

PAK1 Activates $p47^{\text{Phox}}$ and NADPH Oxidase

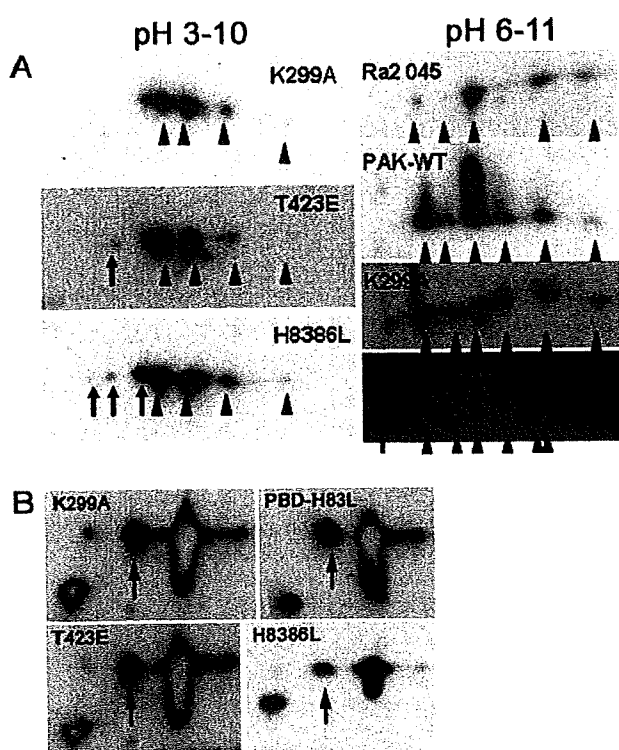


FIGURE 8. Dominant-positive mutants of PAK1 cause phosphorylation of $p47^{\text{Phox}}$, but not RhoGDI, in Ra2 microglia cells. *A*, unstimulated Ra2 cells expressing the indicated PAK1 mutants were lysed in two-dimensional lysis buffer, and isoelectric focusing was performed with IPG strips covering the range of pH 3–10 (left column) or pH 6–11 (right column). After two-dimensional gel electrophoresis and semidry transfer to PVDF membranes, Western blotting was performed with polyclonal rabbit antibodies to $p47^{\text{Phox}}$. Note that expression of PAK1-H8386L (and to a lesser degree PAK1-T423E) causes the appearance or intensification of anodic (more negative) $p47^{\text{Phox}}$ species, marked by arrowheads, indicative of phosphorylation. *B*, Ra2 PAK1 cell lysates were separated by two-dimensional gel electrophoresis and transferred as above, and membranes were subsequently Western-blotted with anti-RhoGDI antibodies. The arrow points to a single phosphorylated species of RhoGDI, which did not seem affected by expression of PAK1 mutants. Western blots in *A* and *B* are representative of 3–5 individual experiments.

AA effects with regard to NADPH oxidase activation may in part be mediated through PKC activation (35), the experiments were performed in the presence of 200 nM BIM-1, which inhibits PKC isoforms with a $K_i \sim 10$ nM. As seen in Fig. 9A, 10 μM AA did not cause a marked respiratory burst in PAK1 wild type or PAK1-T423E Ra2 cells; however, in PAK1-H83L/H86L expressing cells AA caused a sharp immediate peak of superoxide production followed by a longer period with sustained activity (Fig. 9, A and B). Reversely, the PAK1-K299A mutant decreased the magnitude of the AA-induced peak response by $\sim 50\%$. Next, we examined membrane association of a $p47^{\text{Phox}}$ -GFP fusion protein in the different Ra2 PAK protein expressing cells by immunofluorescence. Although association of $p47^{\text{Phox}}$ -GFP with cellular membranes following treatment with 10 μM AA was enhanced over nontreated control cells (data not shown), and $p47^{\text{Phox}}$ -GFP colocalized with a fraction of PAK1 at the membrane (Fig. 9C), by visual inspection $p47^{\text{Phox}}$ -GFP recruitment to the membrane and colocalization with PAK1 seemed independent of the PAK1 mutant expressed (data not shown). The $p47^{\text{Phox}}$ residues phosphorylated by PAK1 *in vitro* have been mapped to serines 303, 304, 320, and 328 (19). We

therefore expressed a $p47^{\text{Phox}}$ mutant with serine to glutamate substitutions on residues 303, 304, and 328 (6) to mimic phosphorylation, and then tested superoxide release as before with luminol-E-CL. Fig. 9, D and E, shows that both $p47^{\text{Phox}}$ wild type and $p47^{\text{Phox}}$ -S303D/S304D/S328D dramatically increased the respiratory burst of PMA or fMLP-stimulated Ra2 cells (~ 10 -fold more than overexpression of gp91 $^{\text{Phox}}$). The wild type $p47^{\text{Phox}}$ protein most efficiently supported the respiratory burst, but notably the $p47^{\text{Phox}}$ -S303D/S304D/S328D caused a basal release of superoxide not normally seen in unstimulated Ra2 microglia (Fig. 9, D and E, and inset in E).

DISCUSSION

We show here that PAK1 regulates fMLP- and PMA-induced phagocyte NADPH oxidase (NOX-2) superoxide production. We decided to undertake an investigation of the *in vivo* role of PAK1 in NADPH oxidase regulation because of the following: (i) PAK1 is known to phosphorylate $p47^{\text{Phox}}$ *in vitro* (21); (ii) PAK1 is Rac1-regulated, thereby constituting a potential link between the Rac and Phox activation arms of NADPH oxidase assembly; and finally (iii) in contrast to other known kinases of $p47^{\text{Phox}}$ (see below), PAK1 is also a major regulator of the actin cytoskeleton, which directly or indirectly supports NADPH oxidase assembly and function (31–33). While this work was under way, it was reported that introduction of the AID fragment of PAK1 (included in PBD used herein) into neutrophils inhibited the respiratory burst *in vivo* (19), and furthermore, adenoviral delivery of PAK1-K299A in endothelial cells inhibited $p47^{\text{Phox}}$ phosphorylation and superoxide production (36). We present here the most extensive evidence for a positive role of PAK1 in NADPH oxidase signaling via $p47^{\text{Phox}}$ phosphorylation by showing the following: (i) fMLP stimulation of microglia activates PAK1 through a VAV1 and Rac1-dependent pathway, and (ii) active PAK1 mutants cause $p47^{\text{Phox}}$ phosphorylation and preactivation, leading to (iii) an increased respiratory burst, an effect (iv) reproduced by a $p47^{\text{Phox}}$ mutant that mimics PAK1 phosphorylation.

Involvement of PAK1 in NADPH Oxidase Activation Is Stimulus-dependent—Constitutively active PAK1-T423E, and in particular PAK1-H83L/H86L, increased fMLP-induced NADPH oxidase activity, and conversely, PAK1-K299A and the PBD-H83L fragment negatively affected activity. PMA activates PKC, which is well known to participate in phosphorylation of $p47^{\text{Phox}}$ (see Ref. 14 and references therein), and is generally considered to phosphorylate all serine residues required for activation of $p47^{\text{Phox}}$ (37). Nevertheless, PAK1 also influenced PMA-induced NADPH oxidase activation, although the effects were less clear-cut than for the fMLP-stimulated response. First, only PAK1-H83L/H86L, and not -T423E, enhanced the PMA-stimulated respiratory burst. Second, PAK1-K299A, which only retarded the fMLP response, reduced the superoxide output following PMA stimulation by 50%, and finally, the strong inhibitory effect of PBD-H83L in the case of fMLP was absent for PMA. The paradoxical, inverted inhibitory effect of PAK1-K299A and PBD on fMLP and PMA-stimulated superoxide production, respectively (compare Fig. 3B with Fig. 6B), can perhaps be explained by the recent uncovering of a binding interaction between $p22^{\text{Phox}}$ and PAK1 (19).

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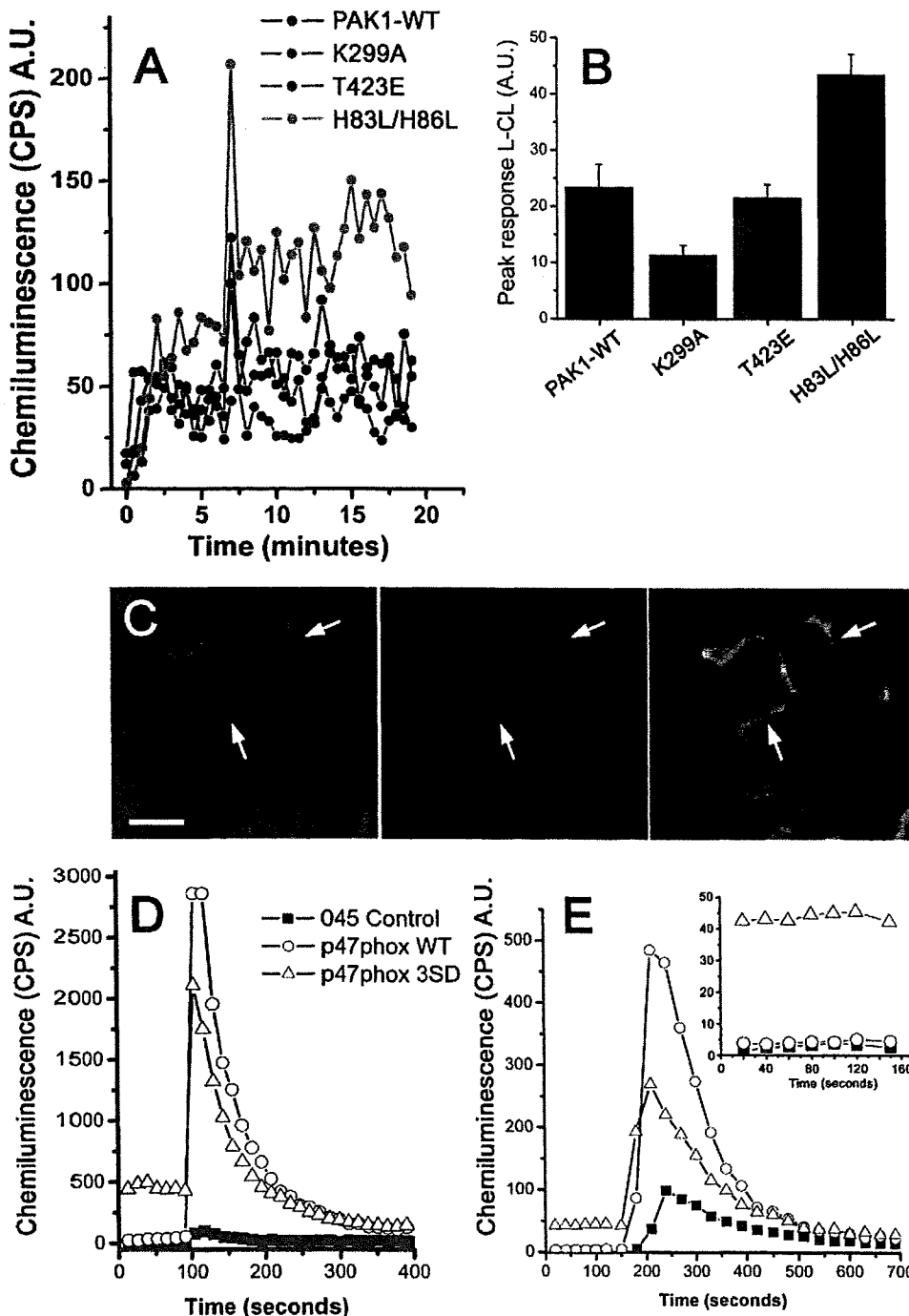


FIGURE 9. AA synergizes with PAK1-H83L/H86L in NADPH oxidase activation and $p47^{Phox}$ mutant S303D/S304D/S328D greatly increases NADPH oxidase activity. *A*, Ra2 $p47^{Phox}$ -GFP cells expressing wild type or mutant PAK1 protein were incubated with 200 nM BIM-1 (a PKC-inhibitor) and then stimulated with 10 μ M arachidonic acid (arrow) for superoxide detection by luminol-E-CL. The graph shows in arbitrary units the mean superoxide production ($n = 3$) of one representative experiment of three, all performed in triplicate. The higher basal superoxide production of PAK1-H83L/H86L cells before AA stimulation (<5 min) as shown in the graph was not consistently observed. *B*, bar graph shows mean \pm S.E. of peak superoxide generation for three independent experiments performed as above (arbitrary units). *C*, Ra2 cells co-expressing PAK1-H83L/H86L and $p47^{Phox}$ -GFP were stimulated with arachidonic acid for 10 min and then fixed for immunofluorescence with anti-Myc tag antibodies. PAK1 (red) can be seen to colocalize with $p47^{Phox}$ -GFP (green) at cell-cell junctions or on the free plasma membrane (arrows). The experiment was performed three times with similar results. Bar, 10 μ m. *D* and *E*, Ra2 045 control, $p47^{Phox}$ wild type, or $p47^{Phox}$ -S303D/S304D/S328D mutant expressing cells were stimulated with fMLP (*D*) or PMA (*E*), and superoxide release was measured with luminol E-CL. The ordinate represents superoxide generation in arbitrary units normalized to Ra2 045 control cells. Traces in each case represent mean of three wells of a single experiment and is representative of four experiments performed. Note that expression of the $p47^{Phox}$ -S303D/S304D/S328D causes superoxide production even under unstimulated conditions (inset in *E*).

PAK is otherwise known to engage in direct binding with its molecular partners in multisubunit complexes, e.g. PAK1-COOL-Pix or PAK2-NEF-VAV1 (17). Therefore PAK1-K299A could exert its negative effect on PMA-induced NADPH oxidase activity, not on the level of (inhibition of) $p47^{Phox}$ phosphorylation but rather via a negative effect on the assembled oxidase by a steric hindrance mechanism, not exerted by the much smaller PBD fragment.

Although PAK1 is recruited to form phagosomes (28, 38, 39), the association is transient (38), and at present the activation status and function of both PAK1 and VAV1 during Fc γ R-mediated phagocytosis of opsonized prey is unclear (40, 41). We found no major effect of PAK1 mutant overexpression on the respiratory burst associated with phagocytosis of IgG-immune complexes, indicating that PAK1 is not required for NADPH oxidase activation downstream of Fc γ R signaling (supplemental Fig. 2).

In PMA-stimulated Ra2 cells, peroxynitrite, the reaction product between superoxide and NO, contributes most significantly to the luminol E-CL signal immediately after stimulation (the oxidative burst) where the ratio between L-NMMA and control cell E-CL drops to ~ 0.5 before stabilizing at ~ 0.75 (supplemental Fig. 1). Importantly, the ratio did not vary between control or the different Ra2 PAK1 cell lines. As the inducible NOS inhibitor 1400W has no inhibitory effect on the luminol E-CL signal in Ra2 cells, we assume the source of NO is either neuronal NOS or endothelial NOS, whose activity can in part be regulated by serine and threonine phosphorylation (42–44). We cannot exclude that PAK mutants differentially activate NOS or cause its uncoupling (to produce superoxide), but our control experiments show that NO production cannot account for the observed differences in the luminol E-CL signal. Further experimentation is needed to estab-

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lish whether PAK1 directly or indirectly contributes to the phosphorylation state, and thereby activity, of NOS.

Can Constitutively Active Rac1 Initiate NADPH Oxidase Activity in Phagocytes?—Several GEFs have been proposed to mediate Rac nucleotide exchange in the setting of respiratory burst induction in neutrophils, including Tiam1, P-Rex, and VAV1 (15, 16, 45). We show here that dominant-negative VAV1-L213A (and expectedly Rac1-N17) inhibited the fMLP-stimulated activation of PAK1 and dose-dependently decreased the microglia respiratory burst. Therefore, VAV1-generated GTP-Rac1 regulates NADPH oxidase activity both by being an essential subunit (3) and by contributing to PAK1 activation. This is supported by our demonstration in HEK293T cells that expression of constitutively active Rac1-V12, but not Rac1-V12/Y40C, which has lost its ability to interact with PAK1 (and p67^{Phox}), is sufficient to induce phosphorylation of p47^{Phox} to a level similar to that of dominant-positive PAK1 mutants alone (supplemental Fig. 3). Similarly, Rac1-mediated PAK1 activation could explain the observed spontaneous activation of NADPH oxidase in a heterologous COS cell system expressing dominant-positive VAV1 or Rac1 (46). However, although Rac1-V12 expression in Ra2 microglia more efficiently increased the PMA-induced superoxide production than Rac1-V12/Y40C (Fig. 7), we observed no spontaneous NADPH oxidase activity above control levels for any of the Rac1-V12 mutants, in contrast to heterologous COS cells (46), indicating additional regulatory elements in phagocyte cells. Both Rac1-V12 dominant-positive mutants inhibited the fMLP-induced respiratory burst *in vivo*, probably because of a requirement for full GDP/GTP cycles as proposed previously for small GTPases Rac2 and Rap1A (47, 48).

Phosphorylation of p47^{Phox} by PAK1—It is currently thought that the primary role of p47^{Phox} is to transport and tether p67^{Phox} to cyt b₅₅₈. Phosphorylation of serines 303, 304, and 328 of p47^{Phox} is a requisite step in the membrane translocation and attachment to cyt b₅₅₈ of p47/67^{Phox} (5, 6, 49) by virtue of phosphorylation-induced uncovering of latent binding sites for p22^{Phox} (4, 50) and phosphoinositol lipids (5, 51) in the membrane. Several kinases have been implicated in p47^{Phox} phosphorylation leading to activation or priming of the NADPH oxidase, including PKC isoforms (14, 52–54), Akt (7, 8), and mitogen-activated protein kinases (10). Although a role for PAK1 in p47^{Phox} mobilization has been suggested by its *in vitro* phosphorylation of p47^{Phox} (21), we show here by two-dimensional gel electrophoresis and Western blotting that expression of PAK1-H83L/H86L, and to a lesser extent PAK1-T423E, caused p47^{Phox} phosphorylation *in vivo*. Careful mapping has demonstrated that serines 303, 304, 320, and 328 (in particular the latter) of p47^{Phox} serve as phospho-acceptor sites for PAK1 *in vitro* (19). Although full activity (eliciting superoxide production) of p47^{Phox} requires additional phosphorylation of Ser-379 (55), the phosphorylation mediated by PAK1 should be sufficient to relieve intramolecular restraints in p47^{Phox} to allow for p22^{Phox} (6) and inositol lipid (5) binding. As shown here, introduction of a p47^{Phox}(S303D/S304D/S328D) mutant as a p47^{Phox} phosphomimetic was sufficient to cause a marked superoxide production in Ra2 cells even under unstimulated conditions. Phosphorylation of p47^{Phox} by PAK1-H83L/H86L

did not cause a consistent basal superoxide release but did cause partial activation of p47^{Phox} because PAK1-H83L/H86L synergized with low concentrations of AA (which cause only partial unfolding and preactivation of p47^{Phox} (5, 34)) in NADPH oxidase superoxide production. Although we found no evidence for PAK1 phosphorylation of RhoGDI, which releases bound Rac1-GDP for subsequent interaction with GEFs (20), we cannot exclude that one or more of the many other substrates of PAK1 (e.g. cytoskeleton-related proteins such as cortactin or filamin (17)) may also influence NADPH oxidase assembly and activation. The important PAK1 substrate LIMK-1, which controls cofilin-dependent actin depolymerization, does not contribute to the PAK1 effects observed in this study.³ Therefore, for the reasons presented above, we greatly favor the idea that the positive effect of PAK1 on NADPH oxidase activity is mainly mediated by direct phosphorylation and preactivation of p47^{Phox}.

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Effects of Aging on Neuroprotective and Neurotoxic Properties of Microglia in Neurodegenerative Diseases

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Key Words

Microglia · Aging · Parkinson's disease

Abstract

The inflammatory process in the brain, which is accompanied by changes in the levels of proinflammatory cytokines and neurotrophins, along with the presence of activated microglia, has recently gained much attention in neurodegenerative diseases. Activated microglia produce either neuroprotective or neurotoxic factors. Many reports indicate that activated microglia promote degeneration of dopamine (DA) neurons in Parkinson's disease (PD). On the other hand, there are several lines of evidence that microglia also have a neuroprotective function. Microglia activated with lipopolysaccharide in the nigrostriatum of neonatal mice protect DA neurons against the PD-producing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), whereas activated microglia in aged mice promote DA cell death by MPTP. These results suggest that the function of activated microglia may change in vivo from neuroprotective to neurotoxic during aging as neurodegeneration progresses in the PD brain.

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Introduction

Parkinson's disease (PD) is characterized by degeneration of dopamine (DA) neurons in the substantia nigra. As a mechanism of neuronal death, the inflammatory process in the brain, which is accompanied by changes in the levels of cytokines and neurotrophins and the presence of activated microglia, has gained much attention in PD, AD, and other neurodegenerative diseases [1, 2]. Activated microglia may play neurotoxic roles by producing proinflammatory cytokines such as tumor necrosis factor (TNF)- α and interleukin (IL)-6. On the other hand, activated microglia may also play neuroprotective roles by producing neurotrophic compounds such as brain-derived neurotrophic factor. Sawada et al. [3] proposed a hypothesis of two-step activation of microglia: cytokines from activated microglia in the substantia nigra and putamen may be initially neuroprotective, but may later become neurotoxic during progression of PD by toxic change of activated microglia. In the present study, the neuroprotective and neurotoxic roles of activated microglia have been examined in relation to aging.

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Neuroprotective and Neurotoxic Roles of Activated Microglia

Activated microglia in the PD brain produce proinflammatory cytokines such as TNF- α and IL-6. Since cytokines have either neuroprotective or neurotoxic effects, a main question is whether activated microglia are neuroprotective or neurotoxic.

Imamura et al. [4] proved, by double immunofluorostaining, the coexistence of cytokines, TNF- α and IL-6, with MHC class II (CR3/43) in ICAM-1- and LFA-1-positive activated microglia in the putamen in PD. Activated microglia were not only observed in the nigrostriatal region with neurodegeneration, but also in other brain regions, such as the hippocampus and cerebral cortex, without significant neurodegeneration. These immunohistochemical observations in PD brains suggest that activated microglia may have either neuroprotective or neurotoxic functions depending upon the brain regions and the stage of disease. Imamura et al. [5] compared the expression of cytokines and neurotrophins in the hippocampus and putamen of postmortem brains from patients with PD or dementia with Lewy bodies (DLB), and normal controls, and suggested that activated microglia in the hippocampus of PD and DLB patients may be different in properties in secreting different kinds and different amounts of cytokines and neurotrophins, such as IL-6 and brain-derived neurotrophic factor, and may be neuroprotective in PD and neurotoxic in DLB.

Neuroprotective and Neurotoxic Functions of Activated Microglia in the Substantia Nigra of Neonatal and Aged Mice, and the PD-Producing DA Neurotoxin 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine

The above data on postmortem brains from PD or DLB patients suggest that activated microglia may have either neuroprotective or neurotoxic functions. It is well known that aging is an important risk factor in PD or DLB.

Sawada et al. [6] have examined the difference between neonatal and aged mice in the *in vivo* effects of microglia activated by systemic administration of lipopolysaccharide (LPS) on nigrostriatal DA neurons treated with the PD-producing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In neonatal mice, microglia activated by treatment with LPS showed a neurotrophic potential toward DA neurons. TH activity and the levels

of DA and the metabolite 3,4-dihydroxyphenylacetic acid as well as those of the proinflammatory cytokines IL-1 β and IL-6 were elevated in the midbrain of LPS-MPTP-treated neonatal mice. The number of DA neurons in neonatal mice was decreased in the MPTP group, but was recovered in the LPS-MPTP group. In contrast, the number of DA neurons in the aged (60-week-old) mice was decreased by a single MPTP administration in the MPTP group, and was further decreased in the LPS-MPTP group, as compared to the saline group. The MPTP group of aged mice showed a slightly increased number of activated microglia in the substantia nigra; in the LPS-MPTP group, the increase was significant. These results indicate that the cell viability of DA neurons was recovered in neonatal mice of the LPS-MPTP group compared with that of the MPTP group. In contrast, the viability of these neurons in the aged mice dropped significantly comparing the LPS-MPTP group with the MPTP group. Activated microglia in the brain may be neuroprotective in neonatal mice, whereas those in the same group of aged mice may be neurotoxic. LPS treatment performed by systemic injection in these experiments may result in involvement of not only microglia, but also many cells, such as astrocytes, vascular endothelial cells, and in particular T cells. However, since microglia are most rapidly activated by LPS, these results suggest that most probably activated microglia in neonatal mice may have a neuroprotective role, in contrast to the neurotoxic effect of activated microglia in aged mice, and that the activated microglia are different in their properties between neonatal and aged brains. The causative factors are still unknown; it is possible that changes of the neurodegenerating potential and microenvironment including components of extracellular matrices and accumulation of oxidized proteins or lipids may affect the activation process and the state of activated microglia.

Conclusion

Studies on the brain of MPTP-treated mice suggest the existence of toxic and neuroprotective subsets of activated microglia. Activated microglia may be neuroprotective in neonatal mice, but neurotoxic in aged mice. Sawada et al. [3] proposed that the function of activated microglia may change *in vivo* from neuroprotective to neurotoxic as degeneration of DA neurons in the substantia nigra progresses in PD. This hypothesis on the toxic change of activated microglia had its origin in the *in vitro* experiments by Vilhardt et al. [7] in collaboration with

Sawada M., which proved that a neuroprotective microglial clone in a culture experiment converted to a toxic microglial clone by transduction of the HIV-1 Nef protein that is related to the pathogenesis of AIDS encephalitis with increasing NADPH oxidase activity. The results in the present review suggest that aging may promote a rapid toxic change in activated microglia by changing the bias of activated microglia.

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A link between stress and depression: Shifts in the balance between the kynurenine and serotonin pathways of tryptophan metabolism and the etiology and pathophysiology of depression

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Abstract

Alteration of tryptophan (TRP) metabolism elicited by proinflammatory cytokines has gained attention as a new concept to explain the etiological and pathophysiological mechanisms of major depression. The kynurenine (KYN) pathway, which is initiated by indoleamine 2,3-dioxygenase (IDO), is the main TRP metabolic pathway. It shares TRP with the serotonin (5-HT) pathway. Proinflammatory cytokines induce IDO under stress, promote the KYN pathway, deprive the 5-HT pathway of TRP, and reduce 5-HT synthesis. The resultant decrease in 5-HT production may relate to the monoamine hypothesis of major depression. Furthermore, metabolites of the KYN pathway have neurotoxic/neuroprotective activities; 3-hydroxykynurenine and quinolinic acid are neurotoxic, whereas kynurenic acid is neuroprotective. The hippocampal atrophy that appears in chronic depression may be associated with imbalances in neurotoxic/neuroprotective activities. Because proinflammatory cytokines also activate the hypothalamo-pituitary-adrenal (HPA) axis, these imbalances may inhibit the hippocampal negative feedback system. Thus, changes in the TRP metabolism may also relate to the HPA axis-hyperactivity hypothesis of major depression. In this article, we review the changes in TRP metabolism by proinflammatory cytokines under stress, which is assumed to be a risk factor for major depression, and the relationship between physiological risk factors for major depression and proinflammatory cytokines.

Keywords: Stress, depression, proinflammatory cytokines, tryptophan, kynurenine, serotonin

Introduction

Changes in the metabolism of the essential amino acid tryptophan (TRP), play an important role in the brain–endocrine–immune system interaction that is hypothesized to be involved in the pathophysiology of major depression. Two main pathways metabolize TRP: one is the kynurenine (KYN) pathway, which is initiated by the enzyme indoleamine 2,3-dioxygenase (IDO) (Hirata et al. 1974), and the other is the serotonin (5-HT) pathway, initiated by the enzyme

tryptophan 5-monooxygenase (Ichiyama et al. 1970; tryptophan hydroxylase (TPH); Figure 1). Most TRP, i.e., ~99% of dietary intake, is metabolized by the former pathway (Stone and Darlington 2002). Immunological challenges such as proinflammatory cytokines (IL-1, IFN- α , IFN- γ , and TNF- α) induce IDO activity (Stone and Darlington 2002; Konsman et al. 2002; Widner et al. 2002; Moffett and Namboodiri 2003; Myint and Kim 2003; Wichers and Maes 2004). Because proinflammatory cytokines can induce depressive symptoms, cytokine-induced

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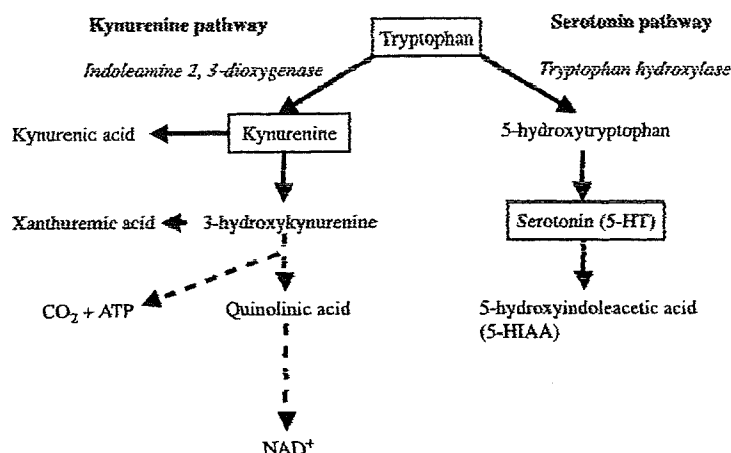


Figure 1. Kynurenine and serotonin pathways of tryptophan metabolism. ATP, Adenosine triphosphate; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; NAD, nicotinamide adenine dinucleotide.

IDO activation may be associated with the etiology of major depression (Konsman et al. 2002; Widner et al. 2002; Wichers and Maes 2004). Activated IDO metabolizes TRP to KYN, and may thereby deprive TPH of its substrate, TRP (Figure 2). Thus, the activated KYN pathway may further inhibit the 5-HT pathway (Konsman et al. 2002; Widner et al. 2002; Myint and Kim 2003; Wichers and Maes 2004). The monoamine hypothesis, one of the main etiological hypotheses of depression, proposes that a decrease in monoamine levels in the central nervous system (CNS) is related to the onset and symptoms of depression (Delgado 2004; Owens 2004). Indeed, lowering 5-HT function by TRP depletion has a mood-lowering effect in the subgroups of recovered

depressed patients, patients with seasonal affective disorder and vulnerable healthy subjects (Van der Does 2001). In the 1970s and 1980s, to clarify the relationship between the amount of KYN metabolites and symptoms of depression, urine levels of xanthurenic acid (XA), which is metabolized from 3-OH KYN (Figure 1), was examined in depressed patients. The excretion of XA after ingestion of TRP was increased in depression, and improvement in XA excretion preceded clinical improvement (Hoes and Sijben 1981). Thus, the induction of IDO activity elicited by proinflammatory cytokines that inhibits 5-HT synthesis may be closely related to the etiological monoamine hypothesis of depression.

The monocyte-T-lymphocyte hypothesis has been proposed to explain the relationship between major depression and immunological activities (Smith 1991; Maes et al. 1995). This theory suggests that the high levels of IL-1 released from activated macrophages directly stimulate the release of corticotrophin-releasing factor (CRF) from the paraventricular nucleus (PVN) in the hypothalamus. Furthermore, IL-6 can also account for hypothalamo-pituitary-adrenal (HPA)-axis abnormalities, due to its direct action on the hypothalamus, again stimulating the release of CRF (Maes et al. 1995). Thus, the HPA-axis hyperactivity hypothesis, which proposes a relationship between HPA-axis hyperactivity and depression (Schule 2007), is closely related to the above-mentioned changes elicited by immunological activation. Both the monoamine and the HPA-axis hyperactivity hypotheses are central to etiological and pathophysiological hypotheses of major depression (Maes et al. 1995; Konsman et al. 2002; Myint and Kim 2003).

Of the metabolites in the KYN pathway (Figure 1), 3-hydroxy kynurenine (3-OH KYN) and quinolinic acid (QUIN) have neurotoxic effects while kynurenic acid (KYNA) has a neuroprotective effect (Myint and

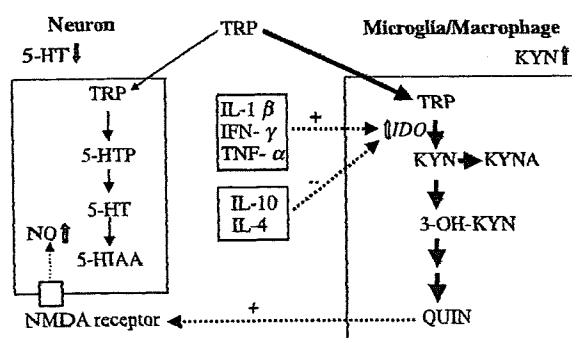


Figure 2. Immunological activation and tryptophan metabolism in the brain. In the macrophages and/or microglia, activated indoleamine 2,3-dioxygenase metabolizes tryptophan. Thus, the kynurenine pathway deprives the serotonin pathway of tryptophan. +, activation; -, inhibition. 3-OH KYN, 3-hydroxykynurenine; 5-HTP, 5-hydroxy tryptophan; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IL, interleukin; KYN, kynurenine; KYNA, kynurenic acid; NMDA, *N*-methyl-*D*-aspartate; NO, nitric oxide; QUIN, quinolinic acid; TNF, tumor necrosis factor; TRP, tryptophan.

Kim 2003; Wichers and Maes 2004). Imbalance in the neurodegenerative and neuroprotective activities of KYN pathway metabolites may be linked to hippocampal atrophy in chronic depression, which plausibly relates to HPA-axis hyperactivity in depression. In gerbils, systemic immune activation induces brain IDO and increased brain and CSF QUIN, whereas brain activity of kynurenine aminotransferase (KAT), which converts KYN to KYNA, and the CSF KYNA level are unchanged. Consequently, the QUIN/KYNA ratio in CSF is increased (Saito et al. 1993). D retrovirus infection increases the QUIN/KYNA ratio in the CSF macaques with simian acquired immune deficiency syndrome (SAIDS) (Heyes et al. 1990). In humans, IFN- α therapy revealed that an increase in the serum KYN/KYNA ratio is significantly associated with the severity of depression (Wichers et al. 2005). In patients with major depression, plasma KYNA and the 1000 \times KYNA/KYN ratio are decreased (Myint et al. 2007). Thus, the neurodegeneration process under stress may be more pronounced due to reinforcement by the neurotoxic effect of high QUIN levels (Myint and Kim 2003).

Although the activation of the KYN pathway has been reported in depression elicited by IFN- α therapy (Bonaccorso et al. 2002; Capuron et al. 2002, 2003; Wichers et al. 2005), the therapy is not the typical cause of clinical depression. The onset of clinical depression is more often elicited by social environment and psychological stress (Kendler et al. 1993; Paykel 1994).

In this article, we review the changes in TRP metabolism under physiological conditions and immunological challenge. An interaction between TRP metabolism and neuroendocrine/immune systems is proposed in relation to the etiological hypotheses of depression. To establish and test this hypothesis, it is important to examine changes in TRP metabolism under physiological conditions which are known to be risk factors for depression and to compare them with those elicited by IFN- α therapy.

Stress and brain tryptophan metabolism

An alteration of TRP metabolism is a good indicator of clinical depression elicited by immunological challenges (Bonaccorso et al. 2002; Capuron et al. 2002, 2003; Wichers et al. 2005) but stress also alters brain TRP metabolism.

Brain tryptophan uptake in response to acute stress and immunological challenge

Stress as well as immune challenges are known to elevate brain TRP levels. In animals, immobilization stress, food deprivation, exercise, and footshock increase brain TRP levels. Furthermore, immunological challenges such as IL-1 and lipopolysaccharide

(LPS), which are known to activate the HPA axis, increase the brain TRP in mice (Dunn 1988a) and rats (Kabiersch et al. 1988). Because stress-related changes in brain TRP occur even in adrenalectomized rats (Curzon et al. 1972) and mice (Dunn 1988b), they are not dependent on adrenocortical activation. Activation of the sympathetic nervous system is responsible for the increase in brain TRP (Dunn and Welch 1991).

Two related mechanisms increase TRP level in the brain (Figure 3). TRP is the only essential amino acid that binds to albumin (Pardridge 1998). In plasma, most TRP binds to albumin, so free TRP is only a small fraction (McMenamy 1965). One mechanism regulating brain TRP levels is the level of circulating free fatty acid (FFA), under control of the sympathetic nervous system. Stress and physical exercise (Chaouloff 1997) increase plasma FFA level via the sympathetic outflow, and then FFA binds to albumin. Because this binding reduces TRP affinity for albumin (McMenamy 1965), the increase in FFA levels inhibits TRP binding to albumin and increases free TRP levels in the plasma (Curzon et al. 1973; Strüder et al. 1999; Figure 3). The other mechanism regulating brain TRP levels is the competition between TRP and the so-called "large neutral" amino acids (LNAA), which include aromatic amino acids and branched-chain amino acids, at the uptake site of the blood-brain barrier (BBB). The plasma TRP/LNAA ratio, rather than the TRP level, regulates TRP uptake activity at the BBB (Fernstrom and Wurtman 1972; Chaouloff 1997; Strüder et al. 1999; Figure 3). Thus, elevation of the TRP/LNAA ratio increases the brain uptake of TRP, and thereby increases brain TRP level.

Finally, stress may increase the brain TRP level. Our recent data (Miura et al. in press) suggest that 20-min novelty stress in mice, namely, exposure to an environment that the animal has not experienced before, elevates brain TRP levels. Thus, psychological stress possibly increases the brain TRP uptake in humans.

Acute stress and alteration of brain tryptophan metabolism

Acute psychological as well as physiological stress may alter brain TRP metabolism to activate the brain KYN pathway by induction of IDO.

IDO activity in the brain is present in neurons as well as in microglia and astrocytes (Guillemin et al. 2004; Roy et al. 2005).

In rats, physiological stress such as footshock increases brain TRP, KYN, KYNA and 3-OH KYN levels of rats (Pawlak et al. 2000). Brain TRP levels range from about 10 to 40 nmol/g, whereas those of KYN range from about 400 to 1200 pmol/g. Further, brain levels of KYNA range from about 20 to 40 pmol/g, and those of 3-OH KYN from about 40 to 80 pmol/g

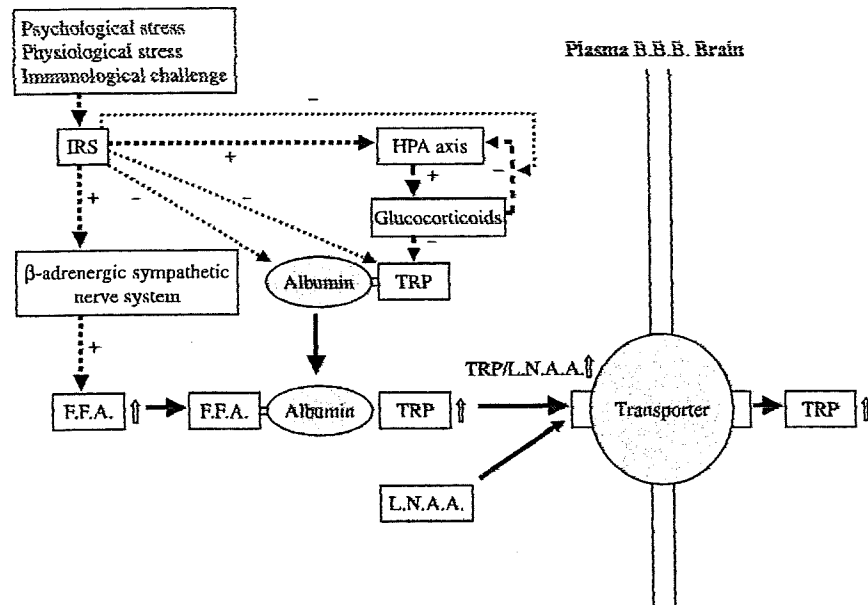


Figure 3. Regulation of plasma level and brain uptake of tryptophan. Stress and immune challenges elevate the brain TRP level. Stimulation of the β -adrenergic system increases FFAs. FFAs bind to albumin, releasing TRP from albumin. Increased plasma TRP elevates the TRP/LNAAs ratio. Thus, the transporter system in the BBB for the uptake into the brain selects TRP rather than LNAAs. Another plasma TRP modulation relates to inflammation. The IRS activates the HPA axis to increase glucocorticoids, consuming TRP and other peripheral amino acids to produce plasma-positive APPs and to reduce plasma negative APPs such as albumin that bind TRP. Thus, HPA axis activity may also play a role in the modulation of plasma TRP levels. APPs, acute-phase proteins; BBB, blood-brain barrier; FFA, free fatty acid; HPA axis, hypothalamo-pituitary-adrenal axis; IRS, inflammatory response system; LNAA, large neutral amino acid; TRP, tryptophan.

(Pawlak et al. 2000). Thus, brain TRP levels are far greater than those of KYN, KYNA, and 3-OHKYN. As physiological stress is able to increase brain TRP levels via increase of TRP uptake across the BBB, the increase in TRP transfer with stress may compensate for the decrease in TRP elicited by the conversion of TRP to KYN. Thus, brain IDO activity may be effective in physiological stress conditions without apparent immunological challenge. Our recent data (Miura et al. in press) suggest that 20-min novelty stress, i.e., exposure of animals to a condition they have never experienced before, elevated brain KYN levels as well as TRP levels without changing the KYN/TRP ratio. Thus, IDO may play a role in the elevation of the KYN level, accompanied by an increased TRP level elicited by acute psychological stress. Further, novelty stress decreased the 5-HT/TRP ratio. Thus, novelty stress shifted the balance between the KYN and the 5-HT pathways away from 5-HT.

However, influences of chronic and/or repeated stress on brain TRP metabolism, which may relate to the etiology of human depression, should be examined further.

Stress and proinflammatory cytokines

Exposure to stressors such as psychological stress activates the production of proinflammatory cytokines in humans. Psychological stress, specifically, an

academic examination for medical students, significantly increases blood proinflammatory cytokines (Maes et al. 1998).

The brain-immune system interaction forms a bidirectional communication network, i.e., from the peripheral immune system to the brain and *vice versa* (Black 1994; Maier 2003). Peripherally activated proinflammatory cytokines released from macrophages signal to the brain via both humoral and neural routes. Because proinflammatory cytokines are relatively large and hydrophilic molecules, they are unlikely to cross the BBB by passive diffusion. They pass through the circumventricular organs (CVOs) and choroid plexus, and enter the brain parenchyma. At the CVOs, IL-1 β induces, as a second messenger, the formation of prostaglandin E₂, a small lipophilic molecule that is able to diffuse freely (Maier 2003). Furthermore, in addition to passive diffusion across the BBB, proinflammatory cytokines may enter the brain by active transport mechanisms (Banks et al. 1995; Watkins et al. 1995; Schiepers et al. 2005). Saturable transport mechanisms have already been identified for IL-1 β , IL-1 α , IL-6, and TNF- α (Banks et al. 1995). IL-1 β also acts on glial cells in the CVOs, meninges, and choroid plexus to stimulate the production of IL-1 β . Thus, IL-1 β further induces *de novo* IL-1 β production by glial cells in a paracrine manner in the brain. This process can propagate the IL-1 β signal to distant regions (Maier 2003).

Cytokine signaling to the brain by neural routes involves the vagus nerve. IL-1 β released from cells located near vagal afferent terminals can activate nerve impulses. However, the vagus may not be the only nerve that can provide this communication function (Maier 2003).

In addition, the newly synthesized IL-1 β in the brain activates both the HPA axis and the sympathetic nervous system in response to peripheral immune stimulation (Maier 2003). Furthermore, cytokines that are produced in the brain enter the peripheral circulation.

An overlap between neurochemical changes elicited by stressors and immune challenges has been frequently noted. Stressors may activate peripheral host-defensive responses, if they elicit bidirectional, immune-brain and brain-immune communication. Stressors such as inescapable shock activate macrophage functions for several days. Novel environmental stress such as exposure to an "open field" increases IL-6 release from macrophages in rats (LeMay et al. 1990). Psychological stress increases IL-1, IL-6, sIL-2R, and cortisol concentrations in human subjects (Schulte et al. 1994). In the brain, acute stress indeed increases the IL-1 β content (Minami et al. 1991; Shintani et al. 1995; Nguyen et al. 1998). Moreover, central and peripheral proinflammatory cytokine expression elicited by acute stress is region- and cytokine-specific (O'Connor et al. 2003). Previous exposure to a stressor increases LPS-stimulated IL-1 β production (Johnson et al. 2002). Thus, stress may sensitize microglia to respond in a heightened fashion to a subsequent peripheral immune stimulus (Frank et al. 2007).

Furthermore, CRF may play a role in the interaction between a stressor and the production of proinflammatory cytokines (Leonard 2005). As CRF receptors are present on most immune cells such as the resident macrophages of the mouse spleen, human monocytes, macrophages, and T-lymphocytes (Schäfer et al. 1997), stressors may increase proinflammatory cytokines via CRF receptor stimulation (Leonard 2005).

Proinflammatory cytokines and tryptophan metabolism

Proinflammatory cytokines induce IDO activity. Subsequent activation of the KYN pathway results in TRP depletion that inhibits cell growth and proliferation, and the 5-HT biosynthesis (Figure 2).

As described above, metabolites in the KYN pathway have a neurotoxic or neuroprotective activity (Figure 1). 3-OH KYN and QUIN have a neurotoxic effect, whereas KYNA has a neuroprotective effect (Myint and Kim 2003; Wichers and Maes 2004). Some of the destructive properties of 3-OH KYN might be caused by its metabolism to 3-hydroxyanthranilic acid, which can undergo auto-oxidation with the formation of superoxide anions (Okuda et al. 1996; Stone and

Darlington 2002; Wichers and Maes 2004). QUIN has the property of being a selective *N*-methyl-D-aspartate (NMDA) receptor agonist (Stone and Darlington 2002; Myint and Kim 2003; Wichers and Maes 2004). A recent *in vitro* study reported that QUIN reduces antioxidant defenses in the cerebral cortex (Leipnitz et al. 2005). In contrast, KYNA may have neuroprotective properties because it has the properties of a NMDA receptor antagonist and could have agonist properties on nicotinic acetylcholine (nACh) receptors such as the α 7-nACh receptor (Stone and Darlington 2002; Myint and Kim 2003). The ratio of QUIN/KYNA is increased in the CSF of macaques with SAIDS (Heyes et al. 1990). Systemic immune activation in gerbils induces brain IDO, with no change in KAT activity, so the ratio of QUIN/KYNA in CSF increases (Saito et al. 1993). Human microglia as well as macrophages produced KYN and QUIN in cultures activated by IFN- γ (Heyes et al. 1996). These findings suggest that immunological challenges including a direct application of IFN- γ activate the KYN pathway and QUIN production in the CNS, but do not affect KYNA production.

Thus, the balance of neurotoxic and neuroprotective activities of these metabolites of TRP may play an important role in the pathogenesis of neurodegenerative disease involving depression.

Proinflammatory cytokines and the HPA axis

As described above, proinflammatory cytokines activate the HPA axis (Maier 2003; Figure 4). IL-1 is a potent stimulus of CRF synthesis, which supports the activation of the HPA axis (Black 1994; Leonard 2001). The production of prostaglandins (PGs) of the E2 series (PGE2s) at the level of the BBB transmits messages from the cytokines (Konsman et al. 2002). Synthesis of PGE2 is dependent on the induction of cyclooxygenase 2 (COX-2) and PGE synthase expressed in endothelial cells of the cerebral blood vessels and possibly perivascular macrophages. PGE2 activates the CRF neurons of the PVN. These processes activate the HPA axis (Konsman et al. 2002).

Further, the proinflammatory cytokine IFN- γ induces IDO expression in hypothalamic and pituitary cells (Tu et al. 2005). The activated IDO in these cells may produce 3-OH KYN and QUIN, which may result in neurodegenerative changes in the HPA axis. Hippocampal atrophy and subsequent inhibition of the negative feedback activity in the HPA axis in chronic depression might be associated with chronic exposure to these neurotoxic products of TRP.

Proinflammatory cytokines and brain monoamines

Proinflammatory cytokines act on brain monoamine systems in a cytokine- and region-specific manner in a

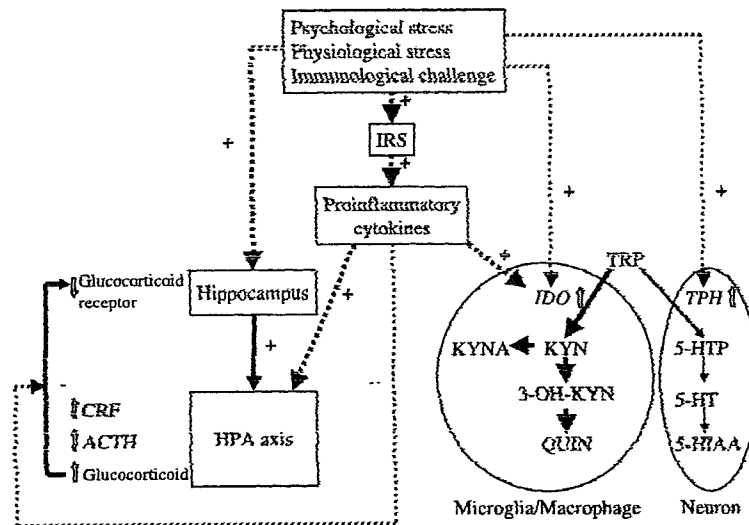


Figure 4. Regulation of brain KYN pathway and HPA axis by stress and immunological challenges. Stress and immune challenges activate the KYN pathway and the HPA axis. Proinflammatory cytokines activate the KYN pathway via induction of IDO, whereas they stimulate the HPA axis. Furthermore, proinflammatory cytokines attenuate the negative feedback control of the HPA axis. ACTH, adrenocorticotropic hormone; CRF, corticotropin releasing factor; 5-HIAA, 5-hydroxyindoleacetic acid; HPA axis, hypothalamo-pituitary-adrenal axis; 5-HT, serotonin; 5-HTTP, 5-hydroxy tryptophan; IDO, indoleamine 2,3-dioxygenase; IRS, inflammatory response system; KYN, kynurenine; KYNA, kynurenic acid; 3-OH KYN, 3-hydroxykynurenine; QUIN, quinolinic acid; TRP, tryptophan; TPH, tryptophan hydroxylase.

complex way (Zalcman et al. 1994). As described above, proinflammatory cytokines may reduce the activity of 5-HT pathway by promoting the KYN pathway due to activation of IDO. Although a single i.p. $\text{INF-}\alpha$ injection has no significant effect on the levels of monoamines and their metabolites or monoamine turnover, repeated i.p. injection significantly decreases both dopamine (DA) and 3,4-dihydroxyphenylacetic acid levels in the mouse brain (Shuto et al. 1997). Acute intracerebroventricular infusion of $\text{INF-}\alpha$ increases 5-HT turnover in the prefrontal cortex and increases DA turnover in the hippocampus, while pre-treatment with diclofenac, a non-steroidal anti-inflammatory drug prevents these neurochemical responses in rats (De La Garza II et al. 2003). Chronic i.p. administration of $\text{INF-}\alpha$ to rats reduces DA levels in the prefrontal cortex, decreases 5-HT levels, and increases 5-HT turnover in the amygdala (De La Garza II et al. 2005). In other experiments, although chronic i.p. administration of $\text{INF-}\alpha$ to rats increases 5-HT turnover in the frontal cortex, hippocampus, amygdala, thalamus, hypothalamus, and brainstem, these treatments do not alter 5-HT transporter mRNA level (Sato et al. 2006). Acute i.p. injection of $\text{IL-1}\alpha$ to rats increases 5-HT release from the CA1 region of the hippocampus, whereas this treatment decreases norepinephrine (NE) release (Broderick 2002). IL-2 increases hypothalamic and hippocampal NE utilization and DA turnover in the prefrontal cortex, whereas IL-6 induces profound elevations of 5-HT and mesocortical DA activity in the hippocampus and prefrontal cortex (Zalcman et al. 1994).

Thus, although the effects of cytokines on brain monoamines are complex, these changes in the activities of brain monoamine systems elicited by proinflammatory cytokines may be related to the etiology and pathophysiology of depression.

Proinflammatory cytokines and depression

It is now generally accepted that psychological stress and psychiatric illness can compromise immune functions (Leonard 2001). There is strong evidence to suggest that changes in cytokine levels outside the brain cause changes in cytokine expression and activity in the brain. Conversely, cytokine changes in the brain elicit responses outside the brain (Dantzer et al. 1999). Proinflammatory cytokines induce neuroendocrine and behavioral changes similar to depression in animal models (Dantzer et al. 1999), and immune therapy using proinflammatory cytokines causes depressive symptoms in human subjects (Bonaccorso et al. 2002; Capuron et al. 2002, 2003; Wichers et al. 2005). In addition, changes in the immune system activity, especially the increased levels in proinflammatory cytokines, are found in human depressive patients (Smith 1991; Maes et al. 1995). Thus, depression may now be considered a disorder that is closely related to dysfunction in the immune-endocrine system (Leonard 2001).

Proinflammatory cytokines induce neuroendocrine and behavioral changes in animals that appear similar to those in depressed patients (Dantzer et al. 1999). In terms of neurochemical changes, proinflammatory

cytokines increase the production of CRF, which can escape glucocorticoid negative feedback. In terms of behavioral changes, the anhedonia and helplessness that may be produced by proinflammatory cytokines are considered two essential features of a major depressive episode with melancholia.

An *in vitro* study has shown that the antidepressants clomipramine, sertraline, and trazodon at concentrations of 10^{-6} M (similar to the plasma concentrations during clinical treatment) markedly reduce the proinflammatory cytokine IFN- γ , but increase the immune-inhibiting cytokine IL-10 release from human blood (Song 2000). Other studies showed that different antidepressants significantly inhibit proinflammatory cytokine secretion from human monocytes activated by LPS and increase the production of anti-inflammatory cytokines (Xia et al. 1996; Kubera et al. 2000). In contrast, *ex vivo* data collected in depressed patients are much less demonstrative, probably because of the heterogeneity of the patients and their clinical conditions (Haack et al. 1999). However, these actions of antidepressants in *in vitro* studies may help to explain the suppressive effects of antidepressants on proinflammatory cytokine production *in vivo* and on the immune function in depression (Song 2000; Castanon et al. 2002). The existence of 5-HT receptors on human immunocytes suggests a potential mechanism for the immune activity of antidepressants (Maes 2001). 5-HT significantly suppresses the production of IFN- γ , whereas the common activity of other antidepressants is to increase IL-10 secretion (Maes 2001). Thus, the suppressive effects of antidepressive drugs on the IFN- γ /IL-10 ratio are probably not related to the direct effects of 5-HT. Another possible mechanism of 5-HT revolves around cAMP. 5-HT stimulates 5-HT receptors on immunocytes and increases the intracellular levels of cAMP by a direct activation of adenylyl cyclase. Increased intracellular cAMP levels are considered to play a critical role in the production of IL-10 and IFN- γ (Maes 2001). Indeed, tricyclic antidepressants (TCAs) as well as selective serotonin reuptake inhibitors (SSRIs) significantly elevate intracellular cAMP levels in T-lymphocytes and monocytes (Xia et al. 1996). However, the exact mechanisms by which antidepressants exert their effects on proinflammatory cytokine production are still unknown.

Proinflammatory cytokines and the monoamine hypothesis of depression

As described above, the increase in proinflammatory cytokines in depression and the depressive symptoms following their administration may change brain monoamine system activity. Proinflammatory cytokines have been implicated in the alteration of presynaptic 5-HT as well as NE activities in the

brain regions assumed to be involved in major depression, including the prefrontal cortex, hypothalamus, hippocampus, and amygdala (Zalcman et al. 1994; Broderick 2002; De La Garza II et al. 2003, 2005; Schiepers et al. 2005; Sato et al. 2006). Thus, possible explanations for the association between proinflammatory cytokines and dysfunction of monoaminergic systems in depression have been proposed. Proinflammatory cytokines regulate 5-HT transporter activity (Ramamoorthy et al. 1995; Mössner et al. 1998) and/or the number or sensitivity of postsynaptic 5-HT receptors, including the 5-HT_{1A} and 5-HT_{2A} receptors (Abe et al. 1999). In addition, proinflammatory cytokines stimulate COX-2 activity and thereby increase the synthesis of PGE₂. Thus, an increase in the plasma and cerebrospinal fluid PGE₂ levels reported in depressed patients may contribute to dysfunction in the release and uptake of brain monoamines by impeding the release of the amines (Leonard 2001).

Thus, activation of the inflammatory response system (IRS) and administration of proinflammatory cytokines may induce depressive symptoms through, among other mechanisms, their modulation of the 5-HT system (Bonaccorso et al. 2000).

Proinflammatory cytokines and HPA axis-hyperactivity hypothesis of depression

The best-documented changes in biological psychiatry are the increase in the activity of the HPA axis and glucocorticoid resistance in depressed patients (Dantzer et al. 1999). Proinflammatory cytokines might play a causal role in depression mainly because they provide another stimulatory input to the CRF system. The monocyte-T-lymphocyte hypothesis (Smith 1991; Maes et al. 1995) proposes that activated macrophages play a role in the clinical onset and pathophysiology of major depression, and suggests that high levels of IL-1 released from activated macrophages directly stimulate CRF release by the PVN neurones in the hypothalamus. According to this theory, cytokines would increase the reactivity of the HPA axis to external stressors by inducing the expression of vasopressin, another ACTH secretagogue, in parvocellular CRF neurons of the PVN. The chronically increased CRF release can by itself alter the production and action of brain cytokines (Dantzer et al. 1999). Proinflammatory cytokines activate the HPA axis and increase the release of glucocorticoids, which have strong immunosuppressive effects. Thus, the immunosuppression observed in major depression may be due to HPA axis hyperactivity elicited by proinflammatory cytokines and/or stress. The reciprocal relationships between HPA axis activity and the immune system may participate in the pathophysiology of major depression (Maes et al. 1995).

Depression and tryptophan metabolism

HPA axis activity and TRP availability, including plasma TRP level and TRP entry into the brain

The synthesis of 5-HT in the brain is directly dependent on brain TRP levels. In animals, tryptophan 2,3-dioxygenase (TDO; Hayaishi et al. 1957), another metabolizing enzyme of TRP, is induced by hydrocortisone. Although there is no direct evidence of TDO induction by steroids in humans, this effect may mediate a reduction in TRP availability in response to hypercortisolaemia (Porter et al. 2004; Figure 3). As described above, brain TRP levels are mainly regulated by two mechanisms, competitive binding to plasma albumin with FFA and competition with LNAA at the BBB (Figure 3). Because glucocorticoid treatment induces hepatic and brain tyrosine aminotransferase, which is the major tyrosine-degrading enzyme (Hirota et al. 1985), tyrosine levels may also be altered by glucocorticoid levels (Porter et al. 2004). Further, tyrosine is one of the competing LNAAs at the BBB. Thus, glucocorticoid levels may regulate plasma TRP levels and TRP entry into the brain in a complex manner.

Glucocorticoids are known to attenuate the plasma TRP level and the TRP/LNAA ratio. However, the effects of glucocorticoids on TRP availability in healthy volunteers remain controversial. In healthy volunteers, dexamethasone significantly decreases plasma TRP and tyrosine levels as well as the plasma TRP/LNAA ratio (Maes et al. 1990a). One study reported that dexamethasone does not alter plasma TRP levels (Porter et al. 1999), whereas another study revealed that hydrocortisone significantly decreases plasma TRP (Bhagwagar et al. 2002). In depressive patients, dexamethasone also significantly decreases plasma TRP levels and the plasma TRP/LNAA ratio (Maes et al. 1990a,b). In recovered depression patients, hydrocortisone also suppresses plasma TRP levels (Bhagwagar et al. 2002). Furthermore, patients with major depression and melancholia exhibit significantly lower TRP availability than patients with minor depression (Maes et al. 1990b). Thus, acute administration of glucocorticoids may suppress the availability of TRP (Figure 3).

In addition, cortisol responses to dexamethasone are significantly and negatively associated with TRP availability (Maes et al. 1987, 1990c). However, there is no correlation between TRP/LNAA ratios and basal cortisol output (Maes et al. 1990b,c; Porter et al. 2004). Some other studies suggested that there is no significant difference in the plasma basal TRP levels between controls and depressed subjects (Cowen and Charig 1987; Price et al. 1991), although these studies did not measure LNAA levels or the TRP/LNAA ratio. Thus, the suppression of TRP availability in depression may relate to a dysfunction of the HPA axis

feedback mechanism rather than to the basal level of glucocorticoids (Porter et al. 2004).

The mechanism underlying the attenuation in TRP availability in depression remains unknown. However, the direction of alteration in TRP availability reveals a decrease contrary to the increase in response to acute stress (Figure 3). Acute stress and immunological challenges elevate plasma TRP and the TRP/LNAA ratio, whereas glucocorticoid treatment and depression suppress these measures. These findings suggest that peripheral TRP availability in response to acute stress and immunological challenge changes via the β -adrenergic system in the normal physiological condition but via the HPA axis in depression, although the possibility that the TRP changes caused by stress or proinflammatory cytokines have nothing to do with the onset of depression remains to be examined. Furthermore, these changes in depression may be associated with HPA axis feedback mechanism dysfunction rather than a deficiency in the basal level of glucocorticoids (Porter 2004). Stress-induced peripheral immune activation, or IRS, may induce peripheral IDO and/or TDO, and may consume TRP and other peripheral amino acids to produce positive acute-phase proteins (APPs) such as haptoglobin and C-reactive protein (Schiepers et al. 2005). In addition, the inflammation process reduces plasma-negative APPs such as albumin, which bind to TRP (Figure 3). These alterations may modulate the increase in TRP availability in response to acute stress.

Prolonged and/or repeated exposure to stressful life events often precedes the onset of clinical depression. Thus, prolonged adaptation to these stresses may have a consumed ability to protect against new stress on the body and brain, i.e., allostatic load (McEwen 2004). Breakdown of the physiological adjustment system which increases the plasma TRP level, TRP/LNAA ratio, and brain TRP uptake in response to acute stress may result in the suppression of TRP availability.

Clinical major depression and tryptophan metabolism

All the above-described results suggest that proinflammatory cytokines induce depression. Some studies have indicated a correlation between the severity of depressive symptoms and serum TRP decrease and/or KYN increase in depression induced by cytokine therapy (Bonaccorso et al. 2002; Capuron et al. 2002, 2003). In the study of women in pregnancy and delivery, depression and anxiety symptoms in the early puerperium are causally related to an increased catabolism of TRP into KYN (Maes et al. 2002; Kohl et al. 2005). These changes in the plasma TRP and KYN/TRP ratio were significantly related to those in the IL-6 level (Maes et al. 2002).

In patients with major depression, the secretion of proinflammatory cytokines from activated macrophages and the synthesis of APPs from the liver are

increased (Leonard 2001). In studies evaluating plasma TRP and LNAA together with indices of immune functions, patients with depression show decreased plasma TRP levels and TRP/LNAA ratios. Plasma TRP levels negatively correlate to IL-6 production (Maes et al. 1993). Furthermore, depressed subjects have significantly lower serum TRP and albumin levels (Maes et al. 1996). These studies suggest that lower TRP availability to the brain in depression is related to immune responses and lower serum albumin (Maes et al. 1993, 1996).

In recent years, imbalance in the neurodegenerative and neuroprotective activities of KYN pathway metabolites has been revealed as an aspect of depression. IFN- α therapy revealed that a long-term increase of the serum KYN/KYNA ratio, reflecting neurotoxic challenge, is significantly associated with a higher total Montgomery Asberg Depression Rating Scale (MADRS) score (Wichers et al. 2005). Further, plasma KYNA and the neuroprotective ratio, $1000 \times$ KYNA/KYN, are decreased in patients with major depression (Myint et al. 2007). These studies suggest that the amount and ratio of the neuroprotective metabolite, KYNA, of the KYN pathway are lower in depressive patients. Indeed, patients with major depression who experienced no apparent immunological challenge as well as depressive patients induced by IFN- α showed a decrease in the KYNA level. Thus, a shift in the balance between the neurotoxic and neuroprotective activities of the KYN pathway toward the former may be associated with the etiology of depression.

Effects of antidepressants on the altered tryptophan metabolism

Although clinical findings support the alteration of TRP metabolism in depression, there is only scant evidence directly indicating the effects of antidepressants in restoring these changes in TRP metabolism. One study reported that paroxetine, an SSRI, did not suppress changes in KYN and neopterin levels elicited by IFN- α therapy in patients with malignant melanoma (Capuron et al. 2003). Further studies evaluating the responses of TRP metabolism to different types of antidepressants in patients with depression as well as in animal models are needed to clarify the role of TRP metabolism in the etiology and/or pathophysiology of depression.

Summary

In the present review, we explained how changes in TRP metabolism may be involved in the etiology and pathophysiology of depression. Because physiological and/or psychological stress as well as immunological challenges activate the IRS, and then induce proinflammatory cytokine expression both in the periphery

and in the brain, these cytokines may induce and activate peripheral and brain IDO. The activated IDO metabolizes TRP into KYN, and shifts the balance between the KYN and 5-HT pathways of TRP metabolism to the former. Thus, the TRP available for 5-HT synthesis is reduced, and the subsequent 5-HT depletion in the brain may result in depression. The 5-HT shortage caused by IDO activation as well as presynaptic and postsynaptic modulation of the 5-HT system by proinflammatory cytokines may be associated with the monoamine hypothesis of depression. Furthermore, downstream of the activated KYN pathway, the balance of neuroprotective/neurotoxic metabolites may be important; 3-OH KYN and QUIN are neurotoxic, whereas KYNA is neuroprotective. Thus, an imbalance in these metabolites may cause neurodegenerative changes such as hippocampal atrophy in depression. Also, the activation of the HPA axis elicited by proinflammatory cytokines may be closely related to the HPA axis-hyperactivity hypothesis of depression. IRS activation elicited by stress and immunological challenges may thus provide the link between the monoamine and HPA axis-hyperactivity hypotheses of depression via proinflammatory cytokine-induced changes in TRP metabolism. Further accumulation of evidence that directly reveals that IDO induction, a shift in the balance between the KYN and 5-HT systems toward the former, and/or a shift in the balance between the neurotoxic and neuroprotective activities of the KYN pathway toward the former, elicited by physiological and psychological risk factors for depression such as prolonged and repeated exposure to stressful life events would provide support for the importance of the activation of the KYN pathway in the etiology of depression.

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In vivo imaging of microglial activation using a peripheral benzodiazepine receptor ligand: [^{11}C]PK-11195 and animal PET following ethanol injury in rat striatum

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Abstract

Objective To investigate whether [^{11}C]PK-11195, a specific peripheral benzodiazepine receptors (PBRs) ligand for positron emission tomography (PET), can show activated microglia in a rat brain injury model.

Methods On day 1, ethanol was injected into the rat's right striatum (ST) using a stereotaxic operative procedure. On day 3, head magnetic resonance imaging (MRI) scans for surgically treated rats were performed to evaluate ethanol injury morphologically. On day 4, dynamic PET scans (17 injured rats and 7 non-injured controls) were performed for 60 min with an animal PET scanner under chloral hydrate anesthesia following a bolus injection of [^{11}C]PK-11195 through tail vein. Because PBRs are present throughout the brain, there is no suitable receptor-free reference region. The reference tissue model may not be applicable because of low target to background ratio for low affinity of [^{11}C]PK-11195 to PBRs. We evaluated the PBRs binding with regions of interest

(ROIs)-based approach to estimate total distribution volume (V). We used an integral from 0 min to 60 min (V_{60}) as an estimate of V . On the coronal PET image, ROIs were placed on bilateral ST. Differences in right/left ST V_{60} ratios between lesioned and unlesioned control rats were compared using unpaired t tests. Immunohistochemical staining was performed for confirming the presence of activated microglia following decapitation on the PET experiment day.

Results The right/left ST V_{60} ratios in lesioned rats (1.07 ± 0.08) were significantly higher than those in unlesioned control rats (1.00 ± 0.06 , $P < 0.05$). On immunohistochemical staining, activated microglia were exclusively observed in the injured right ST but not in the non-injured left ST of the injury rats and the bilateral ST of the non-injured control rats.

Conclusions These results suggest that [^{11}C]PK-11195 PET imaging would be a useful tool for evaluating microglial activation in a rat brain injury model.

Keywords Animal PET · Microglia · [^{11}C]PK-11195 · Peripheral benzodiazepine receptors · Rat

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Introduction

In the central nervous system (CNS), microglia are major glial components and become activated in response to a wide variety of pathological stimuli such as brain injury and degeneration [1–3]. There is increasing evidence that microglia play an active part in degenerative CNS diseases. In Alzheimer's disease (AD), activated microglia appear to be involved with the plaque formation [2]. In the normal adult CNS, microglia constitute relatively stable cell population [4]. The activated microglial cell