Annu. Rev. Pharmacol. Toxicol. 1993. 33:91–108
Copyright © 1993 by Annual Reviews Inc. All rights reserved

ANNUAL REVIEWS Further
Quick links to online content

ANTI-INFLAMMATORY PEPTIDE AGONISTS

Edward T. Wei and Holly A. Thomas
School of Public Health, University of California, Berkeley, California 94720

KEYWORDS: CRF, neurotensin, mystixin, permeability, edema

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org
by Central College on 12/09/11. For personal use only.

INTRODUCTION

The aim of this review is to summarize recent studies suggesting that certain peptides act as agonists to inhibit inflammation, defined by Cotran et al (1) as the reaction of vascularized living tissue to local injury. In previous volumes of this journal, reviews have appeared on drugs which act as antagonists of inflammatory mediators such as platelet-activating factor, leukotrienes, and bradykinin (2–4). Specific antagonists, by design, work one-on-one against substances that promote inflammation, and the efficacy of a single antagonist may be limited if more than one mediator is released during tissue injury (3). An agonist, a term introduced by Reuse (5) to describe a chemical that activates biological events, would be more efficacious than an antagonist if it could suppress convergent processes initiated by more than one inflammatory mediator. The concept of drugs as anti-inflammatory agonists was discussed by Svensjo & Persson in 1985 (6). These authors showed that clinically applied asthma drugs such as the β2-adrenergic agonist terbutaline and the xanthine drug theophylline acted on specific receptors in the microvasculature to shut off plasma protein leakage induced by the inflammatory mediators histamine and bradykinin (7). The experimental model employed for demonstrating these phenomena was the hamster cheek pouch preparation, in which intravital microscopy was used to quantify changes in the microcirculation (8).

Leakage of plasma proteins from small blood vessels, as studied in the hamster cheek pouch model, represents the earliest phase of inflammation. The leakage begins after injury, reaches a peak by 5 to 10 min, and phases out within 15 to 30 min. This stage of increased vascular permeability has been called the immediate-transient response (1). The mechanisms of leakage

0362-1642/93/0415-0091$02.00

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

are attributed to substances released by injured tissues, such as histamine, that are thought to produce changes in endothelial cells of postcapillary venules. In response to inflammatory mediators, these cells contract, such that inter-cellular gaps develop, allowing fluids and solutes of the blood to pass into the interstitial spaces (9). In more severe injuries, direct damage to the microcirculation results in the immediate-sustained response, in which vascular leakage can persist for several hours or continue for days until thrombosis occurs or the vessels are repaired (1). The studies reviewed here suggest that the immediate changes in vascular permeability after local injury can be inhibited by certain peptides. The prototype peptide is corticotropin-releasing factor (CRF), but the list of active agents is rapidly growing. These peptides reduce vascular leakage in the acute phase of inflammation and appear to act as anti-inflammatory agonists.

Corticotropin-Releasing Factor

Corticotropin-releasing factors are hormones that control, via the hypothalamus-pituitary-adrenal axis, the secretion and synthesis of corticosteroids from the adrenal glands (10, 11). Certain peptides, such as vasopressin, may exert physiological control of adrenocorticotropic hormone (ACTH) secretion from the pituitary and could be called corticotropin-releasing factors, but, in this review, the term CRF is restricted to the 41-residue peptides first characterized in 1981 from ovine hypothalami by Vale et al (12). Synonyms for CRF are CRF-41, CRH, and corticoliberin. The amino acid sequences of CRF from various species are shown in Table 1. The sequence of human CRF was deduced from cDNA studies and found to be identical to that of rat CRF (13, 14). The CRF of hoofed animals differed from that of human by seven (ovine) to eight (bovine) amino acids (15), but the pig and sucker fish sequences differed from the human/rat sequence by only two out of 41 residues (16, 17). Peptides with structures similar to CRF were discovered in the skin of *Phyllomedusa sauvagei* frogs and in the urophysis of *Castotomus commersoni* fish (18, 19; Table 1). Although these peptides, sauvagine and urotensin I, were at least equipotent to CRF in releasing ACTH from isolated rat corticotrophs (20), the endogenous functions of sauvagine in the skin of the tree-frog (*Phyllomedusa* species that live in arid regions of South America) and of urotensin I in the urophysis of fish remain unknown. The physiology of CRF in mammals was recently reviewed (10, 21).

CRF, sauvagine, and sucker fish urotensin I possess the unusual property of being able to prevent, in experimental animals, the increased vascular permeability that occurs in tissues shortly after injury (Table 2; 22, 23). For example, CRF reduced swelling (measured as increases in tissue volume), edema (measured as increases in wet weight), and protein extravasation

Table 1 Peptides of the corticotropin-releasing factor superfamily

Peptide	Species	Sequence[a,b]			
		10	20	30	40
CRF	Human/rat	SEEPPISLDL	TFHLLREVLE	MARAEQLAQQ	AHSNRKLMEII
CRF	Pig	SEEPPISLDL	TFHLLREVLE	MARAEQLAQQ	AHSNRKLMENF
CRF	Sucker fish	SEEPPISLDL	TFHLLREVLE	MARAEQLAQQ	AHSNRKMMEIF
CRF	Sheep/goat	SQEPPISLDL	TFHLLREVLE	MTKADQLAQQ	AHSNRKLLDIA
CRF	Cow	SQEPPISLDL	TFHLLREVLE	MTKADQLAQQ	AHNNRKLLDIA
Sauvagine	Frog	>EGPPISIDLS	LELLRKMIEI	EKQEKEKQQA	ANNRLLLDTI
Urotensin I	Sucker fish	NDDPPISIDL	TFHLLRNMIE	MARIENEREQ	AGLNRKYLDEV
Urotensin I	Carp	NDDPPISIDL	TFHLLRNMIE	MARNENQREQ	AGLNRKYLDEV

[a] The carboxyl termini of these peptides are amidated.
[b] Single letter abbreviations for amino acids: S, T, P, A. G; Ser, Thr, Pro, Ala, Gly; M, L, I, V; Met, Leu, Ile, Val; E, D, N, Q; Glu, Asp, Asn, Gln; R, K, H; Arg, Lys, His; F, Y, W; Phe, Tyr, Trp; >E; pyroglutamyl.

(measured as leakage of protein-bound dye from the vascular compartment) in the anesthetized rat's pawskin after exposure of the paw to heat or to extreme cold (24–26). CRF inhibited dye extravasation in mucous membranes after exposure to formaldehyde or substance P, in skeletal muscle after a scalpel incision, and in brain cortex after a freeze lesion (23, 27, 28). Pulmonary edema produced by intravenous injection of epinephrine was also prevented by CRF (29). In these experiments, pretreatment of animals with human/rat CRF at doses of 30 to 60 μg/kg generally reduced vascular leakage by 40 to 60%. The drug effects were eye-catching and apparent to the untrained observer.

The pharmacological properties of CRF are illustrated by some of the experiments on heat-induced vascular leakage (22, 24, 25). When the paws of pentobarbital-anesthetized rats (200 to 300 g body weight) were immersed in 58°C water for 0.5 to 5 min, the normal paw volume of about 1.5 ml was increased by between 0.5 and 1.5 ml within 30 min, the swelling being due to an increase in water content of the paw. After Evans blue dye was administered to the animal, exposure of the paw to heat increased its dye content from 3 μg/paw to about 350 μg/paw. These indices of immediate vascular leakage were reduced by peptides of the CRF superfamily with the following dose-response and time-response characteristics: (*a*) Sauvagine, sucker fish urotensin I, and human/rat CRF injected intravenously (i.v.) 10 min before heat exposure inhibited dye leakage with ED_{50} values of 0.44,

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

1.5, and 5.9 nmol/kg, respectively (22). (*b*) The anti-inflammatory actions of these peptides were fully antagonized by α-helical-CRF(9–41), a synthetic competitive antagonist of CRF at its receptor (30). (*c*) A dose of 28 μg/kg human/rat CRF injected subcutaneously 1 to 2 hr before heat reduced swelling by over 50%. Pretreatment with this dose at 4 hr before heat was also effective, but not at 12 hr (25). (*d*) Intravenous injection of 28 μg/kg CRF produced a rapid drop in blood pressure, but this precipitous drop was not observed after subcutaneous administration. After subcutaneous injection, blood pressure was normal at the time of heat exposure when anti-inflammatory effects were observed (31). (*e*) Intravenous injection of CRF after exposure of the rat's paw to heat immediately halted the progress of swelling and dye leakage, but, as noted above, CRF given by this route lowered blood pressure (22).

CRF inhibited heat-induced vascular leakage in adrenalectomized or hypophysectomized rats (32), showing that its ability to inhibit vascular leakage was independent of its stimulatory action on the pituitary-adrenal axis. Intravenous injections of ACTH (1–24) (0.5 mg/kg), human β-endorphin (1 mg/kg) and dexamethasone (0.5 mg/kg), substances that mimic stimulation of the pituitary-adrenal axis, were ineffective in the same bioassay (24). The anti-inflammatory actions of CRF also appeared to be unrelated to its actions on peripheral sensory nerves (33). Drugs such as morphine, FK33–824 (a potent synthetic enkephalin analog) and ethylketocyclazocine, like CRF, inhibited, at low doses, vascular leakage induced by antidromic stimulation of sensory nerves, a process called neurogenic inflammation (31, 34), yet these drugs, in contrast to CRF, were ineffective in inhibiting vascular leakage induced by exposure of the rat's paw to heat (≥58°C) (24). The general properties of CRF's inhibitory action on vascular leakage—independence of adrenal activation and hypotension—apply to most of the conditions of injury listed in Table 2. The relative potency of CRF for suppressing leakage in different vascular beds, based on dose estimates, is pulmonary edema>skin, mucous membrane, muscle edema>brain edema.

Pain, like increased vascular permeability, is a sign of inflammation, and it is interesting to note that CRF shows positive activity in tests of antinociception. CRF inhibited the abdominal constrictor response to intraperitoneal injection of phenylbenzoquinone in mice (31), the paw withdrawal response of anesthetized rats to 48°C water (24), and the pawlick response of rats in the hot-plate test (35). The rate of firing of wide dynamic range trigeminal neurons in anesthetized rats, stimulated by noxious heat applied to the whisker pad, was reduced by intravenously administered CRF (33). Neurophysiological studies suggest that CRF interacts with peripheral sensory nerves to decrease afferent transmission of nociceptive signals (31, 33). However, the presence of CRF or CRF binding sites in peripheral sensory neurons has not yet been demonstrated (36, 37). Hargreaves et al showed that

Table 2 Situations in which CRF attenuates vascular leakage

Tissue	Conditions or agent producing leakage
Skin (abdomen, paw)	Immersion in warm (48 to 58°C) or cold (−20°C) solutions Exposure to concentrated inorganic acids Antidromic stimulation of saphenous nerve Intradermal injection of inflammatory mediators
Trachea (mucous membranes)	Formaldehyde vapors Antidromic stimulation of vagus Subcutaneous substance P injection
Esophagus (mucous membranes)	Subcutaneous substance P injection
Skeletal muscle	Surgical incision Local injection of substance P
Brain cortex and meninges	gold probe (−50°C) applied to skull
Lung alveoli	Intratracheal instillation of formalin Intravenous epinephrine injection

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

CRF was active in rats against carrageenan-induced hyperalgesia and edema (38). These authors suggested that CRF, peripherally administered at high doses, might produce morphine-like analgesia by stimulating release of the proopiomelanocortin-derived peptide β-endorphin (39). In preliminary studies in humans, it has been reported that CRF reduced postoperative dental pain (35), attenuated elevations of intracranial pressure after neurosurgery (40), and inhibited the development of the flare response to intradermal histamine (41). These observations require more trials in a controlled setting before the results can be accepted.

The precise sites at which and biochemical mechanisms by which CRF inhibits vascular leakage in peripheral tissues remain to be established, in part because the molecular processes underlying rapid gap formation in endothelial cells have not yet been characterized (42). A localized action of CRF on blood vessels has been demonstrated in the standard hamster cheek pouch preparation used to examine vascular permeability. Topical suffusion of CRF onto the mucosa suppressed vascular leaks induced by histamine or bradykinin (43, 44). Displaceable binding sites for iodinated-Tyr^{o}CRF were found on blood vessels and on epithelial cells in close proximity to sites of vascular leakage (28, 45). Because CRF prevented the effects of inflammatory mediators on skin and mucous membranes, it was thought at first that the postcapillary

venules were the primary and exclusive sites of CRF action (28, 46). This idea, however, had to be rejected when further experiments showed that CRF inhibited pulmonary edema induced by epinephrine injections (29) and brain edema induced by a freeze lesion (23). The microvessels of the lung alveoli and of the brain cortex, in contrast to the postcapillary venules of the skin and mucous membranes, are known to be relatively insensitive to mediators such as histamine and serotonin (47), yet CRF was effective in these vascular beds. CRF acts as a functional antagonist of inflammatory mediators that affect postcapillary venules (48), but its efficacy appears to be more generalized. The versatile ability of CRF to inhibit vascular leakage may perhaps be explained by a hormonal mechanism in which endothelial cell-cell or cell-substratum adhesion processes are enhanced. An increased attachment of cells to each other or to basement membranes may fortify tissues against edematogenic forces and account for the wide-ranging anti-inflammatory actions of CRF. The limited evidence for this idea is discussed in the next section on mystixins and intermediate filaments.

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

Modified CRF-related Peptides: Mystixins

Analysis of the secondary structure of peptides of the CRF superfamily has shown that these molecules occur in aqueous solutions predominantly as random coils. At concentrations greater than 1 μM, segments of these peptides tend to aggregate into amphiphilic α-helical conformations (49–51). In the search for segments of CRF that might account for its anti-inflammatory activities, crude peptides corresponding to the 11-residue carboxyl terminus of human/rat CRF, AHSNRKLMEII-NH_2, were synthesized, tested, and found to have anti-inflammatory activity (Table 3; 52). Further characterization of the structures within the crude peptide mixture revealed that substitution of the glutamic acid residue (E) with an anisolylated glutamic acid derivative (*, Table 3) increased overall anti-inflammatory potency. The anisole derivative was apparently a by-product of the temperature-dependent Friedel-Crafts acylation reaction that occurs during hydrogen fluoride cleavage of glutamyl-containing peptides (53, 54). Several peptides containing the anisolylated glutamic acid derivative were made with D-amino acid substitutions (denoted by the lower case of the single letter code) in order to determine if potency or efficacy could be further enhanced, perhaps by increasing the metabolic stability of the peptides. A search of the protein information database (*Protein Identification Resource,* National Biomedical Research Foundation, Georgetown University, Washington, D.C.) revealed that the sequence -RKLLE- was present in many endogenous intermediate filament proteins (52, 55, 56). An analog, lATyRKLL*Il-NH_2, having sequence similarity to the -IATYRKLLE- portion of cytoskeletal filaments (56), was therefore synthesized and tested.

Table 3 Anti-inflammatory potencies of mystixins

Synthetic peptide[b]	ED_{50} (95% C.L.)[a] mg/kg i.v.	
	Heat-edema	Pulmonary edema[c]
aHSnRKLLEIl-NH_2	2.2 (1.4–3.6)	—
lATyRKLLEIl-NH_2	1.9 (1.1–3.8)	
AHSNRKLM*II-NH_2[d]	0.88 (0.52–1.49)	—
aHSnRKLM*Il-NH_2	0.24 (0.09–0.60)	—
aHSnRKLL*Il-NH_2	0.11 (0.04–0.29)	0.04 (0.03–0.07)
lATyRKLL*Il-NH_2	0.05 (0.02–0.12)	0.04 (0.02–0.07)

[a] The ED_{50} and 95% confidence limits were estimated according to Litchfield & Wilcoxon (101).

[b] Lower case denoted D-amino acid,

[c] –, not tested

[d] *, is an anisolylated glutamic acid derivative; methoxybenzene (anisole) reacts with the free carboxyl group of glutamic acid to yield a γ-methoxybenzoyl-α-aminobutyric acid residue in place of Glu(E).

The peptides listed in Table 3 were assayed in the heat-induced edema model wherein the anesthetized rat's paw was immersed in 58°C water for 1 min and the increase in paw weight taken as an index of increased vascular permeability (57). In preliminary tests the peptides containing the anisolylated derivative were found to have hypotensive effects lasting 10 to 40 min (52). These peptides were injected 1 hr before exposure to heat to reduce the possibility that hypotension might contribute to the observed effects. The results showed that the D-amino acid-modified peptide aHSnRKLM*Il-NH_2 was about three times more potent than the similar CRF-sequence (AHSNRKLM*II-NH_2) containing only L-amino acids. Replacement of Met with Leu in position 8 yielded undecapeptides with increased activities, the most potent being lATyRKLL*Il-NH_2. aHSnRKLL*Il-NH_2 and lATyRKLL*Il-NH_2 were also tested in the epinephrine-induced pulmonary edema model and shown to have ED_{50} values of 40 μg/kg i.v. The greater sensitivity of lung tissues to the inhibitory actions of CRF on vascular leakage was noted previously (29).

The sequence -IATYRKLLE-, and similar sequences containing -RKLLE-, occur within the coil region of the intermediate filament proteins keratin, lamin, desmin, and vimentin of many species (52, 55, 56). These proteins are functional components of the cytoskeleton that maintain cell-cell and cell-substratum adhesions (56, 57). Some studies implicate intermediate filaments in the regulation of vascular permeabilty (58). For example, compounds that alter vascular permeability such as bradykinin and EGTA change intermediate filament content and assembly in cultured endothelial cells (59). The anti-inflammatory activity of the synthetic peptide lATyRKLL*Il-NH_2 suggests the possibility that the corresponding peptide

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

sequence within the intermediate filaments might be a factor in the modulation of cell adhesion and hence affect vascular permeability. The short anti-inflammatory peptides containing the sequence -RKL(M/L)xI(I/l)amide were called mystixins because their powerful mode of action is unknown, mysterious, and intriguing (52).

Neurotensin and Related Peptides

When the abilities of peptides of the CRF superfamily to reduce heat-induced edema were compared in vivo, the frog skin peptide, sauvagine, was found to be more potent than sucker fish urotensin I or CRF (22). The results with mystixins suggested that the double basic residues -Arg-Lys- were important for the anti-inflammatory activity of small peptides. Thus, it was of interest to examine other frog skin peptides with double basic residues. Xenopsin, an octapeptide containing -Lys-Arg- isolated from the skin of the African frog (*Xenopus laevis*) by Araki et al in 1973 (60), was found to inhibit heat-induced edema (61) and, because it shows sequence similarity (Table 4) to neurotensin (NT), peptides of this family were studied in greater detail (62, 63).

Xenopsin, NT, NT(8–13), and *NA*cNT(8–13) administered intravenously to rats attenuated the swelling evoked by heat applied to the pawskin (61; Table 4). Typically, a dose of NT or a related peptide of 10 nmol/kg inhibited heat-induced swelling by 60% to 80%. On a molar basis, the NT family peptides were about equipotent with CRF but somewhat more efficacious in reducing vascular leakage. Xenopsin and NT, like CRF and mystixins, also produced significant hypotension lasting for 30 to 60 min after intravenous injection. The hypotension produced by the hexapeptides NT(8–13) and *NA*cNT(8–13) was of shorter duration, lasting less than 10 min, yet these peptides were as effective as NT in suppressing swelling after heat exposure (61). In the same test system, sodium nitroprusside injected at 1 mg/kg i.v. produced a greater degree of hypotension but did not affect heat-induced edema (61).

For characterizion of the effects of NT-like peptides on vascular beds of

Table 4 Potencies of neurotensin (NT) family peptides as inhibitors of heat-induced edema

Peptide	Sequence	ED_{50}(95% C.L.) nmol/kg i.v.	ED_{50} in μg/kg i.v.
Xenopsin	>EGKRPWIL	0.9 (0.5–1.7)	0.9
NT	>ELYENKPRRPYIL	1.5 (0.8–2.8)	2.5
NT(8–13)	RRPYIL	2.1 (1.4–4.2)	1.6
*NA*cNT (8–13)	Ac–RRPYIL	1.9 (1.0–3.8)	1.8
Neuromedin N	KIPYIL	10 (5 – 22)	7.5

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

tissues other than skin, *NA*cNT(8–13) was selected as the prototype because it has weak hypotensive activity relative to the parent tridecapeptide, but retains many of the other pharmacological properties of NT (64). Some studies in fact indicated that NT(8–13) has higher affinity for the NT receptor than NT (65). In membranes derived from cells transfected with cDNA of the neurotensin receptor, NT(8–13) was more potent than NT in inhibiting binding of radiolabeled NT (66). The greater potency of NT(8–13) relative to NT was also observed in binding studies on neuroblastoma cells and human brain samples (67). When tested, *NA*cNT(8–13) inhibited vascular leakage in skeletal muscle after a knife cut, in brain cortex after a freeze lesion, and in lung alveoli after an i.v. injection of epinephrine (61). The anti-inflammatory actions of *NA*cNT(8–13) were not blocked by α-helical-CRF(9–41), indicating that its effects were independent of the CRF receptor (61).

NT was discovered and isolated from bovine hypothalami after investigators noticed that injection of purified fractions of hypothalamic extracts produced cutaneous vasodilatation, hypotension, and cyanosis in the anesthetized rat (62, 63). Early on it was recognized that intravenous administration of NT increased vascular permeability (62). Large doses of NT in the rat increased extravasation of Evans blue dye from the plasma, produced edema in limbs, and increased the hematocrit (62). The mechanism of increased vascular permeability was thought to involve histamine because NT elevated plasma levels of histamine in the rat (63), released histamine from minced rat skin (68), and released bioassayable histamine from the perfused rat hindquarter (69). Thus, actions of NT in the microcirculation were generally viewed as pro-inflammatory and not anti-inflammatory. The only report to suggest a local inhibitory action of NT on histamine release was that of Foreman et al (70), who showed that intradermal injections of NT reduced the wheal and flare reactions to substance P in human skin.

It is not unusual, however, for a substance to exhibit pro-inflammatory actions in one set of test conditions but manifest anti-inflammatory actions under other circumstances. This was elegantly demonstrated by Rampart & Williams (71) for prostacyclin (PGI_2). Local administration of prostacyclin enhanced the edema produced by inflammatory mediators, but intravenously infused prostacyclin reversed the effects of these mediators. Neurotensin, vasoactive intestinal peptide (VIP), and calcitonin gene-related peptide (CGRP) appeared to be pro-inflammatory because, when injected into the human skin, the triple response of Lewis (reddening of the skin, wheal and flare) was elicited (72). However, when these peptides were injected systemically in animals, vascular leakage resulting from inflammation produced by mediators or by tissue injury was suppressed (73, 74). The uncertainties about pro-inflammatory versus anti-inflammatory activity also

apply to CRF, for which a pro-inflammatory role on carrageenan-induced leukocytosis has been postulated (75).

The problems of characterizing substances as pro-inflammatory or anti-inflammatory result, in part, from the influence of the route of drug administration on local hemodynamics (71). Frequently, test compounds both produce vasodilatation and have anti-permeability effects, actions that are exerted at different loci within the microcirculation—the arterioles for vasodilatation and the venules for antipermeability effects (9). Certain prostaglandins, VIP and CGRP, injected intradermally, enhanced the edematogenic actions of inflammatory mediators, presumably by producing local arteriolar vasodilatation and increasing hydrostatic pressure within the venule, the site of leakage (72). On the other hand, slow suffusion of CGRP (and of CRF) onto blood vessels in the hamster cheek pouch preparation revealed antipermeability effects on venules (74). Intravenous or subcutaneous administration of test substances in the intact organism has the advantage of distributing the compound evenly within tissues. Also, these routes may be useful if receptors activating processes that reduce leakage face the vascular lumen and are therefore more accessible from the bloodstream. In studies in vivo, however, a false impression of an antipermeabilty effect may occur if there is extensive vasodilation, because this reduces the hydrostatic pressure necessary to produce edema (76) (as mentioned above, the antiedema effects of CRF were independent of hypotension). With the neurotensin-related peptides, a long-lasting antiedema effect was evident with doses of hexapeptide neurotensin fragments that produced only a transient hypotension (61). Thus, we conclude that the systemic anti-inflammatory actions of neurotensin-related peptides were independent of vasodilatation and were anti-inflammatory and not pro-inflammatory.

Vasoactive Intestinal Peptide, Antiflammins, Calcitonin Gene-related Peptide and Other Peptides

A number of other peptides have been reported to possess anti-inflammatory properties (Table 5). VIP is a 28-residue peptide that was first isolated from porcine intestines (77). A similar 38-residue peptide, called pituitary adenyl cyclase-activating peptide (PACAP), has also been described (78). Said (73) summarized the evidence for anti-inflammatory actions of VIP in the upper and lower respiratory tract. VIP inhibited the bronchoconstrictor and vasoconstrictor actions of inflammatory mediators in the lungs. In models of lung injury produced by hydrochloric acid, oxidants or platelet-activating factor, VIP reduced indices of lung injury such as wet:dry lung weight ratio and protein content in bronchoalveolar lavage (79). An impressive action of VIP was its ability to retard pulmonary edema in the isolated perfused lung (80).

Three nonapeptides (Table 5), with similar sequences and corresponding

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

Table 5 Various peptides reported to have anti-inflammatory activities

Peptide[a]	Species	Sequence
		10 20
VIP	Human/pig/rat	HSDAVFTDNY TRLRKQMAVK KYLNSILN–NH_2
Uteroglobin (39–47)	Rabbit	MQMKKVLDS
Lipocortin 1 (246–254)	Human	HDMNKVLDL
Lipocortin 5 (204–212)	Human	SHLRKVFDK
α-MSH		Ac–SYSMEHFRWG KPV–NH_2
		30
CGRP	Human	ACNTATCVTH RLADFLSRSG GVGKNNFVPT NVGSKAF–NH_2
α-ANP (1–28)	Human/dog	SLRRSSCFGG RMDRIGAQSG LGCNSFRY
ANF	Rat	SLRRSSCFGG RIDRIGAQSG LGCNSFRY

[a] VIP, vasoactive intestinal peptide; MSH, melanocyte-stimulating hormone; CGRP, calcitonin gene-related peptide; ANP, atrial natriuretic polypeptide; ANF, atrial natriuretic factor.

to residues 39–47 of rabbit uteroglobin, residues 246–254 of human lipocortin 1 and residues 204–212 of human recombinant lipocortin 5 (hrLC5), respectively, were reported to exhibit anti-inflammatory effects in in vivo models of carrageenan-induced edema in rabbits and rats (81–83). The first two peptides were dubbed antiflammin 1 and antiflammin 2 by Miele et al (81) because of their apparent anti-inflammatory properties. At first, the activity of antiflammins 1 and 2 was attributed to their observed in vitro inhibition of phospholipase A_2, a critical enzyme in the arachidonic acid cascade and inflammation. Other investigators were, however, unable to demonstrate direct inhibition of phospholipase A_2 by or anti-inflammatory activities of antiflammins 1 and 2 (84, 85). Nevertheless, antiflammin 2 has been shown to reduce neutrophil aggregation, chemotaxis, synthesis of platelet-activating factor, and localized inflammatory reactions in the rat skin (86, 87). Peretti et al (83) reported that the nonapeptide from hrLC5 did not directly inhibit porcine phospholipase A_2 activity in a radiochemical assay, yet this peptide reduced phospholipase A_2-dependent processes in vitro, and direct injection of the peptide into the rat hindpaw reduced carrageenan-induced swelling. It

is likely that when more analogs of these peptides are tested a clearer picture of their anti-inflammatory potential will emerge.

Evidence for the anti-inflammatory properties of CGRP is quite recent. Raud et al (74) reported that synthetic human CGRP inhibited the edema-promoting actions of histamine, leukotriene B_4 and 5-hydroxytryptamine in the hamster cheek pouch mucosa, human skin, and rat paw. In contrast to the conclusions of other investigators who view CGRP as pro-inflammatory (72), Raud et al (74) suggested that release of CGRP from sensory nerves may function as an endogenous anti-inflammatory mechanism. Other peptides reported to exhibit anti-inflammatory properties include α-melanocyte-stimulating hormone (α-MSH) and α-MSH derivatives (88), and atrial natriuretic peptide (89–92). *N*-(Fluorenyl-9-methoxycarbonyl) amino acids, intermediates used in peptide synthesis, were also found to be active in some standard tests of anti-inflammatory activity (93).

DISCUSSION

The vascular tree is a complex network of vessels designed to maintain, at its outermost subdivisions, a surface area between blood and tissues for the exchange of gases and nutrients and for the drainage of waste products (94, 95). During the early stages of inflammation, the sensitive mechanisms regulating microvascular perfusion are altered so that vascular patency is diminished, blood contents leak into tissues, and hemostasis may develop (1). In the whole organism, severe and abrupt injury to the microcirculation distorts tissue architecture, impedes delivery of oxygen to cells, and causes extensive fluid loss from the vascular compartment, leading to edema, electrolyte imbalance, shock, and other circulatory disorders (96). The search for and identification of agents that modulate the immediate responses of inflammation may generate drugs with clinical benefit.

In defining the "anti-inflammatory agonist" activity of peptides, it is useful to explicitly state which stage of inflammation is being investigated in the experimental model. Traditionally, pharmacologists have been interested in chronic inflammation because of its importance in persistent conditions such as arthritis, asthma, and related diseases. Bioassays for agonists that suppress the acute phase of inflammation have a shorter time course and are, in principle, faster to conduct, because simple nonselective methods of traumatic injury can be utilized to produce immediate indices of tissue response. We recommend two bioassays as rapid screening tools. The first is measurement of heat-induced swelling and edema. For example, in the anesthetized rat's paw, swelling is readily quantified by the fluid displacement method (97) or by plethysmography (98), and edema by gravimetric comparison of the heated and nonheated paws. A second test to consider is the measurement of a drug's

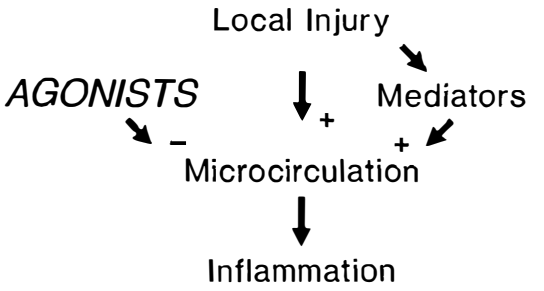
Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

actions against epinephrine-induced pulmonary edema (99, 100). This bioassay yields information about the types of vascular beds acted upon by the drug. Other tests—for example, measurement of antinociception or carrageenan-induced edema—may complement and confirm anti-inflammatory potential. Additional tests of vascular leakage in standard preparations such as the hamster cheek pouch model (8) may help to establish whether a drug's action is exerted locally on postcapillary venules. By necessity, these bioassays have to be conducted in the intact animal, because in vitro models for measuring vascular leakage in the immediate phase of inflammation have yet to be validated.

With regard to the search for prototypes of anti-inflammatory agonists, it may be useful to note that the primary structures of some of the active peptides reviewed in this chapter share common features and may offer clues about structure-activity relationships. For example, CRF(31–41), mystixins, neurotensin-related peptides, antiflammin 1, and VIP have double basic residues, that is, -Arg-Lys-, -Lys-Arg-, -Arg-Arg-, or -Lys-Lys-. A second common feature is the presence of at least two hydrophobic residues, namely, leucine, isoleucine, methionine, or valine. The amino acid sequences and activities of mystixins and neurotensin-related peptides suggest that potency is enhanced by the presence of an aromatic residue between the double basic residues and the two hydrophobic residues at the carboxyl terminus. These common structural attributes suggest the possibility of a unitary biological mechanism for efficacy. A total mass of six residues in a peptide, as exemplified by NT(8–13), may be sufficient for bioactivity. For the endogenous CRF and neurotensin family peptides and the synthetic mystixins, anti-inflammatory activity can be demonstrated at intravenous doses of less than 100 μg/kg.

In summary, we reviewed evidence that certain peptides have the ability to activate biological events which inhibit the immediate manifestations of inflammation. This concept is diagrammed below.



ACKNOWLEDGMENTS

This work was supported in part by National Institute on Drug Abuse Research Grant DA-00091, the Northern California Occupational Health Center, the Chevron Risk Assessment Research Program, and the University of California Toxic Substances Teaching and Research Program.

See note added in proof on page 108.

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

Literature Cited

1. Cotran, R. S., Kumar, V., Robbins, S. L. 1989. Inflammation and repair. In *Robbins: Pathologic Basis of Disease,* ed. S. L. Robbins, R. S. Cotran, V. Kumar, 2:39–86. Philadelphia: Saunders. 4th ed.
2. Saunders, R. N., Handley, D. A. 1987. Platelet-activating factor antagonists. *Annu. Rev. Pharmacol. Toxicol.* 27: 237–55
3. Snyder, D. W., Fleisch, J. H. 1989. Leukotriene receptor antagonists as potential therapeutic agents. *Annu. Rev. Pharmacol. Toxicol.* 29:123–43
4. Bathon, J. M., Proud, D. 1991. Bradykinin antagonists. *Annu. Rev. Pharmacol. Toxicol.* 31:129–62
5. Reuse, J. J. 1948. Comparisons of various histamine antagonists. *Br. J. Pharmacol.* 3:174–80
6. Persson, C. G. A., Svensjo, E. 1985. Vascular responses and their suppression: Drugs interfering with venular permeability. *In Handbook of Inflammation,* ed. I. L. Bonta, M. A. Bray, M. J. Parnham, 5:61–82. Amsterdam/New York/Oxford: Elsevier/North Holland
7. Persson, C. G. A. 1988. Plasma exudation and asthma. *Lung* 166:1–23
8. Svensjo, E. 1990. The hamster cheek pouch as a model in microcirculation research. *Eur. Respir. J.* 3:595s–601s
9. Ryan, G. B., Majno, G. 1977. Acute inflammation. *Am. J. Pathol.* 86:185–276
10. Owens, M. J., Nemeroff, C. B. 1991. Physiology and pharmacology of corticotropin-releasing factor. *Pharmacol. Rev.* 43:425–73
11. Jones, M. T., Gillham, G. 1988. Factors involved in the regulation of adrenocorticotropic hormone/β-lipotropic hormone. *Physiol. Rev.* 68:743–818
12. Vale, W., Spiess, J., Rivier, J. 1981. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. *Science* 213:1394–97
13. Shibahara, S., Morimoto, Y., Furutani, Y., Notake, M., Takahashi, H., et al. 1983. Isolation and sequence analysis of the human corticotropin-releasing factor precursor gene. *EMBO J.* 2:775–79
14. Rivier, J., Spiess, J., Vale, W. 1983. Characterization of rat hypothalamic corticotropin-relcasing factor. *Proc. Natl. Acad. Sci. USA* 80:4851–55
15. Esch, F., Ling, N., Bohlen, P., Baird, A., Benoit, R., et al. 1984. Isolation and characterization of the bovine hypothalamic corticotropin-releasing factor. *Biochem. Biophys. Res. Commun.* 122:899–905
16. Patthy, M., Horvath, J., Mason-Garcia, M., Szoke, B., Schlesinger, D. H., et al. 1985. Isolation and amino acid sequence of corticotropin-releasing factor from pig hypothalami. *Proc. Natl. Acad. Sci. USA* 82:8762–66
17. Okawara, Y., Morley, S. D., Burzio, L. O., Zwiers, H., Lederis, K., Richter, D. 1988. Cloning and sequence analysis of cDNA for corticotropin-releasing factor precursor from the teleost fish *Catostomus commersoni. Proc. Natl. Acad. Sci. USA* 85:8439–43
18. Erspamer, V., Erspamer, G. F., Improta, G., Negri, L., Castiglione, R. D. 1980. Sauvagine, a new polypeptide from *Phyllomedusa sauvagei* skin: occurrence in various *Phyllomedusa* species and pharmacological actions on rat blood pressure and diuresis. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 312: 265–70
19. Lederis, K., Fryer, J., Rivier, J., MacCannell, K. L., Kobayashi, Y., et al. 1985. Neurohormones from fish tails. II: Actions of urotensin I in mammals and fishes. *Recent Prog. Horm. Res.* 41:553–76
20. Rivier, C., Rivier, J., Lederis, K., Vale, W. 1983. In vitro and in vivo ACTH-releasing activity of ovine CRF, sauvagine and urotensin 1. *Regul. Pept.* 5:139

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

21. Chadwick, D. J., Marsh, J., Ackrill, K., eds. 1992. Corticotropin-releasing factor. *Ciba Found. Symp.* 172. Chichester: Wiley
22. Wei, E. T., Kiang, J. G. 1989. Peptides of the corticoliberin superfamily inhibit thermal and neurogenic inflammation. *Eur. J. Pharmacol.* 168:81–86
23. Wei, E. T., Gao, G. C. 1991. Corticotropin-releasing factor: an inhibitor of vascular leakage in rat skeletal muscle and brain cortex after injury. *Regul. Pept.* 33:93–104
24. Kiang, J. G., Wei, E. T. 1987. Corticotropin-releasing factor inhibits thermal injury. *J. Pharmacol. Exp. Ther.* 243:517–20
25. Wei, E. T., Serda, S., Tian, J. Q. 1988. Protective actions of corticotropin-releasing factor on thermal injury to rat pawskin. *J. Pharmacol. Exp. Ther.* 247:1082–85
26. Serda, S. M., Wei, E. T. 1991. Corticotropin-releasing factor inhibits the acute inflammatory response of rat pawskin to cold injury. *Cryobiology* 28:185–90
27. Wei, E. T., Kiang, J. G. 1987. Inhibition of protein exudation from the trachea by corticotropin-releasing factor. *Eur. J. Pharmacol.* 140:63–67
28. Gao, G. C., Dashwood, M. R., Wei, E. T. 1991. Corticotropin-releasing factor inhibition of substance P-induced vascular leakage in rats: possible sites of action. *Peptides* 12:639–44
29. Serda, S. M., Wei, E. T. 1992. Epinephrine-induced pulmonary edema in rats is inhibited by corticotropin-releasing factor. *Pharmacol. Res.* 26:85–91
30. Rivier, J., Rivier, C., Vale, W. 1984. Synthetic competitive antagonists of corticotropin-releasing factor: effect on ACTH secretion in the rat. *Science* 224:889–91
31. Wei, E. T., Kiang, J. G., Buchan, P., Smith, T. W. 1986. Corticotropin-releasing factor inhibits neurogenic plasma extravasation in the rat paw. *J. Pharmacol. Exp. Ther.* 238:783–87
32. Wei, E. T., Wong, J. C., Kiang, J. G. 1990. Decreased inflammatory responsiveness of hypophysectomized rats to heat is reversed by a corticotropin-releasing factor (CRF) antagonist. *Regul. Pept.* 27:317–23
33. Poree, L., Dickenson, A. H., Wei, E. T. 1989. Corticotropin-releasing factor inhibits the response of trigeminal neurons to noxious heat. *Brain Res.* 502:349–55
34. Lembeck, F., Holzer, P. 1979. Substance P as a neurogenic mediator of antidromic vasodilitation and neurogenic plasma extravasation. *Arch. Pharm.* 310:175–83
35. Hargreaves, K. M., Mueller, G. P., Dubner, R., Goldstein, D., Dionne, R. A. 1987. Corticotropin-releasing factor (CRF) produces analgesia in humans and rats. *Brain Res.* 422:154–57
36. Skofitsch, G., Jacobowitz, D. M. 1985. Distribution of corticotropin-releasing factor-like immunoreactivity in the rat brain by immunohistochemistry and radioimmunoassay: Comparison and characterization of ovine and rat/human CRF antisera. *Peptides* 6: 319–36
37. Berkenbosch, F., Linton, E. A., Tilders, F. J. H. 1986. Colocalization of peptide histidine isoleucine amine and corticotropin-releasing factor immunoreactivity in neurons of the rat hypothalamus: a surprising artefact. *Neuroendocrinology* 44:338–46
38. Hargreaves, K. M., Dubner, R., Costello, A. H. 1989. Corticotropin-releasing factor (CRF) has peripheral site of action for antinociception. *Eur. J. Pharmacol.* 170:275–79
39. Hargreaves, K. M., Flores, C. M., Dionne, R. A., Mueller, G. P. 1990. The role of pituitary beta-endorphin in mediating corticotropin-releasing factor-induced antinociception. *Am. J. Physiol.* 258:E235–42
40. Wen, X. L., Guo, Z. G., Datta, N. 1991. The use of corticotropin releasing factor (CRF) for the treatment of postoperative cerebral edema. A preliminary study. *China Med. J.* 104:557–61
41. Wei, E. T., Gao, G. C., Sessler, D. I. 1990. *Clin. Pharmacol. Ther.* 47:192 (Abstr.)
42. Hurley, J. V. 1984. Inflammation. In *Edema*, ed. N. C. Staub, A. E. Taylor, pp. 463–88. New York: Raven. 774 pp.
43. Joyner, W. L., Wei, E. T. 1989. Mechanism for the anti-inflammatory effect of corticotropin-releasing factor (CRF). *FASEB J.* 3:272
44. Joyner, W. L., Macek, B. A., Wei, E. T. 1990. Inhibition of the bradykinin induced inflammation by corticotropin-releasing factor (CRF). *FASEB J.* 4: 1123
45. Dashwood, M. R., Andrews, H. E., Wei, E. T. 1987. Binding of [^{125}I]Tyr-corticotropin-releasing factor to rabbit aorta is reduced by removal of the endothelium. *Eur. J. Pharmacol.* 135: 111–12
46. McDonald, D. M. 1990. The ultra-

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

structure and permeability of tracheobronchial blood vessels in health and disease. *Eur. Respir. J.* 12:57s–585s (Suppl.)

47. Gabbiani, G., Majno, G. 1980. Pathophysiology of small vessel permeability. In *Microcirculation*, ed. G. Kaley, B. M. Altura, 3:143–63. Baltimore: Univ. Park Press
48. Kiang, J. G., Poree, L., Wei, E. T. 1987. Anti-inflammatory activity of corticotropin-releasing factor. II. Mechanisms of action. *Proc. West. Pharmacol. Soc.* 30:63–65
49. Montecucchi, P. C., Gozzini, L. 1982. Secondary structure prediction of sauvagine, a novel biologically active polypeptide from the frog. *Int. J. Pept. Protein Res.* 20:139–43
50. Pallai, P. V., Mabilia, M., Goodman, M., Vale, W., Rivier, J. 1983. Structural homology of corticotropin-releasing factor, sauvagine, and urotensin I: Circular dichroism and prediction studies. *Proc. Natl. Acad. Sci. USA* 80: 6770–74
51. Lau, S. H., Rivier, J., Vale, W., Kaiser, E. T., Kezdy, F. J. 1983. Surface properties of an amphiphilic peptide hormone and its analog: Corticotropin-releasing factor and sauvagine. *Proc. Natl. Acad. Sci. USA* 80: 7070–74
52. Thomas, H. A., Ling, N., Wei, E. T. 1992. Corticotropin-releasing factor as anti-inflammatory agonist. In *Hans Selye Symp. Neuroendocrinol. Stress, 3rd*, ed. Y. Tache, C. Rivier, Montreal, Canada, Oct. 11–14
53. Stewart, J. M., Young, J. D. 1984. *Solid Phase Peptide Synthesis*, pp. 42–43. Rockford, IL: Pierce Chem. Co.
54. Chibber, B. A. K., Urano, S., Castellino, F. J. 1990. Synthesis, purification, and properties of a peptide that enhances the activation of human [Glu1]plasminogen by tissue plasminogen activator and retards fibrin polymerization. *Int. J. Pept. Protein Res.* 35:73–80
55. Hanukoglu, I., Fuchs, E. 1983. The cDNA sequence of a type II cytoskeletal keratin reveals constant and variable structural domains among keratins. *Cell* 33:915–24
56. Quax, W., Egberts, W. V., Hendriks, W., Quax-Jeuken, Y., Bloemendal, H. 1983. The structure of the vimentin gene. *Cell* 35:215–23
57. Nagle, R. B. 1988. Intermediate filaments: a review of the basic biology. *Am. J. Surg. Pathol.* 12(Suppl. 1):4–16
58. Goldman, G., Welbourn, R., Alexander, S., Klausner J. M., Wiles, M., et al. 1991. Modulation of pulmonary permeability in vivo with agents that effect the cytoskeleton. *Surgery* 109: 533–38
59. Alexander, J. S., Patton, W. F., Yoon, M. U., Shepro, D. 1991. Cytokeratin filament modulation in pulmonary microvascular endothelial cells by vasoactive agents and culture confluency. *Tissue Cell* 23:141–50
60. Araki, K., Tachibana, S., Uchiyama, M., Nakajima, T., Yashuhara, T. 1973. Isolation and structure of a new active peptide "xenopsin" on the smooth muscle, especially on a strip of fundus from a rat stomach, from the skin of *Xenopus laevis*. *Chem. Pharm. Bull.* 21:2801–4
61. Gao, G., Wei, E. T. 1992. Xenopsin, neurotensin, and neurotensin-related peptides inhibit vascular leakage in the rat after tissue injury. *J. Pharmacol. Exp. Ther.* In press
62. Carraway, R. E., Leeman, S. E. 1973. The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. *J. Biol. Chem.* 248:6854–61
63. Leeman, S. E., Carraway, R. E. 1982. Neurotensin: discovery , isolation, characterization, synthesis and possible physiological roles. *Ann. NY Acad. Sci.* 400:1–16
64. Granier, C., Van Rietschoten, J., Kitabgi, P., Poustis, C., Freychet, P. 1982. Synthesis and characterization of neurotensin analogues for structure/activity relationship studies. *Eur. J. Biochem.* 124:117–25
65. Kitabgi, P., Checler, F., Mazalla, J., Vincent, J. P. 1985. Pharmacology and biochemistry of neurotensin receptors. *Rev. Clin. Basic Pharmacol.* 5: 397–486
66. Tanaka, K., Masu, M., Nakanishi, S. 1990. Structure and functional expression of the cloned rat neurotensin receptor. *Neuron* 4:847–54
67. Gilbert, J. A., McCormick, D. J., Penning, M. A., Kanba, K. S., Enloe, L. J., et al. 1989. Neurotensin(8–13): comparison of novel analogs for stimulation of cyclic GMP formation in neuroblastoma clone N1E-115 and receptor binding to human brain and intact N1E-115 cells. *Biochem. Pharmacol.* 38:3377–82
68. Cochrane, D. E., Emigh, C., Levine, G., Carraway, R. E., Leeman, S. E. 1982. Neurotensin alters cutaneous vascular permeability and stimulates histamine release from isolated skin. *Ann. NY Acad. Sci.* 400:396–97

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.

69. Kerouac, R., St-Pierre, S., Rioux, F. 1984. Histamine release by neurotensin in the rat hindquarter: structure activity studies. *Peptides* 5:695–99
70. Foreman, J. C., Jordan, C. C., Piotrowski, W. 1982. Interaction of neurotensin with the substance P receptor mediating release from rat mast cells and the flare in human skin. *Br. J. Pharmacol.* 77:531–39
71. Rampart, M., Williams, T. J. 1986. Polymorphonuclear leukocyte-dependent plasma leakage in the rabbit skin is enhanced or inhibited by prostacyclin, depending on the route of administration. *Am. J. Pathol.* 124:66–73
72. Brain, S. D., Williams, T. J. 1988. Neuropharmacology of peptides in skin. *Sem. Dermatol.* 7:278–83
73. Said, S. I. 1991. Neuropeptides (VIP and tachykinins): VIP as a modulator of lung inflammation and airway constriction. *Am. Rev. Respir. Dis.* 143: S22–24
74. Raud, J., Lundberg, T., Brodda-Jansen, G., Theodorsson, E., Hedqvist, P. 1991. Potent anti-inflammatory action of calcitonin gene-related peptide. *Biochem. Biophys. Res. Commun.* 180: 1429–35
75. Karalis, K., Sano, H., Redwine, J., Listwak, S., Wilder, R. L., et al. 1991. Autocrine or paracrine inflammatory actions of corticotropin-releasing hormone in vivo. *Science* 254: 421–23
76. Grega, G. J., Adamski, S. W. 1988. The role of venular endothelial cells in the regulation of macromolecular permeability. *Microcirc. Endothelium Lymphat. 4:143–67*
77. Said, S. I. 1986. Vasoactive intestinal peptide. *J. Endocrinol. Invest.* 9:1919–200
78. Ogi, K., Kimura, C., Onda, H., Arimura, A., Fujino, M. 1990. Molecular cloning and characterization of cDNA for the precursor of rat pituitary adenylate cyclase activating polypeptide (PACAP). *Biochem. Biophys. Res. Commun.* 173:1271–79
79. Berisha, H., Foda, H., Sakakibara, H., Trotz, M., Pakbaz, H., et al. 1990. Vasoactive intestinal peptide prevents lung injury due to xanthine/xanthine oxidase. *Am. J. Physiol.* 259: 151–55
80. Berisha, H., Foda, H., Pakbaz, H., Said, S. I. 1988. *Clin. Res.* 36:855A (Abstr.)
81. Miele, L., Cordella-Miele, E., Facchiano, A., Mukherjee, A. B. 1988. Novel anti-inflammatory peptides from the region of highest similarity between uteroglobin and lipocortin I. *Nature* 335:726–30
82. Miele, L., Cordella-Miele, E., Facchiano, A., Mukherjee, A. B. 1990. Inhibition of phospholipase A2 by uteroglobin and antiflammin peptides. In *Biochemistry, Molecular Biology, and Physiology of Phospholipase A2 and Its Regulatory Factors*, ed. A. B. Mukherjee, pp. 137–60. New York: Plenum
83. Perretti, M., Becherucci, C., Mugridge, K. G., Solito, E., Silvestri, S., et al. 1991. A novel anti-inflammatory peptide from human lipocortin 5. *Br. J. Pharmacol.* 103: 1327–32
84. Binsbergen, J. V., Slotboom, A. J., Arsman, A. J., Hass, G. H. 1989. Synthetic peptide from lipocortin I has no phospholipase A2 inhibitory activity. *FEBS Lett.* 247:293–97
85. Marki, F., Pfeilschifter, J., Rink, H., Wiesenberg, I. 1990. Antiflammins: two nonapeptide fragments of uteroglobin and lipocortin I have no phospholipase A2-inhibitory and anti-inflammatory activity. *FEBS Lett.* 264:171–75
86. Camussi, G., Tetta, C., Bussolini, F., Baglioni, C. 1990. Antiinflammatory peptides (antiflammins) inhibit synthesis of platelet-activating factor, neutrophil aggregation and chemotaxis, and intradermal inflammatory reactions. *J. Exp. Med.* 171:913–27
87. Camussi, G., Tetta, C., Baglioni, C. 1990. Antiflammins inhibit synthesis of platelet-activating factor and intradermal inflammatory reactions. See Ref. 82, pp. 161–72
88. Hiltz, M. E., Catania, A., Lipton, J. M. 1991. Anti-inflammatory activity of a-MSH(11–13) analogs: influences of alteration in stereochemistry. *Peptides* 12:767–71
89. Inomata, N., Ohnuma, N., Furuya, M., Hayashi, Y., Kanai, Y., et al. 1987. Alpha-human atrial natriuretic peptide (alpha-hANP) prevents pulmonary edema induced by arachidonic acid treatment in isolated perfused lung from perfused guinea pig. *Jpn. J. Pharmacol.* 44:211–14
90. Imamura, T., Ohnuma, N., Iwasa, F., Furuya, M., Hayashi, Y., et al. 1988. Protective effect of alpha-human atrial natriuretic polypeptide (alpha-hANP) on chemical-induced pulmonary edema. *Life Sci.* 42:403–14
91. Naruse, S., Takei, R., Horikawa, Y., Tanaka, C., Ebisu, T., et al. 1990.

Effects of atrial natriuretic peptide on brain oedema: the change of water, sodium, and potassium contents in the brain. *Acta Neurochir. Suppl.* 51:118–21

92. Nakao, N., Itakura, T., Yokote, H., Nakai, K., Komai, N. 1990. Effect of atrial natriuretic peptide on ischaemic brain oedema. *Acta Neurochir. Suppl.* 51:201–3
93. Burch, R. M., Weitzberg, M., Blok, N., Muhlhauser, R., Martin, D., et al. 1991. N-(Fluorenyl-9-methoxycarbonyl) amino acids, a class of anti-inflammatory agents with a different mechanism of action. *Proc. Natl. Acad. Sci. USA* 88:355–59
94. Clough, G. 1991. Relationship between microvascular permeability and ultrastructure. *Prog. Biophys. Mol. Biol.* 55:47–69
95. Staub, N. C., Taylor, A. E., eds. 1984. *Edema*. New York: Raven. 774 pp.
96. Messmer, K., Kreimeier, U. 1989. Microcirculatory therapy in shock. *Resuscitation* 18:S51–S61 (Suppl.)
97. Arturson, G., Jakobsson, O. P. 1985. Oedema measurements in a standard burn model. *Burns* 12:1–7
98. van Arman, C. G. 1979. Oedema and increased vascular permeability. *Handb. Exp. Pharmacol.* 50(Part 2):44–74
99. Shigei, T., Sakuma, A., Enomoto, T., Oh-Ishi, S., Hatano, R. 1967. Pulmonary edema induced by adrenaline and related amines in rats, and its modification by various pretreatments. *Jpn. J. Pharmacol.* 17:591–602
100. Malik, A. B., Minnear, F. L., Popp, A. J. 1983. Catecholamine-induced pulmonary edema. *Gen. Pharmacol.* 14: 55–60
101. Litchfield, J. T., Wilcoxon, F. 1949. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* 96:99–113

Note Added in Proof

In a separate line of inquiry, E.V. Fuchs and associates (A. Letai, P.A. Coulombe, E. Fuchs, Do the ends justify the mean? Proline mutations at the ends of the keratin coiled-coil rod segment are more disruptive than internal mutations. *J. Cell Biol.* 116:1992, 1181–195) have reported that the highly conserved –RKLLE– region of intermediate filaments seems critical for maintaining normal cell architecture.

Annu. Rev. Pharmacol. Toxicol. 1993.33:91-108. Downloaded from www.annualreviews.org by Central College on 12/09/11. For personal use only.