Article

Amyloid Beta Peptide ($A\beta_{1-42}$) Reverses the Cholinergic Control of Monocytic IL-1β Release

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Supplemental Table S1: Lactate dehydrogenase (LDH) concentrations in cell culture supernatants of human monocytic U937 cells.

Reagents	Mean ± SD	n
/	**2.6** ± 1.2 %	15
LPS	2.9 ± 1.3 %	15
LPS, $A\beta_{1-42}$ 1 μM	1.0 ± 0.0 %	4
LPS, $A\beta_{1-42}$ 5 μM	0.7 ± 1.5 %	4
LPS, $A\beta_{1-42}$ 10 μM	1.7 ± 1.0 %	4
LPS, $A\beta_{42-1}$ 1 μM	4.0 ± 1.2 %	4
LPS, $A\beta_{42-1}$ 5 μM	4.5 ± 1.3 %	4
LPS, $A\beta_{42-1}$ 10 μM	5.5 ± 1.3 %	4
LPS, BzATP	2.4 ± 1.5 %	15
LPS, BzATP, ACh 7.5 μM	2.3 ± 1.6 %	6
LPS, BzATP, ACh 7.5 μM, $A\beta_{1-42}$ 0.5 μM	2.8 ± 1.2 %	6
LPS, BzATP, ACh 7.5 μM, $A\beta_{1-42}$ 1 μM	3.7 ± 0.8 %	6
LPS, BzATP, ACh 7.5 μM, $A\beta_{1-42}$ 2 μM	3.5 ± 1.0 %	6
LPS, BzATP, ACh 7.5 μM, $A\beta_{1-42}$ 5 μM	4.5 ± 1.3 %	4
LPS, BzATP, ACh 7.5 μM, $A\beta_{1-42}$ 10 μM	4.5 ± 0.6 %	4
LPS, BzATP, ACh 7.5 μM, $A\beta_{42-1}$ 1 μM	3.2 ± 1.5 %	4
LPS, BzATP, ACh 7.5 μM, $A\beta_{42-1}$ 5 μM	3.2 ± 0.5 %	4
LPS, BzATP, ACh 7.5 μM, $A\beta_{42-1}$ 10 μM	3.7 ± 2.2 %	4
LPS, BzATP, Nic 10 μM	3.2 ± 1.0 %	4
LPS, BzATP, Nic 10 μM, $A\beta_{1-42}$ 1 μM	4.0 ± 1.4 %	4
LPS, BzATP, Nic 10 μM, $A\beta_{1-42}$ 5 μM	5.7 ± 2.2 %	4
LPS, BzATP, Nic 10 μM, $A\beta_{1-42}$ 10 μM	6.2 ± 2.9 %	4
LPS, BzATP, ACh 10 μM	2.7 ± 1.0 %	4
LPS, BzATP, ACh 10 μM, $A\beta_{1-42}$ 5 μM	4.2 ± 1.3 %	4
LPS, BzATP, PC 100 μM	2.0 ± 1.4 %	4
LPS, BzATP, PC 100 μM, $A\beta_{1-42}$ 5 μM	3.2 ± 1.5 %	4
LPS, BzATP, CRP 5 μg/ml	1.7 ± 1.0 %	4
LPS, BzATP, CRP 5 μg/ml, $A\beta_{1-42}$ 5 μM	2.7 ± 0.5 %	4
LPS, BzATP, GPC 10 μM	3.8 ± 1.1 %	5
LPS, BzATP, GPC 10 μM, $A\beta_{1-42}$ 5 μM	8.0 ± 2.4 %	5
LPS, BzATP, DPPC 100 μM	1.4 ± 1.7 %	5
LPS, BzATP, DPPC 100 μM, $A\beta_{1-42}$ 5 μM	7.4 ± 1.7 %	5

U937 cells were primed with lipopolysaccharide (LPS) from *Escherichia coli* for 5 h. The P2X7 receptor agonist 2(3)-O-(4-benzoylbenzoyl)adenosine-5-triphosphate (BzATP; 100 μM) was added for another 30 min in the presence or absence of amyloid-β peptide ($A\beta_{1-42}$), reverse $A\beta_{42-1}$ and different nicotinic agonists. LDH release into the cell culture supernatant is given as % of the total release. ACh, acetylcholine; C-reactive protein, CRP; dipalmitoylphosphatidylcholine, DPPC; glycerophosphocholine, GPC; nicotine, Nic.

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Supplemental Table S2: Absolute values of interleukin-1β (IL-1β [pg/ml]) in cell culture supernatants of human peripheral blood mononuclear cells (PBMCs).

	IL-1β [pg/ml]												
Reagents	**LPS**	**LPS + BzATP**	**LPS + BzATP + PC**	**LPS + BzATP + PC + Aβ1-42**	**LPS + BzATP + PC + RgIA4**	**LPS + BzATP + PC +ArIB**	**LPS + BzATP + Aβ1-42**	**LPS + BzATP + RgIA4**	**LPS + BzATP + ArIB**	**LPS + PC**	**LPS + Aβ1-42**	**LPS + RgIA4**	**LPS + ArIB**
Donor 1	**8**	468	135	259	406	423	370	-	-	-	-	-	-
Donor 2	190	381	274	486	311	-	471	419	458	-	-	-	-
Donor 3	49	240	7	149	148	247	384	206	429	26	41	31	40
Donor 4	48	234	26	505	361	299	427	378	681	42	101	67	71
Donor 5	149	1307	203	1312	1299	1298	1067	1284	1263	90	82	168	168
Donor 6	110	2167	99	775	1044	1577	674	1518	2079	52	65	54	72
Donor 7	333	2332	631	1980	2221	2092	1544	1955	2421	156	182	179	166
n =	7	7	7	7	7	6	7	6	6	5	5	5	5
Median	110	468	135	505	406	861	471	852	972	52	82	67	72

Human PBMCs were primed with lipopolysaccharide (LPS) from *Escherichia coli* (5 ng/ml; LPS-pulse). The P2X7 receptor agonist 2(3)-O-(4-benzoylbenzoyl)adenosine-5-triphosphate (BzATP; 100 μM) was added for another 30 min in the presence or absence of amyloid-β peptide (Aβ1-42; 5 μM), phosphocholine (PC; 200 μM) or conopeptides [V11L, V16D]ArIB (ArIB; 500 nM) and RgIA4 (50 nM). IL-1β release into the cell culture supernatant is given as pg/ml.

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Supplemental Table S3: Lactate dehydrogenase (LDH) concentrations in cell culture supernatants of human peripheral blood mononuclear cells (PBMCs).

Reagents	Mean ± SD	n
LPS	2.4 ± 2.8 %	7
LPS, PC 200 μM	1.8 ± 1.5 %	5
LPS, $A\beta_{1-42}$ 5 μM	2.0 ± 1.9 %	5
LPS, RgIA4 50 nM	1.2 ± 0.8 %	5
LPS, ArIB 500 nM	1.2 ± 1.1 %	5
LPS, BzATP	5.1 ± 2.0 %	7
LPS, BzATP, DMSO 0.5 %	5.5 ± 2.5 %	4
LPS, BzATP, PC 200 μM	2.8 ± 1.8 %	7
LPS, BzATP, PC 200 μM, $A\beta_{1-42}$ 5 μM	6.3 ± 2.2 %	7
LPS, BzATP, PC 200 μM, RgIA4 50 nM	4.7 ± 1.6 %	7
LPS, BzATP, PC 200 μM, ArIB 500 nM	4.7 ± 1.0 %	6
LPS, BzATP, $A\beta_{1-42}$ 5 μM	5.8 ± 2.4 %	6
LPS, BzATP, RgIA4 50 nM	4.8 ± 1.7 %	6
LPS, BzATP, ArIB 500 nM	3.8 ± 2.5 %	6

Human PBMCs were primed with lipopolysaccharide (LPS) from *Escherichia coli* (5 ng/ml; LPS-pulse). The P2X7 receptor agonist 2(3)-O-(4-benzoylbenzoyl)adenosine-5-triphosphate (BzATP; 100 μM) was added for another 30 min in the presence or absence of amyloid-β peptide ($A\beta_{1-42}$; 5 μM), phosphocholine (PC; 200 μM) or conopeptides [V11L, V16D]ArIB (ArIB; 500 nM) and RgIA4 (50 nM). LDH release into the cell culture supernatant is given as % of the total release.