(a) |
| | −5 | >3(b) | 2·8(1·8–6·1) | >3(a) |
| Mequitazine | 0 | 6·4(3·7–21) | >10(c) | >10 |
| | −5 | 9·2(4·5–29) | 9·3(2·9–25) | >30 |
| Mepyramine | 0 | 5·9(3·7–16) | >10(b) | >10 |
| | −5 | ~10 | 6·7(1·3–13) | >10 |
| Ketotifen | 0 | 1·3(0·91–1·9) | 5·6(3·2–16) | ~10 |
| | −5 | 0·28(0·19–0·40) | 0·51(0·34–0·88) | 5·2(3·5–11) |
| Disodium cromoglycate | 0 | 2·7(1·9–3·7) | >30 | >30 |
| | −5 | 17(8·0–29) | >30 | >30 |
| FPL 55712 | 0 | 17(11–47) | >20 | >20 |
| CV 3988 | 0 | >5 | >5 | >5 |

At the corresponding dose an inhibition of (a): 20–30%; (b): 30–40%; (c): 40–50% was observed

Table 3. Effects of repeated administrations of LG 30435 (3 μmol kg$^{-1}$ i.v.) and disodium cromoglycate (30 μmol kg$^{-1}$ i.v.) on passive cutaneous anaphylaxis in the rat.

| Treatment Time of administration (min) | | | Spot diameter (mm, mean values ± s.e., n = 6) | | |
|---|---|---|---|---|---|
| −20 | −10 | 0 | Antigen | Histamine | 5-HT |
| Vehicle | Vehicle | Vehicle | 14 ± 1·2 | 14 ± 1·3 | 14 ± 0·5 |
| Vehicle | Vehicle | LG30435 | 2·0 ± 0·6(c) | 6·3 ± 0·6(b) | 12 ± 0·5(a) |
| LG 30435 | LG 30435 | LG 30435 | 2·7 ± 0·5(c) | 3·2 ± 0·5(c,d) | 12 ± 0·4(a) |
| Vehicle | Vehicle | Vehicle | 12 ± 1·2 | 14 ± 0·7 | 15 ± 0·3 |
| Vehicle | Vehicle | DSCG | 0·5 ± 0·2(c) | 13 ± 0·6 | 15 ± 0·8 |
| DSCG | DSCG | DSCG | 6·3 ± 1·3(a,e) | 14 ± 0·6 | 14 ± 0·6 |

a) $P < 0·05$, b) $P < 0·01$, c) $P < 0·001$ vs vehicle; d) $P < 0·05$, e) $P < 0·01$ vs single treatment.

Table 4. Effects on passive lung anaphylaxis in the rat

| Substance | Dose $\mu$mol kg$^{-1}$ i.v. | Time of administration (min) | n | Bronchoconstriction (% of response to total occlusion of the tracheal cannula, mean values ± s.e.m.) |
|---|---|---|---|---|
| Vehicle | — | — | 33 | 53 ± 5 |
| LG 30435 | 3 | −2 | 7 | 4 ± 2(b) |
| | 1 | −2 | 7 | 22 ± 4(a) |
| | 0·3 | −2 | 7 | 49 ± 11 |
| Mequitazine | 3 | −2 | 4 | 63 ± 4 |
| Mepyramine | 10 | −5 | 6 | 53 ± 10 |
| Ipratropium bromide | 3 | −5 | 4 | 53 ± 13 |
| Methysergide | 1 | −5 | 6 | 4 ± 2(b) |
| Disodium cromoglycate | 3 | −1 | 6 | 1 ± 1(b) |
| FPL 55712 | 10 | −1 | 6 | 6 ± 3(b) |
| Brotizolam | 10 | −5 | 5 | 54 ± 22 |

a) $P < 0.01$, b) $P < 0.001$ versus vehicle

effect on the 5-HT-induced spot formation. The potency of LG 30435 against PCA and histamine appeared generally greater than those of mequitazine and mepyramine, but inferior to that of ketotifen. The potency of LG 30435 against PCA is very close to that of DSCG. FPL 55712 exerted a dose-dependent protection from PCA, while CV 3988 showed no effect. Methysergide 3 $\mu$mol kg$^{-1}$ did not affect the antigen- and histamine-induced spots, while it produced a 80% inhibition of the spot induced by 5-HT (data not shown).

As appears in Table 3, LG 30435 did not show self inhibition, when three equal doses were administered 10 min apart. Predosing with DSCG, on the contrary, resulted in loss of inhibition of the final dose, administered with challenge.

*PLA in the rat*

Anaphylactic bronchoconstriction in rats was markedly less intense than in guinea-pigs (Table 4). It peaked between 1·5 and 3 min after the administration of antigen and it was generally short-lasting (3–6 min). So the data reported refer exclusively to the peak response. Also in this case LG 30435 produced a dose-dependent inhibition of bronchonconstriction, with an ED50 of 0·91 (0·56–1·42) $\mu$mol kg$^{-1}$, while antihistamines, antiacetylcholine drugs and PAF-antagonists (brotizolam, a triazolobenzodiazepine, is a potent PAF antagonist, Braquet & Godfroid 1986) were ineffective. By contrast, methysergide, DSCG and FPL 55712 caused almost complete inhibition.

## Discussion

Our results demonstrate that LG 30435 possesses antiallergic properties in three animal models. LG 30435 was a potent inhibitor of bronchoconstriction in guinea-pig PLA. In this model the anaphylactic response is known to be mediated predominantly by the heat-stable $IgG_1$ antibodies, especially when high amounts of antigen and Freund's complete adjuvant are employed to sensitize the animals (Fügner 1985), as in our case. Lewis et al (1983) have shown that bronchoconstriction of a similar PLA model is partly antihistamine-resistant: this latter component increases with increasing doses of antigen challenge, between 0·1 and 100 mg kg$^{-1}$ of egg albumin. Our data seem in good agreement: in fact with a challenging dose of 5 mg kg$^{-1}$ about 60% of the anaphylactic response is resistant to a relatively high dose of mepyramine. LG 30435 seems capable of inhibiting a higher proportion of this response, as shown in Fig. 1. Therefore, histamine antagonism does not seem to be the sole mechanism of its antiallergic action. We have also shown that FPL 55712 has a modest inhibitory effect on guinea-pig PLA and that, in the presence of mepyramine, this compound is capable of suppressing the anaphylactic bronchoconstriction. However, the dose of FPL 55712 needed to produce such an effect is close to that shown to produce inhibition of the bronchoconstriction induced by various agonists (Lewis et al 1983). Therefore, it is difficult to establish whether peptido-leukotrienes are, together with histamine, major determinants of bronchonconstriction in our PLA model. Another autacoid that could be invoked as a mediator of allergic bronchoconstriction is PAF. This phospholipid was first shown to be released from basophils following anaphylaxis in rabbits immunized to produce only IgE antibodies (McManus et al 1979). However, more recently, the release of PAF and its inactive precursor/metabolite lyso-PAF from guinea-pig isolated lungs was demonstrated following antigen challenge in an IgG-dependent model (Fitzgerald et al 1986). Moreover, in a similar IgG-dependent guinea-pig model, the histamine- and leukotriene-independent component of anaphylaxis in-vitro and in-vivo was reported to be mediated by PAF (Darius et al 1986). Therefore we tried to find out whether this was the case with our PLA model. The PAF antagonist CV 3988 at the dose of 1 $\mu$mol kg$^{-1}$ i.v. inhibited by 70% the bronchoconstriction induced by i.v. PAF in the anaesthetized guinea-pig (unpublished results). At a dose five times higher this compound was unable to affect PLA and, when given in combination with mepyramine, did not increase its protective action. Therefore it should be concluded that PAF is not a major mediator of bronchoconstriction in our PLA model and that the inhibitory action of LG 30435 is due to histamine antagonism and possibly to inhibition of the effects of peptido-leukotrienes and/or to yet unidentified mechanisms, such as an interference with the activation of phospholipase A2 by a calmodulin-dependent mechanism, since our quarternary phenothiazine appears to bind to calmodulin and to inhibit

phosphodiesterase in the presence of calmodulin (Renzetti & Criscuoli 1986). A direct smooth muscle relaxant effect of LG 30435 on the airways should be excluded, since this compound was incapable of counteracting the already established bronchoconstriction induced by bombesin, which was readily reversed by salbutamol and aminophylline.

We have also shown that LG 30435 inhibits the IgE-dependent PCA in rats. Histamine antagonism might be sufficient to explain such an effect, since antihistamines are capable of affecting reagin-mediated rat PCA, characterized by release of histamine and other vasoactive mediators from mast cells (Fügner 1985). Moreover LG 30435 is capable of inhibiting compound 48/80-induced histamine release from rat peritoneal mast cells with an IC50 of $1{\cdot}1 \times 10^{-4}$ M, and is 2·6 times more potent than DSCG (Renzetti & Criscuoli 1986). This property, which is not uncommon among antihistamines, but is not related to the ability to antagonize histamine at $H_1$-receptors (Church 1985), may contribute to the antiallergic action of LG 30435 in the rat PCA. While CV 3988 was completely inactive in this model, ruling out the involvement of PAF, FPL 55712 was found to be active, as reported also by other authors (Sheard et al 1984). However, this may be due again to the unspecific effects of this compound, which seems to retain in part the mast cell stabilizing effects of its parent drug, DSCG (Sheard et al 1984). Therefore, the effects of LG 30435 should be ascribed to its antihistamine and, possibly, mast cell stabilizing action.

Finally LG 30435 was a potent inhibitor of PLA in rats, another IgE-mediated response. This property is independent of its antihistamine, antiacetylcholine and anti-PAF actions, but it is probably related to its anti-5-HT and mast cell stabilizing effects. The contribution of peptido-leukotrienes in this model cannot be clearly established, due again to the lack of specificity of FPL 55712, as discussed above. The relevance of our animal models to human allergic reactions is questionable. In fact, guinea-pig PLA can be criticized, because it is an IgG-dependent phenomenon, contrary to human allergy. In fact DSCG is inactive in guinea-pig models, while it is effective in man, as well as in experimental anaphylactic responses mediated by IgE antibodies (Fügner 1985). Rat PCA and PLA are IgE- mediated models and various clinically relevant drugs can be demonstrated thereby. However, it has been recognized that the rat PCA test may not be an indicator of potential activity in human asthma. A number of analogues of DSCG have been identified, which were up to 200 times more potent than the reference compound in the rat PCA, but none of them was found as effective as DSCG clinically (Church & Gradidge 1980). Finally, rat mast cells are rich in 5-HT, a mediator which plays a great part in rat PLA, while it is not contained in human mast cells or basophils and is not therefore a primary mediator of human allergic disease (Church 1985; Schellenberg 1985).

Thus the possibility of extrapolating our data to human asthma may be disputed, but a single animal model representing the features of the human allergic reactions remains to be described. Therefore, the potential therapeutic value of LG 30435 in human asthma can only be evaluated in clinical trials.

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