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.

1 Compound FPL 55618 was kindly donated by Mr P. Sheard of Fisons Ltd Pharmaceutical Division, Loughborough, Leicestershire, LE11 OQY England.
2 This study was supported by grants from the Medical Research Council of Canada and St. Joseph's Hospital Foundation.

3 F. Haas and E.H. Bergofsky, J. clin. Invest. *51*, 3154 (1972).
4 J.M. Kay, J.C. Waymire and R.F. Grover, Am. J. Physiol. *226*, 178 (1974).
5 P. Sheard, personal communication.
6 J.M. Kay, Am. Rev. resp. Dis. *121*, 993 (1980).
7 J.S.G. Cox, J.E. Beach, A.M.J.N. Blair, A.J. Clarke, J. King, T.B. Lee, D.E.E. Loveday, G.F. Moss, T.S.C. Orr, J.T. Ritchie and P. Sheard, Adv. Drug Res. *5*, 115 (1970).
8 E. Schueller, M. Peutsch, L.G. Bohacek and R.K. Gupta, Can. J. med. Technol. *29*, 137 (1967).
9 W. Aherne, J. med. Lab. Technol. *27*, 160 (1970).
10 N.P. Marengo, Stain Technol. *19*, 1 (1944).
11 J.S.G. Cox, Br. J. Dis. Chest *65*, 189 (1971).
12 I.P.F. Mungall, Thorax *31*, 94 (1976).
13 G.R. Barer, C.J. Emery, F.H. Mohammed and I.P.F. Mungall, Q. Jl exp. Physiol. *63*, 157 (1978).
14 J.M. Kay and R.F. Grover, Prog. Resp. Res. *9*, 157 (1975).
15 P. Howard, G.R. Barer, B. Thompson, P.M. Warren, C.J. Abbot and I.P.F. Mungall, Resp. Physiol. *24*, 325 (1975).
16 R.J. Porcelli and E.H. Bergofsky, Physiologist *20*, 75 (1977).
17 E.H. Bergofsky, A. Rev. Physiol. *42*, 221 (1980).
18 A. Williams, D. Heath, J.M. Kay and P. Smith, Thorax *32*, 287 (1977).
19 A. Tucker, I.F. McMurtry, A.F. Alexander, J.T. Reeves and R.F. Grover, J. appl. Physiol. respir. environm. Exercise Physiol. *42*, 174 (1977).

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

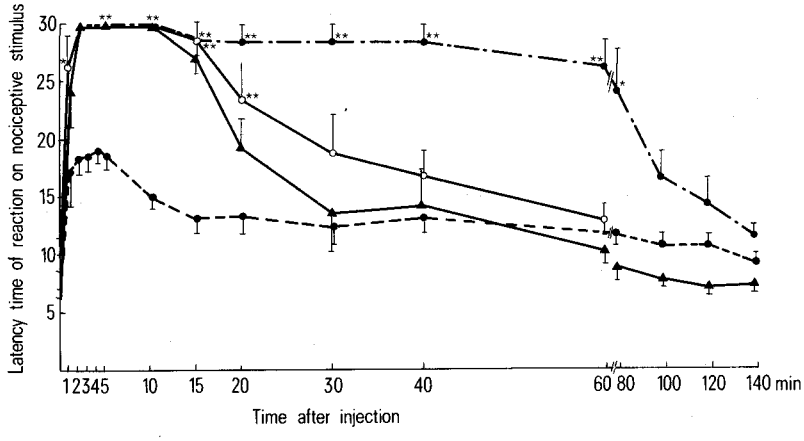
Z.S. Herman, Z. Stachura, Ł. Opiełka, I.Z. Siemion and E. Nawrocka

Department of Pharmacology, Silesian School of Medicine, Marksa 38, 41-808 Zabrze (Poland), and Institute of Chemistry of Wrocław University, Joliot-Curie 14, 50-383 Wrocław (Poland), 26 March 1980

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. * $p < 0.005$; ** $p < 0.001$.

chloride solution and injected in a dose of 200 µg in 10 µl into the lateral brain ventricle according to the method described elsewhere[7]. The correctness of the site of injection was checked visually after the killing of each animal. Corresponding control animals received the same volume of the solvent. Additional control animals were injected with tuftsin produced by Serva, in a dose of 200 µg. All solutions injected into the lateral brain ventricle had a pH of 7.4. In a group of 3 rats, naloxone (Endo Lab.) 1 or 2.5 mg/kg i.p. was injected 30 min before TU or Arg-TU. Analgesic activity was measured 140 min after injection (figure). As an additional control, morphine hydrochloride was injected intracerebro-ventricularly (i.c.v.) in a dose of 10 µg. In 5 animals, naloxone at a dose of 1 or 2.5 mg/kg was injected 30 min prior to morphine. 14 rats were used in each group and the results evaluated statistically using Student's t-test.

Both preparations of TU (WU and Serva) elicited marked analgesia during the 30 min immediately after injection. Arg-TU had a more potent analgesic effect, and it persisted for 140 min (figure). Naloxone did not prevent TU or Arg-TU elicited analgesia.

Morphine produced a maximal increase in latency time to 30 sec which persisted for 2 h. This effect was antagonized completely by both doses of naloxone. Arg-TU evoked abnormal behavior in that 5 min after injection of Arg-TU, rats were in recumbent position with stretched limbs. 10 min after injection, barrel rotations occurred (repeated rotation around the animal's longitudinal axis); this lasted for 30 min. 40 min after injection, clonic convulsions were observed which disappeared after 20 min. This abnormal behavior was not antagonized by naloxone. 1 h after injection, the behavior of rats injected with Arg-TU was not different from the behavior of rats injected with saline solution although analgesic activity was still present. The Serva tuftsin appeared to be less, but not significantly less, potent than the WU tuftsin. Serva tuftsin was used as a control for WU tuftsin synthesized by us. The results presented indicate that TU and Arg-TU have an evident effect on the central nervous system. The barrel rotations that appeared after Arg-TU were similar to the phenomenon described after injection of arginine-vasopressin in rats[8]. The absence of an antagonistic effect of naloxone on TU or Arg-TU analgesia suggests that these substances do not act on opiate receptors. Therefore, 2 suggestions can be made: a) Tuftsin and its analogues may represent a new class of substance of interest in the search for new analgesics. b) It may be speculated that there is a link between immunobiological responses and the central nervous system, since there is the possibility that TU continuously synthesised for stimulation of phagocytes could affect the central nervous system.

1 V.A. Najjar and K. Nishioka, Nature *228*, 672 (1970).
2 K. Nishioka, A. Constantopoulos, P.S. Satok and V.A. Najjar, Biochem. biophys. Res. Commun. *47*, 172 (1972).
3 V.A. Najjar and C. Constantopoulos, J. reticuloendoth. Soc. *12*, 197 (1972).
4 D. Konopińska, E. Nawrocka, I.Z. Siemion, S. Ślopek, S. Szymaniec and E. Kłonowska, Int. J. Pept. Protein Res. *9*, 71 (1977).
5 E. Nawrocka, I.Z. Siemion, S. Ślopek and S. Szymaniec, Int. J. Pept. Protein Res., in press (1981).
6 J.P. O'Callaghan and S.G. Holtzman, J. Pharmac. exp. Ther. *192*, 497 (1975).
7 Z.S. Herman, Psychopharmacologia *16*, 369 (1970).
8 R.L. Delanoy, A.J. Dunn and R. Walter, Life Sci. *24*, 651 (1979).

Granuloma formation in rat liver after hepatic vein ligation

K. Weinbren, S. Mason and S. Sen

Royal Postgraduate Medical School, DuCane Road, London W12 OHS (England), 21 April 1980

Summary. After occlusion of the hepatic vein draining 1 lobe of the rat liver, macrophage granulomas develop which are reproducible and apparently related to a heat-labile macrophage mobilising factor.

The development of a granulomatous reaction has been described in several different circumstances, some of which involve localization of known antigens of low diffusibility[1], some represent reactions to metallic[2] and other[3] compounds and some relate to familiar pathogens[4]. Hypersensitivity is associated with granuloma formation in many instances but the exact mechanism has not been satisfactorily determined[5]. Often an irritant is responsible for the formation of the granulomatous reaction and the development and resorption of the lesion are to some extent dependent on the potency and ineradicability of the irritant[6].

We wish to report the regular development of macrophage granulomas in rat liver lobes subjected to occlusion of the hepatic veins and with no exposure to known toxins, bacterial pathogens or deliberately introduced antigens.

Methods. Male Wistar rats were maintained on 'Research' rat cubes (Parkes 41B diet) and subjected to surgical procedures at 150-300 g b.wt. All operations were performed under ether anaesthesia and the animals were not fasted preoperatively or before death.

1. The hepatic vein draining the left lobe of the liver was ligated in 95 rats and the animals killed and the livers examined at 24 h intervals up to 15 days and then at longer intervals to 12 weeks.

2. To effect necrosis of part of a liver lobe, the hepatic artery and portal vein to the medial half of the median lobe (which normally has 2 branches of the portal vein and 2 branches of the hepatic artery supplying it) were ligated in 8 rats which were killed between 8 and 10 days.

3. A fragment of fresh omental lobe (5×2 mm) was implanted into the left lobe through a 5 mm capsular incision in 10 rats. These were killed at 8 days.

4. In order to implant necrosed autologous tissue, the omental lobe was infarcted by afferent vascular ligation 21 days before implantation in 6 rats, 14 days before implantation in 6 rats and 7 days before implantation in 6 rats. All animals bearing implants of tissue which had undergone prior in vivo necrosis were killed at 8 days after implantation.

5. Implantation was carried out of omental lobe tissue which had been subjected to immersion in water at 100 °C for 2 min in 6 rats and in water at 60 °C for 2 min in 6 rats. All animals were killed at 8 days. All tissues were fixed in