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SIGNALING PATHWAYS OF INTERLEUKIN-1 ACTIONS IN THE BRAIN: ANATOMICAL DISTRIBUTION OF PHOSPHO-ERK1/2 IN THE BRAIN OF RAT TREATED SYSTEMICALLY WITH INTERLEUKIN-1β

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Abstract—Interleukin-1β is released at the periphery during infection and acts on the nervous system to induce fever, neuroendocrine activation, and behavioral changes. These effects are mediated by brain type I IL-1 receptors. *In vitro* studies have shown the ability of interleukin-1β to activate mitogen-activated protein kinase signaling pathways including p38, c-Jun N-terminal kinase and extracellular signal-regulated protein kinase 1 and 2 (ERK1/2). In contrast to other mitogen-activated protein kinases, little is known about ERK1/2 activation in the rat brain in response to interleukin-1β. The aim of the present study was therefore to investigate spatial and temporal activation of ERK1/2 in the rat brain after peripheral administration of interleukin-1β using immunohistochemistry to detect the phosphorylated form of the kinase. In non-stimulated conditions, phosphorylated ERK1/2 immunoreactivity was observed in neurons throughout the brain. Administration of interleukin-1β (60 μg/kg, i.p.) induced the phosphorylation of ERK1/2 in areas at the interface between brain and blood or cerebrospinal fluid: meninges, circumventricular organs, endothelial like cells of the blood vessels, and in brain nuclei involved in behavioral depression, fever and neuroendocrine activation: paraventricular nucleus of the hypothalamus, supraoptic nucleus, central amygdala and arcuate nucleus. Double labeling of phosphorylated ERK1/2 and cell markers revealed the expression of phosphorylated ERK1/2 in neurons, astrocytes and microglia. Since phosphorylated ERK1/2 was found in structures in which type I IL-1 receptor has already been identified as well as in structures lacking this receptor, activation of ERK1/2 is likely to occur in response to both direct and indirect action of interleukin-1β on its target cells. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: interleukin-1β, extracellular signal-regulated kinase 1 and 2, inflammation, brain, neuroanatomy.

The proinflammatory cytokine interleukin-1β (IL-1β), that is released by activated innate immune cells upon injury or infection, acts on the nervous system to induce fever, neuroendocrine activation, and "sickness behavior," in the form of behavioral changes typical of disease (Dantzer, 2001). Intracerebral administration of the IL-1 receptor antagonist (IL-1ra) attenuates fever, activation of the hypothalamo–pituitary–adrenal axis and the reduction in social interactions induced by peripheral administration of lipopolysaccharide (LPS), a widely used model of inflammation (Bluthe et al., 1992; Luheshi et al., 1996). However, given its molecular weight and hydrophilic profile, IL-1β cannot passively cross the blood–brain barrier (BBB) formed by brain vascular cells and astrocytes.

Central action of IL-1β is mediated by brain IL-1 receptors that are identical to those cloned from blood cell lineages. Based on *in situ* hybridization, the type I IL-1 receptor (IL-1RI) is present mainly on brain barrier-related cells, including the leptomeninges, non-tanycytic portions of the ependyma, the choroid plexus, and vascular endothelium. Low to moderate levels of IL-1RI mRNA are expressed in only a few neuronal cell groups, including the basolateral nucleus of the amygdala, arcuate nucleus of the hypothalamus, trigeminal and hypoglossal motor nuclei and area postrema (Ericsson et al., 1995). The expression of IL-1RI at the protein level appears, however, to be more restricted, since functional IL-1RI are present mainly on endothelial cells of brain venules (Konsman et al., 2004).

In a previous study we confirmed the presence of IL-1RI on endothelial cells and demonstrated that peripheral IL-1β administration induces activation of the signaling pathway leading to the activity of the transcription factor nuclear factor kappa B (NFκB) in these same cells (Nadjar et al., 2003). This finding thus provides information about molecular target of IL-1 action on its receptors localized at the level of the BBB. However despite the elucidation of the signal transduction pathways activated by IL-1β at this

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Abbreviations: ACA(d/v), anterior cingulate area (dorsal part/ventral part); AI(d/p/v), agranular insular area (dorsal part/posterior part/ventral part); AP, area postrema; ARH, arcuate nucleus of the hypothalamus; AUD(d/p/v), auditory areas (dorsal/primary/ventral); BBB, blood–brain barrier; CEA(l/c), central amygdala (lateral/capsular); ChP, choroid plexus; CRF, corticotropin-releasing factor; CVO, circumventricular organ; ERK1/2, extracellular signal-regulated protein kinase 1 and 2; GFAP, glial fibrillary acidic protein; IB4, Isolectin B4; IL-1β, recombinant rat interleukin-1 beta; IL-1RI, type I IL-1 receptor; IL-1RI-ir, type I IL-1 receptor immunoreactivity; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; ME(ext/in), median eminence (external part/internal part); MEA(ad/pd/pv), medial nucleus amygdala (anterodorsal/posterodorsal/posteroventral); MEPO, median preoptic nucleus; MO(p/s), motor area (primary/secondary); MPO, medial preoptic area; NeuN, neuronal nuclei; NFκB, nuclear factor kappa B; NTS(co/ge/l/m), nucleus of the solitary tract (commissural part/gelatinous part/lateral part/medial part); OVLT, vascular organ of the lamina terminalis; PB, phosphate buffer; p-ERK1/2, phosphorylated ERK1/2; PIR, piriform area; PVH(ap/dp/lp/mpd/mpv/pml/pmm/pv), paraventricular nucleus of the hypothalamus (anterior parvicellular part/dorsal parvicellular part/lateral parvicellular part/medial parvicellular part, dorsal zone/medial parvicellular part, ventral zone/posterior magnocellular part, lateral zone/posterior magnocellular part, medial zone/periventricular part); SFO, subfornical organ; SON, supraoptic nucleus; SSp, somatosensory area (primary part/supplemental part); SSs, supplemental somatosensory area.

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Table 1.

	NaCl						rrIL-1β					
	15 mn (n=3)	30 mn (n=3)	1 h (n=3)	2 h (n=3)	4 h (n=2)	6 h (n=4)	15 mn (n=4)	30 mn (n=4)	1 h (n=4)	2 h (n=4)	4 h (n=4)	6 h (n=4)
ACAd	+	+/−	+	+/−	+	−	+	+	+/−	+/−	+/−	+/−
ACAv	+/−	+/−	+	+/−	+	−	+	+/−	+/−	+/−	+/−	−
ACB	−	+/−	−	−	−	−	−	−	−	−	−	−
AId	+	−	+	+/−	+	+/−	+/−	+/−	+/−	−	+	−
AIp	+/−	+/−	−	+/−	+	−	+/−	+	−	+/−	−	+/−
ALv	+	+/−	+/−	+/−	+	+/−	+/−	+	+	+/−	++	−
AP	−	+/−	+/−	−	−	+/−	+/−	+/−	++	++	+++	+++
ARH	+/−	+	+	+/−	−	+/−	+	++	+	+	++	++
AUDd	+	+/−	+/−	+/−	+	+/−	−	+/−	−	−	−	+
AUDp	+	+	+/−	+/−	+	+/−	−	+/−	−	−	−	+
AUDv	+	+	+	+/−	+	+	+/−	+/−	+/−	+/−	+/−	+
AVP	−	+	−	−	−	+/−	+/−	+	+/−	−	+/−	+
bv	+/−	+/−	+/−	+/−	+	+	+	+	+/−	+/−	+	−
CEAc	−	+/−	−	−	−	+/−	+/−	+	+/−	+/−	+	+/−
CEAl	−	−	+/−	−	−	+/−	+/−	+	+	+/−	+	−
ChP	+/−	−	−	−	−	−	+	+/−	+/−	+	−	++
COAa	−	+/−	+/−	+/−	−	+/−	−	+/−	+	−	−	−
DMHa	−	+/−	+/−	−	−	−	−	−	−	+/−	−	−
DMX	+/−	+/−	−	−	−	−	−	−	+/−	−	+/−	−
ECT	+/−	+/−	+/−	−	−	−	+/−	+	+/−	+/−	+	+
ENTl	+/−	+/−	+/−	−	+	+/−	−	+/−	−	+/−	−	−
EpC	−	−	−	−	++	−	−	−	+/−	+/−	+	+
IG	−	−	+/−	+/−	−	−	+/−	+	+/−	+/−	−	−
ILA	+/−	−	+/−	−	+	−	+/−	+/−	+/−	+/−	++	−
LH	−	+/−	+/−	+/−	−	+	+/−	+/−	+	+	++	+/−
LRNm	−	+	−	−	+	−	+/−	+/−	−	+/−	+/−	+/−
MEAad	−	−	−	−	−	−	−	+	+	+/−	−	+/−
MEApd	−	+/−	−	−	−	−	−	+	+	+/−	++	+
MEApv	−	−	−	−	−	+/−	−	+/−	+	+/−	+/−	−
MEext	+/−	+/−	+	+/−	++	+	+/−	+	+++	++	+	+++
MEin	+/−	+/−	+/−	+/−	++	+/−	+/−	−	+	+	++	+
meninges	−	+	−	+/−	+	+/−	++	+	+	++	+/−	+
MEPO	−	+/−	+	−	++	+/−	+	+/−	+/−	+/−	+	++
MOp	+	+	+/−	+/−	+	+/−	+	+	+/−	+/−	+/−	−
MOs	+	+	++	+	−	+	+	+/−	+/−	+/−	+/−	+/−
MPO	−	−	+	−	−	+/−	+/−	+	+/−	+/−	−	++
NTSco	+/−	+	−	−	−	−	−	−	+/−	+/−	+/−	+
NTSge	−	+/−	−	−	−	−	−	+/−	+/−	−	−	+
NTSl	−	+/−	−	−	−	−	+/−	−	−	−	−	+/−
NTSm	+/−	+	−	−	+	+/−	+/−	+/−	+/−	+	+	++
OVLT	−	+/−	+/−	−	+++	+/−	++	+	+/−	+	+/−	++
PAA	+/−	+/−	+/−	+/−	+	−	−	−	+	+	−	−
PERI	+	+	+	+/−	+	+	+	+	+	+/−	+	−
PIR	+	+/−	+/−	+/−	−	+/−	+/−	+/−	++	+/−	+/−	−
PL	+/−	−	−	−	+	−	−	+/−	+/−	−	+	−
PMv	+/−	−	−	−	−	−	−	+/−	+	−	−	−
PTLp	+/−	−	−	−	−	−	−	−	−	−	−	−
PVa	+/−	+/−	+/−	−	−	−	−	+/−	+	+	+	+
PVHap	+/−	+	+/−	−	+	−	+/−	−	+++	+++	+++	++
PVHdp	−	−	−	−	+	−	−	+	++	++	++	+++
PVHlp	−	+/−	−	−	−	−	−	++	++	++	+++	++
PVHmpd	+/−	+/−	+/−	−	+	−	+/−	++	++	+++	+++	++
PVHmpv	+/−	−	+/−	−	+/−	−	−	++	++	+++	+++	++
PVHp	+/−	+	+	−	−	−	+	++	+	+	+	+
PVHpml	−	−	−	−	−	−	+	++	++	+++	+++	++
PVHpmm	+/−	−	+/−	−	+	−	+/−	++	++	++	+++	+
PVHpv	+/−	+/−	+/−	−	−	−	+/−	+	+	+/−	+	+
RPA	+/−	+/−	−	−	+	+/−	+/−	−	−	−	−	−
RSPd	+/−	−	+/−	−	−	−	−	−	−	−	−	−