Cellular/Molecular

Angiotensin II Blocks Nicotine-Mediated Neuroprotection against β-Amyloid (1-42) via Activation of the Tyrosine Phosphatase SHP-1

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We showed recently that nicotine activates the growth-promoting enzyme Janus kinase 2 (JAK2) in PC12 cells and that preincubation of these cells with the JAK2-specific inhibitor AG-490 blocked the nicotine-induced neuroprotection against β-amyloid (1-42) [Aβ (1-42)]. These results provided direct evidence for linkage between JAK2 and the α7 nicotinic acetylcholine receptor-induced neuroprotection in PC12 cells. We also showed that preincubation with angiotensin II (Ang II), functioning via the angiotensin II type 2 (AT$_2$) receptor, blocked both the nicotine-induced activation of JAK2 and its neuroprotection against Aβ (1-42). Recently growth-inhibitory effects of the AT$_2$ receptor have been reported to be mediated by the activation of protein tyrosine phosphatases (PTPases) and that AT$_2$ receptor stimulation is associated with a rapid activation of the PTPase SHP-1 (the cytoplasmic tyrosine phosphatase that contains Src homology 2 domains), a negative regulator of JAK2 signaling. Therefore, the potential biological significance of AT$_2$ receptor-induced effects on both the nicotine-induced activation of JAK2 and its neuroprotection against Aβ (1-42) led us to investigate whether SHP-1 activation could be involved in this process. We found that Ang II induced the activation of SHP-1 and that an antisense against SHP-1 not only augmented the nicotine-induced tyrosine phosphorylation of JAK2 but also blocked the Ang II neutralization of the nicotine-induced neuroprotection. These results demonstrate that nicotine-induced tyrosine phosphorylation of JAK2 and neuroprotection against Aβ (1-42) in PC12 cells are blocked by Ang II via AT$_2$ receptor-induced activation of SHP-1.

Key words: nicotine; neuroprotection; JAK2; phosphatydylinositol-3-kinase; SHP-1; PC12 cells; Alzheimer's disease; Ang II

Introduction

The cholinergic deficit in Alzheimer's disease (AD) has been clearly established and is the basis for the current symptomatic strategy. There is an early and significant depletion of high-affinity nicotinic receptors in Alzheimer's patient's brains (Court et al., 2001), and a number of studies have shown cognitive improvement in rodent, primates, and humans after administration of ligands targeting nicotinic acetylcholine receptor (nAChR) (Newhouse et al., 2001). In addition to their known symptomatic effects, neuronal nicotinic ligands have shown neuroprotective activity *in vitro* (Donnelly-Roberts et al., 1996) and *in vivo* (Ryan et al., 2001), suggesting an additional potential for disease modification.

The α7 nAChR forms functional homomeric ligand-gated ion channels that promote rapidly desensitizing Ca^{2+} influx, is widely expressed throughout the mammalian brain, and has been implicated in sensory gating, cognition, and neuroprotection (Seo et al., 2001). In addition, nicotine-induced neuroprotection against β-amyloid [Aβ (1-42)]-induced toxicity is suppressed by α-bungarotoxin, and the selective α7 nAChR agonist anabaseine-

derived 3-(4)-dimethylaminocinamylidine exerts cytoprotective effects (de Fiebre et al., 1995; Kem, 2000). Furthermore, a recent study has reported that the levels of phosphorylated Akt, an effector of phosphatidylinositol 3-kinase (PI-3-K), are increased by nicotine, and the nicotine-induced cytoprotective effects are suppressed by the PI-3-K inhibitors (LY294002 and wortmannin) (Kihara et al., 2001). These findings suggest that the α7 nAChR transduces signals to PI-3-K in a cascade, which ultimately contributes to a neuroprotective effect against Aβ (1-42).

We recently provided evidence for the nicotine-induced complex formation between the α7 nAChR and the tyrosine kinase enzyme Janus kinase 2 (JAK2), which results in subsequent activation of PI-3-K and Akt (Shaw et al., 2002). We also provided evidence that nicotine interaction with the α7 nAChR is "dominant" over Aβ (1-42) interaction with the receptor and that the Aβ (1-42)-induced apoptosis is prevented through the nicotine-induced activation of JAK2. Nicotine neuroprotective effects can be neutralized through activation of the angiotensin II (Ang II) type 2 (AT$_2$) receptor as evidenced by the reversal of JAK2 phosphorylation and inhibition of nicotine-induced neuroprotection (Shaw et al., 2002).

The AT$_2$ receptor exerts growth-inhibitory effects in cultured cells and *in vivo*, one of which has been proposed to be programmed cell death (Horiuchi et al., 1998; Lehtonen et al., 1999). Despite growing interest in AT$_2$ receptor-mediated apoptosis,

relatively little is known about the molecular basis of this process. Recently, growth-inhibitory effects of the AT_2 receptor have been reported to be mediated by the activation of protein tyrosine phosphatases (PTPases) (Horiuchi et al., 1998), and, in rat pheochromocytoma PC12 cells, AT_2 receptor stimulation is associated with a rapid activation of SHP-1 ((the cytoplasmic tyrosine phosphatase that contains Src homology 2 domains) (Horiuchi et al., 1998). However, at present, no functional role has been demonstrated for SHP-1 activation by the AT_2 receptor, and it is interesting to note that SHP-1 has been shown to function as a negative regulator of JAK2 signaling (Marrero et al., 1998). Therefore, the potential biological significance of AT_2 receptor-induced effects on both the nicotine-induced activation of JAK2 and its neuroprotection against Aβ (1-42) led us to investigate whether SHP-1 activation could be involved in this process.

Materials and Methods

Materials. Molecular weight standards, acrylamide, SDS, N-N'-methylene-bisacrylamide, N,N,N',N'-tetramethylenediamine, protein assay reagents, and nitrocellulose membranes were purchased from Bio-Rad (Hercules, CA). Protein A/G-agarose was obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and DMEM, fetal bovine serum, trypsin, and all medium additives were obtained from Mediatech (Herndon, VA). Monoclonal antibody to phosphotyrosine (PY20) and SHP-2 were procured from Transduction Laboratories (Lexington, KY). Poly-(ADP-ribose) polymerase (PARP) antibodies were purchased from New England Biolabs (Beverly, MA). Anti-phosphotyrosine JAK2 and JAK2 antibodies were obtained from Biosource (Camarillo, CA). The Pierce Supersignal substrate chemiluminescence detection kit was obtained from Pierce (Rockford, IL). Goat anti-mouse IgG and anti-rabbit IgG were acquired from Amersham Biosciences (Princeton, NJ), and Tween 20, nicotine, Aβ (1-42) peptide, anti-Aβ (1-42), anti-α7 nAChR, and all other chemicals were purchased from Sigma (St. Louis, MO).

Isolation and culture of PC12 cells. PC12 rat pheochromocytoma cells were maintained in proliferative growth phase in DMEM (Invitrogen, Gaithersburg, MD) supplemented with 10% horse serum, 5% fetal calf serum (Atlanta Biologicals, Norcross, GA), and antibiotics (penicillin–streptomycin) according to routine protocols (Bencherif et al., 1996).

Western blotting studies of JAK2. The phosphorylation of JAK2 proteins was determined in serum-starved PC12 cells stimulated with 10 μM nicotine as described previously (Shaw et al., 2002). At the end of stimulation, cells were washed twice with ice-cold PBSV (phosphate-buffered saline with 1 mmol/l Na_3VO_4). Each dish was then treated for 60 min with ice-cold lysis buffer (20 mmol/l Tris-HCl, pH 7.4, 2.5 mmol/l EDTA, 1% Triton X-100, 10% glycerol, 10 mmol/l $Na_4P_2O_7$, 50 mmol/l NaF, 1 mmol/l Na_3VO_4, and 1 mmol/l PMSF), and the supernatant fraction was obtained as cell lysate by centrifugation at 58,000 × g for 25 min at 4°C. Samples were resolved by 10% SDS-PAGE, transferred to a nitrocellulose membrane, and blocked by 60 min incubation at 22°C in TTBS (TBS with 0.05% Tween 20, pH 7.4) plus 5% skimmed milk powder. The nitrocellulose membrane was incubated overnight at 4°C with affinity-purified anti-phospho-specific JAK2 antibodies. The nitrocellulose membranes were washed 10 min twice with TTBS and incubated with goat anti-rabbit IgG horseradish peroxidase conjugate. After extensive washing, the bound antibody was visualized on a Kodak Biomax film (Eastman Kodak, Rochester, NY) using a Pierce Supersignal substrate chemiluminescence detection kit.

Immunoprecipitation studies of SHP-1. The cell lysate prepared as described above was incubated with 10 μg/ml anti-SHP-1 monoclonal antibodies at 4°C for 2 hr and precipitated by addition of 50 μl of protein A/G-agarose at 4°C overnight. The immunoprecipitates was recovered by centrifugation and washed three times with ice-cold wash buffer (TBS, 0.1% Triton X-100, 1 mmol/l PMSF, and 1 mmol/l Na_3VO_4). Immunoprecipitated proteins were dissolved in 100 μl of Laemmli sample buffer, and 80 μl of each sample was resolved by SDS-PAGE. Samples were transferred to a nitrocellulose membrane and blocked by 60 min incubation at room temperature (22°C) in TTBS plus 5% skimmed milk pow-

der. The nitrocellulose membrane was then incubated overnight at 4°C with 10 μg/ml affinity-purified anti-phosphotyrosine antibodies, and the bound antibodies were visualized using a Pierce Supersignal chemiluminescence detection kit.

SHP-1 tyrosine phosphatase activity assay. SHP-1 activity was determined as described previously (Marrero et al., 1998). Briefly, SHP-1 proteins were immunoprecipitated with anti-SHP-1 antibodies from PC12 cell lysates, and the immunocomplexes were washed three times with ice-cold wash buffer and then three times with phosphatase buffer (50 mM HEPES, 60 mM NaCl, 60 mM KCl, 0.1 mM PMSF, 10 μg/ml aprotinin, and 10 μg/ml leupeptin, pH 7.4). Phosphatase activity was measured by monitoring the rate of formation of p-nitrophenol by dephosphorylation of p-nitrophenyl phosphate. Immunocomplex pellets were resuspended in 100 μl of phosphatase buffer containing 1 mg/ml BSA, 5 mM EDTA, and 10 mM dithiothreitol. The reaction was initiated by the addition of p-nitrophenylphosphate (10 mM final concentration). After the described period of incubation at room temperature, the reaction was stopped by the addition of 1 M NaOH, and absorbance of the sample was determined at 410 nm in a spectrophotometer.

Antisense against SHP-1. An antisense oligonucleotide that targets the translational start site of the murine SHP-1 coding sequence (5′-ACCTCACCATCCTTGGGGT-3′) has been found to significantly reduce SHP-1 expression in human erythroleukemic SKT6 cells (Sharlow et al., 1997). Therefore, we tested the effect of this SHP-1 antisense phophorothiorate oligonucleotide on SHP-1 expression in PC12 cells. Briefly, for oligonucleotide transfections, PC12 cells were washed and resuspended in Optimem I medium at 1×10^6 cells/ml. Oligonucleotides in Optimem I medium and 1% FBS (20 or 40 μmol/l) were incubated with DMRIE-C (15 μg; Invitrogen) for 30 min at 23°C. Liposome–oligonucleotide complexes were then incubated for 5 hr at 37°C with 7.5×10^5 cells (total volume, 1.5 ml), and an equal volume of Optimem I medium containing 8% FBS was added. For assays of SHP-1 expression, PC12 cells were collected, washed at 0°C in Optimem I medium, and lysed at 4°C in 20 mmol/l Tris-HCl, pH 7.4, 2.5 mmol/l EDTA, 1% Triton X-100, 10% glycerol, 10 mmol/l $Na_4P_2O_7$, 50 mmol/l NaF, 1 mmol/l Na_3VO_4, 1 mmol/l PMSF, and 2.2 μg/ml aprotinin (3.0×10^6 cells/0.25 ml per sample). The cleared supernatant was then incubated with 10 μg/ml anti-SHP-1 monoclonal antibodies at 4°C for 2 hr and precipitated by addition of 50 μl of protein A/G-agarose at 4°C overnight. The immunoprecipitates was recovered by centrifugation and washed three times with ice-cold wash buffer (TBS, 0.1% Triton X-100, 1 mmol/l PMSF, and 1 mmol/l Na_3VO_4). Immunoprecipitated proteins are dissolved in 100 μl of Laemmli sample buffer, and 80 μl of each sample was resolved by SDS-PAGE. Samples were transferred to a nitrocellulose membrane and blocked by 60 min incubation at room temperature (22°C) in TTBS plus 5% skimmed milk powder. The nitrocellulose membrane was then incubated overnight at 4°C with 1 μg/ml anti-SHP-1 antibodies, and the bound antibodies were visualized using a Pierce Supersignal chemiluminescence detection kit.

Assessment of PC12 cell apoptosis. Apoptosis was determined by assessing the cleavage of the DNA-repairing enzyme PARP using a Western blot assay. PARP (116 kDa) is an endogenous substrate for caspase-3, which is cleaved to a typical 85 kDa fragment during various forms of apoptosis. PC12 cells were treated with 0.1 μM Aβ for 8 hr in the presence or absence of 10 μM nicotine and/or 100 nM Ang II and/or SHP-1 antisense and/or 10 μM of the JAK2-specific inhibitor AG-490 (Meydan et al., 1996; Dicou et al., 2001). The cells were collected, washed with PBS, and lysed in 1 ml of SDS-PAGE sample buffer boiled for 10 min. Total cell lysates (30 μg of protein) were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked for 1 hr at 25°C with 5% nonfat dry milk in TBST (25 mM Tris-HCl, pH 7.5, 0.5 M NaCl, and 0.05% Tween 20). Membranes were incubated with primary PARP antibody specific for the 85 kDa fragments for 2–3 hr at 25°C, rinsed with TBST, and incubated with secondary antibody for 1 hr at 25°C. Immunodetection was performed with appropriate antibody using an enhanced chemiluminescence system (Amersham Biosciences).

Caspase 3 enzyme activity was determined with a fluorogenic substrate for caspase-3 in crude PC12 cell extracts. The caspase 3 fluorogenic peptide Ac-DEVD-AMC (Promega, Madison, WI) contains the specific
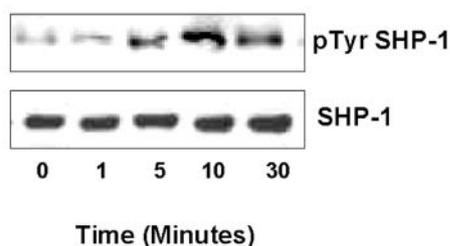
Figure 1. Angiotensin II-induced tyrosine phosphorylation of SHP-1. PC12 cells were incubated for 24 hr in serum-free medium before exposure to Ang II (100 nM) for the times indicated. Cells were lysed, and SHP-1 was immunoprecipitated from lysates with 10 μg/ml anti-SHP-1 monoclonal antibodies at 4°C for 2 hr and precipitated by addition of 50 μl of protein A/G-agarose at 4°C overnight. Immunoprecipitated proteins were resolved by SDS-PAGE and transferred to a nitrocellulose. Immunoprecipitates were then immunoblotted with anti-phosphotyrosine antibody. Shown are representative immunoblots of three immunoblots. pTyr, Tyrosine-phosphorylated.

caspase 3 cleavage sequence (DEVD) coupled at the C terminal to the fluorochrome 7-amino-4-methyl coumarin. The substrate emits a blue fluorescence when excited at a wavelength of 360 nm. When cleaved from the peptide by the caspase 3 enzyme activity in the cell lysate, free 7-amino-4-methyl coumarin is released and can be detected by its yellow–green emission at 460 nm. Appropriate controls included a reversible aldehyde inhibitor of caspase 3 to assess the specific contribution of the caspase 3 enzyme activity (data not shown). Fluorescence units were normalized relative to total protein concentration of the cell extract. We performed the assays in triplicate and repeated the experiments three times. In addition, we measured the decrease in PC12 cell number using a Coulter counter (model ZM; Coulter, Hialeah, FL).

Data analysis. All statistical comparisons were made using Student's *t* test for paired data and ANOVA. Significance was $p < 0.05$.

Results

Ang II-induced activation of SHP-1 and its effects on nicotine-induced tyrosine phosphorylation of JAK2

At present, no functional role has been demonstrated for SHP-1 activation by the AT_2 receptor, and it is interesting to note that SHP-1 can function as a negative regulator of JAK2 signaling (Marrero et al., 1998). Therefore, the potential biological significance of AT_2 receptor-induced programmed cell death led us to investigate whether SHP-1 activation could be involved in this process. First, we discovered that Ang II induced both the tyrosine phosphorylation (Fig. 1) and the activation (Fig. 2) of SHP-1. Because there are no specific pharmacological inhibitors of SHP-1 available, we used antisense probes to suppress the expression of SHP-1 in PC12 cells. An antisense oligonucleotide that targets the translational start site of the murine SHP-1 coding sequence (5′-ACCTCACCATCCTTGGGGT-3′) has been found to significantly reduce SHP-1 expression in human erythroleukemic SKT6 cells (Sharlow et al., 1997). PC12 cells were treated with the sense or antisense phophorothioate oligonucleotides for various times, SHP-1 was immunoprecipitated, and the immunoprecipitates were immunoblotted with anti-SHP-1 antibody. As shown in Figure 3, the antisense (but not the sense) oligonucleotide was effective in completely inhibiting SHP-1 expression within 6–8 hr. We then determined the effects of treatment with antisense oligonucleotides on the nicotine-induced activation of JAK2 via SHP-1, because we showed previously that preincubation of PC12 cells for 8 hr with Ang II blocked the nicotine-induced tyrosine phosphorylation of JAK2 via the AT_2 receptor (Shaw et al., 2002). PC12 cells were stimulated with nicotine in the presence or absence of Ang II lysed, and JAK2 was then immunoprecipitated from the lysates with anti-JAK2 antibody. Im-
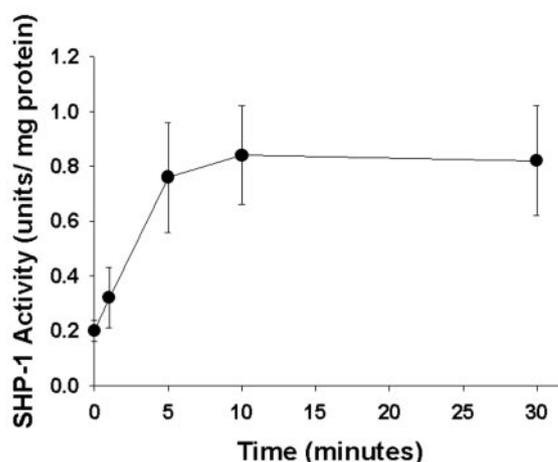
Figure 2. Angiotensin II-induced activation of SHP-1. PC12 cells were incubated with Ang II (100 nM) for the times indicated. The cells were lysed, and SHP-1 was immunoprecipitated from lysates with an anti-SHP-1 antibody. Immunoprecipitates were assayed for SHP-1 activity by measuring the rate of formation of *p*-nitrophenol from *p*-nitrophenylphosphate. Data represent the mean ± SE from six experiments.
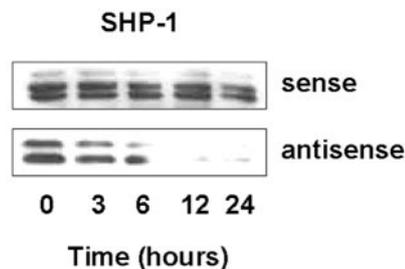
Figure 3. Effect of SHP-1 sense and antisense oligonucleotides on SHP-1 expression in PC12 cells. PC12 cells were treated with SHP-1 sense and antisense oligonucleotides for the times indicated and lysed. SHP-1 was immunoprecipitated from the lysates with anti-SHP-1 antibody. Precipitated SHP-1 proteins were then immunoblotted with specific anti-SHP-1 antibody. Shown are representative immunoblots of three immunoblots.
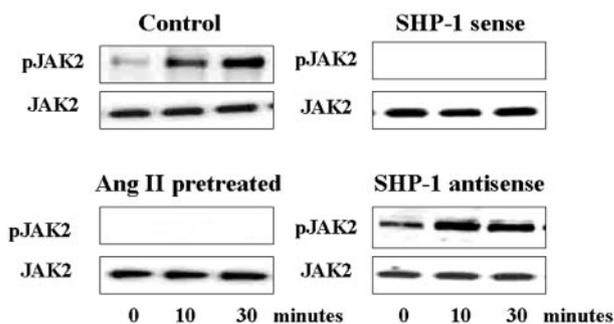
Figure 4. Effects of angiotensin II pretreatment with either SHP-1 antisense or sense on nicotine-induced activation of JAK2. Cells preincubated with Ang II for 8 hr in the presence or absence of SHP-1 antisense or sense oligonucleotides were stimulated with nicotine for the time indicated. Cells were immunoblotted with phospho-specific and non-phospho-specific anti-JAK2. Shown are representative immunoblots of three immunoblots. p, Phosphorylated.

munoprecipitated proteins were separated by gel electrophoresis, transferred to nitrocellulose, and then immunoblotted with anti-phosphotyrosine antibody. Control cells were exposed to SHP-1 sense oligonucleotide. As shown in Figure 4, when cells were exposed to the SHP-1 antisense, JAK2 tyrosine phosphorylation was augmented and the Ang II blockage of the nicotine-induced tyrosine phoaphorylation of JAK2 was obliterated. These results
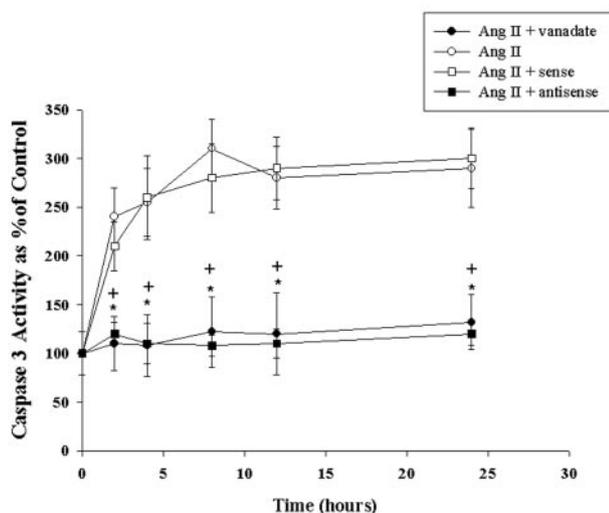
Figure 5. Effects of SHP-1 antisense on the angiotensin II-induced activation of caspase-3. PC12 cells were incubated for the duration shown with Ang II in the presence of either SHP-1 antisense or SHP-1 sense or vanadate. Caspase-3 activities are shown as the mean ± SE of six independent cultures.

1. **Control**
2. **Nicotine**
3. **ABeta (1-42)**
4. **AG-490**
5. **Angiotensin II**
6. **ABeta (1-42) + Nicotine**
7. **ABeta (1-42) + Nicotine + AG-490**
8. **ABeta (1-42) + Nicotine + Angiotensin II**
9. **ABeta (1-42) + Nicotine + Angiotensin II + SHP-1 sense**
10. **ABeta (1-42) + Nicotine + Angiotensin II + SHP-1 antisense**

Figure 6. Effects of SHP-1 antisense on nicotine-induced protection against Aβ- and Ang II-induced apoptosis. PARP expression was measured from lysates of cells treated for 8 hr by Aβ (1-42) peptide and/or Ang II in the presence or absence of nicotine and/or SHP-1 antisense. Shown is a representative immunoblot of three immunoblots.

suggest that SHP-1 is the PTPase that is activated by Ang II and dephosphorylates JAK2 after nicotine-induced JAK2 phosphorylation in PC12 cells.

Assessment of PC12 cell apoptosis
Caspase 3 is expressed in PC12 cells and is known to be involved in apoptosis (Shaw et al., 2002). We examined caspase 3 activity after Ang II-induced apoptosis. We used the fluorescent peptide substrate Ac-DEVD-AMC to measure caspase 3-like activity in cell lysates. As shown in Figure 5, the caspase 3-like activity that resulted in the cleavage of the peptide substrate Ac-DEVD-AMC is evident after 2 hr of Ang II treatment and increased over time until it reached a peak after 8 hr of treatment. However, the Ang II-induced activation of caspase 3 was blocked significantly in the presence of SHP-1 antisense ($+p < 0.01$) or vanadate ($*p < 0.01$) (Fig. 5). Coincubation with the SHP-1 sense had no effect on the Ang II-induced activation of caspase 3.

Apoptosis was also determined by assessing the cleavage of the DNA-repairing enzyme PARP using a Western blot assay. PC12 cells are treated with 0.1 μM Aβ for 8 hr in the presence or absence of nicotine. As shown in Figure 6, PARP (116 kDa) was cleaved to its 85 kDa fragment after Aβ (1-42) treatment. The Aβ (1-42)-induced cleavage of PARP was blocked by nicotine and prevented by AG-490 and Ang II (Fig. 6). However, in the presence of SHP-1 antisense, the Ang II blockage of the nicotine anti-apoptotic effects against Aβ (1-42)-induced cleavage of PARP was aborted (Fig. 6). These results support our main hypothesis, which states that JAK2 plays a central role in the nicotinic α7 receptor-induced activation of JAK2 in PC12 cells, which ultimately contributes to nicotine-induced neuroprotection, and that Ang II blocks these effects through the AT$_2$-mediated receptor activation of the PTPase SHP-1.

Discussion
In this study, we found that the nicotine neuroprotective effects, which can be neutralized through activation of the Ang II AT$_2$ receptor, are reversed by nullifying the PTPase SHP-1 as evidenced by the usage of an antisense against SHP-1. The SHP-1 antisense not only augmented the nicotine-induced tyrosine

phosphorylation of JAK2 but also blocked the Ang II neutralization of the nicotine-induced neuroprotection against Aβ (1-42)-induced cleavage of PARP.

Several reports have documented the apoptotic effects of Ang II through AT$_2$ receptors. AT$_2$ receptors are expressed in PC12 and have been shown to inhibit the JAK–STAT (signal transducer and activator of transcription) signaling cascade (Kunioku et al., 2001). In contrast to nicotine-induced neuroprotection against Aβ (1-42), pretreatment of cells with Ang II blocks the nicotine-induced activation of JAK2 via the AT$_2$ receptor and completely prevents nicotine-mediated neuroprotective effects, further suggesting a pivotal role for JAK2 phosphorylation (Shaw et al., 2002). Our findings, in this study, are again consistent with the opposite roles on cell viability that exists between the α7 nAChR and the AT$_2$ receptor, with activation of the AT$_2$ receptor overriding the potential survival benefit through the α7 nAChR. These results and the convergence of these pathways on phosphorylated JAK2 suggest that recruitment of nicotinic α7 receptor-mediated neuroprotection against Aβ (1-42) may be optimized under conditions in which the AT$_2$-mediated inhibition is minimized by blocking the AT$_2$-induced activation of SHP-1. The findings in this study identify novel molecular mechanisms that are fully consistent with the role attributed to α7 nAChR-induced activation of JAK2 and subsequent neuroprotective effect, as well as the AT$_2$-induced activation of SHP-1 and its alleged role in apoptotic events.

SHP-1 is a soluble tyrosine phosphatase that participates in the negative regulation of the tyrosine kinase JAK2 (Marrero et al., 1998). It has been reported recently that stimulation of AT$_2$ receptors rapidly activates SHP-1 in N1E-115 and AT$_2$-transfected Chinese hamster ovary cells (Horiuchi et al., 1998; Lehtonen et al., 1999). In the present study, we also document that AT$_2$ receptors activate SHP-1 in PC12. Moreover, the onset of SHP-1 activation clearly precedes the onset of JAK2 inhibition and apoptosis, thus suggesting that SHP-1 is an upstream, proximal effector in AT$_2$ signaling. In addition, to establish a functional link between AT$_2$-mediated activation of SHP-1 and inactivation of JAK2, we used an antisense oligonucleotide of SHP-1 and demonstrated that preventing SHP-1 activation abrogates AT$_2$-induced JAK2 inhibition. Furthermore, the termination of AT$_2$ receptor-mediated apoptosis in PC12 cells via transfection of the SHP-1 antisense oligonucleotides additionally supports the concept that the proapoptotic effect of AT$_2$ is associated with the inhibition of JAK2 via a signaling pathway involving the activa-

tion of SHP-1. Altogether, these observations suggest that SHP-1 is a transducer of the proapoptotic signals mediated by the AT_2 receptor, probably through negative regulation of the α7 nAChR signaling pathway.

Nicotinic neurotransmission is compromised in the brains of AD patients, and accumulating evidence suggests that nAChR-selective ligands can offer neuroprotective effects in a number of *in vitro* models, including neuronal death resulting from β-amyloid toxicity, NMDA-mediated cytotoxicity, or growth factor deprivation, and in *in vivo* models, including chemically induced neurotoxicity (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine models and systemic kainic acid-induced excitotoxic effects). Nicotinic ligands reduce β-amyloid aggregation and toxicity and inhibit amyloid deposition in transgenic mice with APPsw (amyloid precursor protein containing the Swedish mutation) (Nordberg et al., 2002). A recent report has demonstrated that the α7 nAChR is also an essential regulator of inflammation and is required for inhibition of cytokines release (Wang et al., 2003). The physiological mechanism coined "the cholinergic anti-inflammatory pathway," which has been proposed to have major implications in immunology and therapeutics, remains unknown. The induction and resolution of inflammatory processes are the complex outcome of interplay between pro-inflammatory and anti-inflammatory cytokines. Pleiotropic cytokines, such as interleukin-6 (IL-6) and IL-10, have been shown to activate the JAK–STAT pathway and act in opposition to effects mediated by the pro-inflammatory cytokines IL-1 and tumor necrosis factor-α (Ahmed and Ivashkiv, 2000). It is conceivable from these findings that multifaceted therapeutic potential targeting cognitive deficits, neuroprotection, and inflammation in neurodegenerative diseases can be recruited through a single pharmacology targeting the α7 nAChR. It remains to be established whether similar pathways are operative for these various end points *in vivo* and whether the negative influence of AT_2 stimulation is clinically relevant. However, the putative beneficial effects of angiotensin converting enzyme inhibitors in Alzheimer's disease and the observation of selective upregulation of AT_2 receptor density (Ge and Barnes, 1996) and biosynthetic enzymes (Savaskan et al., 2001) concurrent with downregulation of nAChR in the temporal cortex of some AD patients (Court et al., 2001) is consistent with the opposite effects on cell viability observed in our studies through activation of AT_2 and α7-nAChR.

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