during primary aggregation. Aggregation and 5-HT release were inhibited by agents (PGE_1 0.1 μM; PGD_2 0.1 μM; adenosine 1 μM) which increase intracellular cyclic AMP, and by dibutyryl cyclic AMP (0.5 mM). Secondary aggregation and the associated release of 5-HT were blocked by indomethacin (0.1 mM), but this inhibitory effect could be readily overcome by increasing the concentration of agonist. 2-n-amylthio-AMP (10–100 μM) blocked secondary but not primary aggregation responses. 2-n-amylthio-AMP is apparently a specific ADP antagonist: it inhibits primary aggregation induced by ADP but not by adrenaline, vasopressin or 5-HT, and its inhibitory effect (unlike that of PGD_2, PGE_1 or adenosine) is not blocked by 9-(tetrahydro-2 furyl) adenine (SQ 22536), an inhibitor of platelet adenylate cyclase (Harris, Phillips & Goldenberg, 1975).

The actions of the prostaglandin analogues Wy 17,186, 16,16 dimethyl PGE_2 and azo-PGH_2 are similar, and are more complex than previously recognized. They directly induce a primary aggregation response and stimulate secondary aggregation and release by an indomethacin sensitive process, possibly triggered by platelet-platelet contact in the primary phase. In addition, they induce secondary aggregation and release by an indomethacin insensitive process. The secondary aggregation responses appear to be mediated by released ADP. In these respects, the complicated behaviour of these compounds resembles that of the native endoperoxides (Salzman, unpublished observations).

We thank Dr D. Harris, Squibb, for SQ 22,536; Dr K. Kikugawa, Kohjin Company for 2-n-amylthio AMP; Dr R.L. Fenichel, Wyeth, for Wy 17,186; and Dr J.E. Pike, Upjohn Company, for the other prostaglandins. This study was supported by grants from the M.R.C., the Arthritis and Rheumatism Council and the American N.H.L.I.

References

COREY, E.J., NICOLAU, K.C., MACHIDA, Y., MALMSTEN, C.L. & SAMUELSSON, B. (1975). Synthesis and biological properties of a 9,11-azo-prostanoid: highly active biochemical mimic of prostaglandin endoperoxides. *Proc. Nat. Acad. Sci.*, **72**, 3355–3358.

GORDON, J.L. & MacINTYRE, D.E. (1976). Stimulation of platelets by bis-enoic prostaglandins. *Br. J. Pharmac.*, **58**, 298–299P.

HARRIS, D.N., PHILLIPS, M.B. & GOLDENBERG, H.J. (1975). Effect of substituted purines on the inhibition by PGE_1 of ADP-induced aggregation of human platelets and on the increase in cyclic AMP induced in platelets by PGE_1. *Fed. Proc.*, **34**, 617.

Levels of prostaglandin F and E in cerebrospinal fluid of cats during pyrogen-induced fever

A.S. MILTON, SUSAN SMITH & K.B. TOMKINS

Department of Pharmacology, University of Aberdeen

Prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) occur in cat brain (Horton & Main, 1967) however, Feldberg, Gupta, Milton & Wendlandt (1973) using bioassay failed to detect either prostaglandin in cat cerebral spinal fluid (CSF). Since $PGF_{2\alpha}$ is pyrogenic in cats (Ewen, Milton & Smith, 1976) we have measured the levels of PGF in the CSF of afebrile and febrile animals by the more sensitive techniques of radioimmunoassay.

Pyrogen (the O-somatic antigen of *Shigella dysenteriae*) dissolved in sterile, pyrogen-free 0.9% saline was injected intravenously (20 μg/kg) in a volume not exceeding 0.4 ml. Control animals were injected with a similar volume of sterile 0.9% saline. Two samples of CSF (approximately 0.5 ml) were collected from the cisterna magna before injection and four samples were collected afterwards. The interval between successive CSF samples was 75 minutes. Rectal temperature was recorded continuously.

Samples of CSF were extracted with ethyl acetate and assayed for PGE (after alkaline conversion to prostaglandin B) and PGF by a double antibody technique (Dighe, Emslie, Henderson, Simon & Rutherford, 1975) without prior separation.

Two distinct types of febrile responses to pyrogen injection were observed. In some animals which had not previously received pyrogen the fever developed after a long latency (90 min), whereas in animals which had received pyrogen before, the fever developed after a short latency (30 min). The level of PGF and PGE measured in CSF taken during the two types of fever and during control experiments are shown in the Table.

In the short latency fevers significant increases of both PGEs and PGFs which paralleled the increases in deep body temperature were seen, and these results are compatible with both prostaglandins having a role

Table 1 Control and febrile PGE and PGF levels (in pg/ml) in cat CSF

	Sample No.	*Control*	*Long latency*	*Short latency*
PGE	1	137 ± 17	144 ± 24	180 ± 48
	2	137 ± 17	124 ± 4	185 ± 58
	3	138 ± 18	126 ± 6	1756 ± 1355**
	4	142 ± 11	259 ± 101	455 ± 193**
	5	129 ± 10	331 ± 154	1773 ± 682**
PGF	1	84 ± 4	80	109 ± 39
	2	92 ± 8	93 ± 13	130 ± 21
	3	115 ± 24	210 ± 56	759 ± 174***
	4	116 ± 35	181 ± 56	1303 ± 400***
	5	80	142 ± 42	1428 ± 578***
	6	115 ± 35	80	769 ± 170***

Comparison with control values.
Students 't' test *** $P < 0.025$
** $P < 0.05$
Values are the mean ± s.e. mean of 4 observations. Controls $n = 8$.

in pyrogen-induced fever. However, it should be realized that the precursors of these prostaglandins, namely prostaglandins G and H and the thromboxanes are themselves pharmacologically-active and may also be involved in fever.

During the long latency fevers, no significant increases in either the PGE or the PGF levels were found. The reasons for these differences between the short latency and long latency responses have not yet been determined.

This work was supported by a grant from the Medical Research Council. The O-somatic antigen of *Shigella dysenteriae* was kindly supplied by W.H.O. Laboratories, Hampstead, and prostaglandins by Dr John Pike, Upjohn Company.

References

DIGHE, K.K., EMSLIE, H.A., HENDERSON, L.K., SIMON, L. & RUTHERFORD, F. (1975). The development of antisera to prostaglandins B_2 and $F_{2\alpha}$ and their analysis using solid-phase and double antibody radioimmunoassay methods. *Br. J. Pharmac.*, **55**, 503–514.

EWEN, LYNDA, MILTON, A.S. & SMITH, SUSAN. (1976). The effect of prostaglandin $F_{2\alpha}$ and prostaglandin D_2 on body temperature of conscious cats. *J. Physiol. (Lond.)*, **258**, 121P.

FELDBERG, W., GUPTA, K.P., MILTON, A.S. & WENDLANDT, S. (1973). Effect of pyrogen and antipyretics on prostaglandin activity in cisternal CS.F. of unanaesthetized cats. *J. Physiol. (Lond.)*, **234**, 279–303.

HORTON, E.W. & MAIN, I.H.M. (1967). Identification of prostaglandins in the central nervous system of the cat and the chicken. *Br. J. Pharmac.*, **30**, 582–602.

Sex differences in guinea-pig brain prostaglandins and the effect of indomethacin

A. BENNETT, ERIKA M. CHARLIER, BHARTI RAJA & I.F. STAMFORD

Department of Surgery, King's College Hospital Medical School, London SE5 8RX

Adult albino guinea-pigs were stunned and their brains removed quickly. Groups of 2–6 brains were pooled, cut into approximately 2 mm cubes and washed thoroughly with Krebs solution. Samples (1–5 g) were homogenized (30 s; immediately or after incubation at 37°C) in Krebs solution alone or with indomethacin or acid ethanol (Bennett, Stamford & Unger, 1973). The prostaglandin (PG)-like material was extracted (Unger, Stamford & Bennett, 1971) and assayed on rat gastric fundus (Bennett *et al.*, 1973). Results were compared using the Mann-Whitney U test unless otherwise stated.

Using Krebs solution alone the biological activities (medians and semiquartile ranges, ng PGE_2 equivalents/g fresh tissue) were: male 5.8 (4.5 to 17)