Plasma Prostaglandins of the E and F Series in Rabbits during Fever Induced by Newcastle Disease Virus, *E. coli*-Endotoxin, or Endogenous Pyrogen

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Prostaglandins of the E and F series in rabbit plasma were estimated by radioimmunoassay after chromatographic separation during fever following the intravenous injection of Newcastle disease virus, *E. coli*-endotoxin, or endogenous pyrogen. Although concentrations of prostaglandins from both series are increased considerably by the three pyrogens, there seemed to be no correlation between prostaglandin concentrations in plasma and body temperature. These findings were supported by the fact, that after the injection of tritiated prostaglandin E_1 into the carotid arteries of normal or feverish animals no radioactivity was detectable in cerebrospinal fluid. The enhanced prostaglandin concentrations in plasma night be responsible for various pathological effects observed during fever such as diarrhoea and abortion.

Introduction

Both prostaglandin E_1 (PGE_1) and prostaglandin E_2 (PGE_2) injected into the cerebral ventricles of an unanesthetized animal produce a prompt elevation of body temperature [1]. In rabbits a high correlation has been discovered between fever following the injection of *E. coli*-endotoxin [2, 3], Newcastle disease virus, or endogenous pyrogen [4] and increased concentrations of prostaglandins of the E series (PGE) in cerebrospinal fluid (CSF). In contrast, the concentrations of prostaglandins of the F series (PGF) in CSF remained largely unchanged during fever induced by these pyrogens [4]. Although PGE and PGF are natural constituents of brain tissue [5, 6], it is not clear whether the higher concentrations of PGE found in CSF during fever are originally synthesized in the adjoining brain regions – especially the hypothalamus – or in other organs too. During the endotoxin shock in dogs higher plasma levels of prostaglandins of the E and A series were found [7, 8]. It is therefore of interest to investigate a possible relation of plasma prostaglandins to thermoregulation during fever.

Material and Methods

Pyrogens

The strain of Newcastle disease virus (NDV) was the same as in previous experiments and propagated in the same manner [9]. *E. coli*-endotoxin (ECE) extracted with phenol/water [10] was provided by Dr. S. Kanoh, Osaka, Japan. Serum containing endogenous pyrogen (EP) was obtained from other rabbits by sterile heart puncture at the height of fever after injection of NDV [9].

Animals

Male and female rabbits from uniform breeding (cross between Widder and Deutscher Riese) weighing between 2.0 and 2.5 kg, received the various pyrogens intravenously (i. v.) after their rectal temperature had stabilized between 38 and 39 °C. Control animals received corresponding volumes of the pyrogen-free solvents or of normal serum. Rectal temperature was recorded thermoelectrically with a thermistor probe (Hartmann and Braun, Frankfurt/M., No. 7377-103), inserted about 7 cm deep into the rectum, and monitored continuously by a multichannel recorder (Hartmann and Braun, Frankfurt/M., No. ARB6/144K).

Estimation of prostaglandins

One-ml samples of plasma were extracted with 3.0 ml of petroleum ether to remove neutral lipids. The aqueous layer was then adjusted to pH 4.0 by addition of 0.1 N HCl and extracted with ethylacetate. The organic phase was evaporated to dryness and the sample residues were applied to silicic acid columns, which were prepared by suspending silicic acid (100 mesh, Serva, Heidelberg) in a mixture of 60:40 benzene/ethyl acetate (0.25 g/ml). Prostaglandin fractions were obtained by develop-

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ing the columns serially with 60:40 benzene/ethyl acetate (fraction I: PGA, PGB), 60:40:2 benzene/ethyl acetate/methanol (fraction II: PGE), 60:40:20 benzene/ethyl acetate/methanol (fraction III: PGF) [11].

To check recovery during the extraction and chromatographic separation procedure, 32 ng (10 nCi) of (^{3}H)PGE_1 and 60 ng (10 nCi) of (^{3}H)$PGF_{1\alpha}$ (New England Nuclear Corp., Boston, Mass., U.S.A.) were each added to one parallel sample. The PGE and PGF fractions were analysed by radioimmunoassay, using the method of Orcyzyk and Behrman [12]. PGE_1 and $PGF_{1\alpha}$ as standard substances were the kind gift of Dr. J. E. Pike, Upjohn Comp., Kalamazoo, Mich., U.S.A. (^{3}H)PGE_1 and (^{3}H)$PGF_{1\alpha}$ were obtained from New England Nuclear Corp., Boston, Mass., U.S.A., and antisera against PGE and PGF from Calbiochem, Lucerne, Switzerland. Radioactivity was counted in a Nuclear Chicago scintillation counter using a toluene-based scintillation mixture.

Experimental procedures

In the first experiment 3 groups of rabbits (Tab. I) each received NDV (2000 hemeagglutinating units/kg), ECE (20 μg/kg), or EP (10 ml of serum/kg). Two hours after the injection of NDV and ECE or one hour after the injection of EP, when temperature had reached its maximum, blood was obtained by sterile heart puncture after light pentobarbital anaesthesia (50 mg/kg, i. v.), collected with Na-EDTA, and immediately centrifuged (7000 × *g*, 4 °C) The plasma was stored at − 25 °C. Hemolytic samples (hemoglobin concentrations > 30 mg/100 ml [13]) were discarded.

In a second experiment groups of 3 rabbits each received NDV, ECE, or EP in the same dose. Rectal temperature was recorded continuously for 7 hours. At intervals according to Figs 1 – 3, 3 ml of blood were drawn from the ear veins and the plasma levels of PGE and PGF estimated as described above.

In the third experiment, 3 rabbits with normal temperature (38.6 ± 0.5 °C, 0.9% pyrogen-free NaCl, i. v.) and 3 animals with fever (40.3 ± 0.4 °C, NDV, i. v.) each received 1.5 μg (0.5 nCi) (^{3}H)PGE_1/kg intravenously. After 1, 2, and 5 min the cisterna magna of one animal from each group was punctured immediately following pentobarbital narcosis (50 mg/kg) and the total CSF was aspirated. The CSF samples were extracted with ethyl acetate [4] and the organic phase was checked for radioactivity by liquid scintillation counting.

To exclude passage of the labeled prostaglandins through the pulmonary circulation, the same dose of (^{3}H)PGE_1 was injected in a parallel experiment into the right common carotid artery after the animals had been anaesthetised (pentobarbital, 50 mg/kg i. v.). At time intervals as in the previous experiment, CSF was collected and checked for radioactivity as described above.

Results

The initial rise of temperature after the injection of the different pyrogens was associated with slight shivering and skin vasoconstriction [4]. As shown in Table I at fever maximum (2 hours after the injection) PGE plasma concentrations from rabbits treated with NDV exceeded those of the control animals about 2-fold, whereas plasma levels of PGF remained largely unchanged. 2 hours after the injection of ECE we found 7-fold higher PGE and 3-fold higher PGF concentrations than in the normal animals (Tab. I). 80% of the rabbits in this group had severe diarrhoea, which was never observed after application of EP. The injection of serum containing EP after 1 hour was followed by a 5-fold increase of PGE concentrations in plasma but no change of PGF in comparison to the concentrations found after the injection of normal serum (Tab. I).

		Maximal rectal temperature [°C] Mean ± s.e.	Prostaglandin concentrations PGE [pg/ml] Mean ± s.e.	PGF [pg/ml] Mean ± s.e.
Controls (solvents)	(n=14)	38.7 ± 0.4 [1, 2]	171 ± 72 [3, 4]	338 ± 64 [5, 6]
NDV	(n=15)	40.0 ± 0.5 [1]	487 ± 57 [3]	357 ± 71 [5]
ECE	(n=18)	40.3 ± 0.4 [2]	1240 ± 107 [4]	1207 ± 160 [6]
Controls (normal serum)	(n= 6)	38.2 ± 0.6 [7]	192 ± 72 [8]	383 ± 83 [9]
EP	(n= 6)	40.5 ± 0.7 [7]	1067 ± 98 [8]	443 ± 74 [9]

(1–4, 6–8: p<0.001; 5, 9: p<0.6.)

Table I. Maximal rectal temperature after the injection of Newcastle disease virus (NDV), *E. coli*-endotoxin (ECE), or serum containing endogenous pyrogen (EP) and plasma concentrations at the time of fever maximum of prostaglandin E (PGE) and prostaglandin F (PGF).

Estimation of PGE and PGF concentrations during a period of 7 hours after the injection of the three pyrogens indicated that there was no correlation between PGE levels in plasma and the development of body temperature (Figs 1 – 3). When

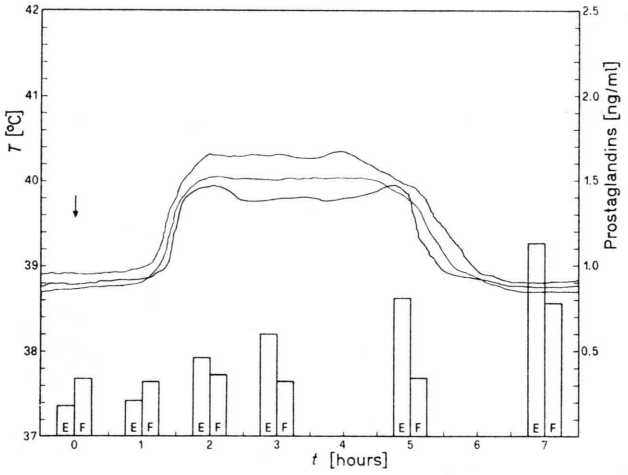

Fig. 1. Rectal temperature and plasma concentrations of prostaglandin E (E) and prostaglandin F (F) after the injection of Newcastle disease virus ($n=3$). Prostaglandin concentrations are means of 3 animals.

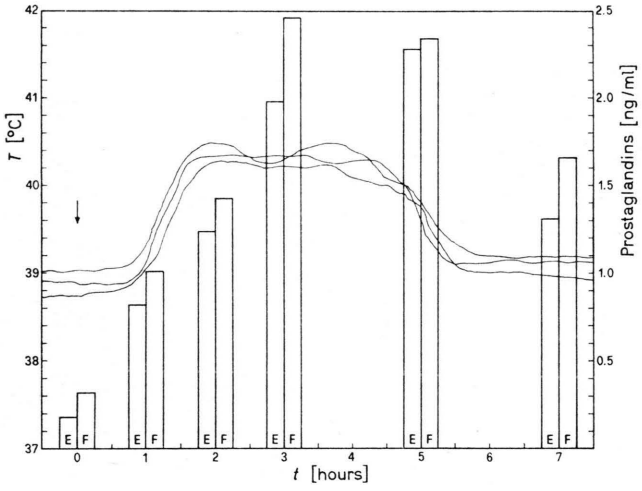

Fig. 2. Rectal temperature and plasma concentration of prostaglandin E (E) and prostaglandin F (F) after the injection of *E. coli*-endotoxin ($n=3$). Prostaglandin concentrations are mean of 3 animals.

temperature at 7 hours after the injection of the different pyrogens had already normalised, PGE plasma concentrations were still considerably higher than before the injection. In the NDV-treated animals we even found the highest PGE concentrations at the end of the observation period (Fig. 1).

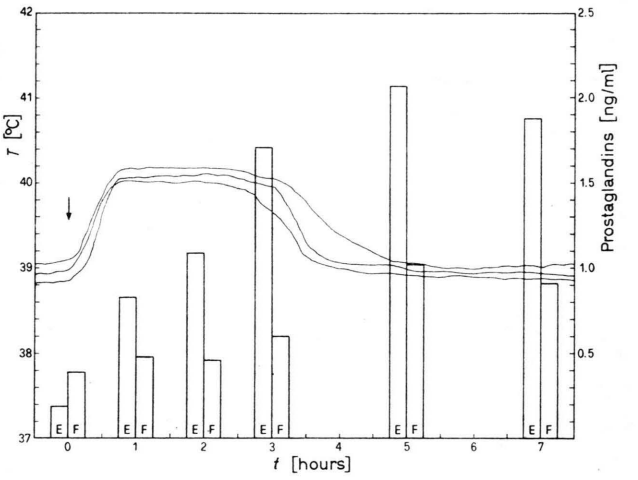

Fig. 3. Rectal temperature and plasma concentrations of prostaglandin E (E) and prostaglandin F (F) after the injection of serum containing endogenous pyrogen ($n=3$). Prostaglandin concentrations are mean of 3 animals.

Enhanced levels of PGF in plasma were found after the injection of ECE, 5 hours after the injection of EP, and 7 hours after the injection of NDV (Figs 1 – 3).

1, 2, or 5 min after the injection of (^{3}H)PGE$_1$ into the ear veins or the common carotid artery no radioactivity was found in CSF, in the animals with normal temperature as well as in those with fever following the injection of NDV.

Discussion

The concentrations of PGE and PGF in plasma of normal rabbits estimated by radioimmunoassay are of the same magnitude as those of humans [11] and dogs [7], obtained by radioimmunoassay, and those of human females [14], analysed by gas-chromatographic-mass spectrographic method, or those of rats [15], quantified by an isotope derivative method using 2-amino-[^{35}S]-thiazole as the reagent.

Although injections of NDV, *E. coli*-endotoxin, or endogenous pyrogen all increased PGE and PGF levels in plasma, there was no correlation between PGE plasma concentrations and temperature. Each pyrogen induced a different prostaglandin pattern in plasma. This might imply, that prostaglandin biosynthesis is stimulated in different cell types by the various agents.

Under physiological conditions prostaglandins of the E and F series are metabolised very effectively in the lung mainly by 15-hydroxy-prostaglandin de-

hydrogenase[16] and approximately 95% of injected prostaglandins would disappear following a single circulation through the lungs[17, 18]. Relatively high concentrations of this enzyme have been detected in kidney and spleen as well[19]. Nakano and Prancan[20] have shown that lungs and kineys during endotoxin shock inactivate PGE_1 at considerably slower rates than normal organs do. Therefore the enhanced plasma levels of PGE and PGF following the injection of the three pyrogens might be due to an impairement of prostaglandin metabolism as well as to enhanced synthesis in different cell systems[21] and release into circulating blood.

[^{3}H]PGE_1 was injected in a dose, which after mixing in plasma induced the natural concentration occurring during fever to rise 15-fold. The lack of radioactivity in CSF indicates that the blood-brain barrier is not permeable for physiologically occurring quantities of PGE in normal as well as in feverish animals. Holmes and Horton[22] observed that after the injection of 10 μg tritiated PGE_1 into the vertebral artery or the common carotid artery of anaesthetized cats no area of the brain (forebrain, midbrain, cerebellum, medulla, and pons) contained radioactivity equivalent to as much as 0.1 μg prostaglandin.

Intravenous application of prostaglandins of the E and F series produces diarrhoea[23] and abortion[24, 25]. The high concentrations of PGE and PGF in plasma might therefore be responsible for these pathological effects, which have been observed in various infectious diseases[26, 27].

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