Absence of endotoxin-fever but not hyperthermia in Brattleboro rats

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February 8, 1983

Summary. I.c.v. administration of bacterial endotoxin produced a fever in the Long-Evans rat but not in the Brattleboro rat. Similar administration of arachidonic acid, prostaglandin E_2, prostacyclin, dibutyryl cAMP, norepinephrine, morphine and β-endorphin caused hyperthermia in both Long-Evans and Brattleboro rats. Variable doses of exogenous arginine vasopressin (AVP) when centrally administered with endotoxin caused fever in the Brattleboro rat. It is suggested that AVP may play an important role in the production and release of endogenous pyrogen.

Fever produced by bacterial endotoxin is said to be mediated by metabolites of arachidonic acid such as prostaglandin (PG) of the E series[1]. However, evidence incompatible with this view has also accumulated[2]. For example, PG antagonist (e.g., SC-19220) which effectively attenuated PG-induced hyperthermia in rabbits, had no effect on hyperthermia induced by sodium arachidonate and pyrogen[3]. Lesioning of the preoptic-anterior hypothalamus results in the inability of the rabbit to respond to PGE_1 with a fever while the fever due to endogenous pyrogen is unaffected which suggests that other pathways not including PGE may be involved in the genesis of fever[4,5]. The neuropeptide, arginine-vasopressin (AVP), has been implicated as an endogenous antipyretic agent in sheep since AVP perfused through the septal area of the brain was found to block the fever due to i.v. administration of endotoxin[6]. In addition to PG, biogenic amines and cAMP have been implicated in the production of fever[7,8]. A variety of endogenous peptides capable of producing opiate-like effects have been isolated from brain tissue[9,10] and peptide and non-peptide opioids have been implicated in thermoregulation[11]. Rats of the Brattleboro strain that are homozygous for hereditary hypothalamic diabetes insipidus have no vasopressin[12]. After comparing the responses of Brattleboro rats and the parent strain, the Long-Evan rat, to bacterial endotoxin and PGE_2, Eagen et al.[13] reported that although both of them produced fever with PG, only the Long-Evans responded with fever to endotoxin. The objects of this study were to a) compare the effect of bacterial endotoxin, arachidonic acid, PGE_2, prostacyclin (PGI_2), dibutyryl cAMP (DBC), norepinephrine (NE), morphine and β-endorphin (BE) on temperature in the Long-Evans and Brattleboro rats b) find out the effect of AVP on endotoxin-induced temperature response in Brattleboro rats and c) determine the effect of AVP on pyrogen-fever in Long-Evans rats.

Methods. Male Long-Evans and Brattleboro rats weighing 300–400 g were used. The rats were anesthetized with 0.8 ml/kg, i.m. of a mixture consisting of Ketamine 50 mg/kg, xylazine 5 mg/kg and acepromazine 1 mg/kg and were placed in a rat head holder (David Kopf Instruments, No. 320). After exposure and the retraction of the superior sagittal sinus, a single cannula was inserted into a lateral ventricle according to the coordinates: (derived from the atlas of De Groot[14]) 0.8 mm posterior to bregma, 2.5 mm lateral, vertical until cerebrospinal fluid rose in the cannula. Dental acrylic was used to secure the cannula. Injection sites were histologically verified at post-mortem. The volume of injection was always 10 µl. At least 1 week was allowed for recovery before animals were used for experiments. Animals used once were randomly used again only one more time, but after a 7-day period. Injections were made at the same time of day (10.00 h) to avoid diurnal variation in temperature. Arachidonic acid sodium salt, *Salmonella typhosa* endotoxin, NE, DBC (Sigma), morphine sulfate (Merck), β-endorphin (Peninsula) and AVP (Calbiochem) were dissolved in sterile, non-pyrogenic 0.9% NaCl solution. The sodium salt of PGI_2 (Upjohn), stored at −9 °C, was dissolved in 0.9% NaCl solution and was adjusted to pH 9.0 with NaOH immediately before use. PGE_2 (Sigma) was stored at −4 °C in 95% ethanol. Shortly before use, the ethanol was evaporated by blowing nitrogen over the solution and PG residue was redissolved in saline solution. The animals were placed in cages where they could move freely 2 h before the beginning of experiments carried out at an environmental temperature of 21 ± 1 °C. Body temperatures were measured with copper constantan thermocouples inserted approximately 6 cm into the rectum and connected to an Autodata Nine Analog/Digital Data acquisition system (Acurex Corp., CA). Statistical significance was assessed with Student's t-test, and value for $p < 0.05$ was considered significant.

Results. 0.5–3 µg of endotoxin induced fever in the Long-Evans rats while the same dose produced an insignificant rise in temperature in Brattleboro rats (table 1). PGE_2 (0.5–2 µg), arachidonic acid (30–100 µg), PGI_2 (30–100 µg), DBC (30–100 µg), NE (30–100 µg), morphine (30–100 µg) and BE (10–50 µg) caused hyperthermia in Long-Evans and

Table 1. The effect of various i.c.v. treatments on rectal temperature of Long-Evans and Brattleboro rats

	Long-Evans rats Mean Δ temperature (°C)	Brattleboro rats Mean Δ temperature (°C)
0.9% saline	0.1 ± 0.04 (n = 10)	0.1 ± 0.08 (n = 9)
Endotoxin 0.5 µg	0.8 ± 0.18 (n = 5)*	0.2 ± 0.10 (n = 5)
Endotoxin 1.0 µg	1.2 ± 0.10 (n = 5)*	0.3 ± 0.08 (n = 5)
Endotoxin 3.0 µg	1.6 ± 0.20 (n = 5)*	0.4 ± 0.22 (n = 5)
PGE_2 0.5 µg	0.5 ± 0.05 (n = 5)*	0.6 ± 0.10 (n = 5)*
PGE_2 1.0 µg	1.0 ± 0.12 (n = 5)*	1.2 ± 0.08 (n = 5)*
PGE_2 2.0 µg	1.4 ± 0.15 (n = 5)*	1.6 ± 0.20 (n = 5)*
Arachidonic acid 30 µg	0.4 ± 0.10 (n = 5)	0.5 ± 0.08 (n = 5)*
Arachidonic acid 50 µg	0.9 ± 0.15 (n = 5)*	0.8 ± 0.08 (n = 5)*
Arachidonic acid 100 µg	1.3 ± 0.18 (n = 5)*	1.2 ± 0.15 (n = 5)*
PGI_2 30 µg	0.5 ± 0.12 (n = 5)*	0.4 ± 0.08 (n = 5)
PGI_2 50 µg	0.8 ± 0.12 (n = 5)*	0.7 ± 0.06 (n = 5)*
PGI_2 100 µg	1.2 ± 0.18 (n = 5)*	1.4 ± 0.20 (n = 5)*
DBC 30 µg	0.5 ± 0.08 (n = 5)*	0.4 ± 0.04 (n = 5)
DBC 50 µg	0.8 ± 0.12 (n = 5)*	0.9 ± 0.08 (n = 5)*
DBC 100 µg	1.1 ± 0.06 (n = 5)*	1.3 ± 0.15 (n = 5)*
NE 30 µg	0.4 ± 0.10 (n = 5)	0.5 ± 0.10 (n = 5)*
NE 50 µg	0.7 ± 0.10 (n = 5)*	0.8 ± 0.04 (n = 5)*
NE 100 µg	1.1 ± 0.04 (n = 5)*	1.0 ± 0.09 (n = 5)*
Morphine 30 µg	0.6 ± 0.12 (n = 5)*	0.5 ± 0.10 (n = 5)*
Morphine 50 µg	0.9 ± 0.04 (n = 5)*	0.8 ± 0.06 (n = 5)*
Morphine 100 µg	1.4 ± 0.15 (n = 5)*	1.3 ± 0.12 (n = 5)*
BE 10 µg	0.6 ± 0.04 (n = 5)*	0.7 ± 0.18 (n = 5)*
BE 30 µg	1.1 ± 0.04 (n = 5)*	1.0 ± 0.04 (n = 5)*
BE 50 µg	1.5 ± 0.20 (n = 5)*	1.4 ± 0.08 (n = 5)*

PGE_2, prostaglandin E_2; PGI_2, prostacyclin; DBC, dibutyryl CAMP; NE, norepinephrine; BE, β-endorphin. *Significantly different from 0.9% saline value: $p < 0.05$.

Brattleboro rats (table 1). In Brattleboro rats, variable doses of AVP (0.5–2 μg) did not produce any significant change in body temperature (table 2) but doses above 2 μg, for example 5 μg induced a 0.6±0.12 °C fall in temperature (n=5). In additional test groups, 0.5–2 μg of AVP injected 30 min before endotoxin (1 μg) significantly $p < 0.05$ increased the febrile response (table 2). In Long-Evans rats, variable doses of AVP (0.25–1 μg) had no effect on body temperature in control animals (table 3). However, it significantly ($p < 0.05$) attenuated pyrogen-fever when it was given 30 min after pyrogen (table 3).

Discussion. The primary difference between Long-Evans and Brattleboro rats is the genetic defect that prevents AVP production in Brattleboro rats[12]. Both Long-Evans and Brattleboro rats develop hyperthermia with arachidonic acid, PGE_2, PGI_2, DBC, NE, morphine and BE, which indicates that the induction of hyperthermia by these agents was not different in these strains and that AVP did not play a significant role in the production of hyperthermia. The absence of fever in Brattleboro rats to bacterial endotoxin confirms the results of Eagen et al.[13] that Brattleboro rats are incapable of developing a fever in response to central and peripheral injections of endotoxin in amounts that will produce fever in their parent strain, the Long-Evans rats. The lack of febrile response to endotoxin is probably not due to an increased ability of the Brattleboro rats to detoxify the bacterial endotoxin as has been postulated for the absence of fever following the 1st exposure of the Wistar rats to i.v. injections of endotoxin[15], since Eagen et al.[13] have demonstrated that daily intracerebral injections of endotoxin did not cause fever in the Brattleboro rats.

AVP was antipyretic since it reduced fever due to pyrogen in Long-Evans rats at a dose which had no significant effect on normal body temperature of control animals. Similar findings have been already reported in rats, sheep and guinea-pigs[6,16,17]. Therefore it is less likely that the absence of pyrogen-fever in Brattleboro rats is due to the absence of AVP, since it is an endogenous antipyretic in mammals[18] and the lack of it in Brattleboro rats should lead to uncontrolled fever.

Vasopressin neurons, transplanted from normal rat fetuses into the 3rd ventricle of adult Brattleboro rats, alleviate the polydipsia and polyuria of the hosts[19]. In our experiments, exogenous AVP when administered with endotoxin caused fever in Brattleboro rats. It is suggested that AVP may play an important role in the production of endogenous pyrogen (EP) from endotoxin rather than the genesis of fever since central administration of endogenous pyrogen induces virtually identical fever in Long-Evans and Brattleboro rats[20] and that the mediators of fever such as arachidonic acid and its metabolites, DBC and NE induced hyperthermia in Long-Evans and Brattleboro rats. It is believed that EP produced and released by cells of the reticulo endothelial system (RES) during inactivation of exogenous bacteria acts centrally within the rostral diencephalan to activate the heat conservation/production mechanisms which subserve the febrile response[21]. The functional integrity of the RES during some forms of shock (e.g.) endotoxin shock may be critically dependent upon the release of endogenous AVP[22]. Thus, the role of AVP may be in the production and release of EP.

Table 2. Effect of i.c.v. pretreatment of arginine vasopressin on the temperature response-induced by i.c.v. administration of endotoxin in Brattleboro rats

Treatment	Mean Δ temperature (°C)
0.9% saline	0.1 ± 0.08 (n = 10)
0.9% saline + endotoxin 1 μg	0.3 ± 0.14 (n = 5)
Arginine vasopressin 0.5 μg + 0.9% saline	0.1 ± 0.12 (n = 6)
Arginine vasopressin 1.0 μg + 0.9% saline	0.2 ± 0.06 (n = 6)
Arginine vasopressin 2.0 μg + 0.9% saline	0.2 ± 0.16 (n = 6)
Arginine vasopressin 0.5 μg + endotoxin 1 μg	0.6 ± 0.10 (n = 5)*
Arginine vasopressin 1.0 μg + endotoxin 1 μg	1.0 ± 0.15 (n = 5)*
Arginine vasopressin 2.0 μg + endotoxin 1 μg	1.3 ± 0.18 (n = 5)*

*Significantly different from endotoxin 1 μg value: $p < 0.05$.

Table 3. Effect of i.c.v. arginine vasopressin on fever-induced by i.c.v. administration of endotoxin in Long-Evans rats

Treatment	Mean Δ temperature (°C)
0.9% saline	0.15 ± 0.08 (n = 5)
Endotoxin 1 μg + 0.9% saline	1.1 ± 0.12 (n = 5)
0.9% saline + arginine vasopressin 0.25 μg	0.1 ± 0.04 (n = 5)
0.9% saline + arginine vasopressin 0.5 μg	0.1 ± 0.18 (n = 5)
0.9% saline + arginine vasopressin 1.0 μg	0.2 ± 0.20 (n = 5)
Endotoxin 1μg + arginine vasopressin 0.25μg	0.6 ± 0.18 (n = 5)*
Endotoxin 1μg + arginine vasopressin 0.5 μg	0.3 ± 0.20 (n = 5)*
Endotoxin 1μg + arginine vasopressin 1.0 μg	0.2 ± 0.10 (n = 5)*

*Significantly different from endotoxin 1 μg value: $p < 0.05$.

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0014-4754/83/121343-02$1.50+0.20/0