Plasma haptoglobin response to intracerebroventricular administration of cytokines in rats

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ABSTRACT

To examine effects of brain cytokines on hepatic acute phase protein synthesis, the plasma level of haptoglobin (Hp) was measured after intracerebroventricular (icv) administration of human recombinant interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF)- α in rats. Icv injection of IL-1 or TNF (100 ng/rat, 3 times at every 3 h) produced a significant increase in plasma Hp level 12–24 h after the first injection. Neither intraperitoneal injection of these cytokines at the same doses nor icv injection of IL-6 affected the plasma Hp level. These results indicate that brain IL-1 and TNF, but not IL-6, can induce the acute phase response of some plasma proteins.

In the acute phase of inflammatory process, some plasma proteins, so-called acute phase proteins (APPs), are synthesized in the liver. Haptoglobin (Hp), α 1-acid glycoprotein and α 2-macroglobulin (α 2-MG) are typical APPs in the rat (5). It appears to be established now that APP synthesis in the liver is controlled directly by circulating inflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor α (TNF- α) (3, 5). Although these cytokines were originally identified in peripheral cells, they are also found to be synthesized in the brain, suggesting a significant role of brain cytokines under certain pathophysiological conditions (11). In fact, injection of a minute amount of these cytokines into the brain induces typical acute phase responses such as fever (6, 12), induction of slow-wave sleep (14), and activation of both sympathetic nervous (1, 16) and adrenocortical systems (9, 13, 17).

There are also reports suggesting that these cytokines may participate in controlling hepatic APP synthesis through their action in the brain. Bailey *et al.* (2), for example, reported that intracerebroventricular (icv) injection of crude 'leukocytic endogenous mediator', which probably contains various cytokines, to rats induced an increase in plasma α 2-MG level as well as fever and hypo-

ferremia. Furthermore, Mashburn *et al.* (7) demonstrated in guinea pigs that intrapreoptic injection of recombinant IL-1 increased plasma levels of copper and protein-bound *N*-acetylneuraminic acid, suggesting indirectly that the plasma ceruloplasmin, another APP, and acute phase glycoproteins are under the influence of central IL-1. Despite these early studies, there are few reports demonstrating directly that recombinant cytokines given into the brain can actually induce APP synthesis. To clarify whether IL-1, IL-6 and TNF- α can act at the level of the brain to influence hepatic APP synthesis, in the present study, we examined plasma Hp response to icv injection of these cytokines. The plasma level of IL-6, the most effective inducer of Hp synthesis, was also examined in some experiments.

Female Wistar rats weighing 180–210 g were anesthetized with sodium pentobarbital (40 mg/kg, ip) and implanted with a stainless steel cannula (0.4 mm o.d.) in the right lateral cerebral ventricle, and used after 2 weeks of recovery period. Human recombinant IL-1 β (2×10^7 U/mg protein, Otsuka Pharmaceutical, Tokushima, Japan), TNF- α (2.55×10^6 U/mg protein, Dainippon Pharmaceutical, Osaka, Japan) and IL-6 (5×10^6 U/mg protein, Tosoh, Ayase, Japan) were diluted with sterilized

20 mM phosphate-buffered saline (PBS) and injected intraperitoneally (500 μ l) or intracerebroventricularly (10 μ l) at doses indicated. Zero to 72 h after the first injection, blood samples (100 μ l) were collected from the tail vein into heparinized glass pipettes, and diluted with 50 mM PBS. After centrifugation, plasma samples were used for assays of Hp and IL-6.

Plasma Hp concentration was evaluated by an enzyme-linked immunosorbent assay. Briefly, the plasma samples were diluted by up to 2,400-fold with 50 mM PBS and applied to micro-wells. After blocking the wells with 1% egg albumin, peroxidase-labeled goat anti-human Hp immunoglobulin (Binding Site, Birmingham, England Lot. G2070R) and the enzyme substrate were added. The wells were incubated at 37°C for 90 min, and the absorbance at 405 nm was measured. Hp contents were calculated using human Hp phenotype 1-1 (Sigma, St. Louis, MO, U.S.A.) as a standard, because purified rat Hp is not commercially available. The minimal plasma Hp concentration detectable by this method was about 2.4 μ g/ml. The concentration of plasma IL-6 was measured using an IL-6-dependent cell line MH60.BSF2 (a gift from Dr T. Matsuda, Osaka University, Suita, Japan) as previously described (8). The minimal plasma IL-6 concentration detectable was about 7.5×10^{-2} U/ml (15 pg/ml).

To examine the effect of brain cytokines, first, we injected 40–150 ng recombinant human IL-1 β , IL-6 and TNF- α intracerebroventricularly once, and obtained blood samples 24 h later. No noticeable change was found in plasma Hp level compared with vehicle controls (data not shown). Next, beginning at zero time, each rat was injected three times with 100 ng of IL-1 β , IL-6, or TNF- α at 3 h intervals resulting in a total cytokine administration of 300 ng/animal. As shown in Fig. 1, plasma Hp level increased significantly 24 h after the first injection of IL-1 and TNF. The stimulatory action of these two cytokines would not be a consequence of some nonspecific effect of the treatment, because a similar injection of vehicle (PBS) or IL-6 did not produce any noticeable increase in plasma Hp level. IL-6 was not effective even at much higher doses of 300–900 ng/rat for at least 48 h (data not shown). The stimulatory effect of central IL-1 on plasma Hp response is not unexpected, because there was a report suggesting that plasma ceruloplasmin, another APP, is under the influence of central IL-1 in the guinea pig (7). Furthermore, De Simoni *et al.* (4) demonstrated that icv admin-

Fig. 1 Plasma haptoglobin (Hp) levels after intracerebroventricular injection of interleukin (IL)-1, IL-6, tumor necrosis factor (TNF) or vehicle (PBS). One hundred ng of each cytokine was injected three times at 3 h intervals. Hp levels were measured 24 h after the first injection. Values are expressed as relative to those at time 0, and are means $\pm$ SE for 7 rats. The Hp concentration at time 0 was 17.0 ± 1.1 μ g/ml as equivalent to human Hp phenotype 1-1. * $P < 0.05$ compared with vehicle control

istration of IL-1 induces a high circulating level of IL-6, which is considered to play the most crucial role in acute phase protein synthesis as a hepatocyte-stimulating factor.

Fig. 1 shows that TNF- α , as well as IL-1, is also effective in inducing plasma Hp response. Since this is the first evidence for a possible role of brain TNF in the regulation of APP response, plasma Hp and IL-6 responses to TNF were examined in detail. Fig. 2 shows the time course of the responses. Plasma Hp level increased rapidly to a peak at 12 h, and then decreased gradually to basal level. When the same dose (100 ng) of TNF was injected intraperitoneally under the same schedule (3 times at every 3 h) as for the icv injection, plasma Hp level did not show any significant change. Again, icv injection of vehicle (PBS) had no effect. It is thus quite unlikely that the Hp responses induced by central TNF is due to a passage of TNF into the circulation through the blood-brain barrier. Plasma IL-6 level was also increased 12 h after the icv injection of TNF, whereas it was not affected by the intraperitoneal injection. Thus, centrally given TNF induces a concomitant increase in the plasma levels of Hp and IL-6 at doses which are ineffective when given peripherally, suggesting that brain TNF stimulates hepatic Hp synthesis through the rise of plasma IL-6, as IL-1 probably does. It should be noted, however, that Hp synthesis is stimulated not only by IL-6 but also by other cytokines. Recent studies have indicated that Hp is

Fig. 2 Time courses of the change in plasma Hp (A) and IL-6 (B) levels after intracerebroventricular or intraperitoneal injection of TNF (100 ng/rat, 3 times within 6 h). Arrows show the injection time. Values are means $\pm$ SE for 5–7 rats, and expressed as in Fig. 1. The IL-6 concentration at time 0 (0 h) was 0.32 ± 0.04 U/ml. * $P < 0.05$ compared with the value at time 0 and vehicle control at respective times

classified as a type 1 APP, at least in the rat, of which synthesis is stimulated by IL-1 and TNF in synergy with IL-6 (3). In preliminary experiments, we observed a slight but consistent increase in plasma TNF level after icv injection of IL-1. Further studies are needed to clarify the most important peripheral factor for Hp synthesis induced by central cytokines.

It has been demonstrated that some cytokines such as IL-1 and TNF have potent central actions to produce broad spectrum of inflammatory host defense responses such as fever (6, 12), anorexia (10), adrenocortical activation (9, 13, 17) and slow-wave sleep induction (14). The present findings extend the list of central actions of these cytokines and indicate the existence of a pathway from the brain to the liver, probably through the increase in plasma IL-6. The pathophysiological significance of this pathway is not clear at present, but it might explain how infections or lesions confined to the central nervous system result in systemic alterations in acute phase responses including APP induction. In fact, recent reports have demonstrated that the concentration and distribution of IL-1 and TNF in the central nervous system

change dramatically in response to a small and local lesion of the brain (15, 18) or to peripheral administration of bacterial endotoxin (11).

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