Tachyphylaxis due to the D-Phe^7 analogue of substance P

J. W. GROWCOTT, N. N. PETTER*, *Biology and Chemistry Departments, I.C.I. Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, U.K.*

The identification of an antagonist of substance P (SP), H-Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}- NH_2, would be of considerable importance in elucidating the role of the peptide as a transmitter substance. It has been reported that the D-Phe^7 analogue of SP (D-Phe^7 SP) shows weak antagonism of the effect of SP on the guinea-pig isolated ileum (Yamaguchi et al 1979). Claims for antagonist activity have been made previously (Stern et al 1961; Saito et al 1975) but have not been substantiated, thus we have attempted to confirm this recent observation.

D-Phe^7 SP was synthesized in our laboratories, and synthetic SP purchased from Beckmann, Inc., Switzerland. The biological system used closely resembled that described by Yamaguchi et al (1979), who have yet to publish exact protocols. Thus female guinea-pigs (Duncan-Hartley, 300–350 g) were killed by cervical dislocation and the terminal ileum removed. Segments from the caecal end were suspended in a 10 ml organ bath containing Tyrode solution and gassed with 95% oxygen, 5% carbon dioxide. D-Ph^7 SP was incubated with the tissue for 10 min before the start of the log-dose response curves.

D-Phe^7 SP was found to be approximately one-twentieth as active as SP as a spasmogen, the EC50's being $5{\cdot}8$ ($\pm 3{\cdot}7$) $\times 10^{-8}$ M ($n = 3$) and $2{\cdot}4$ ($\pm 2{\cdot}1$) $\times 10^{-9}$ M ($n = 3$), respectively (Fig. 1). This is in contrast to Yamaguchi et al (1979) who reported the synthetic analogue to be only approximately one-fiftieth the potency of SP as an agonist.

In our experiments, designed to investigate the antagonist properties of this analogue further, it was found that only a supramaximal concentration of D-Phe^7 SP (1×10^{-6} M) produced a significant shift to right of the SP dose-response curve. Threshold and EC50 concentrations of D-Phe^7 SP ($1{\cdot}0 \times 10^{-9}$ and $1{\cdot}3 \times 10^{-8}$ M respectively), caused very little change in the sensitivity of the tissue to SP (Fig. 2). At the supramaximal con-

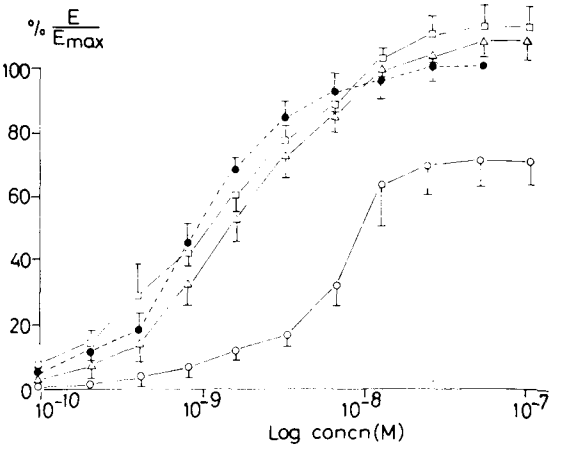

FIG. 2. The effect of D-Phe^7 SP on the log dose-response curve for substance P in the guinea-pig isolated ileum. Substance P control, (●). Substance P in the presence of $1{\cdot}0 \times 10^{-9}$ M (□); $1{\cdot}3 \times 10^{-8}$ M (△); $1{\cdot}0 \times 10^{-6}$ M (○), D-Phe^7 SP. D-Phe^7 SP was incubated with the tissue for 10 min before the start of each dose-response curve. Vertical bars indicate mean and standard deviation ($n = 3$).

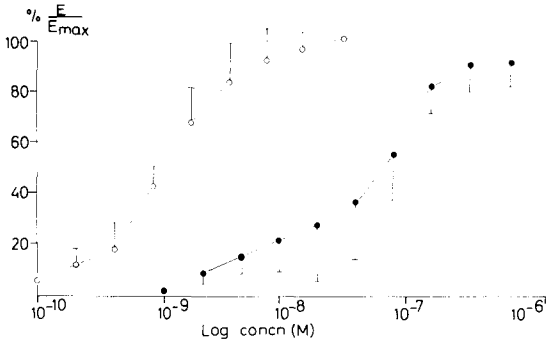

FIG. 1. Log dose-response curves for substance P (○), and D-Phe^{7}SP (●) in the guinea-pig isolated ileum. Vertical bars indicate mean with standard deviation ($n = 3$).

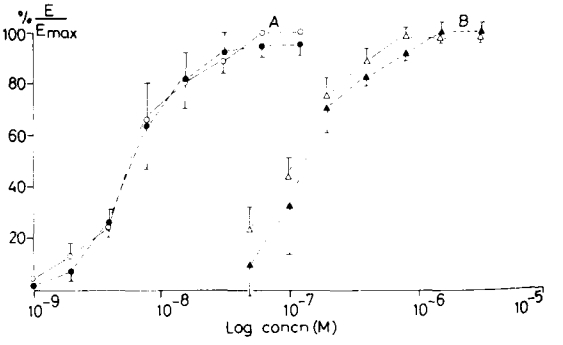

FIG. 3. The effect of D-Phe^7 SP on the log dose-response curves for, A, acetylcholine, B, histamine in the guinea-pig isolated ileum. Acetylcholine control (○). Acetylcholine in the presence of $1{\cdot}0 \times 10^{-6}$M D-Phe^7. SP (●); histamine control (△); histamine in the presence of $1{\cdot}0 \times 10^{-6}$ M D-Phe^7 SP (▲). D-Phe^7 SP was incubated with the tissue for 10 min before the start of each dose-response curve. Vertical bars indicate mean with standard deviation ($n = 2$).

* Correspondence.

centration of D-Phe[7] SP, tissue sensitivity to acetylcholine and histamine was unaltered (Fig. 3). The results suggest that a specific tachyphylaxis or desensitization of the tissue to the effect of SP had been caused by D-Phe[7] SP. The same effect could be demonstrated for a supramaximal concentration of SP against SP, but not with EC50 or threshold concentrations. Moreover, these effects were observed using the 10 min incubation period as outlined by Yamaguchi et al (1979) with which desensitization occurred while it was not possible to demonstrate this phenomenon with the 2 min incubation period routinely used in this laboratory.

A similar claim for SP-antagonist activity has subsequently been made for Ile[8]-SP (Rackur et al 1979). Although this peptide has not been investigated using the present protocol, it is tempting to speculate that a similar desensitization may also be responsible for the apparent antagonist activity of this analogue.

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Aggregation of rat polymorphonuclear leucocytes in vitro

FIONA M. CUNNINGHAM, M. E. SHIPLEY, M. J. H. SMITH*, *Biochemical Pharmacology Research Unit, Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London SE5 8RX, U.K.*

The intravenous administration of either zymosan-activated serum (ZAS) or formylmethionyl-leucyl-phenylalanine (FMLP), a synthetic chemotactic peptide, causes a transient but profound neutropenia in the rat (Cunningham & Smith 1980). Complement derived peptides and FMLP may induce changes in the neutrophil membrane which in turn lead to increased margination and aggregation, the neutrophil aggregates being trapped in the pulmonary capillary bed. The neutropenia therefore results from a combination of margination and aggregation. Similar effects have been described in other species following the intravenous injection of cytotaxins, inulin-treated plasma, or agents known to activate complement in vivo. e.g. cobra venom factor (CVF) (O'Flaherty et al 1977; Craddock et al 1977a). These observations suggest that the neutropenia seen in man during haemodialysis (Craddock et al 1977a) and nylon fibre filtration leucopheresis (Hammerschmidt et al 1978) may be due to margination plus aggregation, these effects being caused by the release of complement-derived peptides after contact of blood with the Cellophane membrane or nylon fibres.

Although the ion dependency of aggregation and adherence vary, aggregation being both calcium and magnesium-dependent whereas adherence is dependent upon magnesium ions (O'Flaherty et al 1978b), it seems likely that the alterations in structure and function of the neutrophil (PMN) membrane occurring during aggregation and adherence both in vivo and in vitro are similar (Craddock et al 1979). During haemodialysis there is evidence that, having undergone these changes, altered PMNs are able to produce endothelial damage in the lungs, reflected by changes in arterial Po_2, diffusing capacity and an increased flow of protein-rich lymph occurring during the period of neutropenia (Craddock et al 1977b). There is, however, no penetration of PMNs across the vascular endothelium except in the presence of an extravascular source of activated complement, generated for example by alveolar macrophages, when neutrophils migrate into the interstitium and play an essential role in the inflammatory reaction (Henson et al 1979). It seems likely that in other circumstances a localized inflammatory process may be associated with intravascular activation of PMNs at the site of inflammation. This in turn may increase their tendency to adhere to the vascular endothelium and to each other and may lead to increased permeability of the vascular endothelium before PMN migration into the tissue. In the tangled web of processes comprising the acute inflammatory reaction it is not yet possible to ascertain the relative importance of such phenomena but a study of PMN aggregation may provide new insights into the response.

We have, therefore, studied the effect of FMLP and ZAS on the aggregation of PMNs in vitro. An in vitro model of aggregation could provide a useful method of investigating structure activity relationships in potential anti-inflammatory drugs with regard to their actions on polymorph function.

Methods and materials. Peritoneal exudates containing 84 ± 4% PMNs were elicited in female Wistar/OLA albino rats (150–200 g) by the injection of 6 ml of 12% sodium caseinate. Cells were harvested after 24 h by the intraperitoneal injection of 20 ml Hanks Balanced Salt Solution (HBSS) containing 5U ml^{-1} heparin (Cunningham et al 1979). The cells were then washed once in HBSS and resuspended at a concentration of $1{\cdot}0–1{\cdot}3 \times 10^7$ cells ml^{-1} in Eagle's Minimum Essential Medium (MEM) buffered to pH 7·4 with 30mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid). Cell suspensions were allowed to equilibrate at 37 °C for 30 min before use. In an experiment com-

* Correspondence.