Acute hypothalamo-pituitary-adrenal axis response to LPS-induced endotoxemia: Expression pattern of bradykinin type B1 and B2 receptors

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Online supplement

Supplemental Results:

Real-time quantitative PCR:

Supplemental Figure 1 shows quantitative real-time PCR in 3 h LPS-induced kinin B1R and B2R mRNA levels (increase fold change) in the hypothalamus [A], pituitary [B] and adrenal glands [C]. A significant increase in the B1R mRNA level was found in the hypothalamus, whereas in pituitary and adrenal glands mRNA levels of both kinin receptors were increased significantly three hours after LPS injection.

Methodology: cDNA was synthesized from 1 µg total RNA with MMLV reverse transcriptase (Promega, Southampton, UK) using random hexamer nucleotides. Real-time quantitative PCR was performed using SYBR Green Master Mix (Applied Biosystems, Foster City, USA) on ABI Prism 7900 sequence detection system with 10 ng cDNA and 100 nM primers. Gene expression was normalized to TBP (TATA box binding protein) and expressed as relative value to vehicle group using the comparative threshold cycle method ($2^{-\Delta\Delta Ct}$). The primer sequences as follows, kinin B1R fw: 5`-TGA GCA TCC CCA CAT TCC TT-3`, rev: 5`-ACG TTC AAC TCC ACC ATC CT-3`(Acc # AI009899.1), kininB2R fw: 5`-AGG AGA AGT TGG TGG GCT AC-3`, rev: 5`-TCA TCA GGG TGT CGA CGA AA-3` (Acc # L26173.1) and TBP fw: 5`-ACT GTT TCA TGG TGC GTG AC-3`, rev: 5`-AGC ATA AGG TGG AAG GCT GT-3`(Acc # NM_001004198.1).

Effect of LPS given ip on B1R and B2R mRNA levels in the brain stem regions:

Supplemental Figure 2 shows LPS-induced B1R and B2R mRNA levels in the dorsal medulla (DM) and ventral medulla (VM) of the brain stem. A significant increase in the

B1R mRNA level was found both in DM and VM of the brainstem six hours after LPS injection.

Specificity of antibody against B1R: When compared to kidney sections from vehicle-treated WT [supplemental Figure 3 (A)], LPS treatment induced an up regulation of B1R in WT and B2R-KO mice, respectively [supplemental Figure 3 (B) and (D)], whereas no immunopositive signal was found in kidney tissue from B1R-KO mice [supplemental Figure 3 (C)]. The immunopositive signal for B1R was absent in vehicle-treated B1R-KO or B2R-KO mice (data not shown). These data verified the specificity of the antibody against B1R used here.

Specificity of antibody against B2R: Immunofluorescence staining of adrenal glands with B2R antibody revealed strong B2R immunopositive cells in the adrenal medulla and cortex of WT and B1R-KO mice. In case of B1R-KO, the B2R immunopositive signal was even stronger in adrenal medulla as well as in cortex compared to WT (supplemental Figure 4 upper panel and middle panel, respectively). However, there was no B2R immunopositive signal in the adrenal gland of B2R-KO mouse (supplemental Figure 4 lower panel). This data demonstrate the specificity of the antibody used against B2R.

Supplemental Figure Legend:

Supplemental Figure 1

Real-time quantitative PCR analysis of mRNA levels (fold change) in kinin B1R and B2R in [A] hypothalamus, [B] pituitary and [C] adrenal glands 3 h after LPS (5 mg/kg, ip) vs. vehicle treated control rats. Values are mean ± SEM. *p<0.05, ***p<0.001 vs. vehicle. n= 3/group.

Supplemental Figure 2

RT-PCR analysis of mRNA levels: [A] Kinin B1 (upper panel) and [B] B2 (lower panel) receptor mRNA levels normalized to β-actin mRNA in the dorsal and ventral medulla of brainstem 1, 3 and 6 h after a single injection of LPS (5 mg/kg, ip) or 0.9% saline which served as vehicle control in SD rats, as analyzed by RT-PCR. Values are mean ± SEM (n = 4 each group, treatment and each time point). **p<0.01, ***p<0.001 vs. vehicle. n= 4/group/time point.

Supplemental Figure 3

Immunofluorescence images showing the specificity of anti-B1R antibody in mouse kidney. (A) Wild type mice (Bl6) treated with vehicle ip, served as control, (B) wild type, (C) kinin B1R knock-out and (D) kinin B2R knock-out mice, all treated with a single ip injection of LPS and sacrificed after 3h. Specific and strong immunoreactivity for B1R in the kidney following LPS is found in glomerulus and in kidney tubules of wild type (B) and B2R knock-out mice (D), whereas the immunoreactivity is absent in wild type control

(A) and B1R knock-out mice (C), providing evidence for the specificity of the antibody. n= 3/group.

Abbreviations: G= glomerulus; T= kidney tubules; ip= intraperitoneal

Supplemental Figure 4

Immunofluorescence images showing the specificity of anti-B2R antibody in mouse adrenal gland in basal condition in wild type (Bl6) (upper panel), kinin B1R knock-out (middle panel) and kinin B2R knock-out mice (lower panel). Specific and strong immunoreactivity for endogenous B2R is found in the adrenal medulla of wild type and B1R knock-out mice, whereas mild B2R immunoreactivity is detected in adrenal cortical cells. The immunoreactivity is absent in adrenal medulla and cortex of B2R knock-out mice, providing evidence for the specificity of the antibody. n= 3/group.