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Some possible mechanisms of action of an endogenous anti-inflammatory protein

J. BIRD* & D.A. LEWIS

Department of Pharmacy, The University of Aston, Gosta Green, Birmingham B4 7ET

Inflammatory exudates obtained by implantation of polyester sponges in rats have been shown to be anti-inflammatory (Robinson & Robson, 1966) and the active component is known to be a protein (Billingham, Robinson & Robson, 1969). The mechanism of action of these proteins is not known but mechanisms suggested include lysosomal stabilization (Doherty & Robinson, 1976a), interaction with the complement system (Doherty & Robinson, 1976b) or by a counter irritant mechanism (Atkinson, Boura & Hicks, 1969).

The methods used to produce the inflammatory exudate was that employed by Robinson & Robson (1966) except that aseptic techniques were used throughout. Fractionation was carried out on a Sephadex G-150 column, (Billingham, Robinson & Robson, 1969), producing two pooled samples of material.

In this work we have examined the action of the crude exudate and its fractions on monocytes. The monocytes were isolated from guinea-pigs as previously described (Lewis, Best & Bird, 1977) and incubated with various concentrations of the crude exudate and its fractions (1 to 100 mg/ml). After incubation the degree of stabilisation was assessed by assaying for acid phosphatase (Symons, Lewis & Ancill, 1969). It was found that the crude exudate significantly stabilized the monocytes at all concentrations used. Both of the fractions stabilized the monocytes at lower concentrations, but exhibited lytic properties at the higher concentrations used.

It was found that the exudate possessed inherent proteolytic activity (Rinderknecht, Geokas, Silverman & Haverback, 1968) and is autolytic *in vitro* releasing dialysable peptides.

To determine if this autolytic property was of importance an *in vivo* model was developed. This model consisted of aseptically implanting small dialysis sacs, containing 100 mg of exudate in 0.5 ml saline, into rats and leaving them for 10 days to recover. A carrageenin oedema paw test was then carried out on the animals which were compared to groups of sham operated animals and animals implanted with dialysis sacs containing saline alone. The results showed that implantation of dialysis sacs alone was not anti-inflammatory, but that the exudate containing sacs were anti-inflammatory ($P < 0.05$). This could only be due to low molecular weight molecules produced by proteolysis inside the sacs entering into the rats circulation.

In conclusion, this particular inflammatory exudate exhibited a multivalent mode of action. That is, components within the exudate were capable of stabilizing inflammatory cells and also the exudate was capable of producing dialysable peptides by autolytic action which are either anti-inflammatory themselves or which acted as triggers for the production of an anti-inflammatory substance *in vivo*.

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Stimulation of colonic mucus output in the rat

J.W. BLACK, JEAN E. BRADBURY & J.H. WYLLIE

Department of Pharmacology, University College and Department of Surgery, University College Hospital Medical School, London.

Mucus secreted by the colon provides a vital protective and lubricative lining. However the pharmacology of colonic secretion is a neglected field of investigation.

The colonic mucosa is rich in goblet cells and also in 5-hydroxytryptamine (5-HT). A technique has been developed to explore the possible relation of this amine (and also other pharmacological agents) to colonic mucus output.

The lumen of the colon of the anaesthetized rat was perfused at 20 ml/min by recirculating 20 ml of 6 mM N-acetyl cysteine in 155 mM NaCl. Mucus was estimated as the amount (in mg) of total hexose recovered per hour (Winzler, 1955).

The table shows that 5-HT, L-5-hydroxytryptophan (L5HTP) and L-tryptophan, all significantly increased hexose output from the rat colon, as compared with the response in saline-treated control animals. Carbachol also produced increased hexose output, but isoprenaline and histamine did not.

As the dosage of the effective agonists was increased, mean mucus output rose, and then fell again with high doses of the agonists. The dose of each agonist which produced the greatest mucus output was used in studies of the effects of antagonists. As expected, atropine (i.v. load 5 μmole/kg, infusion 100 nmole kg^{-1} min^{-1}) abolished the effect of carbachol but it had no effect on the response to 5-HT or 5HTP. On the other hand chlorpromazine (i.v. load 3 μmole/kg, infusion 100 nmole kg^{-1} min^{-1}) abolished the response to the indoles but did not affect that to carbachol.

Phentolamine (i.v. 110 nmole kg^{-1} min^{-1}) and morphine (i.v. load 0.1 μmole/kg, infusion 10 nmole kg^{-1} min^{-1}) were ineffective in suppressing the actions of 5-HT and L5HTP on colonic mucus output. However, methysergide (i.v. load 4 μmole/kg, infusion 100 nmole kg^{-1} min^{-1}) did reduce the effect of L5HTP and 5-HT, but it also produced a significant increase in hexose output when given alone.

Table 1

Exp.		*No of Rats*	*Mucus Output mg Hex/h (Mean ± 2 s.e. mean)*	*t*
1	Saline	8	0.28 (0.21–0.37)	
	5HT (10 n mol kg^{-1} min^{-1})	8	1.03 (0.85–1.24)	6.52**
	L5HTP (5 μmol kg^{-1} min^{-1})	8	0.98 (0.69–1.41)	6.30**
	L-Tryptophan (122 μmol kg^{-1} + 12.2 μmol kg^{-1} min^{-1})	8	0.69 (0.52–1.92)	4.51**
2	Saline	8	0.25 (0.19–0.32)	
	Carbachol (30 nmol kg^{-1} min^{-1})	8	1.41 (1.11–1.78)	9.76***
3	Saline	4	0.315 (0.253–0.394)	
	Isoprenaline (30 nmol kg^{-1} min^{-1})	4	0.318 (0.29–0.349)	0.129 N.S.
4	Saline	4	0.449 (0.395–0.511)	
	Histamine (100 nmol kg^{-1} min^{-1})	4	0.400 (0.332–0.481)	1.8 N.S.

** $P < 0.01$.
*** $P < 0.001$.
Asymmetric limits are derived from calculation with logarithmically transformed data.