Lack of pyrogenic tolerance transmission between brain and periphery in the rabbit

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Summary. Successive injections of lipopolysaccharide (LPS) either intravenously (i.v.) or intracerebroventricularly (i.c.v.) induced pyrogenic tolerance to LPS in rabbits. Tolerance was shown by a decrease of the magnitude of the fever response to repeated doses of LPS, irrespective of the route of pyrogen administration. A significantly greater and more dramatic decrease of the fever index, however, was observed in rabbits made tolerant to pyrogen given i.v. than when the pyrogen was given i.c.v. Transmission of the pyrogenic tolerance between brain and peripheral tissues, however, has not been ascertained.
Key words. Fever; endotoxin; tolerance; brain; periphery.

Development of tolerance to repeated peripheral administration of exogenous pyrogens, such as LPS [1] and muramyl dipeptide [2], is associated with as yet poorly-recognized pathophysiological alterations on both sides of blood-brain barrier (BBB), which have resulted in a decrease of the fever index and an alteration in the time-course of the change of body temperature during febrile responses. Available experimental data suggest that in peripheral tissues these changes may relate either to the inhibition of endogenous pyrogen (EP) synthesis [3] or to adaptive changes of sensitivity of target cells for EP [4] – both of these are most likely associated with glucocorticoid action. It has been found that during induction of tolerance by means of several i.v. injections of pyrogen, the level of steroid hormones increased in blood [5]. This indicates the involvement of the brain in the peripherally-induced tolerance process. Changes inside the brain during pyrogenic tolerance may contribute to the activation of the vasopressin-dependent endogenous antipyresis [6] as well as to the enhancement of the hypothalamo-pituitary-adrenal axis (HPA axis). Recent evidence has shown that interleukin-1 (Il-1), a pyrogenic monokine secreted predominantly by mononuclear phagocytes, can stimulate the HPA axis [7, 8], and it provides an intrinsic brain-periphery feedback loop for suppression of the immune response [9]. Such a loop is of considerable physiological importance in controlling the intensity of the immune response.

The present study addresses the questions of i) whether consecutive daily injections of LPS into the lateral cerebroventricle can induce the typical febrile tolerance, and ii) whether there is a possibility of transferring the tolerance between brain and periphery.

Male New Zealand white rabbits weighing 3–4 kg were used throughout the study. Two weeks prior to experimentation stainless steel guide cannulae were implanted in the lateral ventricle of anesthetized rabbits, according to the stereotaxic coordinates of Sawyer et al. [10], and secured with screws and dental cement. Surgical procedures were carried out under halothane anesthesia as described elsewhere [11]. Rectal temperature was continuously measured using a precalibrated thermistor probe inserted 10 cm beyond the anus and taped to the tail. Recordings were performed in unrestrained animals at an ambient temperature of 20 °C.

LPS of *Salmonella abortus equi* (Sigma), dissolved in sterile saline, was injected in the marginal ear vein (i.v.) at a dose of 0.3 µg/kg. The dose of LPS for i.c.v. injection was 20 ng in a volume of 20 µl per animal. Peripheral pyrogenic tolerance was induced in rabbits by four daily i.v. injections of LPS. On the 5th day the rabbits were injected i.c.v. with LPS.

In the second series of experiments tolerance was induced in rabbits by six daily i.c.v. injections of LPS. Then, on the 7th day, the rabbits were treated with LPS given i.v. The temperature of each rabbit was measured and recorded for 30 min before the pyrogen injection and for 6 h afterwards. The fever index was calculated from the area under the curve of the time-course of rectal temperature for a period of 6 h after injection, and expressed as °C · h.

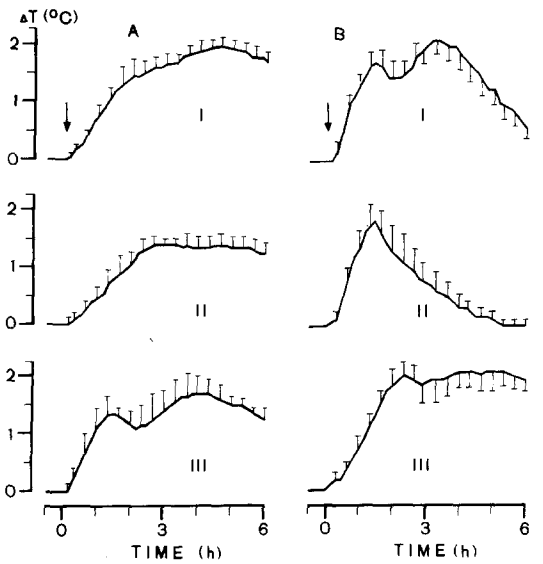

Figure 1. Changes of rectal temperature during induction of tolerance to LPS given i.v. (*B*) and i.c.v. (*A*). I = first day of tolerance; II = last day of tolerance; III = after changing the route of LPS administration. Pyrogen was injected at time zero, (mean ± SD, n = 4).

As shown in figure 1 B, the development of tolerance to LPS given i.v. was related to the changing of the biphasic rise in body temperature to a monophasic one. The consequence of this was a statistically significant decrease in the fever index, comparing the responses to the first and to the fourth administration of LPS (Student's t-test, $p < 0.05$) (fig. 2). In the case of i.v. injection, however, a marked drop of the second phase of fever was already seen after the second dose of LPS, and the decrease was greater after the third injection. The time-course of fever as well as the fever index, calculated for the third response, did not change following the fourth dose. Successive i.c.v. injections of LPS, on the other hand, did not cause such dramatic changes in the time-course of rectal temperature change (fig. 1 A). A progressive decrease of the fever response to successive i.c.v. injections was observed, however, and the decrease in the fever index on the 6th day of the experiment was also statistically significant ($p < 0.05$) (fig. 2).

When rabbits which had received daily i.v. injections of LPS were then given pyrogen i.c.v., they all responded with a fever which had the same characteristics as were observed in naive rabbits (fig. 1 B/III). Similarly, in rabbits made tolerant to LPS injected i.c.v., the fever response had the characteristics of that observed in naive rabbits when the route of LPS injection was changed (fig. 1 A/III). These findings indicate that there is a lack of transfer of the tolerance to pyrogen effects from the periphery into the brain, and vice versa.

Considered together, the results suggest that tolerance to pyrogen effects is produced independently in brain and periphery. This may indicate that, during induction of tolerance in the periphery, there is no circulating factor originating from the peripheral tissues which is able to penetrate across the blood-brain barrier (BBB) and induce a simultaneous tolerance in the brain. Therefore, it seems probable that the induction of tolerance to a given pyrogen in brain and periphery requires a direct challenge of each of these tissues with the pyrogen. This, however, does not rule out an involvement of the brain in modulation of the course of fever, or in the maintenance of refractoriness to pyrogen when the tolerance is induced in the periphery. Activation of the HPA axis[7, 8] and stimulation of endogenous antipyresis[6] support the above notion.

It is tempting to speculate, therefore, that a signalling system exists which 'informs' the brain about tolerance induction in the periphery, but does not produce tolerance in the brain itself. Whether this signalling system is related to humoral factors, and whether it is connected with the action of endogenous mediators of fever, are open questions. Experimental data show that exogenous pyrogens and endogenous proteinaceous intermediates of the febrile response (e.g. Il-1) do not penetrate across the BBB[12, 13], which suggests that these agents are not signalling factors from the periphery that are indispensable for the involvement of the brain in the induction of pyrogenic tolerance. The mechanism by which endogenous pyrogen affects the hypothalamic thermostat in fever onset seems to be a function of the ability of EP to stimulate arachidonic acid turnover in various tissues and production of the prostaglandin E_2 (PGE_2)[14]. It has been observed that PGE_2 activated the liberation of corticotropic releasing factor[15]. It is furthermore thought that PGE_2 can penetrate across the BBB[12]. Although PGE_2 seems to act as a putative signal for induction of the brain-periphery immune-suppressive feedback loop during tolerance, we are not aware of any report indicating that repeated injection of PGE_2 can produce effects characteristic for pyrogenic tolerance, and that PGE_2 itself is an indispensable factor in inducing the tolerance.

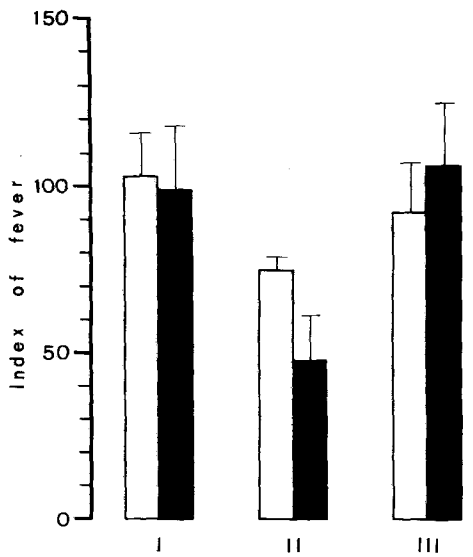

Figure 2. Fever indexes (°C · h) during induction of tolerance to LPS given i.c.v. (open columns) and i.v. (closed columns). I = first day of tolerance; II = last day of tolerance; III = after changing the route of LPS administration, (mean ± SD, n = 4).

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