Cutaneous reaction to prostaglandins E_1,E_2 and $F_2\alpha$ in the bovine

Recent findings have shown that some prostaglandins of the E series induce inflammatory responses in the guinea-pig (Horton, 1963), rat (Kaley & Weiner, 1968; Arora, Lahiri & Sanyal, 1970; Crunkhorn & Willis, 1971a) and man (Bergstrom, Duner & others, 1959; Solomon, Juhlin & Kirschenbaum, 1968; Crunkhorn & Willis, 1971a; Søndergaard & Greaves, 1971; Greaves, Søndergaard & McDonald-Gibson, 1971). We have been interested in the dermal responses of cattle to histamine, 5-HT, kinins, and in passive cutaneous anaphylaxis (Wells & Eyre, 1970), and in view of the inflammatory potency of prostaglandins, and the suggestion by Crunkhorn & Willis (1971a & b) that cutaneous reactions to prostaglandins may be a consequence of liberated histamine, it seemed of interest to investigate these compounds in the bovine species.

Male Guernsey or Jersey calves, 30–50 kg, and aged 4 to 8 wk were depilated using calcium thioglycolate cream (Wells & Eyre, 1970). Five per cent Pontamine blue solution (E. Gurr Ltd., London, England) (0·6 ml/kg, i.v.) was administered immediately before 0·2 ml intradermal injections of various concentrations of prostaglandins (PGs) E_1, E_2, and $F_2\alpha$, compound 48/80, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate monohydrate (5-HT) and bradykinin triacetate were made. All injected compounds were dissolved in isotonic sodium chloride solution. PGs E_1, E_2, and $F_2\alpha$ were freshly prepared by diluting stock solutions containing 100 μg/ml in 95% ethanol. Skin-reactions appeared as ovoid or circular blue wheals and were measured with calipers at 30 min (maximal response). A threshold reading of 1·0 cm was established arbitrarily on the basis that intradermal injections of 0·2 ml saline gave skin-reaction diameters of 0·4–0·8 cm. The results are in Table 1.

Intradermal injections of PGE_1, PGE_2, $PGF_2\alpha$, compound 48/80 and isotonic saline were made in three calves anaesthetized with pentobarbitone. Subcutaneous tissues adjacent to the skin-reactions were removed at 5, 60 and 120 min, fixed in 80% ethanol, stained with toluidine blue (Riley, 1959) and examined for mast cell changes. Mepyramine maleate (5 mg/kg) was administered 30 min before similar intradermal injections in a further two animals.

Histamine, bradykinin, PGE_1 and PGE_2 produced increased permeability within 5 min of intracutaneous injection. The potencies of PGE_1 and PGE_2 were similar to each other on a weight basis, but greater than either histamine or bradykinin. $PGF_2\alpha$ produced no observable increase in vascular permeability although at high

Table 1. *Cutaneous responses to PGE_1, PGE_2, $PGF_2\alpha$, histamine, 5-HT and bradykinin.* Cutaneous responses smaller than the threshold value of 1·0 cm diameter are designated as subthreshold (s.t.). The higher dose (500 μg) was tested only with compound 48/80.

Drugs	No. of animals	Mean diameters of skin-reactions elicited by intradermal injection of drugs in the doses (μg) indicated						
		0·002	0·02	0·2	2·0	20	200	500
PGE_1	5	s.t.	1·3 ± 0·3	1·5 ± 0·3	1·5 ± 0·3	1·6 ± 0·2	2·1 ± 0·2	—
PGE_2	6	s.t.	1·0 ± 0·1	1·4 ± 0·1	1·5 ± 0·2	1·7 ± 0·1	2·1 ± 0·2	—
$PGF_2\alpha$	5	s.t.	s.t.	s.t.	s.t.	s.t.	s.t.	—
Histamine	6	s.t.	s.t.	1·2 ± 0·1	1·7 ± 0·2	2·4 ± 0·2	2·8 ± 0·3	—
Bradykinin	6	s.t.	s.t.	1·1 ± 0·2	1·4 ± 0·3	1·6 ± 0·1	2·2 ± 0·4	—
Compound 48/80	5	s.t.	s.t.	s.t.	1·0 ± 0·1	1·1 ± 0·1	1·3 ± 0·2	1·3*

* Mean of two animals tested.

doses (>100 ng) some skin blanching was observed. 5-HT did not produce permeability changes but manifested itself as an erythematous spot. Admixtures with $PGF_2\alpha$ did not influence the cutaneous permeability threshold of E_1, E_2 or histamine but a mixture of compound 48/80 (500 μg) and $PGF_2\alpha$ (10 μg) produced a ten fold reduction in the threshold response for 48/80.

Subcutaneous mast cells were not disrupted to any observable extent by PGE_1, PGE_2 or $PGF_2\alpha$. There was some granular spilling from cells but apparently no greater than that in saline-injected sites. In addition, compound 48/80 (200 μg) produced only moderate mast cell degranulation in similar tissue biopsy samples. Pretreatment with mepyramine reduced the permeability changes induced by histamine and compound 48/80 10–100 fold, but this antagonist was completely ineffective against the lesions caused by either PGE_1 or PGE_2.

Thus both PGE_1 and PGE_2 effectively increase local vascular permeability in calf skin, at doses that were comparable with those of other suggested inflammatory mediators (histamine and bradykinin). The relatively short onset of action (5 min) corresponds with that described by Crunkhorn & Willis (1971a) in rat skin.

That the threshold doses of E_1 and E_2 were not altered by mepyramine may indicate that these prostaglandins do not exert major vascular permeability changes by indirectly releasing histamine from the mast cell. This suggestion is also supported by the failure of either PGE_1 or PGE_2 (when administered intradermally) to degranulate the subcutaneous mast cells. The failure of $PGF_2\alpha$ to interfere with local vascular changes caused by PGE_1 and PGE_2, yet reducing the response to compound 48/80, further suggests a direct action of the E series. This agrees with the observations of Kaley & Weiner (1971) and Arora & others (1970) who examined PGE_1 in the rat skin. However there is some disagreement on this point because it has been suggested in contrast that the vascular changes induced by PGE_1 and PGE_2 in the rat are mediated via histamine and 5-HT released from mast cells in the skin (Crunkhorn & Willis, 1971a & b). These authors showed that $PGF_2\alpha$ interfered both with the E-series and with compound 48/80-mediated cutaneous reactions in the rat. It has been shown that PGE_1 releases histamine from rat mast cells and from human skin (Von Euler & Eliassen, 1967; Søndergaard & Greaves, 1971).

In conclusion, it seems that both PGE_1 and PGE_2 mediate an inflammatory response in calf skin mainly by direct action. Although Bergstrom (1967) has described the presence of prostaglandins in some bovine tissues, there is no evidence as yet for their presence in the skin of this species, and it remains to be seen whether either PGE_1 or PGE_2 is released during the inflammatory process *per se*.

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Isomeric substrate studies with normal and atypical serum cholinesterase

Atypical and usual serum cholinesterase are structurally different and have been separated by chromatography on a DEAE column (Liddell, Lehmann & Silk, 1962). The evidence indicates that the enzymes differ in their amino-acid content. Clark, Glaubiger & La Du (1968) have shown that the pK of the atypical enzyme is lower than that of the usual enzyme. This, together with information obtained from choline affinity studies, has led these workers to suggest a different ionizable group at the binding site (i.e. anionic site) of the atypical enzyme. They also suggest that results from dephosphorylation studies indicate differences between the esteratic sites of the two enzymes.

It was therefore decided to investigate the isomeric substrate specificity of atypical serum cholinesterase to determine whether detectable changes had taken place in the geometry of the esteratic site.

Homozygous atypical and normal human sera obtained from single individuals were diluted 1 : 50 before use. The rates of hydrolysis of acetyl (ACh), butyryl (BuCH) and benzoyl (BzCh) choline and of D-butyryl-α-(D-Bu α-MeCh), L-butyryl-α-(L-Bu α-MeCh), D-butyryl-β- (D-Buβ- MeCh) and L-butyryl-β-(L-Bu β-MeCh)-methylcholine were determined manometrically at 37° in a Warburg apparatus (Beckett, Harper & Clitherow, 1963). In each case, V_{max} was determined over the substrate concentration range 5×10^{-4} to 5×10^{-2}M using diluted serum (1·5 ml) in a total volume of 3 ml. The data were corrected for non enzymic hydrolysis and the K_m and V_{max} values were determined from Lineweaver and Burke double reciprocal plots.

The substrate characteristics, K_m and V_{max} of the substrates studied with the usual and atypical sera are presented in Table 1. As reported generally, the K_m values are consistently lower, and the maximal rates of hydrolysis consistently higher for normal serum as compared with atypical serum.

With the exception of BzCh, the relative rates of hydrolysis of the acylcholines were similar for both sera as shown in Table 2, and the pattern is similar to that obtained for horse serum and purified horse serum cholinesterase by Beckett, Mitchard & Clitherow (1968).

The data presented in this paper are similar to those obtained by Davies, Morton & Kalow (1960) for the hydrolysis of a homologous series of choline esters by usual and atypical human sera. They too showed a wide variation in the ratio atypical K_m: normal K_m and a relatively constant ratio atypical V_{max}: normal V_{max} for all the aliphatic substrates studied; in both studies the rate of hydrolysis of BzCh was relatively faster by atypical serum than for any other substrate. Many workers (Kalow & Staron, 1957; Clark & others, 1968; Erdos, Foldes & others, 1959) have reported significant differences between cholinesterase characteristics obtained with aliphatic and with aromatic choline esters.