Mast cell stabilizing compound FPL 55618 reduces right ventricular hypertrophy and lung mast cell hyperplasia in chronically hypoxic rats[1]

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Summary. Rats treated with chronic hypobaric hypoxia (21 days, 380 Torr) and mast cell stabilizing compund FPL 55618 had significantly less right ventricular hypertrophy and lung mast cell hyperplasia than rats subjected to chronic hypoxia alone. Right ventricular blood pressure was not reduced.

Lung mast cells (MC) have been implicated in the pulmonary vasoconstrictor response to alveolar hypoxia primarily because they contain vasoactive substances such as histamine and are situated in an ideal sensing location between alveolar spaces and small pulmonary blood vessels[3]. Furthermore, they may degranulate after acute hypoxia[3]and proliferate during chronic hypoxia[4]. We have given the Fisons compound FPL 55618 (8-allyl-5-[3-methylbutoxy]-4-oxo-4H-1-benzopyran-2-carboxylic acid, sodium salt)[5] a potent MC stabilizing compound to rats to see if it would reduce the pulmonary hypertension and right ventricular hypertrophy (RVH) produced by chronic hypobaric hypoxia (CH)[6]. FPL 55618 is a monochromone which is structurally related to the bischromone, disodium cromoglycate[7].

Methods. 2 groups of female Wistar rats (b.wt 149–178 g) were placed in a hypobaric chamber for 21 days. Initially, the pressure was 460 Torr but after 3 days this was reduced to 380 Torr simulating an altitude of 5500 m above sea level. The chamber was opened for 20 min twice weekly for cleaning and replenishment of food and water. 1 group (n = 12) received a normal diet and drinking water. The other group (n = 10) received a normal diet but its drinking water contained FPL 55618 which was commenced the day before placement in the hypobaric chamber. For the first 11 days of the experiment the concentration of FPL 55618 was 0.05% which gave an average daily dose of 63.2 mg/kg b.wt. Thereafter the concentration was reduced to 0.01% (14.1 mg/kg b.wt). After 21 days CH, the rats were removed from the chamber and pulmonary hypertension was evaluated by cannulating the right external jugular vein under ether anesthesia and measuring right ventricular blood pressure. The animals were then killed. The heart and lungs were fixed in formalin after endotracheal instillation of 13 ml of formalin. RVH was assessed by expressing the weight of the free wall of the right ventricle as a percentage of the weight of the left ventricle and interventricular septum and also as a percentage of the final body weight (FBW). Perivascular, peribronchial and alveolar septal MC were counted in histological sections of lung stained with toluidine blue[8]. The MC counts were corrected[9] for section thickness[10] (5.8 μm) and mean diameter of MC (5.8 μm). The area of the sections was measured by projection and weighing of images cut out on paper. Results were expressed as MC per cm^2 lung tissue.

Results. The rats treated with CH and FPL 55618 had significantly less RVH and lower total lung MC than rats subjected to CH alone (table). There was a reduction in the number of perivascular, peribronchial and alveolar septal MC which did not attain statistical significance. There was no difference in right ventricular blood pressure and FBW.

Discussion. It is not clear why the right ventricular blood pressure in the rats given FPL 55618 was not reduced because the lesser degree of RVH in these animals would suggest a lower pulmonary vascular resistance. Perhaps the right ventricular pressure measured outside the hypobaric chamber did not accurately reflect the pulmonary vascular resistance under hypoxic conditions within the chamber. Alternatively, FPL 55618 may have acted directly on the right ventricular myocardium and inhibited the development of hypertrophy. This possibility is considered unlikely because the related compound disodium cromoglycate does not specifically affect the heart and systemic circulation in most animal species including the rat[7]. FPL 55618 is related to disodium cromoglycate which inhibits MC degranulation and release of vasoactive substances by stabilizing the cell membrane[11]. In a previous study disodium cromoglycate 10 mg/kg b.wt i.p. failed to reduce RVH and lung MC count in rats exposed to CH although it caused retardation of growth in both test and control animals[12]. However we used a larger dose of FPL 55618 and this compound is 87 times more effective than disodium cromoglycate in inhibiting passive cutaneous anaphylaxis (PCA) in rats[13]. The ID_{50} of FPL 55618 in rat PCA is 0.024 mg/kg b.wt i.v. given immediately before antigen[5]. One study showed that disodium cromoglycate inhibited acute hypoxic pulmonary vasoconstriction in 3 of 8 intact anesthetized dogs[14]. This was not confirmed in a more recent study in which dog and cat lungs were perfused in vivo at constant pressure or constant blood flow[15]. A recent paper reported that FPL 55618 failed to inhibit acute hypoxic pulmonary vasoconstriction in perfused rat and cat lung preparations[13]. It is probable that in CH the mechanisms responsible for maintaining pulmonary hypertension may not be the same as those which operate during acute hypoxia. Furthermore, the action of disodium cromoglycate on the lung may be more complicated than previously thought. Specifically, i.v. administration of disodium cromoglycate to cats is followed by a marked reduction in circulating blood histamine, a

Final body weight (FBW), right ventricular weight (RV), right ventricular blood pressure and lung mast cells (MC) in rats exposed to chronic hypoxia and chronic hypoxia with compound FPL 55618

	Chronic hypoxia and FPL 55618	Chronic hypoxia	p
FBW (g)	194 ± 13 n = 10	193 ± 24 n = 12	n.s.
RV weight (g)	0.2956 ± 0.0348 n = 10	0.3603 ± 0.0544 n = 12	< 0.005
RV/FBW (%)	0.1518 ± 0.0181 n = 10	0.1871 ± 0.0201 n = 12	< 0.001
RV/(LV + S) (%)	54.9 ± 6.5 n = 10	60.5 ± 5.2 n = 12	< 0.025
$\bar{P}rv$ (Torr)	35.7 ± 8.8 n = 9	35.1 ± 3.9 n = 12	n.s.
$\bar{P}rvs$ (Torr)	44.6 ± 9.0 n = 9	44.9 ± 6.5 n = 12	n.s.
Total lung MC/cm^2	66.5 ± 21.8 n = 10	85.8 ± 20.3 n = 12	< 0.05
Perivascular lung MC/cm^2	42.7 ± 17.1 n = 10	56.0 ± 18.0 n = 12	n.s.
Peribronchial lung MC/cm^2	20.1 ± 8.0 n = 10	25.7 ± 7.5 n = 12	n.s.
Alveolar septal MC/cm^2	3.7 ± 2.7 n = 10	4.1 ± 2.3 n = 12	n.s.

$\bar{P}rv$ = right ventricular mean blood pressure, $\bar{P}rvs$ = right ventricular mean systolic blood pressure, p = evaluation of unpaired t-test, results are given as mean values ± SD.

marked reduction in secretion of histamine by the lung and a reduction in pulmonary vascular resistance[16]. Disodium cromoglycate also appears selectively to make available the H-1 constrictor receptor so that following disodium cromoglycate administration, infused histamine produces a greater vasoconstrictor response than usual[17]. It has been proposed that lung MC may be the cellular mediators[3,4,14] of hypoxic pulmonary hypertension or that may proliferate in response to increased blood pressure in the lungs[18,19]. Our results suggest that lung MC may play a role in the development of chronic hypoxic pulmonary hypertension.

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Tuftsin and D-Arg^3-tuftsin possess analgesic action

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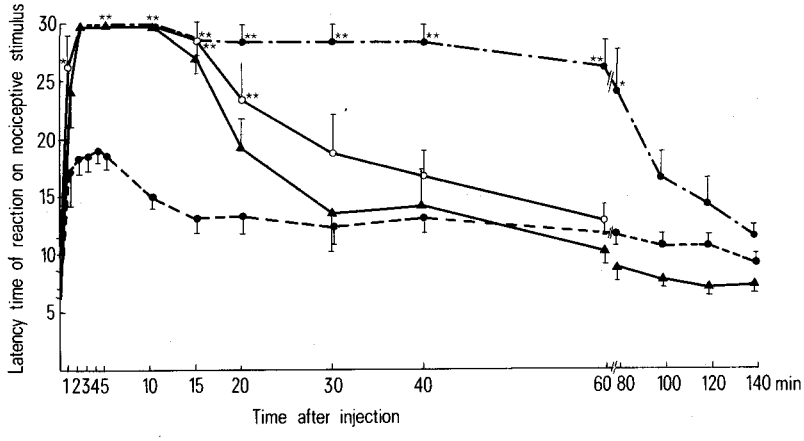
Summary. Tuftsin has marked analgesic activity but is less potent than D-Arg^3-tuftsin. The absence of an antagonistic effect of naloxone on tuftsin and its analogue suggests that these substances do not act on opiate receptors. Tuftsin and its analogues may represent a new class of substances of interest in the search for new analgesics.

Tuftsin is a tetrapeptide, L-threonyl-L-lysyl-L-prolyl-L-arginine, discovered by Najjar and Nishioka[1]. It is produced in spleen and is present in β-globulin[2]. Tuftsin stimulates the phagocytic activity of blood polymorphonuclear leukocytes and particularly the neutrophils[2]. A deficiency of tuftsin causes the syndrome of defective phagocytosis with frequent and prolonged infections[3]. It is the purpose of this communication to report on the analgesic properties of tuftsin and its synthetic analogue which were observed unexpectedly during the screening of several penta- and tetrapeptides for their possible analgesic effects. The synthesis of tuftsin was performed at the Institute of Chemistry of Wrocław University (WU), and is described by Konopińska et al.[4]; that of D-Arg^3-tuftsin is described by Nawrocka et al.[5].

Experiments were performed using male Wistar rats (200–220 g) from the central animal farm of the Silesian School of Medicine. The analgesic activity of the test substances was measured by the reproducible and specific hot-plate procedure described by O'Callaghan and Holtzman[6]. A licking of the fore or hind paws was used as the end-point for the determination of response latencies recorded to the nearest 0.1 sec. If a latency time was 30 sec the rat was removed from the hot plate to avoid heat burn and in this case the latency time was taken as 30 sec. Tuftsin (TU) and D-Arg^3-tuftsin (Arg-TU) were dissolved in 0.9% sodium



Analgesic action of tuftsin and D-Arg^3 tuftsin. WU synthesized in Wrocław University. Results are expressed as mean of latency period of response to pain stimulus in sec ± SD. n = 14. ●———●, Control 0.9% NaCl; ●—·—·—●, D-Arg^3 tuftsin (WU) 200 µg; ○———○, Tuftsin (WU) 200 µg; ▲———▲, Tuftsin (Serva) 200 µg. * $p < 0.005$; ** $p < 0.001$.