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SOME EFFECTS OF INTRAVENOUS PROSTAGLANDIN E_1 AND ENDOTOXIN IN YOUNG CHICKENS

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1 The effects of intravenous prostaglandin E_1 and endotoxin were studied in young chickens (11–17 days old).

2 At a thermoneutral ambient temperature (31°C), intravenous prostaglandin E_1 produced behavioural and electrocortical sleep, increased oxygen consumption and, after an initial fall, elevated body temperature. Below thermoneutrality (16°C), the initial hypothermic effect was more marked and oxygen consumption was lowered.

3 The soporific actions of prostaglandin E_1 were sufficient to counteract dexamphetamine-induced behavioural and electrocortical arousal and vocalization.

4 Intravenous injection of the O-somatic antigen of *Shigella dysenteriae* evoked, after a latent period, long lasting hyperthermia. This indicates that in young chicks the blood brain barrier is probably permeable to endotoxins.

Introduction

Horton (1964) demonstrated that intravenous prostaglandin E_1 produced behavioural sleep in young chicks. This action was presumed to be central since the blood-brain barrier is absent or not fully effective at this age (Waelsch, 1955; Bakay, 1956; Lajtha, 1957); indeed, tritiated prostaglandin E_1 penetrates into the brain after intravenous or intra-arterial injection in young chicks (Holmes & Horton, 1968). When infused into selected brain-sites to bypass the blood-brain barrier, prostaglandin E_1 induced behavioural sleep in young and adult chickens (Nisticò & Marley, 1973; Artunkal & Marley, 1974). The present paper describes more fully the effects of intravenous prostaglandin E_1 on behavioural, electrocortical and metabolic activities and on body temperature. Additionally, the effects of intravenous endotoxin on body temperature were investigated since failure of endotoxin, administered by this route, to elevate body temperature of adult hens (Pittman, Veale, Cockerham & Cooper, 1976) has been attributed to relative impermeability of the blood-brain barrier to endotoxins and/or the endogenous pyrogens produced by them. The effects of prostaglandins E_1, E_2 and of the O-somatic antigen of *Shigella dysenteriae* injected into the hypothalamus are compared in a subsequent paper with their effects when given intravenously.

Methods

Animals

Rhode Island Red pullets of 80–90 g were used (11–17 days old). They were housed under thermoneutral conditions i.e. at 33–34°C for the first week after hatching and for the following 2 weeks at 29–31°C.

Operative procedures

These were performed under halothane anaesthesia. Methods for implanting electrocortical recording electrodes, an intravenous jugular cannula and a thermistor placed subcutaneously between the scapulae have been described (Dewhurst & Marley, 1965; Allen & Marley, 1967). For injections into an internal carotid artery, retrograde injections were made down a polyethylene cannula inserted into the external carotid artery so that its tip lay at the junction with the common and internal carotid arteries.

Experimental procedures

Chicks were tested when recovery was complete, at least 24 h after the operative procedures. About 1 h before the control period, each chick was placed in a soundproof, environment-controlled experimental box

with a one-way screen and facilities for external monitoring of body temperature and electrocortical activity. Electrocortical activity was automatically integrated at 1 min intervals, large amplitude potentials producing high integral counts and alert low voltage electrocortical patterns giving low integrals. Vocalization was recorded and quantitated by the same integrating method. Ambient temperature was maintained at $16 \pm 0.5°C$ or 30–31°C, i.e. below or within the thermoneutral range for chicks at this age (Freeman, 1963; Allen & Marley, 1967); relative humidity was maintained at approximately 60%.

Intravenous and intra-arterial injections were given via a length of polyethylene tubing which passed through the roof of the box to connect with the implanted cannula. Body temperature was monitored continuously on a Grant miniature recorder; results are expressed as actual body temperatures rather than changes in body temperature. Oxygen consumption was measured as described by Allen & Marley (1967).

Drugs

Those used were prostaglandin E_1 (Upjohn & Co. Ltd.), O-somatic antigen of *Shigella dysenteriae* (W.H.O.) and dexamphetamine sulphate (S.K. & F. Ltd.). Prostaglandin E_1 was dissolved in ethanol and sodium carbonate (1 mg prostaglandin E_1 in 0.1 ml 95% ethanol and 0.9 ml sodium bicarbonate (0.2 mg/ml)) and prepared freshly for each experiment.

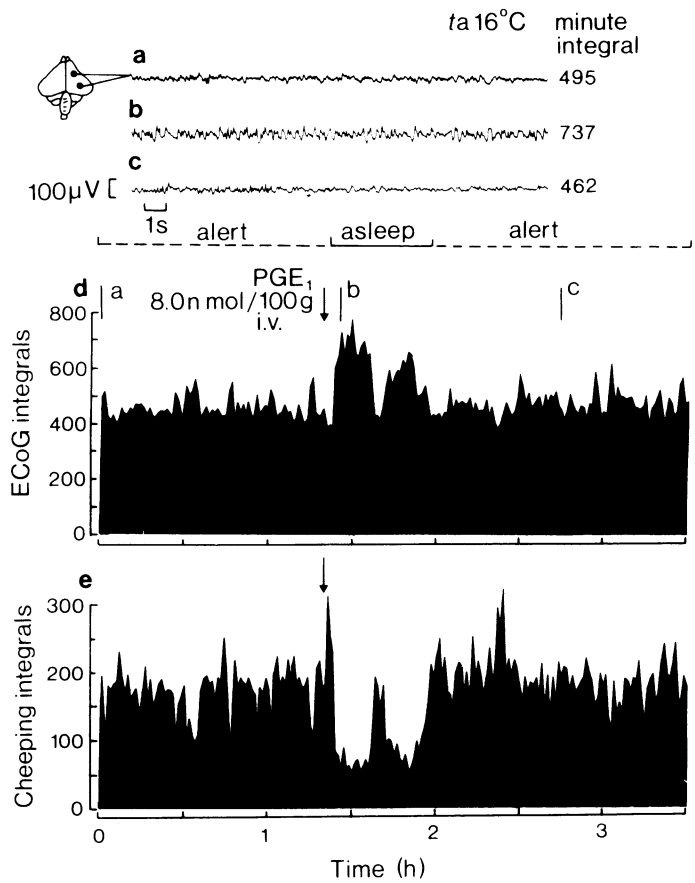

Figure 1 Effects of prostaglandin E_1 (PGE_1) on electrocortical activity and cheeping. Records of electrocortical activity (a–c; upper part) and of integrated electrocortical activity (d; middle) and cheeping (e; lower part). (a–c) Electrocortical activity recorded at times indicated in (d). (a), Control alert electrocortical activity; (b), activity of larger amplitude and slower frequency induced by prostaglandin E_1: (80 nmol/100 g i.v.) coincident with behavioural sleep and a reduction in cheeping (expressed as integrals, e). (c and d), Return of electrocortical activity and integrals to their pre-injection values, respectively. *ta*, ambient temperature, 16°C.

Results

Behaviour, cheeping and electrocortical (ECoG) activity

Horton's (1964) finding that intravenous prostaglandin E_1 induced behavioural sleep was confirmed in 6 chicks at a thermoneutral ambient temperature (31°C) and in 14 chicks tested below thermoneutrality (ambient temperature 16°C). The chicks either squatted or stood in a 'tripod' position with the head lowered, the beak resting on the ground, the eyes closed and the wing and tail feathers relaxed. Additionally prostaglandin E_1 induced the electrocortical changes of slow wave sleep i.e. large amplitude (>100 μV) slow frequency (2 to 4 Hz) waves. As evident in Figure 1, taken from a chick maintained at an ambient temperature of 16°C and following prostaglandin E_1 (8 nmol/100 g i.v.), the control alert ECoG (Figure 1a) changed to the large amplitude, slower frequency pattern associated with sleep (Figure 1b), integrals of ECoG activity being doubled during this period (Figure 1d). Behavioural and electrocortical sleep lasted approximately 30 min, and there was a return to alert ECoG activity on recovery (Figure 1c) with reduction in ECoG integrals. As shown in Figure 1e, loud 'distress' cheeping, which occurs on exposure to cold i.e. 16°C (Collias & Joos, 1953), was substantially diminished during the behavioural and electrocortical sleep

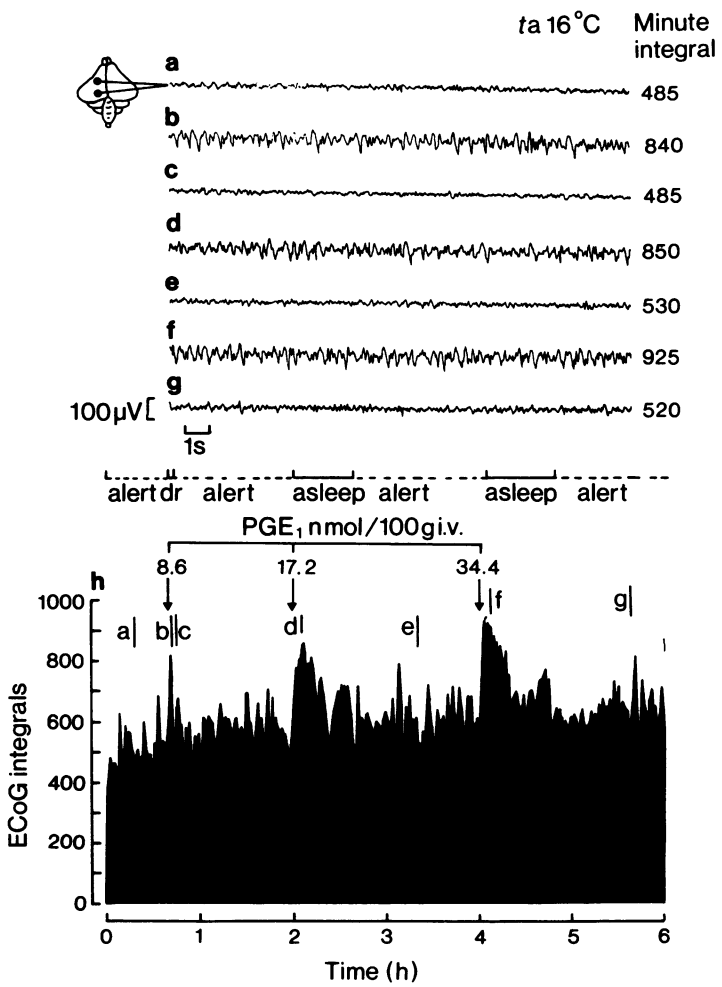

Figure 2 Effects of prostaglandin E_1 (PGE_1) on electrocortical activity (a–g), recorded at times indicated in lower part of figure. Increasing doses of prostaglandin E_1 (8.6, 17.2 and 34.4 nmol/100 g i.v.) produced similar changes in amplitude and frequency of electrocortical activity (b, d and f) but the changes were of increasing duration (h). *ta*, ambient temperature 16°C; dr, drug administered at time indicated.

induced by prostaglandin E_1. In Figure 2, taken from an experiment with another chick at an ambient temperature of 16°C, the electrocortical slow wave sleep changes (Figure 2b,d,f) induced by three doses of prostaglandin E_1 (8.6, 17.2, 34.4 nmol/100 g i.v. respectively) are illustrated, together with the return to alert electrocortical activity (Figure 2c,e,g) on recovery from each of the doses. While the maximum increase in ECoG integrals was similar for the three doses (Figure 2h), the durations during which these were elevated were dose-dependent (Figure 2h). However, mean electrocortical changes (Figure 3) indicated that it was the increase in integrals that was dose-dependent rather than the duration of effect.

The soporific actions of prostaglandin E_1 were intense, a point generally overlooked in comparison to its pyrexic actions, sufficiently so to counteract the central excitant effects of dexamphetamine and *vice versa*. Thus, Figure 4a–e illustrates that the electrocortical and behavioural sleep following prostaglandin E_1 (8.6 nmol/100 g i.v.), were converted to electrocortical (Figure 4c and e) and behavioural arousal by dexamphetamine (2 μmol/100 g i.v.), which in turn were replaced by sleep induced by prostaglandin E_1 (8.6 and then 17.2 nmol/100 g i.v.; Figure 4d and e). Amphetamine also elicits vocalization ('twittering') quantitatively different from that evoked by cold. As shown in Figure 5 taken from an experiment in which the chick exhibited little vocalization during the control period, cheeping was substantially increased by dexamphetamine (2 μmol/100 g i.v.) integrals of cheeping increasing from about 80–100/min to 250–330/minute. Cheeping was sustained for 20 min but was then attenuated by prostaglandin E_1 (16 nmol/100 g i.v.) only to return as the soporific effects of prostaglandin E_1 abated.

Body temperature

At a thermoneutral ambient temperature (31°C), prostaglandin E_1 (16, 32, 128 or 256 nmol/100 g) given either intravenously (9 chicks) or intra-arterially directly to the head (2 chicks) at first produced a dose-dependent hypothermia of between 1° and 2.5°C followed by an elevation of body temperature (Figure 6a and b). In 21 chicks below thermoneutrality (16°C) the initial hypothermic effect was more marked (compare Figure 6c and e).

Oxygen consumption

In the 9 chicks tested at thermoneutrality (31°C), mean oxygen consumption was elevated by 23 to 34% (see Table 1, Figure 6d) by prostaglandin E_1 (16, 32,

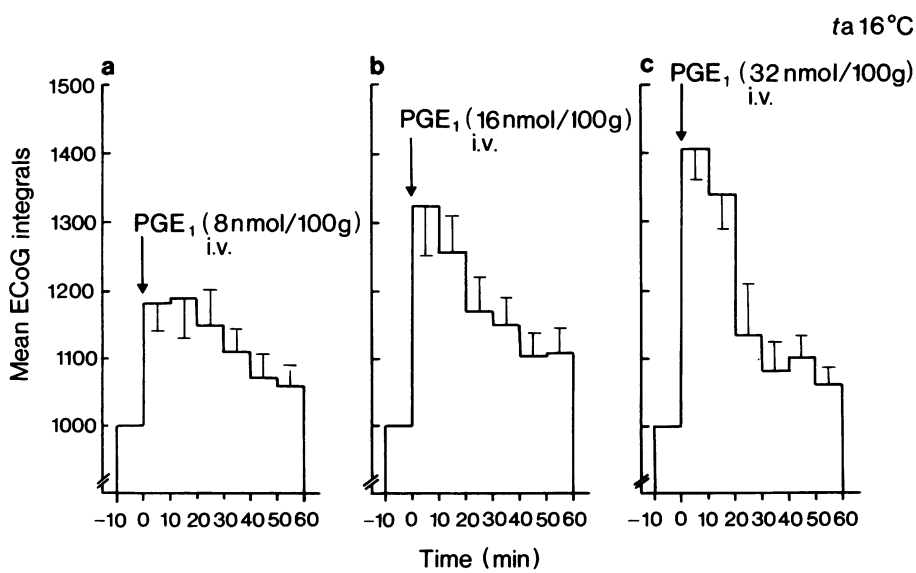

Figure 3 Mean effects of prostaglandin E_1 (PGE_1, 8, 16 and 32 nmol/100 g i.v.) on 10 min integrals of electrocortical activity. Vertical bars indicate s.e. mean. Mean control electrocortical integrals for each chick were normalized to 1000. (a) $n=14$, (b) $n=12$, (c) $n=3$, *ta*, ambient temperature, 16°C.

Table 1 Mean percentage changes, with s.e. mean in oxygen consumption due to intravenous prostaglandin E_1 (PGE_1) in chicks at an ambient temperature (*ta*) of either 31° or 16°C

Dose PGE_1 (nmol/100 g i.v.)	n	*ta 31°C*	n	*16°C*
4			5	−24.24 ± 5.1
8			9	−26.12 ± 3.3
16	4	+23.13 ± 1.4	6	−36.14 ± 2.3
32	3	+23.95 ± 4.8		
64	2	+34.5 ± 9.5		

(+), increases and (−), decreases in oxygen consumption.

128 or 256 nmol/100 g i.v.). In contrast, for the 20 chicks tested below thermoneutrality (16°C), prostaglandin E_1 (8 or 16 nmol/100 g i.v.) produced an immediate marked fall in mean oxygen consumption of from 24 to 36% followed by a slight overshoot above control values (Table 1, Figure 6f); recovery with overshoot of oxygen consumption preceded recovery of body temperature.

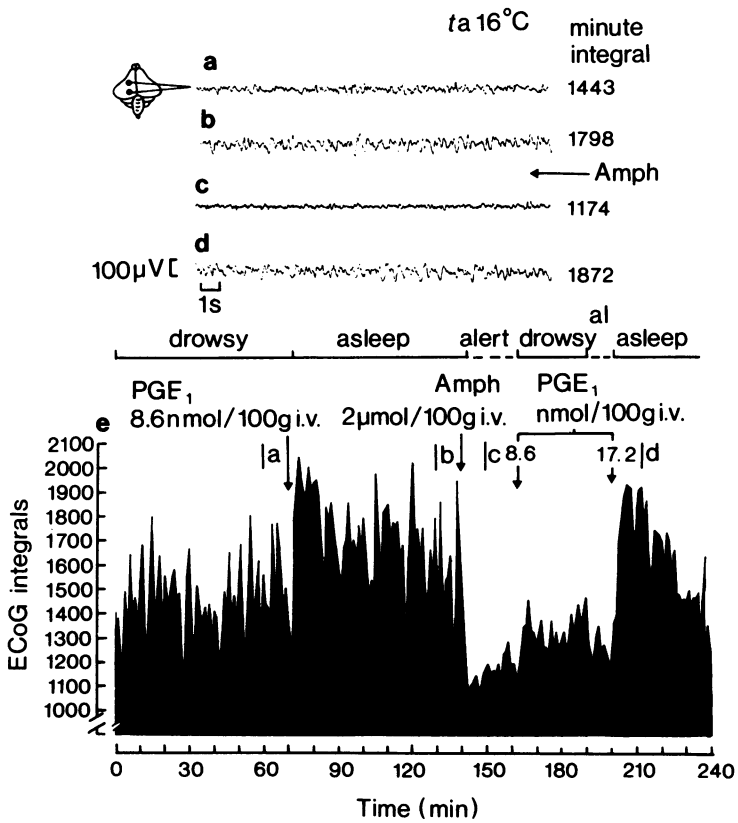

Figure 4 Reversal of the depressant effects of prostaglandin E_1 (PGE_1) (8.6 nmol/100 g i.v.) on electrocortical activity by dexamphetamine (Amph, 2 μmol/100 g i.v.) and of the excitant effects of dexamphetamine by a total of 25.8 nmol/100 g i.v. prostaglandin E_1. al, alert; *ta*, ambient temperature 16°C.

Effects of O-somatic antigen of Shigella dysenteriae *on body temperature*

The O-somatic antigen of *Shigella dysenteriae* (6 μg/100 g i.v.) evoked long-lasting hyperthermia commencing after a latency of approximately 1 h; ambient temperature was 31°C. Maximum elevation of mean body temperature (4 chicks) of 1.15°C was reached 4.5 h after injections and 4.5 h later it was still 0.6°C above the pre-injection values (Figure 7); in contrast, in saline- or vehicle-injected controls there was no elevation of body temperature. There was no evidence of an initial hypothermic response.

Discussion

The behavioural sleep evoked in chicks with intravenous prostaglandin E_1 (Horton, 1964) was shown in the present experiments to be accompanied by appropriate electrocortical changes. These phenomena, dose-dependent inasmuch as larger doses produced correspondingly longer effects, were assumed to be central in origin since similar effects ensued after intrahypothalamic injection (Artunkal, Marley & Stephenson, 1977). These soporific effects were sufficiently intense to replace amphetamine-evoked arousal with behavioural and electrocortical sleep; additionally, cheeping elicited by cold or by amphetamine i.e. by a physiological or a chemical stimulus was suppressed during sleep induced by prostaglandin E_1.

The effects on oxygen consumption and body temperature varied according to ambient temperature. At thermoneutrality (31°C), intravenous prostaglandin E_1 elevated oxygen consumption and after an initial fall, elevated body temperature; in contrast, below thermoneutrality it lowered both. Excluding the initial fall in body temperature at thermoneutrality these effects were similar to those of prostaglandin E_1 injected into the hypothalamus of young chicks and together with the behavioural effects, were assumed therefore to be central in origin. In 2–3 day old chicks, approximately 1/50th of an intravenous injection of

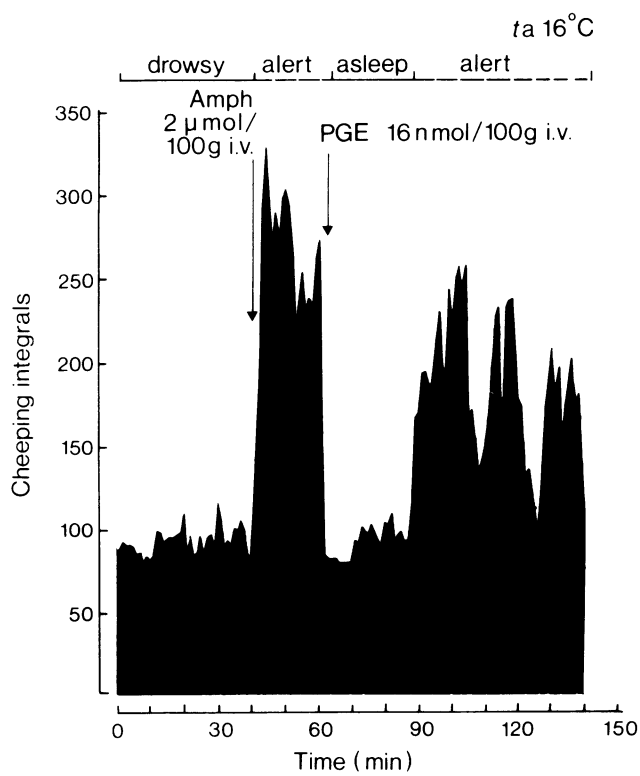

Figure 5 Suppression of dexamphetamine (Amph, 2 μmol/100 g i.v.)-induced vocalization by prostaglandin E_1 (PGE_1, 16 nmol/100 g i.v.). The effects of prostaglandin E_1 persisted for about 30 minutes. *ta*, ambient temperature 16°C.

PGE_1 (2 μg) was recovered in the brain 1 min after injection (Holmes & Horton, 1968). Assuming similar penetration for 11–17 day old chicks, then in the present study 0.1 to 0.5 nmol would be expected to enter the brain within 1 min of injection. This compares with our usual intrahypothalamic dose of 14.3 nmol (Artunkal & Marley, 1974; Artunkal *et al.*, 1977). Nevertheless, while the effects of intravenous prostaglandin E_1 on oxygen consumption (and body temperature) resembled those seen after intra-hypothalamic injection a peripheral site of action cannot be entirely excluded since it has been reported that in 9 week old chicks, prostaglandin E_1 (8.5 nmol/100 g i.v.) produced total inhibition of

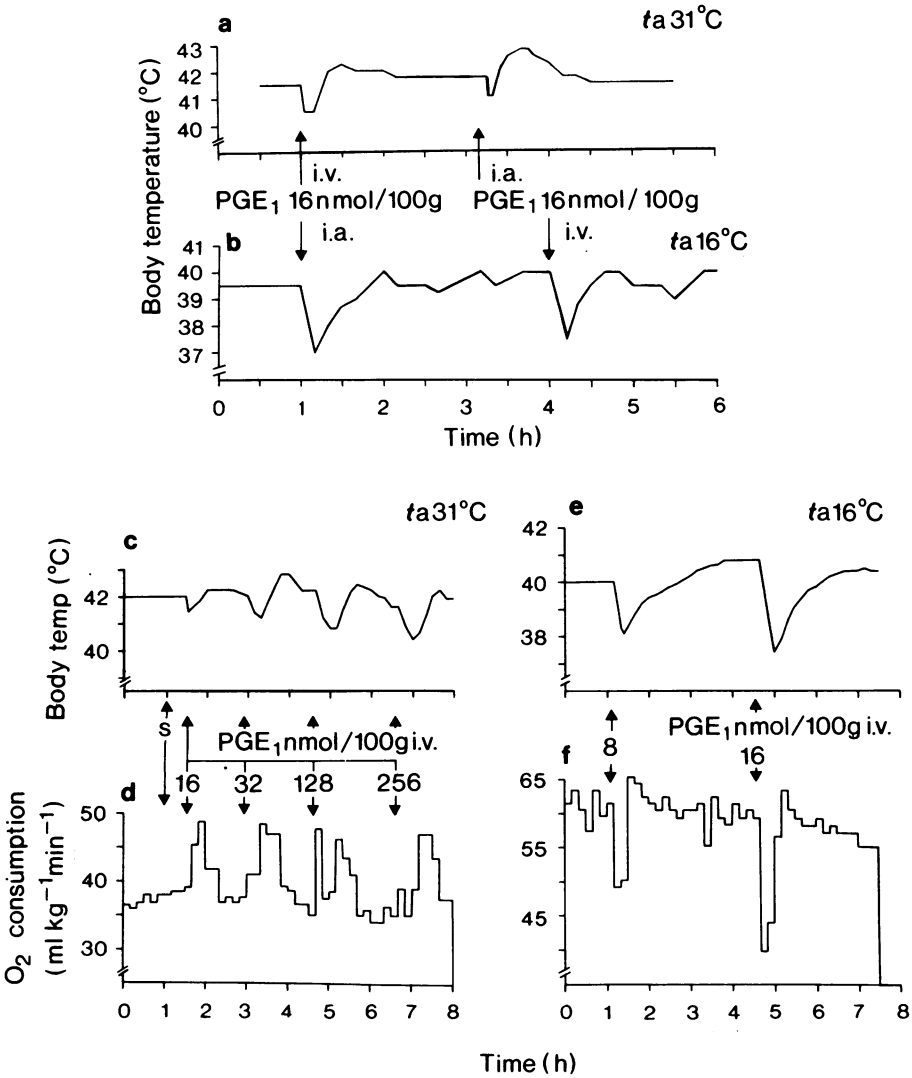

Figure 6 Representative traces comparing the effects of intra-arterial and intravenous prostaglandin E_1 (PGE_1) on body temperature of a chick at an ambient temperature (*ta*) of 31°C (a) and 16°C (b). Reducing ambient temperature to 16°C potentiated the initial hypothermic effect. At thermoneutrality (31°C), lowering of body temperature (c) was associated with an elevation of oxygen consumption (d), whereas below thermoneutrality (16°C), the initial hypothermic effect of prostaglandin E_1 (c) was accompanied by a fall in oxygen consumption (f).

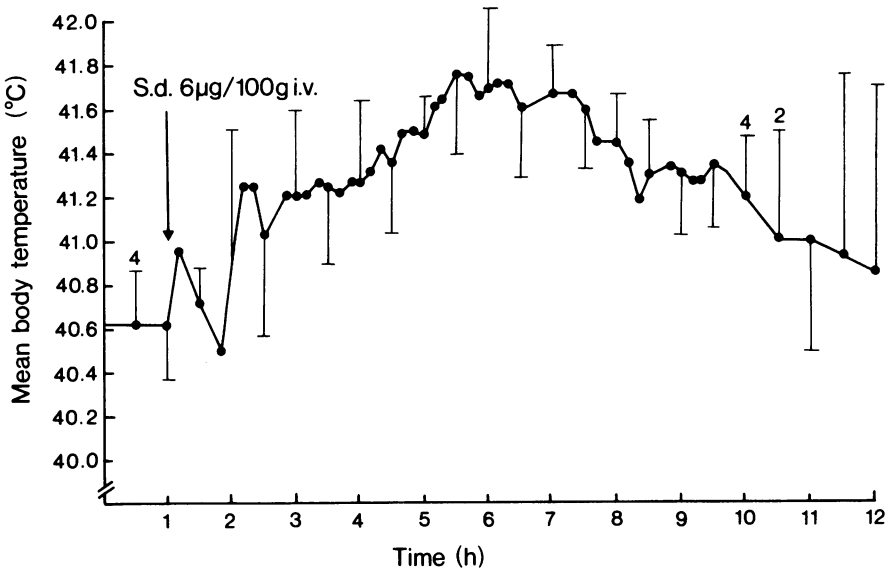

Figure 7 Mean effect of the O-somatic antigen of *Shigella dysenteriae* (S.d. 6 µg/100 g i.v.) on body temperature of 4 chicks (last 4 mean values are from 2 chicks only). Vertical bars represent s.e. mean.

lipolysis induced by placing chicks in an ambient temperature of 2–3°C for 4 h (Wagner, Peterson & Cenedella, 1971). The relevance of this is difficult to assess since prostaglandin E_1 did not affect basal lipolysis at thermoneutrality (Wagner *et al.*, 1971) and under the present experimental conditions (2–3 week old chicks at 16°C), plasma non-esterified fatty acid concentration was not elevated (Marley & Stephenson, 1975).

Prostaglandins of the E series are thought to be the mediators of endotoxin fever (for references, see Feldberg, 1975). Since intravenous injections of endotoxins evoke hypothermia in rats (Feldberg & Saxena, 1975) and adult chickens (Pittman *et al.*, 1976), in contrast to the hyperthermia noted after their central administration, it has been suggested that the blood brain barrier of these species is relatively impermeable to endotoxins. Indeed, no convincing evidence of either endogenous or exogenous pyrogens entering the brain from the periphery has been obtained (Milton, 1976). The finding that in young chicks, in which the blood brain barrier is immature, intravenous endotoxin evoked hyperthermia suggests that at this age in this species endotoxin did enter the brain. Assuming that prostaglandin E_1 mediates endotoxin fever and that prostaglandins penetrate the brain of young chicks then the production of hypothermia after its intravenous injection below thermoneutrality seems paradoxical; this aspect is studied in the ensuing paper.

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