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The Muscarinic Cholinergic Receptor-stimulated Signal Transduction Cascade is Affected by Interleukin-1β

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β-amyloid plaque-mediated glial up regulation of the pro-inflammatory cytokine interleukine-1β (IL-1β) has been assumed to contribute to the impairments of cortical cholinergic neurotransmission observed in Alzheimer's disease (AD). To test for this hypothesis two neuroblastoma cell lines, a murine cholinergic septal cell line SN56 and the human SH-SY5Y cell line, were exposed to IL-1β followed by agonist stimulation of muscarinic acetylcholine receptors. By detecting key molecules of both signal transduction cascades (phosphatidyl inositol breakdown, protein kinases, transduction factors, cholinergic enzyme expression) the downstream levels of interaction of both signal cascades should be revealed.

Key words: interleukine-1β, cholinergic signal transduction, Alzheimer's disease.

Introduction

In AD there is increasing evidence that neurotoxicity is mediated also through inflammatory processes. These processes involve activation of microglia by amyloid-β peptide leading to release of pro-inflammatory cytokines including IL-1β among others. Neurotoxic processes mediated by these cytokines may include direct neuronal death by enhancement of apoptosis, decreased synaptic function, and inhibition of hippocampal neurogenesis.

One of the currently held hypothesis is that an inflammatory cycle drives AD pathology [1, 2]. Feedback and feed forward effects of cytokines on glial cells and neurons amplify initial stimuli into rampant runaway responses.

In this study we show that IL-1β affects the cholinergic transduction cascade.

Materials and Methods

Cell culture. Human neuroblastoma SH-SY5Y cells were cultured in Dubelco's modified Eagle's medium. One day after transfer to culture dishes (10^5 cells/dish) the cells were incubated for 8 days with 10 μM trans-retinoic acid.

Culturing of murine. SN56.B5.G4 cells was performed in the same medium and under similar conditions as described for SH-SY5Y cells (for details, see [3]).

Stimulation experiments. Cultured differentiated cells were incubated with varying concentrations of IL-1β for 1 to 24 h as indicated, followed by stimulation of the cholinergic receptors by varying concentrations of the agonist carbachol for 1 h.

Results and Discussion

Effect of IL-1β on the muscarinic acetylcholine receptor (mAChR)-mediated cascade. Preexposure of SN56 cells to IL-1β for one hour did not affect the carbachol-stimulated formation of inositol phosphates (IP; Table 1), but significantly induced the expression of acetylcholinesterase (AChE) activity (Table 2), while cholineacetyltransferase (ChAT) activity was not affected by IL-1β. Interestingly, stimulation of IL-1β-preexposed cells with 1 mM carbachol resulted also in upregulation of AChE activity but to a lower extent as compared to incubations in the absence of carbachol (Tabe 2), indicating interactive mechanisms.

T a b l e 1. Pre-exposure of SN56 and SH-SY5Y cells to IL-1β affects the mAChR-stimulated formation of IP_3

Treatment	Percentage changes of IP_3 level over basal level	
	SN56	SH-SY5Y
100 mM carbachol	328±47	n.d.
1 mM carbachol	187±12	280±48
1 ng/ml IL-1β	n.d.	150±40
1 ng/ml IL-1β + 100 mM carbachol	294±28	n.d.
1 ng/ml IL-1β + 1000 mM carbachol	n.d.	340±49

n.d. — not determined.

T a b l e 2. Pre-exposure of SN56 and SH-SY5Y cells to IL-1b affects the mAChR-mediated up-regulation of activities of AChE and ChAT

Treatment	Percentage changes over basal level			
	SN56		SH-SY5Y	
	AChE	ChAT	AChE	ChAT
1 ng/ml IL-1β for 1 h	42±5	-4±5	19±10	23±33
100 ng/ml IL-1β for 1 h	n.d.	n.d.	18±9	n.d.
100 mM carbachol	n.d.	n.d.	-39±2	n.d.
1 mM carbachol	18±3	-1±4	8±1	13±41
1 ng/ml IL-1β + 1 mM carbachol	21±2	19±10	-1±4	27±13

Effects of mAChR stimulation. Stimulation of SN56 cells with the non-subtype-selective mAChR agonist carbachol resulted in dose-dependent increases in the level of IP_3 (Table 3), as well as in translocation of protein kinase Cα to the membrane fraction (Table 4).

The activities of AChE and ChAT of SN56 cells were increased after stimulation with carbachol, while with increasing concentrations of carbachol the cholinergic enzyme activities in SH-SY5Y cells approached the control levels (Table 5).

The carbachol-mediated effect on cholinergic enzyme activities could not be prevented by blocking the M1-mAChR subtype with pirenzepine. However, the non-

selective mAChR antagonist atropine alleviated them (Table 6) suggesting an effect through the M2-mAChR signaling cascade.

T a b l e 3. Formation of IP_3 following stimulation of SN56 or SH-SY5Y cells with carbachol for 30 min

Carbachol	Percentage increase of IP_3 over basal level	
	SN56	SH-SY5Y
100 mM	330±80	n.d.
1000 mM	186±26	280±51

T a b l e 4. Translocation of proteinkinase Cα to the membrane fraction following stimulation of SN56 or SH-SY5Y cells with carbachol for 30 min

Carbachol	Percentage of translocated protein kinase Cα over basal level	
	SN56	SH-SY5Y
1 mM	5±4	n.d.
10 mM	34±14	n.d.
100 mM	70±10	n.d.
1000 mM	48±4	300±40

T a b l e 5. Changes in the activities of AChE and ChAT following stimulation of SN56 and SH-SY5Y cells with increasing concentrations of mAChR agonists for 60 min

Treatment	Percentage changes over basal level			
	SN56		SH-SY5Y	
Carbachol	AChE	ChAT	AChE	ChAT
1 mM	116±6	80±8	n.d.	n.d.
10 mM	79±16	40±12	n.d.	n.d.
100 mM	78±4	30±10	-31±6	n.d.
1000 mM	32±4	6±3	8±1	13±5
Talsaclidine 50 mM	n.d.	-6±4	19±10	n.d.

T a b l e 6. Changes of the activities of AChE and ChAT following stimulation of SN56 cells with carbachol for 60 min in the presence of various antagonists of mAChR

mAChR drug	Percentage changes of enzyme activity over basal level	
	AChE	ChAT
10 mM carbachol	80±8	n.d.
+25 mM pirenzepine	110±14	51±7
+50 mM atropine	5±4	4±5
+100 mM atropine	142±15	9±8
+25 mM pirenzepine	58±7	-22±14
+50 mM atropine	-18±11	-27±16

Conclusions. Cholinergic enzyme activities are controlled through both M1- and M2-mAChR activation but in opposite directions: induction of enzyme activity is mediated through M2-mAChR, while stimulation of M1-mAChR inhibits or does not af-

fect activity as compared to the control level (Table 5). This was proved by the stimulation of cells with the M1-mAChR-specific agonist talsaclidine, which kept the cholinergic enzyme activities suppressed (Table 5).

These presented data strongly support the suggestion that chronic IL-1β exposure interferes with the muscarinic cholinergic receptor-mediated signaling cascade which may contribute to the cholinergic deficits in AD.

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