INTERACTIONS BETWEEN BACTERIAL PYROGEN AND PROTEOLIPID EXTRACTED FROM THE CEREBRUM (I)

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Abstract—As proteolipid of the myelin sheath and its parent glial membrane may possibly interact with bacterial pyrogen (lipopolysaccharide, LPS) during penetration into the brain, we investigated the interaction of LPS with proteolipid derived from the cerebrum of rabbits, rats and chickens. Intravenous administration of LPS (1 μ g/kg) produced a febrile response in rabbits, but not in rats and chickens. Marked hyperthermia was observed in these three species after intracisternal administration of LPS (0.01–0.1 μ g/kg). Dinitrophenol (30 mg/kg s.c.) induced a high fever in these three species tested, particularly in the chickens. The pyrogenicity of LPS given intravenously to rabbits was inactivated by incubation of LPS with proteolipid *in vitro*. Inactivation effects of proteolipid extracted from the three species was in the order of: chickens, rats and rabbits. In rats, the inactivation effects of proteolipid from the adult animal were more potent than in the case of newborn animals. The febrile response induced by dinitrophenol and leucocytic pyrogen in rabbits, however, was not suppressed by incubation with proteolipid extracted from the rabbit brain. These results suggest that proteolipids do play an important role in the mechanism of penetration of LPS into the brain.

It is generally accepted that bacterial pyrogen (lipopolysaccharide, LPS) given i.v. directly acts on the thermoregulatory center in the hypothalamus after penetrating the blood brain barrier (1–3). However, the mechanism of penetration of LPS is poorly understood. Bennett et al. (4) and we (5–7) demonstrated that LPS given i.v. was detected in the cerebrospinal fluid of febrile rabbits after i.v. administration of LPS. We also found that pyrogenicity of LPS was markedly potentiated by pretreatment of rabbits with eserine, probably due to the enhancement of permeability of the blood brain barrier (8). There are species differences of animals regarding the febrile response induced by LPS and it is well known that the pyro-

genicity of LPS depends on the development of blood brain barrier, which is closely related to the progress of myelination (9). Thus, it was deemed worthy of investigation to determine whether or not proteolipid of the myelin sheath and its parent glial plasma membrane (10, 11) is affected by the febrile response induced by LPS. The present experiment was an attempt to elucidate the possible interaction between LPS and proteolipid extracted from the cerebrum of rabbits, rat and chickens.

MATERIALS AND METHODS

Experimental animals: Male domestic white rabbits weighing 2.0–2.8 kg, male Wistar rats weighing 150–200 g and male

adult chickens weighing 1.5–2.0 kg were used.

Record of febrile response: Rectal temperature was measured with a thermoelectric apparatus, and the fever index for 4 hr (FI-4) was calculated from the curves of mean value ($\Delta T^{\circ}\text{C}$) in 3 animals of each species using a planimeter, according to the method of Kanoh et al. (12).

Preparation of pyrogens: LPS was prepared from phenol-water extraction of *E. coli* UKT-B strain and purified by ultracentrifugation at $105000\times g$ (13). The purified LPS was dissolved in Folch's chloroform-methanol-water mixture (86:14:1) (14). Leucocytic pyrogen was prepared from polymorphonuclear leucocytes collected from the exudate in the peritoneal cavity of rabbits 18 hr after injection of sterile saline (15).

Preparation of proteolipid and other fractions: Proteolipid was extracted from the white and gray matter of the cerebrum using a chloroform-methanol mixture (14). The extraction was composed of a lower transparent chloroform phase, an upper water phase and a residual fraction, which corresponded to proteolipid fraction, water soluble fraction and insoluble fraction, respectively. Twenty ml of proteolipid fraction and 9.6 ml of water soluble fraction were obtained from 1 g of cerebrum wet wt. and extracted residue was suspended in 20 ml of sterile saline followed by sonication at 20 kc for 10 min.

Incubation procedures: LPS solution (0.1 ml) was incubated with these three fractions (9.9 ml) at 37°C for 30 min *in vitro*, at a concentration of $10\text{ }\mu\text{g/ml}$, and then diluted with sterile saline to $0.1\text{ }\mu\text{g/ml}$ before i.v. administration ($0.1\text{ }\mu\text{g/kg}$) to rabbits. The proteolipid fractions diluted with the lower phase solvent (Folch's solvent) was also used for the comparative studies of inactivation effect. In control experiments, LPS was incubated in lower phase solvent, upper

phase solvent (chloroform-methanol-water; 3:48:47) and saline, respectively. α -2,4-Dinitrophenol (Nakarai Chemicals) was dissolved in the proteolipid fraction at the concentration of 10 mg/ml and incubated at 37°C for 30 min. The organic solvent was then evaporated *in vacuo* and the dried complex was resuspended in saline before injection. Lyophilized leucocytic pyrogen was dissolved in water and incubated with the proteolipid fraction at 37°C for 30 min at the concentration of 10 mg of pyrogen/ml. After incubation, the mixture solution was dialyzed against methanol and then water.

RESULTS

Pyrogenicity of LPS and dinitrophenol:

Intravenous administration of $1\text{ }\mu\text{g/kg}$ of LPS produced a febrile response of over 2°C in rabbits (Fig. 1). The same dose of LPS, however, did not lead to the production of

Fig. 1. Changes in rectal temperature ($\Delta T^{\circ}\text{C}$) after intravenous (i.v.) and intracisternal (i.c.) administration of LPS in rabbits, rats and chickens. Each curve represents the mean response in 3 animals.

Fig. 2. Changes in rectal temperature ($\Delta T^{\circ}\text{C}$) after subcutaneous administration of dinitrophenol (30 mg/kg) in rabbits, rats and chickens. Each curve represents the mean response in 3 animals.

fever in rats and chickens. Marked hyperthermia was observed in rabbits and chickens by intracisternal injection of $0.01 \mu\text{g/kg}$ of LPS. In the rat, $0.1 \mu\text{g/kg}$ of LPS was required to obtain a febrile response over 1.5°C .

Subcutaneous administration of 10 mg/kg of dinitrophenol led to the production of fever in chickens, but not in rabbits and rats. In the chickens, there was a marked febrile response of over 4°C and the animals died within 30 min after 30 mg/kg of the drug had been given s.c. In rabbits and rats, a febrile response of approx. 2.0°C occurred with a dose of 30 mg/kg (Fig. 2).

Treatment of LPS with proteolipid: The pyrogenicity of LPS was determined after incubation of LPS with proteolipid, water soluble and residual fractions from the rabbit cerebrum. As shown in Fig. 3, the febrile response of LPS given i.v. to rabbits completely disappeared after treatment with the proteolipid fraction. Incubation of LPS with the water soluble fraction and residual fraction did not produce to any significant inactivation of the pyrogenicity.

Treatment of dinitrophenol and leucocytic pyrogen with proteolipid: The pyrogenicity of dinitrophenol and leucocytic pyrogen was not affected by incubation with the pro-

Fig. 3. Effects of proteolipid, water soluble and residual fractions extracted from the rabbit cerebrum on LPS induced fever. Dose of LPS: $0.1 \mu\text{g/kg}$ i.v. Each curve represents the mean response in 3 rabbits.

Fig. 4. Effects of proteolipid fractions extracted from the rabbit cerebrum on fever response induced by subcutaneous administration of dinitrophenol (DNP: 20 mg/kg) and intra-cisternal administration of leucocytic pyrogen (LP: 0.1 mg/kg). Each curve represents the mean response in 3 rabbits.

teolipid fraction obtained from the rabbit cerebrum (Fig. 4).

Inactivation effects of proteolipid extracted from different species: Inactivation effects of proteolipid on pyrogenicity of LPS were compared using the proteolipid fraction extracted from the cerebrum of rabbits, rats and chickens. The proteolipid fraction derived

from chickens had the most potent protective effect against the fever reaction of LPS (Fig. 5). Inactivation effects of the fractions were in the order of: chickens, rats and rabbits.

Inactivation effects of proteolipids extracted from adult and newborn rats: The inactivation effects of proteolipid on pyrogenicity of LPS were examined using the proteolipid fractions extracted from the cerebrum of adult, 6-day-old, and 1-day-old rats. Treatment of LPS with the fraction from the newborn animals produced a weaker protection against the pyrogenicity of LPS as compared to the fraction from the adult animals. The order of inactivation effects of the fractions was as follows: adult, 6-day-old and 1-day-old animals (Fig. 6).

Fig. 5. Effects of proteolipid fractions extracted from the cerebrums of rabbits, rats and chickens on LPS-induced fever. Dose of LPS: $0.1 \mu\text{g/kg}$ i.v. FI-4 represents the fever index for 4 hr.

Fig. 6. Effects of proteolipid fractions extracted from the cerebrums of adult, 6-day-old and 1-day-old rats on LPS-induced fever. Dose of LPS: $0.2 \mu\text{g/kg}$ i.v. FI-4 represents the fever index for 4 hr.

DISCUSSION

The intravenous administration of LPS reconfirmed the species differences of the pyrogenicity of LPS. This LPS produced a marked febrile response in rabbits, but not in rats and chickens. On the other hand, intracisternal administration induced a febrile response in all three species. In mice and rats, LPS induced either hyperthermia or hypothermia, depending on the dose and the route of administration (16). Feldberg and Saxena (17) reported that a febrile response was observed in rats after intraventricular administration of LPS but not after i.v. administration, thereby suggesting that LPS had difficulty in passing through the blood brain barrier in rats. Pittman et al. (18) demonstrated that administration of bacterial pyrogen into the anterior hypothalamus of chickens produced a febrile response whereas the i.v. route induced no such response. These reports are in line with our findings in chickens.

Dinitrophenol is a pyrogen which acts peripherally in an uncoupling action of mitochondrial oxidative phosphorylation (16).

As this drug produced a febrile response in rabbits, rats and chickens, there is probably little species differences in the production of heat in the peripheral tissues. Therefore, the species differences in pyrogenicity of LPS may depend to some extent on permeability of the blood brain barrier. De Robertis and Gerschenfeld (19) stated that the glial cell membrane might play an important role in control mechanisms involved in the blood brain barrier. The myelination by glial cell membrane was shown to advance in parallel with growth (9).

In the present study, the pyrogenicity of LPS was totally inactivated by treatment with the proteolipid fraction extracted from the cerebrum, however, the proteolipid fraction failed to inactivate the pyrogenicity of dinitrophenol and leucocytic pyrogen. Inactivation effects of proteolipid fractions derived from the cerebrum was in the order of : chickens, rats and rabbits; and this order corresponded to the lack of response of fever reaction in these three species after i.v. administration of LPS. Proteolipid is a lipophilic protein material (10), and LPS is composed of a lipid A, a pyrogenic principle, and a hydrophilic polysaccharide (13). Therefore, proteolipid probably binds firmly to the lipid A moiety of LPS, however, the differences of inactivating capacity of proteolipid between the species of animals remain obscure.

Our findings lend support to the idea that proteolipid, a main component of myelin sheath and glial cell membrane, may play an important role in the penetration mechanism of LPS through the blood brain barrier.

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