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STUDY ON THE POSSIBLE ENTRY OF BACTERIAL ENDOTOXIN AND PROSTAGLANDIN E_2 INTO THE CENTRAL NERVOUS SYSTEM FROM THE BLOOD

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1 A study has been made of the possible entry of ^{51}Cr-bacterial endotoxin and [5,6,8,11,12,14,15(n)-^{3}H]-prostaglandin E_2 ([^{3}H]-PGE_2) into the CNS of the anaesthetized cat.

2 No radioactivity was detected in perfusates of the preoptic-anterior hypothalamus or in the cerebrospinal fluid (c.s.f.) *in vivo*, or in brain tissue *post mortem* following intracarotid infusion of ^{51}Cr-bacterial endotoxin.

3 Intracarotid administration of [^{3}H]-PGE_2 resulted in the entry of radioactivity into the CNS of endotoxin pretreated cats. Chromatographic analysis indicated the radioactivity in c.s.f. to be associated with PGE_2 and a metabolite similar to 13, 14-dihydro-15-keto PGE_2.

4 Intracarotid administration of 13, 14-dihydro-15-keto [5,6,8,11,12,14(n)-^{3}H]-PGE_2 resulted in the presence of the compound in the CNS of the anaesthetized cat after pretreatment with bacterial endotoxin.

5 It is concluded that PGE_2 and possibly 13,14-dihydro-15-keto PGE_2 but not bacterial endotoxin may enter the CNS from the cerebral circulation to elicit the febrile response to bacterial endotoxin in cats.

Introduction

The pathogenesis of hyperthermia associated with infection including peripheral infection, involves firstly an exogenous pyrogen such as bacterial endotoxin and secondly modification of the activities of central neurones thought to mediate thermoregulation (Cabanac, Stolwijk & Hardy, 1968; Wit & Wang, 1968; Eisenman, 1969). It is uncertain whether this central effect is exerted directly by exogenous pyrogen or indirectly by endogenous mediators such as leucocytic pyrogen (Beeson, 1948; Bennett & Beeson, 1953a, b) and/or prostaglandins of the E series (Milton & Wendlandt, 1970; Feldberg, Gupta, Milton & Wendlandt, 1973). This uncertainty is due in part to the observation that a febrile response is evoked in the cat as in other species, not only by intra-hypothalamic injection of bacterial endotoxin (Villablanca & Myers, 1965), but also by leucocytic pyrogen (Jackson, 1967) and prostaglandin E_1 (Feldberg & Saxena, 1971). In addition, the information available as to whether or not exogenous pyrogens present in blood can enter the CNS is both scant and equivocal (see review by Milton, 1976).

In this study the possible entry of two pyrogens, bacterial endotoxin and prostaglandin E_2 (PGE_2) into the CNS from the cerebral circulation in the anaesthetized cat has been investigated. PGE_2 is thought to be the ultimate mediator of pyrexia in cats (Feldberg *et al.*, 1973).

Some preliminary results of this study have been presented to the 7th International Congress of Pharmacology (Dascombe, 1978).

Methods

Female cats weighing 2.5 to 4.5 kg were anaesthetized with pentobarbitone sodium 40 mg/kg intraperitoneally. The rectal temperature of each cat was monitored continuously and maintained in the range 37 to 39°C by a heating pad placed beneath the animal.

Surgical intervention

The left common carotid was cannulated to the head with the cannula tip in the common carotid artery and the left femoral artery towards the heart. A cannula was introduced towards the head in an internal

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jugular vein or retrogradely into the right external jugular vein in cats receiving an infusion of either [^{3}H]-PGE_2 or 13,14-dihydro-15-keto [^{3}H]-PGE_2. Samples of c.s.f. were obtained from the cisterna magna by the method of Feldberg *et al.* (1973). The guide cannula necessary for this technique had been implanted chronically and aseptically into cats under pentobarbitone anaesthesia at least eight weeks before the study. The left preoptic-anterior hypothalamus (PO/AH) was perfused using a cannula of concentric design in which the inflow tube was positioned inside the outflow tube and extended 1 mm beyond the end of the latter tube. The outflow tube was implanted chronically and aseptically under pentobarbitone anaesthesia at least four weeks before the study.

Experimental procedure

Thirteen cats received an intracarotid injection of benzyl [^{14}C]-penicillin potassium 2 μCi/kg with non-radioactive benzylpenicillin sodium 6 mg/kg, followed by infusion (50 μl/min) into the carotid artery of benzyl [^{14}C]-penicillin potassium 1 μCi $kg^{-1} h^{-1}$ and non-radioactive benzylpenicillin sodium 3 mg $kg^{-1} h^{-1}$. Four cats later receiving ^{51}Cr-endotoxin were injected with [^{3}H]-water 10 μCi/kg then infused with [^{3}H]-water 5 μCi $kg^{-1} h^{-1}$ at the same time as receiving benzylpenicillin. Infusion of benzylpenicillin with or without [^{3}H]-water was terminated after 1 h.

Four cats received 5 to 10 min after the end of infusion of benzylpenicillin, an injection into the carotid artery of ^{51}Cr-endotoxin 20 μg or 100 μg/kg (equivalent to 0.4 to 6.0 μCi/kg) followed by infusion (50 μl/min) of ^{51}Cr-endotoxin 20 μg or 100 μg $kg^{-1} h^{-1}$ respectively. The infusion of bacterial endotoxin was of 60 min duration in one cat and 90 min duration in three others. Five other cats received non-radioactive bacterial endotoxin 20 μg/kg into the cannulated carotid artery followed 5 to 10 min later by an injection of [^{3}H]-PGE_2 5 μCi/kg with non-radioactive PGE_2 10 μg/kg and then infusion of similar doses of tritiated and non-radioactive PGE_2/hour for 90 min. Two cats received similar treatment to the latter five animals, except that they were given 13,14-dihydro-15-keto [^{3}H]-PGE_2 2.5 μCi/kg instead of [^{3}H]-PGE_2 5 μCi/kg with both injection and infusion of non-radioactive PGE_2.

Samples of blood (2 ml) from the femoral artery and of c.s.f. (0.5 ml) were removed under negative pressure at the times indicated in Results. Venous blood from the head (0.5 to 1.0 ml) was collected from cats receiving [^{3}H]-PGE_2 or 13,14-dihydro-15-keto [^{3}H]-PGE_2. Plasma and red blood cell samples were obtained from heparinized blood by centrifugation at 325 *g* for 5 min in an unrefrigerated centrifuge. Red blood cells were washed with 0.9% w/v NaCl solution (saline). Samples of blood were replaced by equivalent volumes of heparinized saline (50 units/ml). The left PO/AH was perfused continuously with artificial c.s.f. 15 μl/min (Merlis, 1940) during infusion peripherally of benzylpenicillin. Perfusion of the PO/AH was stopped after 1 h and the collected perfusate removed, before the PO/AH was again perfused during intracarotid administration of radiolabelled pyrogen.

Animals were killed at the end of pyrogen infusion by injection of pentobarbitone sodium (100 mg/kg) into the cannulated carotid artery. The brain was excised, sectioned and weighed after a single wash with distilled water. The area of brain perfused was verified histologically *post mortem* according to the atlas of Snider & Niemer (1961).

Measurement of radioactivity

The amount of gamma radioactivity in samples was measured with a Packard Auto Gamma Scintillation Spectrometer 5220. Radioactivity due to ^{3}H and ^{14}C was measured with a Packard Tri-Carb Scintillation Spectrometer using a toluene/Triton X-100 scintillant after tissue solubilization and decolourization where necessary. Results have been corrected for counting efficiency, double isotope overspill and, for ^{51}Cr, radiodecay, and are presented as nanoCuries (nCi). Results for *n* experiments are presented as the mean ± s.e.

Radio-labelling of bacterial endotoxin

Shigella flexneri endotoxin 5 to 10 mg was incubated with sodium [^{51}Cr] chromate 1 to 2 mCi dissolved in approximately 4 ml saline for 6 days in the dark at 4°C. Sodium [^{51}Cr] chromate unbound after incubation with endotoxin was removed by Pellicon molecular filtration. The retentate was washed with 15 ml aliquots of saline and molecular filtered until constant minimal amounts of radioactivity were present in the filtrate (<0.2% ^{51}Cr added to incubate). The retentate was dissolved in saline and stored at 4°C in the dark until use within 9 days. No dissociation of radio-isotope from endotoxin was detected by molecular filtration during storage. Electrophoresis of *Shigella flexneri* endotoxin was effected on agarose film (Corning Universal) in a barbitone acetate buffer (I 0.1, pH 8.6) with a running time of 35 min and a constant potential difference of 90 V. The distribution of ^{51}Cr on the film was monitored with a Panax thin layer scanner.

Identification of prostaglandins

Prostaglandin-like material was extracted from biological fluids acidified with HCl to pH 3, into ethyl

acetate and evaporated to dryness under nitrogen. Prostaglandins were identified by thin layer chromatography. Chromatograms were developed in chloroform:methanol:acetic acid:water (90:9:1:0.65 v/v) (Pace-Asciak, 1976) for 45 min at 21 to 23°C on Anasil H plates, and visualized with phosphomolybdic acid 10% w/v ethanol. Chromatograms of [^{3}H]-prostaglandins were divided into 1 cm bands and analysed by liquid scintillation counting.

Determination of residual blood volume in brain tissue

Four cats were anaesthetized and prepared as described under Surgical Intervention. Blood (10 ml) was withdrawn from the femoral artery and labelled with sodium [^{51}Cr] chromate 20 μCi/kg (Amersham, 1978). Radiolabelled erythrocytes were infused over 20 min into the left common carotid artery of the donor animal, after intracarotid injection of *Shigella flexneri* 20 μg/kg ($n = 2$) or saline ($n = 2$). After allowing 20 min for mixing, 2 ml of femoral arterial blood was taken. Animals were killed and the brain removed as described in Experimental procedure. Brain and blood contents of radioactivity were measured and the amount of residual blood in brain tissue determined as equivalent volumes of femoral arterial blood/g wet wt. of tissue (μl/g).

Aseptic techniques

Preparation of ^{51}Cr-endotoxin and ^{51}Cr-erythrocytes was effected under sterile, contaminant pyrogen-free conditions. Glass incubation vessels were prepared as described previously (Dascombe & Milton, 1975). Pellicon filters were attached to incubation vessel closures and immersed in 2% formalin for at least 12 h, rinsed with sterile, pyrogen-free water and enclosed in sterile, pyrogen-free incubation vessels. Sodium [^{51}Cr] chromate, benzyl [^{14}C]-penicillin, non-radioactive benzylpenicillin and 0.9% w/v NaCl solution ('Steriflex', Allen & Hanburys) were commercial sterile, pyrogen-free preparations. Prostaglandins and artificial c.s.f. were sterilized by Millex filtration (pore size 0.22 μm). Transfer and administration of drugs were made with disposable sterile, pyrogen-free syringes and hypodermic needles.

Drugs

The following drugs were used: benzylpenicillin sodium (Beechams); benzyl [^{14}C]-penicillin potassium, sp. act. 135 to 147 μCi/mg and 13,14-dihydro-15-keto [5,6,8,11,12,14(n)-^{3}H]-prostaglandin E_2, sp. act. 210 mCi/mg (Radiochemical Centre, Amersham); pentobarbitone sodium (May & Baker); prostaglandin E_2 (kindly donated by Upjohn Co.); [5,6,8,11,12,14,15(n)-^{3}H]-prostaglandin E_2, sp. act. 438 mCi/mg (Radiochemical Centre, Amersham); *Shigella flexneri* Lipopolysaccharide W (Difco Laboratories); sodium chromate [^{51}Cr]solution B.P., sp. act. 100 to 350 μCi/μg, and [^{3}H]-water (Radiochemical Centre, Amersham).

Results

Studies with benzyl [^{14}C]-penicillin and [^{3}H]-water

Benzyl [^{14}C]-penicillin administered via the carotid artery to 11 cats produced plasma concentrations of ^{14}C equal to 4.86 ± 0.35 nCi/ml at 30 min and 4.05 ± 0.40 nCi/ml at 60 min in femoral arterial blood. C.s.f. sampled at these times contained 0.18 ± 0.01 nCi/ml and 0.25 ± 0.02 nCi/ml respectively (equivalent to 3.9 ± 0.3% and 6.7 ± 0.9% of the plasma concentrations). Perfusate fluid recovered from the PO/AH during intracarotid infusion of benzyl [^{14}C]-penicillin in 8 of the cats contained 13 ± 3 pCi/ml (equivalent to 0.3 ± 0.1% mean plasma content of radionuclide).

[^{3}H]-water injected concurrently with benzyl [^{14}C]-penicillin in 4 cats resulted in plasma ^{3}H concentrations of 25.70 ± 1.85 nCi/ml at 30 min and 27.47 ± 2.66 nCi/ml at 60 min in femoral arterial blood. Plasma concentrations of benzyl [^{14}C]-penicillin in these animals were 5.03 ± 0.15 nCi/ml and 4.00 ± 0.74 nCi/ml respectively. Both ^{3}H and ^{14}C were detected in c.s.f. (Figure 1) and in hypothalamic perfusates. The concentrations of radionuclides in recovered perfusion fluid were 0.83 ± 0.40 nCi/ml for ^{3}H (equivalent to 3.5 ± 1.9% mean plasma content) and 13 ± 7 pCi/ml for ^{14}C (equivalent to 0.3 ± 0.1% mean plasma content).

One of the 11 cats had low concentrations (see previous paragraph) of ^{14}C in c.s.f. and hypothalamic perfusate during intracarotid infusion of benzyl [^{14}C]-penicillin. During the later infusion of [^{3}H]-PGE_2 into this animal, the concentration of ^{14}C in c.s.f. increased to equal that of systemic arterial plasma. The ^{14}C content of the second perfusion sample was 35% greater than the content of the first sample. Samples of c.s.f. and perfusate did not contain visible amounts of red blood cells. These observations were in contrast with those for other animals where the c.s.f. content of benzyl [^{14}C]-penicillin remained less than that of the plasma ($n = 6$) and the ^{14}C content of the second perfusate was 38 ± 9% ($n = 4$) less than that of the first sample. Histological examination *post mortem* showed a lesion between the site in the PO/AH perfused and the third cerebral ventricle. The observations relating to the distribution of [^{3}H]-PGE_2 in this cat have not been included in results presented here.

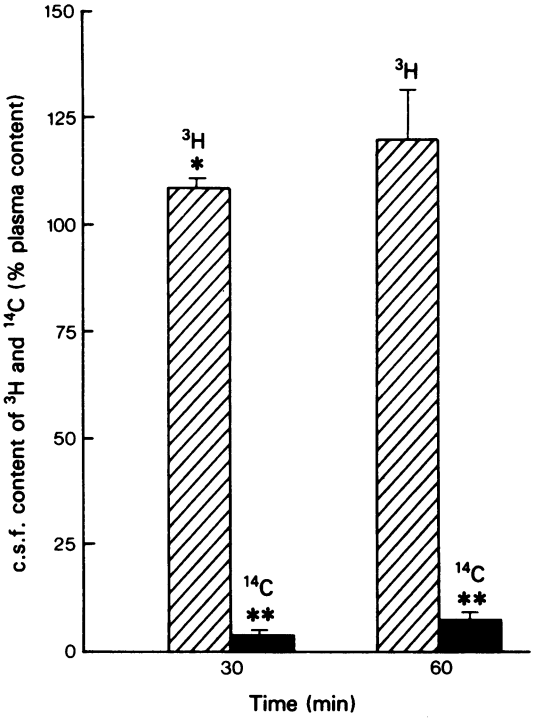

Figure 1 C.s.f. was sampled in anaesthetized cats 30 min and 60 min after intracarotid injection of benzyl [^{14}C]-penicillin (2 μCi/kg) and [^{3}H]-water (10 μCi/kg), during an arterial infusion of these compounds (1 μCi and 5 μCi $kg^{-1}h^{-1}$ respectively) which began at 0.25 min and ended at 60 min. Columns represent mean c.s.f. content ($n = 4$); vertical lines indicate s.e. Significance of the difference from plasma content determined by Student's t test: *$0.05 > P > 0.02$; **$P < 0.001$.

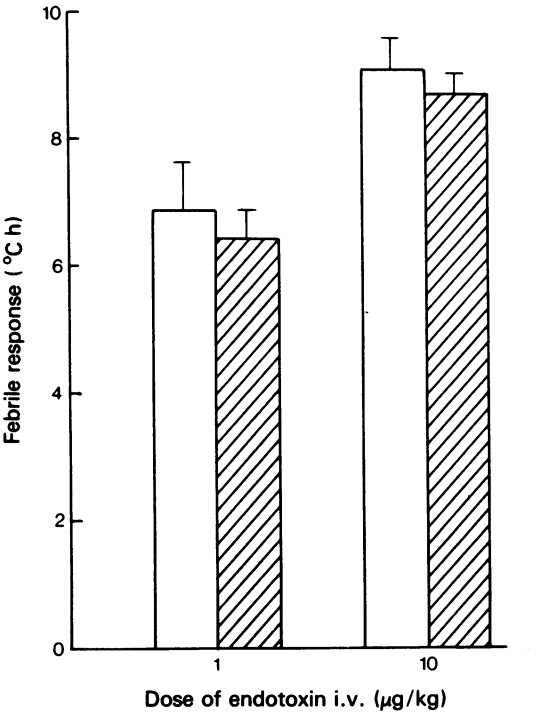

Figure 2 Endotoxin from *Shigella flexneri* was incubated with non-radioactive sodium chromate (1 mg endotoxin: 14 μg sodium chromate) at 4°C for at least 6 days. Intravenous injection of chromium-treated endotoxin (1 and 10 μg/kg) into conscious cats produced febrile responses (°Ch) measured over 6 h, which were not significantly different from control values. Columns represent mean response ($n = 4$) for control (open) and chromate-treated (hatched) endotoxin; vertical lines indicate s.e.

Samples of c.s.f. taken from 2 cats after injection of benzyl [^{14}C]-penicillin contained visible amounts of red blood cells. Examination *post mortem* showed damage caused by the sampling needle to the brain tissue beneath the cisternal membrane. These animals were excluded from the pyrogen study.

Studies with ^{51}Cr-bacterial endotoxin

Endotoxin from *Shigella flexneri* incubated with non-radioactive sodium chromate under similar conditions to those used with sodium [^{51}Cr] chromate, induced febrile responses in the conscious cat similar to those produced by untreated endotoxin (Figure 2). Electrophoresis of the bacterial endotoxin showed a slowly moving polysaccharide-protein fraction and a faster moving fraction staining for protein with Amido Black. ^{51}Cr associated with bacterial endotoxin was found only with the slowly moving component.

Intracarotid injection of ^{51}Cr-bacterial endotoxin in 4 cats produced the plasma concentrations of radionuclide in femoral arterial blood represented in Figure 3. The amount of ^{51}Cr associated with red blood cells was 8.6 ± 1.8% ($n = 11$) of the blood content. No radioactivity was found in c.s.f. sampled at the times indicated in Figure 3, or in perfusates of the PO/AH, although the threshold of detection of radioactivity (=2 × standard deviation of background counts) was 0.2 to 3% of the plasma concentration of ^{51}Cr-endotoxin in systemic arterial blood. Brain tissue contained *post mortem* no more radioactivity than that attributable to the residual blood content in the tissue samples.

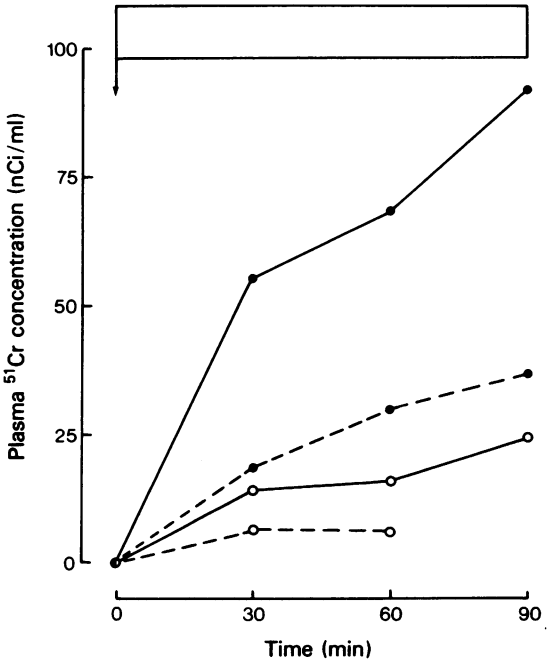

Figure 3 Systemic arterial plasma was sampled in 4 anaesthetized cats after intracarotid injection (↓) of ^{51}Cr-bacterial endotoxin (○, 20 μg/kg or ●, 100 μg/kg) and during intracarotid infusion (shown by open rectangle above figure) of the radiolabelled pyrogen (20 μg or 100 μg $kg^{-1}h^{-1}$ respectively) which began at 0.25 min and lasted 60 min (n = 1) or 90 min (n = 3). ^{51}Cr-endotoxin had a specific activity of either 19.5 μCi/mg (---) or 58.0 μCi/mg (——).

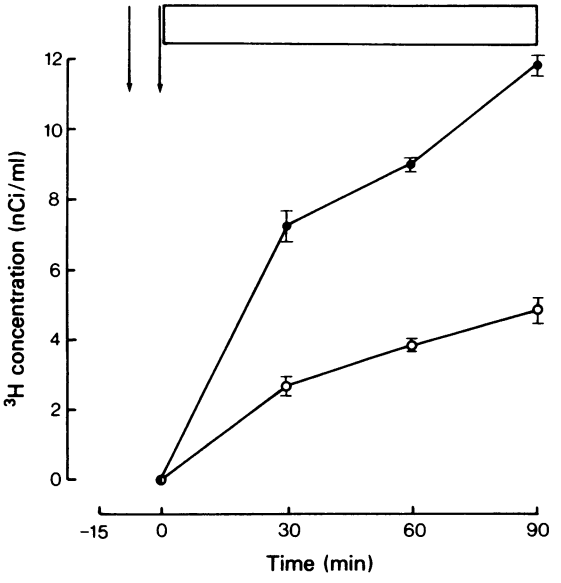

Figure 4 Systemic arterial plasma (●) and c.s.f. (○) were sampled in anaesthetized cats after intracarotid injection of *Shigella flexneri* endotoxin (20 μg/kg) (first arrow). [^{3}H]-prostaglandin E_2 ([^{3}H]-PGE_2, 5 μCi/kg) and PGE_2 (10 μg/kg) were injected into the common carotid artery at the time indicated by the second arrow and were followed by infusion (shown by open rectangle above figure) of similar doses of [^{3}H]-PGE_2 and PGE_2 per hour for 90 min. Points represent the mean value of 4 experiments; vertical lines show s.e.

Studies with [^{3}H]-prostaglandins

Intracarotid injection of [^{3}H]-PGE_2 with non-radioactive PGE_2 resulted in the entry of ^{3}H into c.s.f. in 4 cats pretreated with *Shigella flexneri* endotoxin 20 μg/kg (Figure 4). Two of the 4 animals had a cannula implanted in the PO/AH and perfusates of this region contained 0.04 nCi/ml and 0.11 nCi/ml (equivalent to 0.5 and 1.2% mean plasma content in systemic arterial blood). Chromatography of the radioactive material in c.s.f. and central venous plasma sampled at 90 min (Figure 4) showed the radionuclide to be associated with PGE_2-like material and a second compound with a R_F value similar to that of 13,14-dihydro-15-keto PGE_2. The R_f value of the latter prostaglandin was similar to that of PGB_2. The quantity of radioactivity associated with the 13,14-dihydro-15-keto PGE_2-like compound(s) was about 2.3 × more than that with PGE_2. Brain tissue contained ^{3}H (4.68 ± 0.44 nCi/g, 35 samples from 4 cats) with an apparent uniform distribution in the brain. This was from 14 to 208 × more radionuclide than that attributable to the residual blood content in the different brain regions.

Injection of 13,14-dihydro-15-keto [^{3}H]-PGE_2 with non-radioactive PGE_2 into 2 cats resulted in the entry of ^{3}H into c.s.f. (Figure 5). Perfusates of the PO/AH contained 0.2 and 0.3% of the mean plasma content of ^{3}H in these animals (Figure 5). Chromatography of the radioactive material in c.s.f. sampled at 90 min (Figure 5) showed the radionuclide to be associated with 13,14-dihydro-15-keto PGE_2-like compound(s). Brain tissue contained ^{3}H(2.10 ± 0.19 nCi/g, n = 19), equivalent to 11 to 188 × more radioactivity than that in residual blood.

Discussion

Bacterial endotoxins alter the permeability of cerebral vessels to several dyes (Eckman, King & Brunson, 1958) but evidence relating to the entry of endotoxins themselves into the CNS has remained equivocal. Bennett, Petersdorf & Keene (1957) found animals

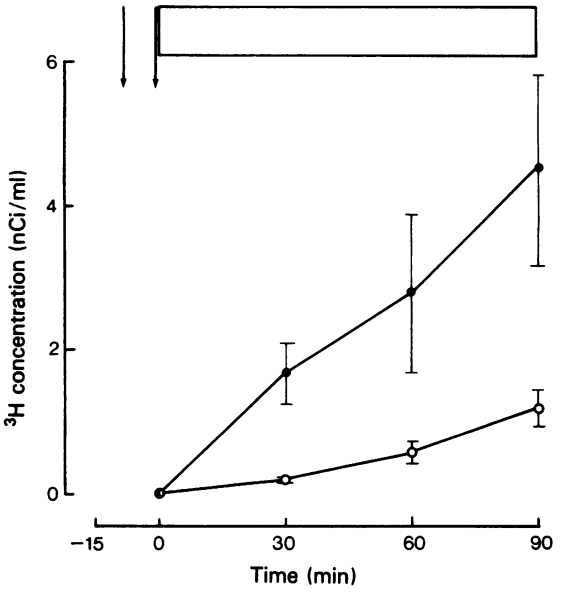

Figure 5 Systemic arterial plasma (●) and c.s.f. (○) were sampled in anaesthetized cats after intracarotid injection of *Shigella flexneri* endotoxin (20 μg/kg) (first arrow); 13,14-dihydro-15-keto [^{3}H]-PGE_2 (2.5 μCi/kg) and PGE_2 (10 μg/kg) were injected into the common carotid artery at the time indicated by the second arrow and were followed by infusion (shown by open rectangle above figure) of similar doses of 13,14-dihydro-15-keto [^{3}H]-PGE_2 and PGE_2 per hour for 90 min. Points represent the mean value and range of 2 experiments.

made tolerant to bacterial endotoxin by repeated daily administration continue to respond to endotoxin by developing fever. The authors suggested this was due to the entry of endotoxin from the peripheral circulation into the CNS where tolerance does not develop. As pointed out by Feldberg & Milton (1978), this evidence is unconvincing for it is probable that tolerance to endotoxin is never complete. Rowley, Howard & Jenkin (1956) detected no ^{32}P-endotoxin in brain tissue after peripheral injection in mice and guinea-pigs. However, dissociation of ^{32}P from the ^{32}P-endotoxin complex was observed both *in vitro* and *in vivo* and it is difficult to assess the amount of intact ^{32}P-endotoxin that remained in the blood. A ^{51}Cr-endotoxin complex apparently stable *in vivo*, was used by Braude Carey, Sutherland & Zalesky (1955a) and Braude, Carey & Zalesky (1955b) and they also reported an absence of bacterial endotoxin from brain tissue following peripheral administration, as did Cooper & Cranston (1963) using an endotoxin labelled with ^{131}I.

These earlier studies monitored only the brain tissue content of endotoxin *post mortem*, and after only a single intravenous injection despite similar observations by all three groups of workers that bacterial endotoxin was removed rapidly from the peripheral circulation. In this study an injection followed by an infusion of ^{51}Cr-endotoxin to the head have been used to maintain raised plasma concentrations of endotoxin in the cerebral circulation. Furthermore, techniques have been used to monitor *in vivo* entry both into the PO/AH, a pyrogen-sensitive region of the CNS (Villablanca & Myers, 1965; Feldberg & Saxena, 1971) and into the c.s.f. The ability of the methods to detect entry of substances into the PO/AH and the c.s.f. was verified by the presence of [^{3}H]-water in samples obtained after intracarotid administration. The possibility that chronic cannulation of the cat brain caused damage to the blood-brain and the blood-c.s.f. barriers that remained until the day of the study cannot be discounted. However, such damage appears to have been minimal if not absent as indicated by low CNS: plasma ratios of benzyl [^{14}C]-penicillin (Weinstein, 1975).

No ^{51}Cr-bacterial endotoxin was detected either in perfusates of the PO/AH or in the c.s.f. although the threshold of detection of radioactivity ($= 2 \times$ standard deviation of background counts) was 0.2 to 3% of the plasma concentration of ^{51}Cr-endotoxin. Brain tissue contained no more radioactivity than that attributable to the content of residual blood. Electrophoresis and molecular filtration studies showed the radionuclide to be associated with bacterial endotoxin before injection into cats, and the small quantities of radioactivity associated with erythrocytes after administration indicates the complex remained intact in the animal (Braude *et al.*, 1955b). The possibility exists, however, that a non-radioactive pyrogenic fraction was released in the animal from the macromolecule of the endotoxin to enter the CNS. There is no experimental evidence for this theory and it must be concluded from the results presented that bacterial endotoxin does not enter the brain of the cat.

In contrast with this latter finding, intracarotid administration of [^{3}H]-PGE_2 resulted in the presence of radioactivity in perfusates of the PO/AH, the c.s.f. and brain tissue in cats pretreated with *Shigella flexneri* endotoxin. Chromatography of the radioactive material present in c.s.f. showed ^{3}H to be associated with PGE_2 and a second compound possibly 13,14-dihydro-15-keto PGE_2. The radiochemical purity of injected [^{3}H]-PGE_2 was 90 to 98% and this was not changed by extraction procedure. It appears, therefore, that the formation of the second compound was due to metabolism of PGE_2. The identification of the metabolite(s) is not definitive because at least one other prostaglandin (PGB_2) was shown to have a similar R_f value to that of 13,14-dihydro-15-keto PGE_2 in the chromatography system used in this study. The observation that 13,14-dihydro-15-keto

PGE_2 enters the brain after intracarotid injection shows that the presence of this metabolite in the CNS may be in part the result of peripheral metabolism. [^{3}H]-PGE_2 (nCi) was injected with non-radioactive PGE_2 (ng) in the ratio 1:2. Chromatography indicated metabolism of the prostaglandin after injection into the cat resulted in samples containing 2/3.3 ng PGE_2/nCi ^{3}H. Hence, from the results presented, the concentrations of exogenous PGE_2 in c.s.f. were 1 to 4 ng/ml and in brain tissue about 3 ng/g, after intracarotid administration of the prostaglandin giving plasma concentrations of 3.5 to 8 ng/ml in systemic arterial blood. These values are based on the assumption the distribution of exogenous non-radioactive PGE_2 was similar to that of [^{3}H]-PGE_2, and do not take into account endogenous PGE_2. Similar amounts of PGE_2 are found in the c.s.f. of febrile cats (Cammock, Dascombe & Milton, 1976), and nanogram doses of PGE_1 produce hyperthermia when injected into the PO/AH in this species (Feldberg & Saxena, 1971). The thermoregulatory response to 13,14-dihydro-15-keto PGE_2 present in the brain is unknown. It is apparent, therefore, that PGE_2 can enter the CNS from the cerebral circulation in amounts thought to mediate the febrile response, when femoral arterial plasma concentrations of the prostaglandin are in the range 3.5 to 8 ng/ml. Concentrations of PGE_2 in brain arterial plasma were presumably greater than this after intracarotid administration of the prostaglandin. Although primary prostaglandins are normally present in plasma in picogram quantities (Dray, Charbonnel & Maclouf, 1976), several workers have reported systemic plasma or serum concentrations of prostaglandins to be raised, up to about 5.4 ng/ml, in endotoxin-treated animals (Skarnes & Harper, 1972; Anderson, Jubiz, Kralios & Tsagaris, 1973; Kessler, Hughes, Bennett & Nadela, 1973). Raised levels of prostaglandins in response to endotoxin may be due to increased synthesis and/or impaired metabolism of the compounds (Nakano & Prancan, 1973). It is conceivable that in the presence of endotoxin, prostaglandin concentrations may be greater at sites responsive to endotoxin, such as the cerebral blood vessels (Eckman *et al.*, 1958), than those in systemic plasma or serum.

In conclusion, the results of this study support and increase the evidence obtained by earlier workers (Braude *et al.*, 1955b; Rowley *et al.*, 1956; Cooper & Cranston, 1963) that bacterial endotoxin does not enter the brain. The results presented here indicate that if prostaglandin concentrations increase in the cerebral circulation in response to bacterial endotoxin, PGE_2 may then enter the brain to cause hyperthermia in cats. This hypothesis does not exclude the possible involvement of centrally produced prostaglandins in the febrile response or the possibility of other endogenous pyrogens entering the brain to act directly or indirectly on thermoregulatory neurones.

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