*J. Physiol.* (1979), **287**, *pp.* 519–533
*With 8 text-figures*
*Printed in Great Britain*



# EFFECTS OF CENTRAL ADMINISTRATION OF PROBENECID ON FEVERS PRODUCED BY LEUKOCYTIC PYROGEN AND $PGE_2$ IN THE RABBIT

BY I. L. CRAWFORD, J. I. KENNEDY, J. M. LIPTON* AND S. R. OJEDA

*From the Departments of Physiology, Neurology and Pharmacology, Southwestern Medical School, University of Texas Health Science Center at Dallas, Dallas, Texas, 75235, U.S.A. and Veteran's Administration Hospital, Dallas, Texas, 75216, U.S.A.*

(*Received 26 June 1978*)

SUMMARY

1. Single intracerebroventricular (I.C.V.) injections of probenecid (PBCD, 0·125–0·5 mg) enhanced and prolonged fever caused by I.V. administration of leukocytic pyrogen (LP) in rabbits resting in neutral (23 °C), cold (10 °C) and hot (30 °C) environments. Similar effects were produced by single I.C.V. injections of PBCD given before $PGE_2$ (0·5 $\mu$g) was injected I.C.V. in the three ambient temperatures.

2. Fever produced by I.V. LP was also prolonged by infusion and by multiple injections of PBCD.

3. PBCD given I.P. (100 mg/kg) enhanced and prolonged fever caused by I.V. injection of *Salmonella typhosa* endotoxin.

4. Hyperthermia produced by I.C.V. $PGE_2$ was not augmented by subsequent PBCD infusion. However, pre-treatment with PBCD followed by $PGE_2$ injection and PBCD infusion caused hyperthermia that was very high and prolonged, and, in some cases, lethal.

5. Acetaminophen (2 mg, I.C.V.) and indomethacin (10 mg/kg, I.V.) lowered body temperature when given during fever induced by LP and prolonged by PBCD infusion.

6. The concentration of PGE in cerebrospinal fluid (C.S.F.) samples taken from the third or lateral ventricles rose or stabilized during PBCD infusions made during LP fever. However, similar changes in PGE concentration also occurred during control infusions when body temperature was low.

7. We conclude that termination of the actions of both central endogenous pyrogen and centrally administered $PGE_2$, and the subsequent reduction of fevers produced by them, require a PBCD-sensitive facilitated transport system. The reduction of PBCD-prolonged LP fevers by antipyretics which block PGE synthesis suggests that prolongation by PBCD of LP fever is not due to blockade of PGE transport in a subsequent step in fever mediation *per se*, but is due to inhibition of transport of LP itself, or of other mediators associated with it.

* To whom reprint requests should be sent.

## INTRODUCTION

Fever results from the action of endogenous pyrogens on central controls of body temperature. However, little is known about how endogenous pyrogens act on the brain to initiate and maintain fever and how the actions of pyrogens once in the brain are terminated in defervescence. Numerous processes might be responsible for termination of pyrogenic effects in the brain but it is especially appropriate to consider transport processes since they provide a primary mechanism for the removal of endogenous chemicals from the central nervous system (Davson, 1976). For example, the removal of central prostaglandins, some of which have been proposed to have a role in mediation of fever (Feldberg & Saxena, 1971; Milton & Wendlandt, 1971), has been shown to occur through saturable, facilitated transport processes across both the choroidal and extrachoroidal regions of the blood-brain barrier (Bito, Davson & Hollingsworth, 1976*a*; Bito, Davson & Salvador, 1976*b*). With the hypothesis that facilitated transport processes are required for termination of the action of pyrogens in the brain we examined the effects of intracerebroventricular administration of probenecid, a drug known to block transport of weak organic acids (Brazeau, 1975), on fever produced by leukocytic pyrogen and $PGE_2$.

## METHODS

*Animals.* Forty-three male albino New Zealand rabbits were used. The animals were individually caged in a 21–23 °C ambient temperature. Food and water were available *ad libitum*. All experiments were performed in an environmental chamber in which the temperature was controlled within 0·5 °C of the setting.

*Surgical procedures.* The rabbits were anaesthetized with ketamine hydrochloride (Vetalar, Parke-Davis; 20 mg/kg, I.M.) and pentobarbitone sodium (Nembutal, Abbott Laboratories; 30 mg/kg, I.P.) and placed in a Kopf rabbit head holder (David Kopf Instruments, Tujunga, Calif.) which had been modified (Lipton & Romans, 1976) and fitted with a horizontal zero plane locator (Crawford, Kennedy & Lipton, 1977). Either a single cannula with a rubber dam (David Kopf Instruments, no. 201) or a cannula made of 20 gauge stainless-steel tubing which was fitted with an obturator and embedded in a threaded pedestal with a plastic cap, was implanted in each rabbit. Cannulae were implanted in the third ventricle in thirty rabbits and in a lateral ventricle in the remaining animals using stereotaxic co-ordinates derived from the atlases of Sawyer, Everett & Green (1954) and Fifková & Maršala (1967). After the superior sagittal sinus was retracted a single cannula was placed in the third ventricle according to the co-ordinates: 2·5 mm anterior to bregma, on the mid line, and down until c.s.f. rose in the cannula (11–13 mm below the brain surface). The co-ordinates used for placing each cannula into a lateral ventricle were: 1·0 mm anterior to bregma, 2–3 mm lateral, vertical until c.s.f. rose in the cannula. Dental acrylic was used to secure the cannula to stainless-steel screws driven into the calvarium. Benzathine penicillin G (Bicillin L-A, Wyeth Laboratories) was given post-operatively (150,000 u., I.M.). Locations of cannulae were determined in histological studies of brain tissue fixed in Bouin's solution.

*Injections and infusions.* Probenecid (PBCD, *p*-dipropylsulphamoyl benzoic acid, Sigma Chemical Co.) was solubilized in non-pyrogenic isotonic saline (Abbott Laboratories) by titration with 1 N-NaOH. Amounts of the base equal molar to the PBCD concentrations (100 $\mu$g/ml.) were required to convert the insoluble acidic drug to its water-soluble Na salt. The same quantity of NaOH was added to saline as a vehicle control. All solutions were passed through sterile membrane filters (0·2 $\mu$m, Millipore) and gas sterilized before use. All single I.C.V. injections of PBCD were made in a volume of 50 $\mu$l. followed by either a 20 $\mu$l. saline flush or a slow infusion of PBCD. Infusions were controlled by varying the speed of a syringe pump. Fever was produced by injecting 0·10 ml. leukocytic pyrogen (LP) into a marginal ear vein. The LP was produced by incubating heparinized rabbit blood, to which *Salmonella typhosa* endotoxin 1 $\mu$g/ml. (Difco

no. 0901) had been added, at 38 °C for 1 hr. The blood was centrifuged at 1500 rev/min for 20 min and the plasma layer removed; saline in volume equal to one half that of red cells was then added. After a further 5 hr of incubation followed by centrifugation for 15 min at 3000 rev/min, the supernatant containing LP was removed. The stock $PGE_2$ was stored in ethanol which was evaporated by a stream of nitrogen, the remaining solid was re-dissolved in saline just before injection. Acetaminophen was dissolved in non-pyrogenic saline. Indomethacin was dissolved in an Na carbonate solution. Precautions taken to reduce the probability of contamination with extraneous pyrogens are described elsewhere (Harris & Lipton, 1977).

*Procedures for measuring concentrations of PGE in c.s.f.* Aliquots (20 $\mu$l.) of c.s.f. obtained from cannulae in the third and lateral ventricles were kept frozen until concentrations were determined. Samples were assayed without previous extraction. Before the assay a volume of 180 $\mu$l. 0·01 M-K phosphate buffer (pH 7·4) containing 0·15 M-NaCl, 0·01% $NaN_3$ and 0·1% bovine serum albumin was added to each sample. This buffer was also used to dilute the [$^3$H]$PGE_2$ standards for the assay. The concentration of $PGE_2$ in the samples was determined by a radioimmunologic assay similar to that described by Jaffe & Behrman (1974) in which dextran-coated charcoal is used to separate the antibody-bound prostaglandin from the free prostaglandin. Antiserum to $PGE_2$ was generously provided by Dr F. Kohen of the Weizman Institute, Israel. Its specificity has been reported previously (Bauminger, Zor & Lindner, 1973). Although it reacts to some extent with PGAs and PGBs, its cross-reactivity with PGF is negligible (less than 0·5%). Since the antiserum does not distinguish between $PGE_2$ and $PGE_1$, values obtained using this serum are expressed as PGE equivalents. The sensitivity of the assay was 7·0 pg of added $PGE_2$. The standard curve was linear between 7 and 125 pg. Intra-assay variation was less than 9%. Three different dilutions of cerebral tissue extracts exhibited inhibition curves that were parallel to those of the $PGE_2$ standards (Ojeda, Naor & McCann, 1978).

*General procedures.* Conscious rabbits were restrained in conventional rabbit holders and a thermistor probe was inserted about 100 mm into the rectum and taped to the tail. The temperature of the dorsal surface of the ear, an indicator of periphal vasomotor tone, was recorded using a thermistor probe. Temperature recordings were made using an automatic temperature recording system (Datalogger, United Systems Corp.) or a polygraph (Grass Instruments). No treatments were given until at least 60 min after the rectal probe was put in place. The thermal response index (TRI), the area under the fever curve, was calculated according to procedures described by Clark & Cumby (1975).

## RESULTS

### *Effects of single* I.C.V. *injections of PBCD on fever produced by LP and $PGE_2$*

Injection of PBCD into the third ventricle 10 min before I.V. injection of LP augmented subsequent fever in rabbits in neutral, hot, and cold environments (Fig. 1). Similar PBCD injections 10 min before $PGE_2$ was given I.C.V. also enhanced and prolonged the ensuing hyperthermias in a dose-related manner in all three environments. The augmentation by PBCD of fevers produced by LP and $PGE_2$ were roughly parallel in the neutral and hot environments and the TRI in the cold showed considerable overlap. In the hot environment elevated base line rectal temperatures tended to limit the maximal thermal response.

In twenty-seven experiments on seven animals LP was given alone or followed by 0·5 mg PBCD I.C.V. at the peak of fever or only PBCD was given. A single injection of 0·5 mg PBCD given at or near the peak temperature caused the fevers to be prolonged (Fig. 2, upper) in all experiments. PBCD alone had either no effect on rectal temperature or decreased it. In five rabbits with fever induced by LP 0·5 mg PBCD was given at the peak of fever, 70–80 min later, and again 1 hr after the second injection. In all animals the injections of PBCD caused transient decreases in rectal temperature followed by rises or stabilization (Fig. 2, lower) such that fever was prolonged for periods of over 12 hr. I.C.V. injections of PBCD diluent in the same

animals given LP on another occasion did not prolong or enhance the febrile response. In a single rabbit 0·5 mg PBCD given I.C.V. 1 hr before LP was injected caused a febrile response that was similar in rate of rise to those seen after LP alone. However, the fever was prolonged and body temperature was still elevated (40·4 °C) when recording was stopped 9 hr after the LP injection.

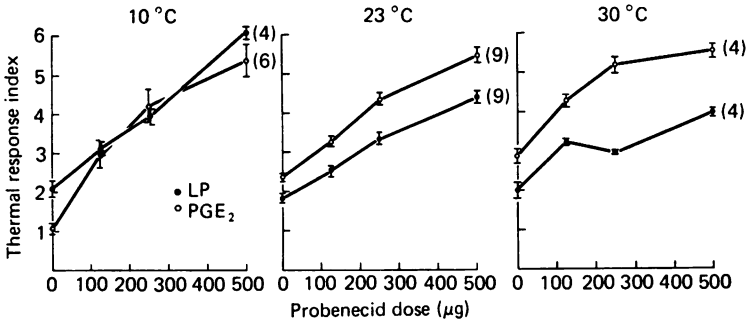


Fig. 1. Augmentation of febrile responses produced by I.V. LP (0·1 ml.) and I.C.V. $PGE_2$ (0·5 μg) by single I.C.V. injections of PBCD given 10 min before LP and $PGE_2$ in three ambient temperatures. Scores represent mean (± S.E. of mean) 5-hr thermal response indexes. Numbers of animals are given in parentheses.

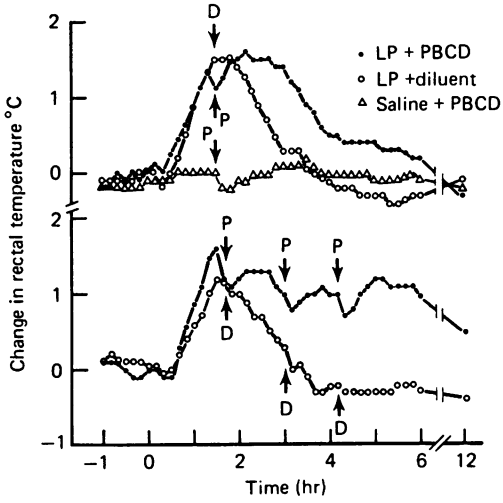


Fig. 2. Augmentation of febrile response produced by LP when PBCD (0·5 mg) is given at fever peak (upper) and when multiple PBCD injections are given (lower). LP or control saline injection given at zero; time of other injections indicated by arrows. P = PBCD injection. D = diluent injection. Examples from a single animal with third ventricular cannula.

### *Effects of peripheral administration of PBCD and endotoxin*

PBCD was injected peripherally (100 mg/kg, I.P.) in four rabbits 1 hr after *Salmonella typhosa* endotoxin (1 mg/kg, I.V.) was given. These PBCD injections made during the chill phase of bacterial endotoxin fever caused rectal temperature to remain at high levels (39·6–40·1 °C) for the duration of the 12 hr recording period

(Fig. 3). Single I.P. injections of PBCD decreased rectal temperature 0·1–1·0 °C and hyperthermia did not ensue. In two rabbits duration of fever produced by endotoxin, as measured from the time of endotoxin injection until rectal temperature returned to base line, was approximately 6 hr, in agreement with fever durations seen earlier (Harris & Lipton, 1977). However, when PBCD was administered at the fever peak, 2 hr after endotoxin was given, rectal temperature rapidly rose an additional 0·7 °C in one rabbit and remained elevated for over 8 hr. In the other rabbit, rectal temperature rapidly rose to 42·7 °C, whereupon the animal developed convulsions and died.

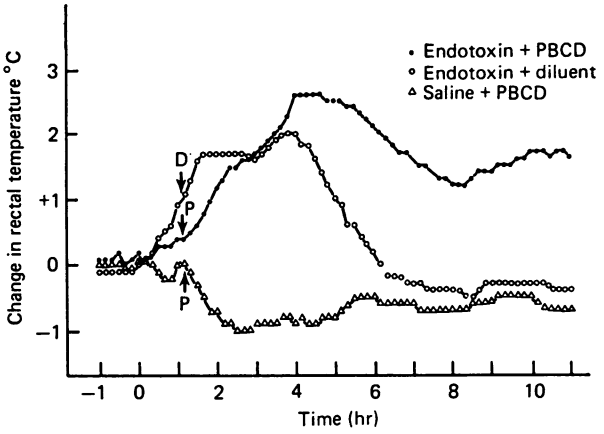


Fig. 3. Enhancement and prolongation of fever caused by I.V. bacterial endotoxin injection (1 mg/kg) by I.P. injection of PBCD (100 mg/kg) in a single rabbit. Endotoxin or saline control injection given at zero, other injections as noted in Fig. 2.

### *Effects of* I.C.V. *infusion of PBCD on LP fever*

Bolus injection plus infusion of PBCD 10 min after LP was given increased peak fever and 5 hr TRIs (Fig. 4, Table 1). The $TRI_5$ values obtained after PBCD were almost twice those seen when control infusions of diluent were given. Although rectal temperatures returned to base line (criterion: within 0·2 °C of base line) within 5 hr after LP injection when the animals received control infusions of diluent, the temperatures in PBCD infusion experiments were still elevated when recording was stopped 9–13 hr after LP injection. When the concentration of the PBCD bolus was reduced by half and the bolus injection and infusion delayed 60–70 min after the LP injection, in two rabbits, the peak fever and the $TRI_5$ was increased (Table 1) and rectal temperature returned to base line in 7 and 9 hr.

Antipyretics reduced LP fevers which had been augmented by PBCD. Acetaminophen (1 mg) given I.C.V. during prolonged fever at 1 hr after PBCD infusion was stopped and again 18 min later, caused vasodilatation in the ears and decreased rectal temperature (Fig. 5, Table 2). Indomethacin (10 mg/kg, I.V.) also caused rapid defervescence when given during PBCD-prolonged fever (Fig. 5, Table 2) at a dose which was found to be antipyretic, but not hypothermogenic, in pilot experiments.

*Effects of PBCD infusion on $PGE_2$ hyperthermia*

An I.C.V. bolus injection of 0·125–0·5 mg PBCD followed by PBCD infusion, did not consistently increase or prolong hyperthermia caused by prior I.C.V. administration of 0·5 $\mu$g $PGE_2$ (Fig. 6, Table 3). Control infusion augmented the fever considerably in some cases suggesting that any possible potentiation of $PGE_2$

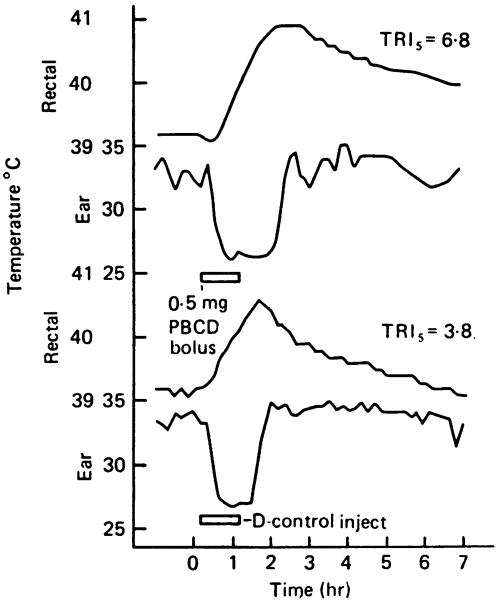


Fig. 4. A single I.C.V. PBCD injection and infusion (50 $\mu$g/min) 10 min after I.V. LP enhanced and prolonged fever (upper). Lower panel shows fever after control injection and infusion of diluent. LP given at zero. Examples from a single rabbit.

TABLE 1. Enhancement and prolongation of LP fever by I.C.V. bolus injection plus infusion of PBCD

| Animal | Delay between LP and PBCD injections (min) | PBCD Bolus (mg) | PBCD Infusion Rate ($\mu$g/min) | PBCD Infusion Duration (hr) | Max. $\Delta T$ (°C) | $TRI_5$ | Fever Duration (hr) |
|---|---|---|---|---|---|---|---|
| R3V-31 | 10 | 0·5 | 50 | 1 | 2·3 | 8·1 | > 13 |
| | 10 | Dil. | Dil | 1 | 1·7 | 4·4 | < 5 |
| R3V-40 | 10 | 0·5 | 50 | 1 | 1·8 | 7·6 | > 11 |
| | 10 | Dil. | Dil. | 1 | 1·4 | 4·1 | < 5 |
| R3V-42 | 10 | 0·5 | 50 | 1 | 1·7 | 6·8 | > 9 |
| | 10 | Dil. | Dil. | 1 | 1·4 | 3·8 | < 5 |
| R3V-29 | 70 | 0·25 | 50 | 1 | 1·2 | 4·1 | 9 |
| | 70 | Dil. | Dil. | 1 | 0·8 | 2·3 | < 5 |
| R3V-47 | 60 | 0·25 | 50 | 1 | 1·7 | 6·6 | 7 |
| | 60 | Dil. | Dil. | 1 | 1·5 | 4·3 | < 5 |

hyperthermia might have been obscured by the effects of diluent infusion. In eleven experiments on 6 rabbits with lateral ventricular cannulae there were also no marked differences in $PGE_2$ hyperthermia after PBCD and after diluent infusions.

Although $PGE_2$ fevers were not differentially affected by PBCD and diluent infusions given after the $PGE_2$ injections, marked hyperthermias were induced by pre-treating the animals with PBCD (Fig. 7). Four rabbits given a bolus injection

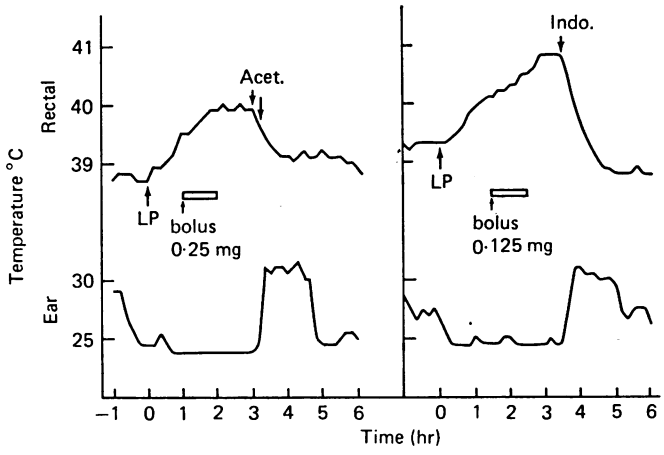


Fig. 5. Injections of acetaminophen I.C.V. (left, 1 mg at each arrow) and indomethacin I.V. (right, 10 mg/kg) reduced LP fever that had been augmented by PBCD.

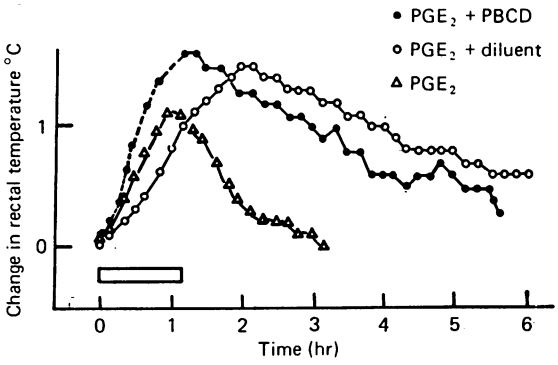


Fig. 6. Effects on rectal temperature of I.C.V. $PGE_2$ (0·5 $\mu$g) alone and followed by: I.C.V. PBCD injection (0·5 mg) and infusion (50 $\mu$g/min); by control injection and infusion of diluent; in a single rabbit.

(0·25 mg) and infusion (12·5–50 $\mu$g/min) of PBCD for 10 min before $PGE_2$ was injected, followed by further infusion for 50–60 min, showed large increases in rectal temperature. In two rabbits rectal temperature rose rapidly and both animals died in marked hyperthermia. Of the two survivors one had a rectal temperature of 40·8 °C and the other of 40·1 °C when recording was stopped 10 hr after the initial PBCD injection.

TABLE 2. Antipyretics reduce LP fever augmented by PBCD

| | | PBCD | | | Antipyretic | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Infusion | | | | | |
| Animal | Delay between LP or saline and PBCD injections (hr) | Bolus (mg) | Rate ($\mu$g/min) | Duration (hr) | Type/Dose | Time given (hr after infusion) | $\Delta T$ at time given | Effect on body temperature |
| R3V-27 | 1·3 (LP) | 0·125 | 50 | 1 | Acet./2 mg | 1 | 1·5 | ↓ |
| | 1·3 (S) | 0·125 | 50 | 1 | Acet./2 mg | 1 | 0·6 | → |
| | 1·3 (LP) | Dil. | Dil. | 1 | Acet./2 mg | 1 | 1·0 | → |
| R3V-45 | 1 (LP) | 0·25 | 50 | 1 | Acet./2 mg | 1 | 1·3 | ↓ |
| | 1 (S) | 0·25 | 50 | 1 | Acet./2 mg | 1 | 0·2 | → |
| | 1 (LP) | Dil. | Dil. | 1 | Acet./2 mg | 1 | 0·8 | → |
| RLV-72 | 1·5 (LP) | 0·25 | 50 | 1 | Acet./2 mg | 1 | 1·1 | ↓ |
| R3V-27 | 1·3 (LP) | 0·125 | 50 | 1 | Indo./10 mg/kg | 1 | 1·4 | ↓ |
| | 1·3 (LP) | 0·125 | 50 | 1 | Indo./10 mg/kg | 1 | 1·3 | ↓ |
| R3V-44 | 0·17 (LP) | 0·3 | 100 | 1 | Indo./10 mg/kg | 2.3 | 1·5 | ↓ |
| R3V-45 | 0·17 (LP) | 0·5 | 100 | 1 | Indo./10 mg/kg | 2 | 1·1 | ↓ |
| R3V-46 | 0·17 (LP) | 0·25 | 50 | 1 | Indo./10 mg/kg | 3·2 | 1·2 | ↓ |

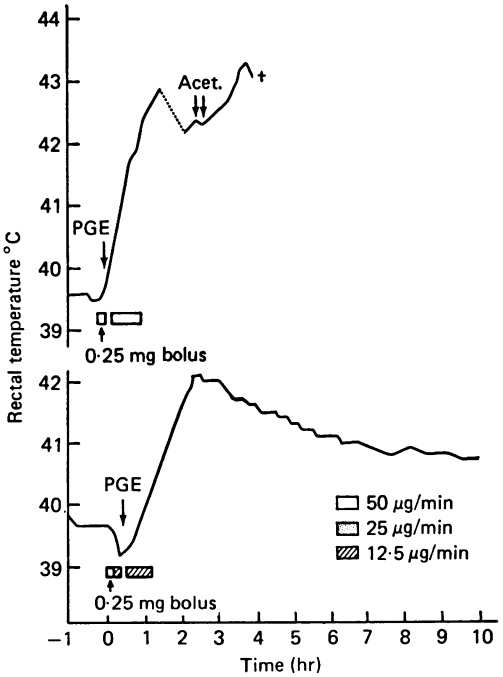


Fig. 7. PBCD bolus injection and infusion before, plus PBCD infusion after, I.C.V. $PGE_2$ (0·5 μg) caused life-threatening (lower) and lethal (upper) hyperthermias. Break in upper record indicates period of artificial cooling. Examples from single animals; 1 mg acetaminophen given I.C.V. at each arrow.

TABLE 3. I.C.V. PBCD injections and infusions given after I.C.V. $PGE_2$ (0·5 μg) did not augment $PGE_2$ hyperthermia

| | | PBCD | | | | |
|---|---|---|---|---|---|---|
| | | | Infusion | | | |
| Animal | Delay after $PGE_2$ (hr) | Bolus (mg) | (μg/min) Rate | Duration (hr) | Max. $\Delta T$ | $TRI_5$ |
| R3V-42 | 0·17 | 0·5 | 50 | 1 | 1·5 | 5·8 |
| | 0·17 | Dil. | Dil. | 1 | 1·6 | 5·8 |
| R3V-44 | 0·17 | 0·5 | 50 | 1 | 1·1 | 4·0 |
| | 0·17 | Dil. | Dil. | 1 | 0·9 | 5·6 |
| | 0·17 | 0·25 | 50 | 0·5 | 1·1 | 3·4 |
| | 0·17 | Dil. | Dil. | 0·5 | 0·7 | 3·2 |
| R3V-45 | 0·17 | 0·5 | 50 | 1 | 1·5 | 5·8 |
| | 0·17 | Dil. | Dil. | 1 | 1·6 | 5·4 |
| | 0·17 | 0·25 | 50 | 0·5 | 1·5 | 10·5 |
| | 0·17 | Dil. | Dil. | 0·5 | 1·2 | 8·2 |
| | 0·5 | 0·125 | 25 | 1·5 | 0·9 | 4·0 |
| | 0·5 | Dil. | Dil. | 1·5 | 1·3 | 5·9 |
| R3V-46 | 0·5 | 0·25 | 50 | 1 | 1·4 | 5·9 |
| | 0·5 | Dil. | Dil. | 1 | 1·3 | 4·8 |
| R3V-47 | 0·5 | 0·125 | 50 | 1·5 | 1·4 | 10·8 |
| | 0·5 | Dil. | Dil. | 1·5 | 1·5 | 8·3 |

*PGE concentration of c.s.f. during PBCD-augmented LP fever*

The effects on body temperature of PBCD and antipyretics in these experiments were generally the same as those described in previous sections: PBCD enhanced and prolonged LP fever, and acetaminophen (2 mg, I.C.V.) and indomethacin (10 mg/kg, I.V.) reduced body temperature when given during the prolonged fevers (Fig. 8). In agreement with previous findings by other investigators (Feldberg & Gupta, 1973; Feldberg, Gupta, Milton & Wendlandt, 1973) we found that PGE concentration of c.s.f. taken from a lateral or the third ventricle increased in fever

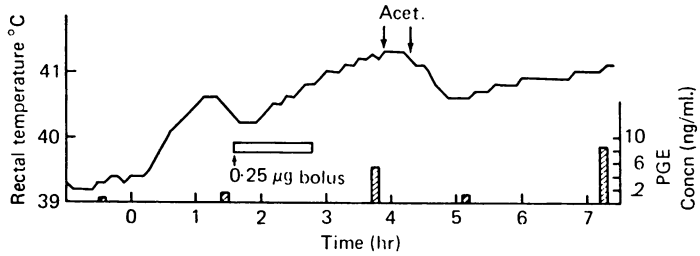


Fig. 8. Effects of LP, PBCD bolus injection and infusion, and acetaminophen on PGE concentration of c.s.f. samples taken from a lateral ventricle in a single rabbit. 1 mg acetaminophen given I.C.V. at each arrow.

TABLE 4. Increase in PGE concentration of c.s.f. during fever

| | PGE concentration (ng/ml.) | | | Rectal temperature (°C) | | |
|---|---|---|---|---|---|---|
| Experiment | Pre-fever | Fever | Change | Pre-fever | At time of sample | Change |
| 1 | 0·64 | 3·34 | 2·70 | 39·3 | 40·1 | 0·8 |
| 2 | 0·30 | 0·57 | 0·27 | 39·0 | 39·8 | 0·8 |
| 3 | 2·12 | 4·51 | 2·39 | 39·0 | 39.8 | 0·8 |
| 4 | 1·12 | 3·26 | 2·14 | 39·1 | 39·7 | 0·6 |
| 5 | 1·10 | 2·56 | 1·46 | 39·1 | 39·7 | 0·6 |
| 6 | 0·44 | 2·57 | 2·13 | 38·9 | 39·7 | 0·8 |
| 7 | 0·60 | 2·08 | 1·48 | 39·0 | 40·4 | 1·4 |
| 8 | 0·62 | 1·84 | 1·22 | 39·3 | 40·7 | 1·4 |
| 9 | 0·74 | 2·44 | 1·70 | 38·9 | 39·6 | 0·7 |
| 10 | 0·55 | 2·55 | 1·70 | 39·5 | 40·6 | 0·7 |
| 11 | 2·04 | 5·13 | 3·09 | 39·7 | 40·4 | 0·7 |
| 12 | 0·66 | 2·07 | 1·41 | 39·0 | 40·2 | 1·2 |

TABLE 5. Effects of antipyretics on fever and PGE concentrations of c.s.f.

| | | PGE concentration (ng/ml.) | | | Rectal temperature (°C) | | |
|---|---|---|---|---|---|---|---|
| Experiment | Antipyretic/Dose | Pre-anti-pyretic | Post-anti-pyretic | Change | Pre-anti-pyretic | Post-anti-pyretic | Change |
| 1 | Acet./2 mg | 2·57 | 0·89 | −1·68 | 40·2 | 39·1 | −1·1 |
| 2 | Acet./2 mg | 2·44 | 0·56 | −1·88 | 40·8 | 38·4 | −2·4 |
| 3 | Acet./2 mg | 4·30 | 1·25 | −3·05 | 40·9 | 39·4 | −1·5 |
| 4 | Indo./10 mg/kg | 5·67 | 1·36 | −4·31 | 41·4 | 40·7 | −0·7 |
| 5 | Indo./10 mg/kg | 1·82 | 0·52 | −1·30 | 40·2 | 38·5 | −1·7 |
| 6 | Indo./10 mg/kg | 3·75 | 1·02 | −2·73 | 40·8 | 39·4 | −1·4 |

(Fig. 3, Table 4) and decreased after antipyretics were given (Table 5). In six of seven experiments PGE concentration of c.s.f. rose during PBCD infusion, as rectal temperature rose or remained almost unchanged (Table 6). However, PGE concentrations of four of seven samples taken after diluent infusion were also increased even though rectal temperature decreased during these treatments. These results indicate that diluent infusion alone can cause the release of central PGEs and that the release of these autacoids is not necessarily associated with increased body temperature. In the three experiments in which PGE concentration of c.s.f. declined with diluent infusion rectal temperature decreased in two and rose in the third.

TABLE 6. Effects of injections and infusions of PBCD and diluent on PGE concentrations of c.s.f. during LP fever

| Experiment | PGE concentration (ng/ml.) | | | Rectal temperature (°C) | | |
|---|---|---|---|---|---|---|
| | Pre-infusion | Post-infusion | Change | Pre-infusion | Post-infusion | Change |
| | | | PBCD | | | |
| 1 | 2·56 | 2·57 | 0·01 | 39·7 | 40·2 | 0·5 |
| 2 | 3·34 | 2·44 | −0·90 | 40·1 | 40·8 | 0·7 |
| 3 | 1·14 | 4·30 | 3·16 | 40·8 | 40·9 | 0·1 |
| 4 | 2·44 | 5·36 | 2·92 | 39·6 | 40·2 | 0·6 |
| 5 | 1·84 | 5·67 | 3·83 | 40·7 | 41·4 | 0·7 |
| 6 | 1·25 | 1·82 | 0·57 | 39·7 | 40·2 | 0·5 |
| 7 | 1·14 | 4·30 | 3·16 | 40·8 | 40·9 | 0·1 |
| | | | Diluent | | | |
| 1 | 2·57 | 1·75 | −0·82 | 39·7 | 40·5 | 0·8 |
| 2 | 0·47 | 1·96 | 1·49 | 39·8 | 39·4 | −0·4 |
| 3 | 2·08 | 12·50 | 10·42 | 40·4 | 39·6 | −0·8 |
| 4 | 5·13 | 4·11 | −1·02 | 40·4 | 39·7 | −0·7 |
| 5 | 12·50 | 4·67 | −7·83 | 40·4 | 39·8 | −0·6 |
| 6 | 4·64 | 5·24 | 0·60 | 39·9 | 38·8 | −1·1 |
| 7 | 2·07 | 12·50 | 10·33 | 40·2 | 39·7 | −0·5 |

## DISCUSSION

Central administration of PBCD prolonged fevers caused by peripheral injections of LP and bacterial endotoxin. These findings suggest that PBCD delays termination of the central action of pyrogens which initiate and maintain fever. Although PBCD has other effects, such as inhibition of certain glycine conjugases, the drug was developed specifically to inhibit the transport of weak organic acids and its pharmacological actions are largely confined to these inhibitory effects (Brazeau, 1975). The results thus suggest that a PBCD-sensitive transport system is essential to termination of the action of pyrogens in the brain. In afebrile animals PBCD did not cause fever, which indicates further that an interaction between central pyrogen and a central transport system is responsible for the prolongation of fever.

LP, the central action of which is presumably responsible for fever production, has not been fully characterized but crude LP obtained from granulocytes of the rabbit has been described as an acidic protein of 10,000–20,000 daltons molecular weight with some components as large as 60,000 daltons (Bodel, Wechsler & Atkins, 1969). Dinarello, Goldin & Wolff (1974) noted that two types of LP can be derived from

human blood: one from monocytes (38,000 D) and the other from neutrophils (15,000 D). Small quantities of proteins up to 900,000 D can enter the brain, via vesicular transport through arterioles in the ventral part of the diencephalon and adjacent brain stem, within as little as three minutes (Westergaard & Brightman, 1973). LP, LP fragments, or other pyrogens may pass more readily through fenestrated endothelia that permit exchange of proteins in certain brain regions such as the wall of the optic recess, the median eminence, and the area postrema, to diffuse through surrounding brain tissue. It is unlikely that PBCD prolongs fever by preventing LP transport into the C.N.S. Rather, since LP is destroyed in the periphery, prevention of its access to central controls should have the opposite result: a reduction in fever duration.

A more likely mechanism of action of PBCD is the blockade of removal of pyrogens from sites of action in the brain. The termination of central pyrogen action may occur at the arterioles where, through reverse transport, pyrogen may be removed from the brain; within the parenchyma where it may be destroyed, bound, or taken up; in the c.s.f. where pyrogen may be destroyed or from which it may be taken up by the arachnoid villi and choroid plexus. More is known about the influence of PBCD on transport processes in the latter site than in the other. The active transport of organic anions from c.s.f. and contiguous extracellular fluid to blood is believed to protect the nervous system from a variety of potentially toxic compounds (Davson, 1976; Fishman, 1966). PBCD has been definitively shown to block transport of organic anion dyes in the choroid plexus. In chick (Cameron, 1953) and dogfish (Rall & Sheldon, 1962) choroid plexus a gradient of dye has been shown to develop from a dilute incubation medium to higher concentrations in the lumen of chloroidal capillaries. There is also considerable, although less direct, evidence that PBCD can block transport of cyclic AMP (Cramer & Lindl, 1972), prostaglandins (Bito *et al.* 1976*a*; Bito *et al.* 1976*b*) neurotransmitter metabolites (Guldberg, Ashcroft & Crawford, 1966; Karoum, Bunney, Gillin, Jimerson, Van Kammen & Wyatt, 1977), penicillin (Fishman, 1966), and other acidic substances, across the choroid plexus. If the activity of the PBCD-sensitive organic anion transport system tends to protect the C.N.S. from toxic substances, it seems reasonable that the same system might also protect the brain from prolonged activity of pyrogens.

Prostaglandins of the E series have been proposed as central mediators of fever largely because they cause rapid increases in body temperature when injected centrally (Feldberg & Saxena, 1971; Milton & Wendlandt, 1971), because the PGE content of c.s.f. rises in fever (Feldberg & Gupta, 1973; Feldberg *et al.* 1973), and because antipyretics are thought to act, at least in part, by blocking prostaglandin synthetase (Vane, 1971). Release of central PGE has been hypothesized as a step in fever mediation beyond the production of LP (Feldberg & Saxena, 1971). However, recent research (Cranston, Hellon & Mitchell, 1975; Cranston, Duff, Hellon, Mitchell & Townsend, 1976) has raised questions as to whether central release of PG, *per se*, is essential in fever. In the present experiments pre-treatment with single injections of PBCD augmented PGE hyperthermia in a dose-related fashion, generally paralleling the effects when PBCD was administered at the initiation of LP fever. Infusion of PBCD after injection of $PGE_2$, however, had little effect on the ensuing hyperthermia. Yet, when central transport was reduced by pre-treatment with PBCD and

continued PBCD infusion after the $PGE_2$ injection, life-threatening and lethal hyperthermias developed. There is very little prostaglandin dehydrogenase activity in the brain (Nakano, Prancan & Moore, 1972) and metabolism of the centrally released autacoids is believed to occur primarily after they are passed from the brain to the lungs. Bito and his colleagues (Bito *et al.* 1976*a*, *b*) have shown that PBCD inhibits transport of PGs across the choroid plexus, thereby blocking their inactivation. These previous findings on the transport of PGE may help to explain the basis for the differential effects on body temperature of PBCD given before and after I.C.V. $PGE_2$ and the nature of the events underlying the prolongation of LP fever by PBCD. The augmentation of PGE hyperthermia by PBCD only when the transport blocker is administered before the autacoid suggests that the normal central inactivation of PGE via PBCD-sensitive transport processes is very rapid. It seems that the inactivating carrier system must be blocked before PGE is administered if enhancement and prolongation of the PGE hyperthermia are to be seen. From the previous reports cited above and the present findings, it would seem that simple blockade of transport of PGE released by LP might account for the prolonged fevers caused by LP and endotoxin. In support of this idea is the finding that PBCD infusion increased or stabilized PGE content of the c.s.f. This latter argument is not conclusive since control infusion had the same effect on some occasions even though there was no change or a decrease in body temperature. To the contrary, evidence against the idea that PBCD prolongs fever by delaying inactivation of centrally released PGE was the finding that acetaminophen and indomethacin both lowered LP fever prolonged by PBCD at a time after the usual response to LP would have ended. These drugs inhibit PG synthesis (Vane, 1971) as well as the production of several other metabolites of arachidonic acid, and are ineffective against hyperthermias caused by I.C.V. and intracerebral administration of PGE. This evidence leads us to conclude that PBCD-prolonged LP fever is not due to accumulation of endogenously released PGE. Rather, it seems more likely that PBCD blocks termination of the effects of LP itself or of some related but unknown central mediator. As described above, PBCD also blocks central inactivation of relatively high doses of exogenous $PGE_2$ and of PGE released into the c.s.f. during diluent infusion but the relation between these effects and the mediation of fever is uncertain.

The prolonged febrile response seen with PBCD infusion is similar to that which occurs when I.C.V. infusion of taurine (2-aminoethanesulphonic acid), in animals given peripheral endotoxin (Harris & Lipton, 1977) or LP (Lipton & Ticknor, 1979) is stopped. This amino acid was proposed to prolong fever by promoting accumulation of endogenous pyrogen in the brain. The mechanism through which taurine might promote the accumulation of pyrogen was not specified but, if inactivation of LP or a related fever mediator requires active transport as the present results suggest, it may be that this amino acid can affect the pyrogen transport system. It is known that amino acids are cleared from the c.s.f. to blood by specific transport mechanisms (Cutler, 1970; Cutler & Lorenzo, 1968) and it may be that pyrogen is extruded from the brain via the same transport processes. Unlike PBCD, taurine does not prolong hyperthermia produced by I.C.V. injections of $PGE_2$. Further study of this difference in effects may help to explain more precisely the nature of the process of central inactivation of pyrogen and the nature of central PGE hyperthermia.

Dr John Pike of the Upjohn Company generously supplied the prostaglandin used in these experiments. This research was supported by the National Institute of Neurological and Communications Disorders and Stroke grant NS-10046 and by the Leland Fikes Foundation.

## REFERENCES

BAUMINGER, S., ZOR, U. & LINDNER, H. R. (1973). Radioimmunological assay of prostaglandin synthetase activity. *Prostaglandins* **4**, 313–324.

BITO, L. Z., DAVSON, H. & HOLLINGSWORTH, J. R. (1976*a*). Facilitated transport of prostaglandins across the blood-cerebrospinal fluid and blood-brain barriers. *J. Physiol.* **256**, 273–285.

BITO, L. Z., DAVSON, H. & SALVADOR, E. W. (1976*b*). Inhibition of *in vitro* concentrative prostaglandin accumulation by prostaglandins, prostaglandin analogues and by some inhibitors of organic anion transport. *J. Physiol.* **256**, 257–271.

BODEL, P., WECHSLER, A. & ATKINS, E. (1969). Comparison of endogenous pyrogens from human and rabbit leucocytes utilizing Sephadex filtration. *Yale J. Biol. Med.* **41**, 376–387.

BRAZEAU, P. (1975). Inhibition of tubular transport of organic compounds. In *The Pharmacological Basis of Therapeutics*, ed. GOODMAN, L. S. & GILMAN, A., pp. 862–863. New York: Macmillan.

CAMERON, G. (1953). Secretory activity of the choroid plexus in tissue culture. *Anat. Rec.* **117**, 115–123.

CLARK, W. G. & CUMBY, H. R. (1975). The antipyretic effect of indomethacin. *J. Physiol.* **248**, 625–638.

CRAMER, H. & LINDL, T. (1972). Probenecid inhibits efflux of adenosine 3′,5′-monophosphate (AMP) from cerebrospinal fluid (CSF) in the rat. *Psychopharmacologia* **26**, Suppl. 49.

CRANSTON, W. I., DUFF, G. W., HELLON, R. F., MITCHELL, D. & TOWNSEND, Y. (1976). Evidence that brain prostaglandin synthesis is not essential in fever. *J. Physiol.* **259**, 239–249.

CRANSTON, W. I., HELLON, R. F. & MITCHELL, D. (1975). A dissociation between fever and prostaglandin concentration in cerebrospinal fluid. *J. Physiol.* **253**, 583–592.

CRAWFORD, I. L., KENNEDY, J. I. & LIPTON, J. M. (1977). A simple 'planilabe' for rapid establishment of the stereotaxic horizontal zero plane in rabbits. *Brain Res. Bull.* **2**, 397–398.

CUTLER, R. W. P. (1970). Transport of lysine from cerebrospinal fluid of the cat. *J. Neurochem.* **17**, 1017–1027.

CUTLER, R. W. P. & LORENZO, A. V. (1968). Transport of L-aminocyclopentanecarboxylic acid from feline cerebrospinal fluid. *Science, N.Y.* **161**, 1363–1364.

DAVSON, H. (1976). The blood-brain barrier. *J. Physiol.* **255**, 1–28.

DINARELLO, C. A., GOLDIN, N. P. & WOLFF, S. M. (1974). Demonstration and characterization of two distinct human leukocytic pyrogens. *J. exp. Med.* **139**, 1369–1381.

FELDBERG, W. & GUPTA, K. P. (1973). Pyrogen fever and prostaglandin-like activity in cerebrospinal fluid. *J. Physiol.* **228**, 41–53.

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

FELDBERG, W. & SAXENA, P. N. (1971). Fever produced by prostaglandin $E_1$. *J. Physiol.* **217**, 547–556.

FIFKOVÁ, E. & MARŠALA, J. (1967). Stereotaxic atlases for the cat, rabbit and rat. In *Electrophysiological Methods in Biological Research*, ed. BURÊS, J., PETŘAŇ, M. & ZACHAR, J., pp. 653–731. New York: Academic.

FISHMAN, R. A. (1966). Blood-brain and CSF barriers to penicillin and related organic acids. *Archs Neurol. Psychiat., Chicago* **15**, 113–124.

GULDBERG, H. C., ASHCROFT, G. W. & CRAWFORD, T. B. B. (1966). Concentrations of 5-hydroxyindolylacetic acid and homovanillic acid in the cerebrospinal fluid of the dog before and during treatment with probenecid. *Life Sci. Oxford* **5**, 1571–1575.

HARRIS, W. S. & LIPTON, J. M. (1977). Intracerebroventricular taurine in rabbits: effects on normal body temperature, endotoxin fever and hyperthermia produced by $PGE_1$ and amphetamine. *J. Physiol.* **266**, 397–410.

JAFFE, B. M. & BEHRMAN, H. R. (1974). Prostaglandin E, A and F. In *Methods of Hormone Radioimmunoassay*, ed. JAFFE, B. M. & BEHRMAN, H. R., pp. 19–34. New York: Academic.

KAROUM, F., BUNNEY, W., JR., GILLIN, J. C., JIMERSON, D. VAN KAMMEN, D. & WYATT, R. J. (1977). Effect of probenecid on the concentration of the lumbar cerebrospinal fluid acidic metabolites of tyramine, octopamine, dopamine and norepinephrine. *Biochem. Pharmac.* **26**, 629–632.

LIPTON, J. M. & ROMANS, W. E. (1976). Modification of rabbit head holder to increase speed and accuracy of stereotaxic surgery. *Brain Res. Bull.* **1**, 159–160.

LIPTON, J. M. & TICKNOR, C. B. (1979). Central effect of taurine and its analogues on fever caused by intravenous leukocytic pyrogen in the rabbit. *J. Physiol.* **287**, 535–543.

MILTON, A. S. & WENDLANDT, S. (1971). Effects on body temperature of prostaglandins of the A, E and F series on injection into the third ventricle of unanaesthetized cats and rabbits. *J. Physiol.* **218**, 325–336.

NAKANO, J., PRANCAN, A. Y. & MOORE, S. E. (1972). Metabolism of prostaglandin $E_1$ in the cerebral cortex and cerebellum of the dog and rat. *Brain Res.* **39**, 545–548.

OJEDA, S. R., NAOR, Z. & MCCANN, S. M. (1978). Prostaglandin E levels in hypothalamus median eminence and anterior pituitary of rats of both sexes. *Brain Res.* **149**, 274–277.

RALL, D. P. & SHELDON, W. (1962). Transport of organic acid dyes by the isolated choroid plexus of the spring dogfish *S. acanthus*. *Biochem. Pharmac.* **11**, 169–170.

SAWYER, C. H., EVERETT, J. W. & GREEN, J. D. (1954). The rabbit diencephalon in stereotaxic coordinates. *J. comp. Neurol.* **101**, 801–824.

VANE, J. R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biol.* **231**, 232–235.

WESTERGAARD, E. & BRIGHTMAN, M. W. (1973). Transport of proteins across normal cerebral arterioles. *J. comp. Neurol.* **152**, 17–44.