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# ANTI-INFLAMMATORY PROPERTY OF 401 (MCD-PEPTIDE), A PEPTIDE FROM THE VENOM OF THE BEE *Apis mellifera* (L.)

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1 Peptide 401, a potent mast cell degranulating factor from bee venom, substantially inhibited the oedema provoked by subplantar injection of carrageenin or intra-articular injection of turpentine in the rat. The $ED_{50}$ of 401 was c. 0.1 mg/kg. The anti-inflammatory effect was assessed by measurement of the increased $^{125}$I-albumin content of an injected site in comparison with an uninjected contralateral site.

2 Peptide 401 also suppressed the increased vascular permeability due to intradermal injection of various smooth muscle spasmogens (histamine, bradykinin, 5-hydroxytryptamine (5-HT), and prostaglandins).

3 Other comparable mast cell degranulating agents (48/80 and melittin) showed little evidence of anti-inflammatory activity when tested at comparable dosage on turpentine arthritis and carrageenin oedema.

4 The anti-inflammatory effects were not abolished by pretreatment with mepyramine and methysergide, which abolished the increased vascular permeability produced by local injection of 401.

5 The anti-inflammatory action of 401 was not affected by regional denervation or pretreatment with phenoxybenzamine, and was reduced but not abolished by adrenalectomy.

6 Measurement of skin temperature, fractional extraction of $^{86}$Rb and blood flow in perfused mesentery gave no evidence that the anti-inflammatory action of 401 was due to reduced tissue perfusion.

7 It is concluded that 401 may exert its anti-inflammatory action directly by making the vascular endothelium anergic to phlogistic stimuli.

## Introduction

The venom of the honey bee *Apis mellifera* (L.) is a complex mixture of pharmacologically and biochemically active agents. Investigations into the composition of the venom were first reported by Langer (1897) and more recently it has been shown by Neumann & Habermann (1954) that most of the biological activity was due to the peptides and proteins in the venom.

The components of the venom can be separated by dialysis into two fractions. The non-dialysable fraction contains the enzymes, phospholipase A and hyaluronidase together with other proteins not known to have enzymatic activity. The dialysable fraction contains low molecular weight peptides, histamine, inorganic ions and other unidentified compounds. Early work on the separation of the components of the venom by chromatography and electrophoresis was carried out by Habermann & Reiz (1965b) and the major constituents have now been purified.

Three basic low molecular weight peptides have been described. Melittin, by weight the principal constituent of the venom (c. 30%) is a peptide of 26 amino acid residues, which has a high surface activity and is a potent haemolysin (Habermann & Jentsch, 1966). Apamin (c. 2% of the venom) contains 18 amino acid residues and is a neurotoxin producing motor abnormalities associated with actions upon the central nervous system (Habermann & Reiz, 1965a). The third peptide (c. 1% of the venom), first isolated by Breithaupt & Habermann (1968), contains 22 amino acid residues and was described as a mast cell degranulating (MCD)-peptide. The primary sequence of this peptide was reported by Haux (1969) and is in agreement with that found by Vernon, Hanson &

Brimblecombe (1969), who also determined the position of the two disulphide bridges in the peptide which they called 401 (Figure 1). Billingham, Morley, Hanson, Shipolini & Vernon (1973) observed that the peptide showed marked anti-inflammatory activity in the rat against carrageenin-induced oedema and that it suppressed developing and established adjuvant arthritis.

The present study has confirmed the anti-inflammatory property of this peptide and has attempted to establish its mechanism of action. A preliminary account of this work was presented to the Society (Hanson, Morley & Soria-Herrera, 1972).

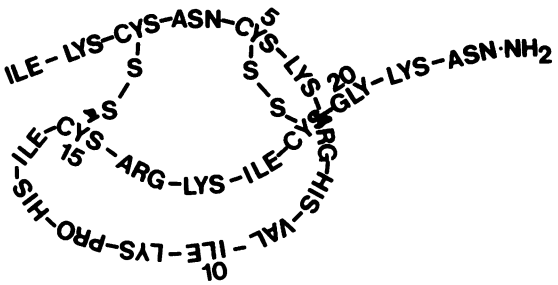


**Fig. 1** Structure of peptide 401.

## Methods

### *Iodination*

Rat serum albumin was labelled with $^{125}I$ by the iodine monochloride method (Macfarlane, 1958). The labelled albumin was separated from unbound iodine by column chromatography (G10 Sephadex). Aliquots were stored at $-20^{\circ}$ C.

### *Inflammatory stimuli*

Joint oedema was provoked by injection of 0.01 ml turpentine oil into the synovial cavity of the right knee joint with a micrometer syringe (AGLA, Burroughs Wellcome). Carrageenin oedema was provoked by sub-plantar injection of 0.1 ml of a solution of carrageenin (1% in 0.9% w/v NaCl solution) into the right hind foot. In all experiments the contralateral uninjected site was used as the control, the responses being expressed as a paired difference. Intradermal injections were made in a volume of 0.1 ml into abdominal flank skin.

### *Vascular permeability measurements*

Wistar strain rats (150-250 g) received an intravenous injection of 0.5 ml of $^{125}I$-labelled rat serum albumin (c. 10 $\mu$Ci/mg, 2.5 mg/ml) mixed with Evans blue dye (2%). Injection was via the sublingual vein under light ether anaesthesia. Immediately following this intravenous injection, the appropriate inflammatory agent was injected. Anti-inflammatory agents were administered by intraperitoneal, subcutaneous or intravenous injection either at the same time as, or at appropriate periods before the application of the inflammatory agent and the isotope injection. After an interval of 1 h for skin tests and 4 h for joint or foot lesions, rats were anaesthetized and 1 ml samples of blood collected by cardiac puncture. In turpentine oedema experiments, the entire knee joint region of each hind limb was removed by a wad punch (diameter 23 mm) and in carrageenin oedema experiments the hackle joint was severed on both hind feet. For measurement of skin lesions, the flank and abdominal skin was removed, spread evenly and discs of skin (diameter 20 mm) encompassing each skin test site were cut out with a wad punch.

Samples were placed in counting vials and counted in an automatic $\gamma$-spectrometer. For each specimen, division of the specimen count by the corresponding blood count (after background subtractions) gave the specimen albumin content in terms of an equivalent blood volume. This makes allowance for differences in the dilution of the isotope caused by differences in the blood volumes of the animals and in individual intravenous injection volumes. For the uninjected skin or joint, the specimen count reflects both intravascular albumin and albumin accumulated as a consequence of normal permeability. For tissues bearing inflammatory lesions the count contains a corresponding component plus a count due to the albumin which has extravasated during the period of increased vascular permeability. The increased extravasation of albumin was therefore calculated by subtraction of the figure for the uninjected site from that of the contralateral injected site.

### *Blood pressure*

In rats and guinea-pigs, blood pressure was recorded under nembutal anaesthesia from the common carotid artery with a Statham strain gauge pressure transducer. Test materials were administered via a cannula in the femoral vein. In the rat, blood pressure was also recorded in the restrained conscious animal. In these animals one carotid was cannulated under phenobarbitone anaesthesia 1-2 h before the subcutaneous injection of 401.

*Mast cell degranulation*

Rat areolar tissue spreads were incubated for 15 min in Tyrode solution containing the various test compounds. Degranulation was assessed by staining the tissue with toluidine blue (1% w/v) and allocating each observed cell a score (0 to 4) according to the extent of degranulation and the cumulative total for 100 cells was expressed as a percentage of maximal degranulation.

*Denervation*

Rats were anaesthetized with ether and a 1 cm portion of the right sciatic nerve was removed above the knee. Animals were used two days after nerve section.

*Adrenalectomy*

Adrenals were removed from the rats under ether anaesthesia. Thereafter, these animals received 0.4% sodium chloride in their drinking water. Animals were used six and nine days after adrenalectomy.

*Fractional extraction of $^{86}$Rubidium*

Animals received an intravenous injection of 0.9% w/v NaCl solution (saline) containing $^{86}$Rubidium via the sublingual vein (under light ether anaesthesia) and were killed by cervical dislocation 60 s after the injection. Samples of various tissues were collected, counted and weighed.

*Isolated perfused mesentery*

The superior mesenteric artery of the rat was cannulated following cervical dislocation and the mesenteric veins were cut. Mesenteric tissue was perfused with a roller pump with an albumin enriched Tyrode solution (Wade & Beilin, 1970), perfusion pressure being recorded by a Statham strain gauge transducer. Close arterial injections of drugs were made via a length of pressure tubing immediately proximal to the cannula.

*Skin temperature recording*

Skin temperature was measured with a disc shaped thermistor (S.T.C.) taped to a depilated area of skin. Temperature was monitored on a flat bed pen recorder (Telsec 700).

*Materials*

401 and melittin were pure peptides isolated from bee venom (Rodopa, Bulgaria) (Hanson, Shipolini & Vernon, unpublished method).

$^{125}$I was carrier-free iodine IMS-4, and $^{86}$Rb was rubidium chloride RGS-1P (Radiochemical Centre, Amersham); rat serum albumin (Sigma); Evans blue (Gurr); turpentine (Boots); carrageenin (Viscarin, Marine Colloids Inc.,); indomethacin (MSD); phenylbutazone (Geigy); bradykinin (Sandoz); 48/80 (Wellcome Trust); sodium salicylate (B.D.H.); prednisolone acetate (Roussel); adrenocorticotrophic hormone (ACTH) (Armour); phenoxybenzamine hydrochloride (Smith, Kline & French); dexamethasone sodium phosphate (Merck Sharp & Dohme Ltd); mepyramine maleate (May & Baker), and methysergide bimaleate (Sandoz).

Concentrations of histamine acid phosphate (B.D.H.) and 5-hydroxytryptamine oxalate (5-HT) (Sigma) are expressed as base.

Prostaglandins $E_1$ and $F_{2\alpha}$ were a gift of Dr J.F. Pike (Upjohn). Serum kallikrein was prepared from guinea-pig serum by the method of Davies & Lowe (1963).

## Results

*Effect of 401 on turpentine and carrageenin oedema*

Intra-articular injection of 0.01 ml of turpentine in the rat causes a severe inflammatory response with an initial oedematous phase occurring in the first few hours. In this oedematous phase, the increase in vascular permeability of the synovial and neighbouring vessels gave a substantial accumulation of extravascular albumin. The time course of this accumulation was determined by giving an intravenous injection of $^{125}$I-albumin to animals at 0, 1, 2, 3 and 5 h after intra-articular turpentine injection and measuring the extravasated albumin 1 h later (Figure 2a).

Subplantar injection of carrageenin in the hind foot of the rat also causes a severe oedema and the time course of this accumulation of extravascular albumin was determined in a similar manner (Figure 2b). Turpentine oedema was also assessed by measuring the joint swelling with a gauge and in carrageenin oedema the paw volume was measured by weighing the hind feet. Figure 2 shows that the results obtained by these methods were comparable and that isotope accumulation provided a sensitive method for measurement of these inflammatory reactions. The measurements of isotope accumulation show that the response is biphasic in both tests and that a period of 4 h includes both phases of the response. Accordingly in both test systems an intravenous pulse of $^{125}$I-albumin of 4 h duration was used to measure the inflammatory reaction.

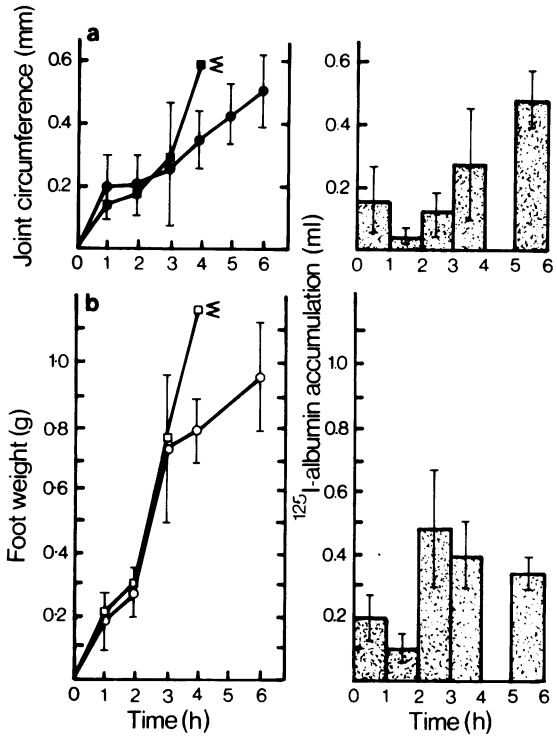


**Fig. 2** Time course of inflammatory reaction in (a) turpentine oedema; (■) $^{125}$I-albumin accumulation in terms of equivalent blood volume (ml); (●) increased joint circumference (mm); (b) carrageenin oedema; (□) $^{125}$I-albumin accumulation in terms of equivalent blood volume (ml); (○) increased weight of foot (g). Histograms show the hourly accumulation of $^{125}$I-albumin from which the cumulative extravasation was calculated in terms of equivalent blood volume (ml). Observations represent the mean of four animals ± s.d.

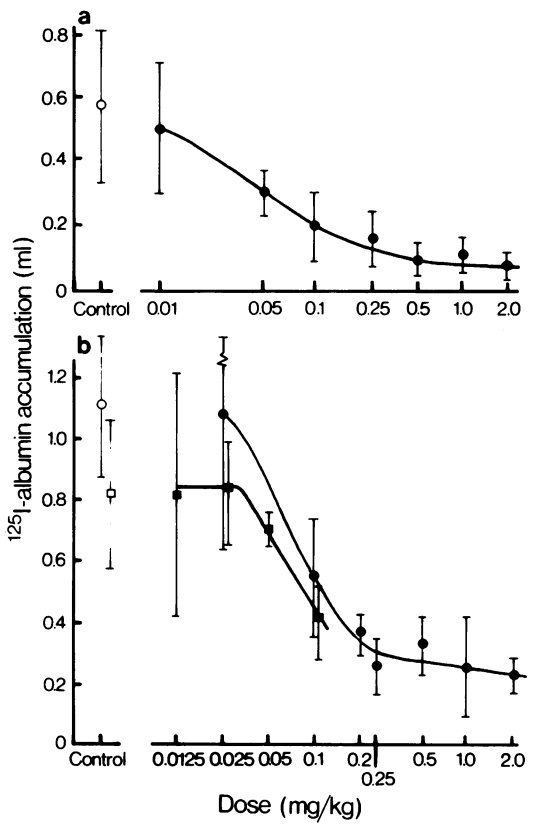


**Fig. 3** Suppression of increased vascular permeability by increasing doses of 401 in (a) turpentine oedema, and (b) carrageenin oedema. $^{125}$I-albumin accumulation 4 h after subcutaneous injection of 401 (●) or saline (○), and in rats treated with mepyramine (2.5 mg/kg) and methysergide (2.5 mg/kg) immediately before intravenous injection of 401 (■) or saline (□). Points represent the mean increased accumulation (in terms of equivalent blood volume ± s.d.) at the injected site compared with the contralateral uninjected site (paired difference) for groups of five animals.

Peptide 401 given subcutaneously immediately before intra-articular turpentine injection suppressed the oedema in a dose-related manner with a maximal effect at c. 1 mg/kg (Figure 3a). This dose reduced albumin accumulation by about 85%. There was a similar suppression of the inflammatory response caused by subplantar injection of carrageenin and a 80% reduction in albumin accumulation was obtained with a dose of 1 mg/kg (Figure 3b).

In these experiments, the inflammatory response was calculated as the increase in $^{125}$I-albumin content of the injected site (knee or foot) over that observed for the contralateral uninjected site. Thus an agent causing an increase in the $^{125}$I-albumin content of the uninjected site (e.g. by systemic inflammatory action) might cause an apparent reduction in the observed difference without having suppressed the inflammatory response at the injection site. There is in fact evidence of some systemic inflammatory action of 401 at higher doses (0.5-2 mg/kg) causing an increase in both albumin accumulation and weight of the uninjected foot (Figure 4). The magnitude of this systemic effect in carrageenin oedema was small when compared with the inflammatory response to turpentine or carrageenin. The anti-inflammatory action of 401 was accordingly accentuated at higher dosage. However, it remained clearly demonstrable at doses

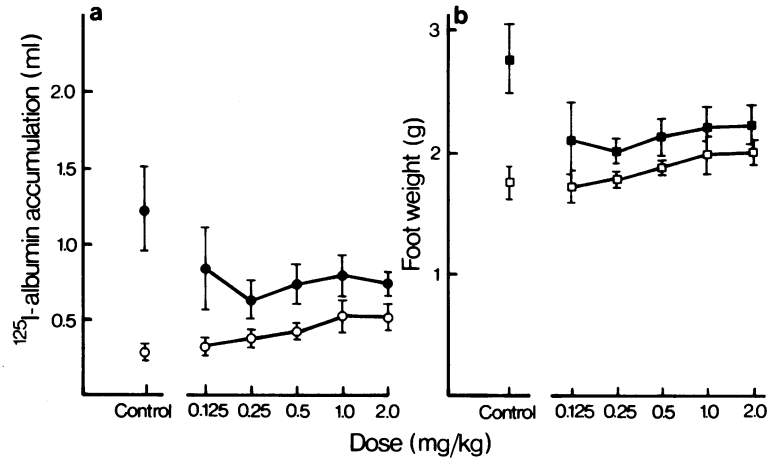


**Fig. 4** Suppression of increased vascular permeability in carrageenin oedema: (●) $^{125}$I-albumin content of the right (injected) foot; (○) $^{125}$I-albumin content of the left (uninjected) foot; (■) weight of the right (injected) foot; (□) weight of the left (uninjected) foot. Points represent the mean of five animals ± s.d.

(0.025-0.25 mg/kg) in which 401 had no systemic effect on the contralateral foot. Use of intravenous vital dye (Evans Blue) gave visual confirmation that 401 reduced the extravasation of albumin at the site of injection of carrageenin or turpentine. The potent anti-inflammatory action of 401 was very striking and compared well with the effects of the established anti-inflammatory agents; mepyramine, indomethacin, phenylbutazone, salicylate, ACTH, prednisolone and dexamethasone. None of these inhibited turpentine oedema to the same extent at the doses indicated (Table 1).

*Mechanism of the anti-inflammatory action of peptide 401*

*(1) Role of mast cell degranulation.* In addition to exhibiting anti-inflammatory action, 401 itself

**Table 1** Suppression of turpentine arthritis

| *Treatment* | *Dose (mg/kg)* | *Response* (ml blood equivalent)* | *% Inhibition of $^{125}$I-alb. accumulation* |
|---|---|---|---|
| Saline (no intra-articular turpentine) | – | –0.01 ± 0.05 ($n$ = 9) | – |
| Saline | – | 0.57 ± 0.19 ($n$ = 22) | – |
| Non-steroidal anti-inflammatory drugs: | | | |
| Mepyramine | 2 | 0.55 ± 0.29 ($n$ = 7) | 4 |
| Indomethacin | 5 | 0.31 ± 0.21 ($n$ = 15) | 46 |
| Phenylbutazone | 50 | 0.25 ± 0.17 ($n$ = 5) | 57 |
| Sodium salicylate | 500 | 0.24 ± 0.06 ($n$ = 11) | 58 |
| Steroidal anti-inflammatory drugs: | | | |
| ACTH | (1 unit) | 0.47 ± 0.09 ($n$ = 5) | 18 |
| Prednisolone | 1 | 0.39 ± 0.06 ($n$ = 3) | 33 |
| Dexamethasone | 0.025 | 0.34 ± 0.01 ($n$ = 4) | 40 |
| 401 | 2 | 0.08 ± 0.04 ($n$ = 10) | 86 |

* Means response ± s.d. ($n$ = no. of animals). Increased $^{125}$I-albumin content of the right (injected) knee in comparison with the left (uninjected) knee.
Administration of drugs: mepyramine maleate was given intravenously 5 min before intra-articular turpentine injection; indomethacin, phenylbutazone and sodium salicylate were given by intraperitoneal injection 15 min before turpentine injection: ACTH, prednisolone and dexamethasone were given by intraperitoneal injection 3 h before turpentine injection and 401 was given subcutaneously at the time of turpentine injection.

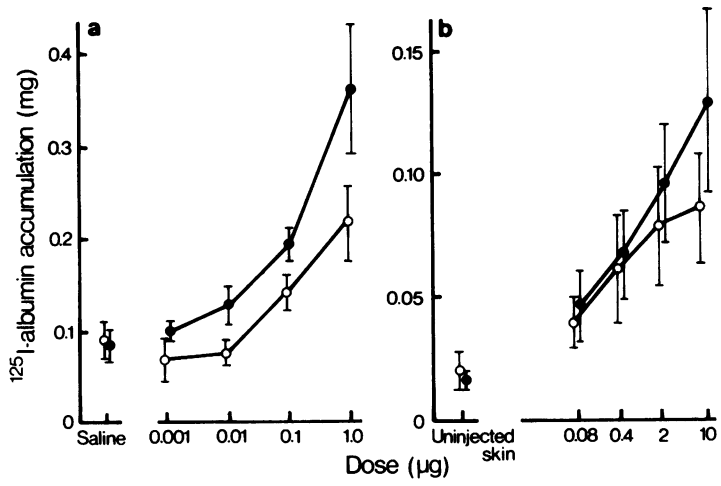


**Fig. 5** $^{125}$I-albumin accumulation following intradermal injection of 401 (•) and melittin (○) in (a) the rat, and (b) the guinea-pig. Points represent the mean accumulation in the skin test sites ± s.d. (a) for groups of four observations (six animals); (b) for groups of 16 observations (four animals).

increases vascular permeability as would be expected from its potency as a mast cell degranulating agent (Breithaupt & Habermann, 1968). It produces increased vascular permeability following intradermal injection in both the rat and the guinea-pig. The inflammatory dose-response relationships (10 ng-100 μg/ml) are shown in Figures 5a and b. Since the local concentration occurring on subcutaneous injection of 401 in anti-inflammatory studies exceeded these doses, the possibility existed that the anti-inflammatory activity of 401 might in some way be associated with its own local inflammatory actions. Melittin, another peptide from bee venom which also causes mast cell degranulation, hypotension (Table 2) and increased vascular permeability (Fig. 5a), and the potent mast cell degranulating synthetic compound 48/80 (Table 2) were therefore compared with 401 for their ability to suppress increased vascular permeability. Melittin (2 mg/kg) showed no significant inhibition of turpentine oedema nor did it reduce responses evoked by intradermal injection of histamine, 5-HT or bradykinin. In contrast, comparable doses of 401 caused marked

**Table 2** Comparison of 401, Melittin and 48/80

| | | *Material* | | |
|---|---|---|---|---|
| *Test* | *Dose* | *401* | *Melittin* | *48/80* |
| Mast cell degranulation | 10 μg/ml | 79 | 83 | 73 |
| (values represent | 1 | 72 | 77 | 64 |
| % degranulation) | 0.1 | 52 | 34 | 50 |
| | 0.01 | 19 | 26 | 58 |
| | 0.001 | – | – | 23 |
| | 0 | 5 | 9 | 6 |
| Blood pressure | 5 μg | – | 22 | not |
| depression in mmHg | 4 | 48, 32 | – | tested* |
| (values represent | 2 | 30, 24 | 12 | |
| single observations) | 1 | 18 | – | |
| Inhibition of turpentine oedema $ED_{50}$ (mg/kg) | – | 0.07 | >2.0 | 1.0 |

* 401 and 48/80 reported to be equipotent (w/w) on rat blood pressure (Breithaupt & Habermann, 1968).

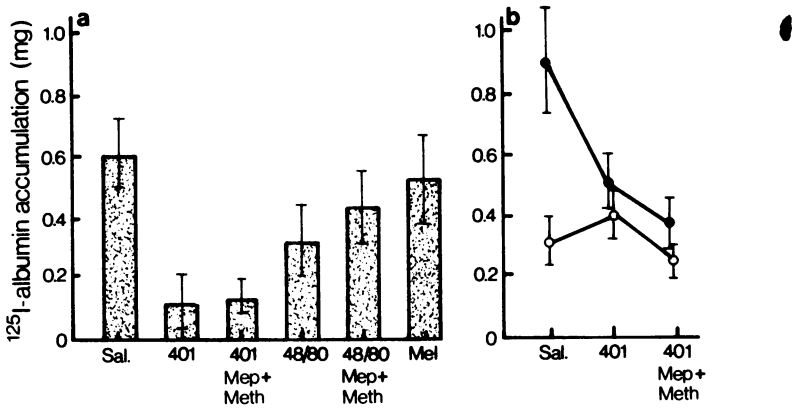


**Fig. 6** The effect of mepyramine and methysergide on the anti-inflammatory action of 401 in turpentine oedema. (a) Increased accumulation of $^{125}$I-albumin following intra-articular injection of turpentine in animals pretreated as follows: subcutaneous saline (sal); subcutaneous 401 (1 mg/kg) (401); subcutaneous 401 (1 mg/kg) following systemic mepyramine (2.5 mg/kg) and methysergide (2.5 mg/kg) (401, Mep + Meth); subcutaneous 48/80 (1 mg/kg) (48/80); subcutaneous 48/80 (1 mg/kg) following systemic mepyramine (2.5 mg/kg) and methysergide (2.5 mg/kg) (48/80, Mep + Meth); and subcutaneous melittin (2 mg/kg) (Mel). Columns represent mean of five animals ± s.d. (b) $^{125}$I-albumin content of right (injected) knee (•) and left (uninjected) knee (○). Points represent mean of six animals ± s.d.

inhibition in these tests. Similarly, 48/80 (1 mg/kg) had no anti-inflammatory action comparable with 401 in turpentine oedema (Table 2) nor did it suppress the increased vascular permeability caused by intradermal histamine, 5-HT and bradykinin. These results demonstrate that the anti-inflammatory action of 401 cannot be simply attributed to its mast cell degranulating property or its inflammatory actions. This conclusion is strengthened by the observation that, in the guinea-pig, neither 401 nor melittin suppressed the increase in vascular permeability in turpentine arthritis, hypersensitivity arthritis and skin reactions of allergic inflammation at doses up to 2 mg/kg, despite the fact that both peptides cause an inflammatory response in this species (Figure 5b).

Pretreatment of the rat with the specific antagonists, mepyramine maleate (2.5 mg/kg) and methysergide bimaleate (2.5 mg/kg) fully suppressed skin reactions to histamine (0.5 μg) and 5-HT (0.2 μg) but not bradykinin (0.3 μg) and caused only slight reduction of responses to intra-articular turpentine or subplantar carrageenin. This pretreatment had little effect on the ability of peptide 401 to reduce the inflammatory response to subplantar carrageenin (Figure 3). In these 401-treated animals the use of mepyramine and methysergide caused a reduction in the count of both injected and uninjected feet (Fig. 6), an effect presumably due to antagonism of systemically released histamine and 5-HT. Whilst this pretreatment was without effect on the anti-inflammatory action of 401, it did cause some reduction in the anti-inflammatory activity of 48/80. Because of its potent mast cell degranulating action, 401 is relatively toxic on intravenous injection. However, pretreatment with mepyramine and methysergide permitted intravenous administration of 401 and in such experiments the anti-inflammatory potency of 401 was comparable with that observed following subcutaneous injection (Figure 3).

*(2) Specificity of action.* The inflammatory responses in the rat to intra-articular injection of turpentine and subplantar carrageenin are complex, possibly involving sequential release of a number of endogenous agents (Di Rosa, Giroud & Willoughby, 1971). Drugs which increased the calibre and permeability of blood vessels by direct action on the vascular wall were therefore used. Peptide 401 at a dose of 1 mg/kg injected subcutaneously totally abolished dye accumulation following intradermal injection of bradykinin (0.2 μg), prostaglandin $E_1$ (0.5 μg), serum kallikrein (100 μg), histamine (0.3 μg), 5-HT (0.1 μg) and 48/80 (0.025 μg). An interval of 1 h or more between the subcutaneous injection of 401 and the subsequent intradermal injections was necessary for demonstration of the anti-inflammatory effect. However, when injected intravenously, 401 (200 μg/kg) was effective immediately and abolished the leakage of blue dye produced by

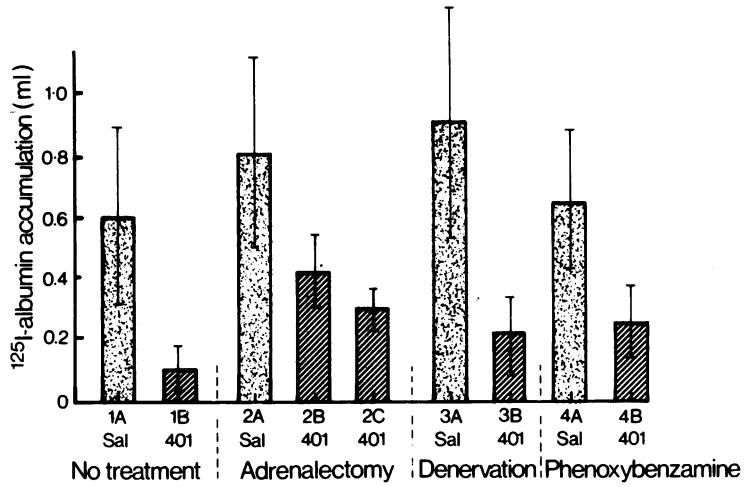


**Fig. 7** The effect of adrenalectomy (2), denervation (3) or pretreatment with phenoxybenzamine (10 mg/kg) (4), on the anti-inflammatory activity of 401. Increased accumulation of $^{125}$I-albumin after intra-articular injection of turpentine into knee joint following treatment with saline 1A, 2A, 3A and 4A or 401 (1 mg/kg) 1B, 2B and C, 3B and 4B. Adrenalectomy was performed six (2A and 2B) and nine (2A and 2C) days before the experiments. Columns represent the mean ± s.d. from five animals.

intradermal injections of bradykinin (0.5 μg), histamine (0.8 μg) and 5-HT (0.2 μg). This suggested that the delay required on subcutaneous injection could be attributed to slow absorption from the injection site.

*(3) Contribution to endogenous anti-inflammatory systems.* The irritant action of 401 raised the possibility that its injection initiated the acute phase of shock (Florey, 1962) and thereby activated endogenous anti-inflammatory mechanisms, particularly the sympathetic and adrenal systems. However, pretreatment of the animals with mepyramine and methysergide prevented the increased vascular permeability on subcutaneous injection of 401 without substantially affecting its anti-inflammatory activity. The possible contribution of neural mechanisms to the anti-inflammatory activity was investigated by studying the effect of 401 on turpentine oedema in denervated limbs. This treatment had no significant effect on the anti-inflammatory action of 401 (Figure 7). Pretreatment of the animals with phenoxybenzamine (10 μg/kg) an agent which specifically blocks α-adrenoceptors, also had little effect on the anti-inflammatory activity of 401 (Figure 7).

The effect of 401 on turpentine oedema was also measured in adrenalectomized animals six and nine days after operation. Although there was some reduction of the anti-inflammatory activity of 401 (Fig. 7) it is clear that the release of adrenal cortico-steroids cannot account for a large part of this anti-inflammatory action.

The release of prostaglandin $F_{2\alpha}$ was considered as a possible mechanism in view of its reported anti-inflammatory action in this species (Willoughby, 1968). However, in doses up to 50 μg/kg any apparent anti-inflammatory action of prostaglandin $F_{2\alpha}$ could be accounted for by the increased count in the contralateral knee. These experiments show that the anti-inflammatory action of 401 cannot merely be attributed to the stimulation of adrenergic or corticosteroid factors or release of prostaglandin $F_{2\alpha}$.

*(4) Vasomotor activity.* Subcutaneous injection of 401 at a dose level at which anti-inflammatory activity is maximal in turpentine arthritis and carrageenin oedema resulted in a sustained fall in blood pressure apparent after 1 h and persisting for several hours (Figure 8). This hypotensive response is reduced by pretreatment with mepyramine (2.5 mg/kg) and methysergide (2.5 mg/kg) which allows intravenous injection of 401. This pretreatment does not affect the anti-inflammatory activity of 401 (Fig. 3) suggesting that hypotension is not responsible for anti-inflammatory activity. Production of an anti-inflammatory effect by selective regional vasoconstriction remains a possibility although the inability of even large doses of 401 (up to 1 mg) to cause detectable vasoconstriction in the isolated perfused mesentery suggests that such vasoconstriction would have to be indirect. No evidence that 401 caused selective vasoconstriction in skin was obtained

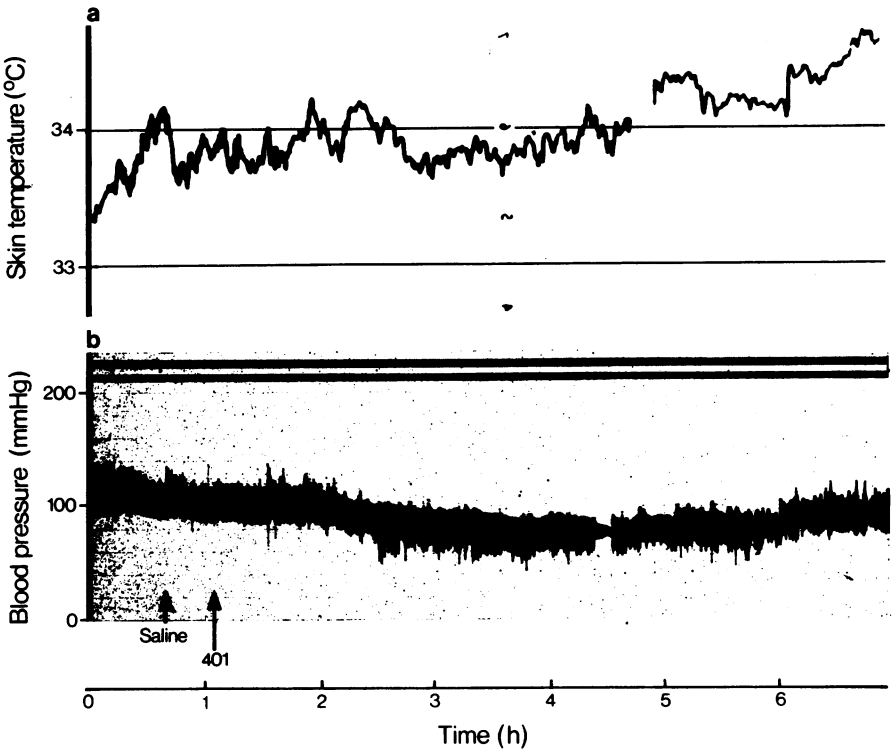


**Fig. 8** (a) Arterial blood pressures following subcutaneous injection of 401 (2 mg/kg) in restrained rat. (b) Flank skin temperature in the same experiment.

either by measurement of skin temperature over several hours following subcutaneous injection of 401 (Fig. 8) or by measurement, 2 h after subcutaneous injection of 401, of the fractional extraction of $^{86}Rb$ in skin tissue.

## Discussion

These results demonstrate that peptide 401 is a potent anti-inflammatory agent in the rat. It is able to reduce or abolish responses to intradermal injection of chemically defined agents such as histamine, 5-HT, bradykinin and prostaglandins, as well as responses to more complex stimuli such as local injection of carrageenin or turpentine, in which several mediators are involved (Di Rosa *et al.*, 1971). In addition to these actions, peptide 401 suppresses the development of adjuvant arthritis and reduces the severity of primary and secondary lesions in established adjuvant arthritis (Billingham *et al.*, 1973).

In addition to its anti-inflammatory action, 401 is a potent mast cell degranulating agent *in vivo* and *in vitro* and this property could account for the hypotensive and vascular permeability effects observed on intravenous or subcutaneous injection. However, it is not possible to attribute the anti-inflammatory action of 401 merely to vasoactive amine release following mast cell degranulation since other mast cell degranulating agents with a different (e.g. melittin) or similar (e.g. 48/80) mechanism of action (Breithaupt & Habermann, 1968) do not exhibit comparable anti-inflammatory activity in the tests employed in this investigation. Also, pretreatment of rats with mepyramine and methysergide to antagonize the actions of histamine and 5-HT reduced the systemic hypotension and abolished the local inflammation produced by 401 without loss of anti-inflammatory activity; the slight reduction in anti-inflammatory activity against carrageenin and turpentine oedema being consistent with specific antagonism of histamine and 5-HT release in these inflammatory reactions. 401 is without anti-inflammatory action in guinea-pigs, although it causes increased vascular permeability on intradermal or subcutaneous injection. It is therefore

unlikely that the anti-inflammatory action of 401 depends upon its irritant properties. Nonetheless, it remains possible that the anti-inflammatory property of 401 may be in some way related to mast cell degranulation or other tissue damage since large doses of prostaglandin $E_1$ (1 mg/day) reduce the severity of adjuvant arthritis (Zurier & Ballas, 1973) and alkylpseudothioureas reduce inflammatory reactions to a wide range of stimuli (Ercoli, Arbona & Tabernero, 1971).

The lack of specificity of action of 401 and more especially its ability to suppress responses to intradermal injection of smooth muscle spasmogens, indicate that 401 does not depend upon depletion of substrates (e.g. kininogen, complement) for its mode of action. Whilst adrenalectomy caused some reduction in the potency of 401, denervation and pretreatment with phenoxybenzamine had little effect. It can be concluded, therefore, that alterations in cortico-steroid release or vasomotor activity can only make minor contributions to the anti-inflammatory action of 401. A modification of prostaglandin synthesis has not been excluded, although for such a mechanism of action to operate, it is necessary to presuppose that skin reactions to histamine, bradykinin and 5-HT depend upon coincident prostaglandin synthesis, at present an unlikely assumption in view of the inability of non-steroidal anti-inflammatory drugs to suppress all of these responses in rats (see Collier, 1969).

Gross measurements of plasma protein extravasation did not differentiate between the effects of altered transmural pressure or altered vessel wall area (e.g. due to vasoconstriction or vasodilatation in the microvascular bed) and altered vascular permeability itself. Nevertheless, persistence of the anti-inflammatory property of 401 in mepyramine- and methysergide-treated animals, together with the inability of 401 treatment to affect fractional extractions of $^{86}Rb$ by skin and the absence of any vasoconstrictor activity of 401, suggest that peptide 401 acts directly on the vessel wall to produce anergy to agents causing increased vascular permeability.

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