dependent manner. The minimum effective dose was not constant, probably due to differing ages of the donors but adrenaline (10 μg/ml) consistently stimulated lipolysis. Propranolol (10 μg/ml) inhibited glycerol release.

During incubation with adrenaline there was an increase in prostaglandin, assayed as prostaglandin E_2, released into the incubation medium (3.0 ± 1.2 ng/g) compared with control (1.0 ± 0.5). Prostaglandin content of control and adrenaline-treated fat were 1.9 ± 0.7 and 1.0 ± 0.2 respectively. Indomethacin completely blocked formation of prostaglandins in the fat and incubation medium. In the presence of dexamethasone (2 μg/ml), although the concentration of prostaglandins in the medium was slightly reduced (2.2 ± 0.5) the prostaglandin content of the fat was raised (7.2 ± 0.9) above that of fat stimulated with adrenaline alone.

Synthesis of prostaglandins was further investigated by incubating [^{14}C]-arachidonic acid with human fat microsomes for 30 min at 37°C. After extraction with diethyl ether, the extracts were subjected to thin-layer chromatography in benzene:dioxan:acetic acid 60:30:3 (Morrison, Nishikawa & Needleman, 1977).

After incubation, two peaks of radioactivity which co-chromatographed with prostaglandin $F_{2\alpha}$ and prostaglandin E_2 were produced. According to the amount of protein present, the conversion to prostaglandins was 0.4–2%. Adrenaline (50 μg/ml) markedly increased the synthesis of prostaglandins. In the solvent system used, 6-keto $F_{1\alpha}$ and prostaglandin $F_{2\alpha}$ have similar mobilities.

These results show that human fat stimulated with adrenaline synthesises prostaglandins during lipolysis. The sensitivity of human fat to adrenaline confirms that catecholamines have a role in regulation of fat mobilization in man. Indomethacin blocked synthesis of prostaglandins in human fat while dexamethasone prevented their release but not their synthesis. As prostaglandin $F_{2\alpha}$ and 6-keto-prostaglandin $F_{1\alpha}$ had the same chromatographic mobility, we are investigating whether this prostacyclin metabolite is also formed by human fat.

This work was supported by grants from the Medical Research Council and the Arthritis and Rheumatism Council. We wish to thank Mr J.A. Southam, Epsom District Hospital, for the samples of human adipose tissue and Dr J.E. Pike of the Upjohn Company for the gift of prostaglandins.

References

CHANG, J., LEWIS, G.P. & PIPER, PRISCILLA J. (1977). Inhibition by glucocorticoids of prostaglandin release from adipose tissue *in vitro*. *Br. J. Pharmac.*, **59**, 425–432.

MORRISON, A.R., NISHIKAWA, K. & NEEDLEMAN, P. (1977). Unmasking of thromboxane A_2 synthesis by ureteral obstruction in the rabbit kidney. *Nature, Lond.*, **267**, 259–260.

SHAW, J.E. & RAMWELL, P. (1968). The release of prostaglandin from rat epididymal fat pad in neurons and hormonal stimulation. *J. Biol. Chem.*, **243**, 1498–1503.

Chemical mediators of vascular responses in inflammation: a two mediator hypothesis

T.J. WILLIAMS (introduced by G.P. LEWIS)

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, London WC2A 3PN

Since the work of Thomas Lewis (Lewis, 1927) on the skin reactions to histamine, putative mediators of inflammation have been evaluated according to their ability to mimic inflammatory reactions. Here, a different approach is suggested, namely that the chemical mediation of increased vascular permeability, and vasodilatation, be considered separately.

Plasma exudation and blood flow changes were measured in rabbit skin using [^{131}I]albumin and ^{133}Xe, as previously described (Williams, 1976a).

Intradermally-injected E-type prostaglandins were found to be potent at increasing blood flow with little vascular permeability-increasing activity (Williams, 1976b). However, bradykinin and histamine increased vascular permeability, but were not potent vasodilators (e.g. bradykinin 10,000 times less potent than prostaglandin E_1 on a molar basis, blood flow measured by ^{133}Xe clearance). In terms of inflammatory oedema, the importance of prostaglandins is that they are potent potentiators of histamine- and bradykinin-induced exudation (Williams & Morley, 1973; Moncada, Ferreira & Vane, 1973). From later observations (Williams, 1976b) it is concluded that prostaglandins do not affect histamine- or bradykinin-induced increased

vascular permeability, but that exudation is increased because of the vasodilator activity of prostaglandins; (the precise physical mechanism underlying this remains undetermined).

The interaction between permeability-increasing mediators and vasodilator mediators, as proposed in an inflammatory reaction, can be simulated by an intradermal injection of a mixture of a kinin-releasing enzyme, kallikrein (mammalian pancreas) and the substrate for prostaglandin E_1 synthesis, dihomo-γ-linolenate; (similar results were obtained with arachidonate). Kallikrein (0.5 µg, 0.005 u) produced some exudation (21.4 ± 4.2 µl), whereas dihomo-γ-linolenate (0.5 µg) produced vasodilatation with little exudation (5.0 ± 1.6 µl). Kallikrein/dihomo-γ-linolenate mixtures produced a potentiated response (136.2 ± 10.7 µl). Exudation potentiation was inhibited either by the prostaglandin synthetase inhibitor, indomethacin (1 µg injected locally 5 min previously: 136.2 to 48.6 ± 6.2 µl), or by the kallikrein inhibitor, aprotinin (1 µg, 7.1 KIU similarly injected: 136.2 to 25.6 ± 8.5 µl). As predicted by the hypothesis, the inhibitory substances could be distinguished by their effects on blood flow: indomethacin inhibited blood flow and (therefore) exudation; aprotinin inhibited exudation only.

Examination of *Bordetella pertussis* oedema provided further support for the hypothesis. Injection of 2.5×10^8 organisms produced exudation and increased blood flow with a peak at 60–90 minutes. Injection of prostaglandin E_1 (0.1 µg), 60 min after *B. pertussis* injection into the same site, resulted in exudation potentiation; (*B. pertussis*/saline = 14.8 ± 3.4 µl, saline/prostaglandin $E_1 = 5.3 \pm 1.6$ µl, *B. pertussis*/prostaglandin $E_1 = 35.1 \pm 3.8$ µl). This demonstrates the presence of an endogenous permeability-increasing mediator. Similarly, bradykinin (0.5 µg) injected 60 min after *B. pertussis* also resulted in exudation potentiation; (*B. pertussis*/saline = 7.8 ± 1.5 µl, saline/bradykinin = 18.4 ± 2.0 µl, *B. pertussis*/bradykinin = 91.8 ± 3.0 µl). This demonstrates the presence of an endogenous vasodilator mediator, the production of which was inhibited by indomethacin (Williams, 1977).

These results suggest that inflammation involves the release (not necessarily concurrently) of mediators important for their permeability-increasing activity, which are probably not products of cyclo-oxygenase activity; and vasodilator mediators, notably E-type prostaglandins. The magnitude of exudation depends on the levels of both types of mediators. (Doses: µg/0.1 ml; results: mean ± s.e. mean, $n = 6$).

This work is supported by the Medical Research Council. Bradykinin was a gift from Sandoz Products Ltd. Excellent technical assistance was provided by M.J. Peck.

References

LEWIS, T. (1927). *The Blood Vessels of the Human Skin and their Responses*. London: Shaw & Sons, Ltd.

MONCADA, S., FERREIRA, S.H. & VANE, J.R. (1973). Prostaglandins, aspirin-like drugs and the oedema of inflammation. *Nature (Lond.)*, **246**, 217–218.

WILLIAMS, T.J. (1976a). Simultaneous measurement of local plasma exudation and blood flow changes induced by intradermal injection of vasoactive substances, using [^{131}I]albumin and ^{133}Xe. *J. Physiol. (Lond.)*, **254**, 4–5P.

WILLIAMS, T.J. (1976b). The pro-inflammatory activity of E-, A-, D- and F-type prostaglandins and analogues 16, 16-dimethyl-PGE_2 and (15S)-15-methyl-PGE_2 in rabbit skin; the relationship between potentiation of plasma exudation and local blood flow changes. *Br. J. Pharmac.*, **56**, 341–342P.

WILLIAMS, T.J. (1977). Potentiation of bradykinin-induced exudation following intradermal injection of particulate or colloidal materials in the rabbit: evidence for prostaglandin release and action in inflammation. *Br. J. Pharmac.*, **60**, 291–292P.

WILLIAMS, T.J. & MORLEY, J. (1973). Prostaglandins as potentiators of increased vascular permeability in inflammation. *Nature (Lond.)*, **246**, 215–217.