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SHORT COMMUNICATION

The Influence of Interleukin-1β on γ-Glutamyl Transpeptidase Activity in Rat Hippocampus

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Summary

Brain infections as well as peripheral challenges to the immune system lead to an increased production of interleukin-1beta (IL-1β), a cytokine involved in leukocyte-mediated breakdown of the blood-brain barrier. The effects of IL-1β have been reported to depend on whether the route of administration is systemic or intracerebral. Using 50-day-old male rats, we compared the effects of IL-1β on brain γ-glutamyl transpeptidase (GGT; an enzymatic marker of brain capillary endothelium) at 2, 24 and 96 h after either an intravenous (i.v.) injection of 5 μg IL-1β or an intracerebroventricular (i.c.v. - lateral ventricle) infusion of 50 ng IL-1β. When the i.v. route was used, the GGT activity underwent small but significant changes; decreasing in the hippocampus 2 h after the i.v. injection, increasing 24 h later and returning to control levels at 96 h. No significant changes in the hippocampal GGT activity were observed at 2 and 24 h following the i.c.v. infusion. The GGT activity in the hypothalamus remained unchanged regardless of the route of IL-1β administrations. Similar changes in GGT activity were revealed histochemically. The labeling was found mainly in the capillary bed, the changes being most evident in the hippocampal stratum radiatum and stratum lacunosum-moleculare. A transient increase in GGT activity at 24 h, together with a less sharp delineation of GGT-stained vessels, may reflect IL-1β induced increased turnover of glutathione and/or oxidative stress, that may in turn, be related to altered permeability of the blood-brain barrier in some neurological and mental disorders, including schizophrenia.

Key words

Pro-inflammatory cytokine • Rat hippocampus • γ-glutamyl transferase • Blood-brain barrier • Reactive oxygen species • Schizophrenia

Changes in the production of interleukin-1β (IL-1β) accompany many neurological and mental disorders (ischemic, traumatic and degenerative brain damage, Rothwell and Luheshi 2000; autism, depression

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and schizophrenia, Pearce 2003). IL-1β belongs to a group of pro-inflammatory cytokines, produced by blood phagocytes and by brain macrophages, microglia and endothelial cells (Zhang *et al.* 1998, Gallová *et al.* 2004). It has been reported to induce a breakdown of the blood-brain barrier (BBB) in both *in vivo* (Bolton *et al.* 1998) and *in vitro* experimental models (de Vries *et al.* 1996). Effects of IL-1β are mediated by specific receptors (IL-1R) located on both luminal and abluminal sides of endothelial cells. Activation of IL-1R results in the production of prostaglandin E_2, nitric oxide (Cao *et al.* 2001) as well as in the generation of reactive oxygen species (Tolando *et al.* 2000, Gallová *et al.* 2004). The damage to the BBB reflected in changes of the activity and/or expression of γ-glutamyl transpeptidase (GGT), an ectoenzyme expressed preferentially on the luminal side of rat brain endothelial cells (Šťastný *et al.* 1997, Zhang *et al.* 1997). GGT is thought to be involved in protection of the brain capillary endothelium from oxidative stress *via* its importance for the regulation of glutathione levels. In contrast to the luminal localization of GGT, the IL-1R is distributed on both sides of the brain endothelial cells. This appears to be in conflict with the reported large difference between the respective effects of intravenous (i.v.) or intracerebroventricular (i.c.v.) administration of IL-1β (Proescholdt *et al.* 2002).

In an attempt to resolve the apparent contradiction we studied effects of IL-1β administration on GGT in the hippocampus and hypothalamus, two regions of the rat brain that have been identified as having high levels of the enzyme (Lisý *et al.* 1983). We hypothesized that the GGT activity, being located on the same (luminal) side as the activated IL-1R, would increase and help to protect BBB during the i.v. administration of IL-1β but would fail to respond against IL-1β approaching the IL-1R from the interstitial space (i.c.v. route).

Experiments were carried out using 50-day-old males (Wistar:Han from BioTest, CR; b.w. 240-260 g) housed in a temperature-controlled environment (21 to 22 °C) under a 12 h light/dark regime and given food and water *ad libitum*. Every effort was made to minimize the number of animals and their possible suffering in accordance with the Declaration of Helsinki. Interleukin-1β (rat IL-1β, recombinant; Sigma, St. Louis, MO) was administered on the day of the experiment between 09:00 and 10:00 h. Animals were injected into the tail vein with 5 μg IL-1β in 0.75 ml sterile 0.9 % NaCl. Control rats were given isotonic saline only. At intervals of 2, 24 and 96 h after the i.v. injection, rats were decapitated and left/right hippocampus (and hypothalamus) were dissected as previously described (Lisý *et al.* 1983, Šťastný *et al.* 1997). Another group of rats was administered IL-1β at 50 ng IL-1β/0.5 μl sterile saline (or the same volume of sterile saline in the controls) into each lateral cerebral ventricle (Skuba and Šťastný 2004). At 2 and 24 h following the i.c.v. infusion, the samples of brain tissue were collected as described above.

Tissue was homogenized in 1 ml of 0.9 % NaCl, centrifuged at 12,000 x *g* for 20 min and resuspended in 1.5-2 ml of distilled water. The resulting suspensions were used for the assay of GGT activity and protein estimation as previously described (Šťastný *et al.* 1997). For histochemical detection of GGT activity, deeply anesthetized animals were decapitated, the brains were frozen in isopentane at –70 ºC and 10 μm slices

Table 1. Changes in the activity of GGT activity (μmol p-nitroanilide. mg^{-1} protein . min^{-1}) in crude membrane fractions prepared from rat hippocampus and hypothalamus after i.v. and/or i.c.v. administration of IL-1β.

Response to i.v. IL-1β administration

Rat brain region	***Control***	***IL-1β (2 h)***	***IL-1β (24 h)***	***IL-1β (96 h)***
Hippocampus	7.90 ± 0.28	6.24 ± 0.50*	9.27 ± 0.51*$^{\#}$	7.74 ± 0.28$^{\#\$}$
Hypothalamus	13.47 ± 0.23	12.34 ± 0.78	13.22 ± 2.45	12.33 ± 0.59

Response to i.c.v. IL-1β administration

Rat brain region	***Control***	***IL-1β (2 h)***	***IL-1β (24 h)***	***IL-1β (96 h)***
Hippocampus	8.91 ± 0.73	7.74 ± 0.27	9.27 ± 0.51	n. d.
Hypothalamus	12.67 ± 0.76	12.35 ± 1.01	12.11 ± 1.88	n. d.

Data are means ± S.E.M. of 3 experiments with activity determination in duplicates. Neuman-Keuls pairwise comparison showed significant differences ($p<0.05$):* vs. control, $^{\#}$ vs. IL-1β (2 h), $^{\$}$ vs. IL-1β (24 h); n.d.= not determined.

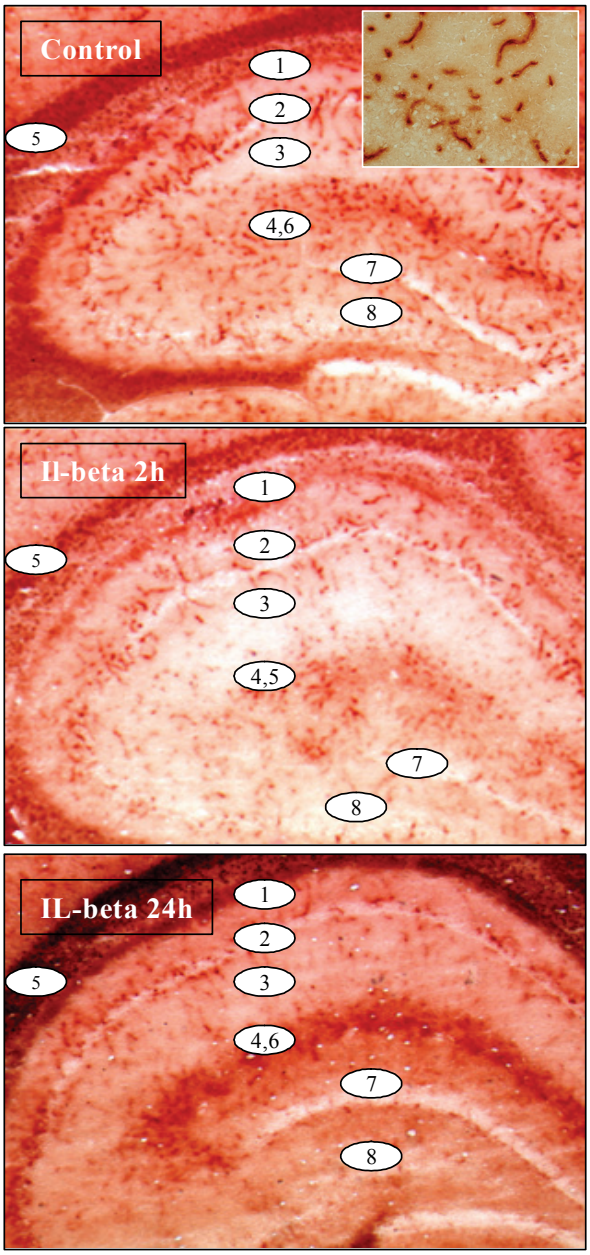

Fig. 1. Histochemical staining of GGT activity in the dorsal part of the hippocampus of control rats (above), 2 h (middle) and 24 h (below) after i.v. injection of IL-1β (cryostat sections, Olympus Provis AX-70, magnification x20). Insert: preferential localization of the enzyme in vascular bed is shown in control animal in the stratum oriens (magnification x400). For GGT activity measured in regions 1-8 see Table 2.

were cut on a cryostat (Brighton, UK). Histochemical activity of GGT was determined by a previously described technique (Lojda 1981, Šťastný *et al.* 2004) using γ-glutamyl-4-methoxy-naphtylamide (Bachem, Bubendorf, Switzer-land) as an enzyme substrate.

The specific activity of GGT in homogenized tissue was found to be lower in the hippocampus compared to the hypothalamus (Table 1). Two changes in the GGT activity – a decrease at 2 h and a rise at 24 h

Table 2. Densitometry of histochemical staining of GGT activity in the dorsal part of the hippocampus of control rats, 2 h and 24 h after i.v. injection of IL-1β.

Region	Control	2 h after IL-1β	24 h after IL-1β
Dorsal hippocampus proper			
(1) Stratum oriens	0.19 ± 0.01	0.15 ± 0.01*	0.42 ± 0.01*#
(2) Stratum pyramidale	0.12 ± 0.04	0.09 ± 0.02	0.32 ± 0.02*#
(3) Stratum radiatum	0.13 ± 0.01	0.06 ± 0.01*	0.31 ± 0.08*#
(4) Stratum lacunosum	0.27 ± 0.02	0.13 ± 0.02*	0.60 ± 0.03*#
(5) Alveus	0.31 ± 0.05	0.25 ± 0.01	0.81 ± 0.02*#
Dentate gyrus			
(6) Stratum moleculare	0.17 ± 0.01	0.14 ± 0.01	0.45 ± 0.01*#
(7) Stratum granulare	0.10 ± 0.04	0.07 ± 0.02*	0.18 ± 0.01*#
(8) Hilus	0.11 ± 0.01	0.08 ± 0.08*	0.32 ± 0.01*#

Values are means ± S.E.M. of 5-12 densitometric measurements. One-Way ANOVA/Kruskal-Vallis test indicated significant differences ($p<0.05$) between different layers of the hippocampus in control as well as IL-1β-treated rats. Dunn´s pairways comparison within individual layers of hippocampus indicated following significant differences ($p<0.05$): * vs. control, # vs. IL-1β (2 h). Numbers 1-8 refer to layers in Fig. 1.

after the i.v. administration of IL-1β – were observed in the hippocampus but not in hypothalamus (Table 1). No changes in the GGT activity following the i.c.v. administration of IL-1β were statistically significant (Table 1). Histochemical localization of GGT in the hippocampus indicated a decrease at the 2 h time point and an increase at 24 h time point in the enzyme activity, being most evident in the hippocampal stratum pyramidale, radiatum and moleculare-lacunosum (Fig. 1; Table 2). At the 2 h interval, the fall of GGT activity appeared preferentially associated with brain capillaries whereas 24 h after the i.v. injection of IL-1β there was an additional increase in GGT activity in the adjacent neuropil. At the 24 h interval, GGT-stained microvessels appeared less sharply delineated from the surrounding brain parenchyma. Furthermore, the present data extend previous findings on the non-homogeneity of the capillary network in different strata of the rat hippocampus (Grivas *et al.* 2003).

The histochemical changes in GGT activity are consistent with previous reports demonstrating that the initial activation of endothelial cells is IL-1β-dependent (Proescholdt *et al.* 2002) and is accompanied by increased microvascular permeability within the first few hours after the cytokine injection (Bolton *et al.* 1998), followed by a period of astrocytes/microglia activation (Proescholdt *et al.* 2002). The activation of glial cells

might be followed by the induction of GGT mRNA with a maximum of GGT activity 24 h after the IL-1β administration, in analogy to the similar changes observed in myeloid cells (Miller *et al.* 1993). Whereas the decrease observed 2 h after IL-1β injection can reflect an interaction of reactive oxygen species (and nitric oxide) with GGT, the subsequent increase observed 24 h after the i.v. injection suggests a possible occurrence of a transient IL-1β/IL-1R-mediated expression of GGT. The resulting changes in the activity of GGT in the capillary wall could be further potentiated by GGT-mediated production of reactive oxygen species and by possible activation of the nuclear transcription factor NF-κB during early stages of the immune and inflammatory responses to the systemic elevation of IL-1β (Accaoui *et al.* 2000).

In summary, the present data are consistent with a role of the endothelial membrane-bound enzyme GGT in helping to maintain the structural and functional integrity of brain microvessels in response to the presence of IL-1β in the blood stream. The changes in the enzyme activity are, however, biphasic suggesting that the process may involve both its interaction with free radical species and subsequent enzyme induction as well as additional mechanisms. It suggests that overproduction of free radicals by IL-1β might be involved in the increased blood-brain barrier permeability observed in patients with schizophrenia (Kirch *et al* 1985).

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