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Potent Neuroprotective Properties against the Alzheimer β-Amyloid by an Endogenous Melatonin-related Indole Structure, Indole-3-propionic Acid*

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Widespread cerebral deposition of a 40–43-amino acid peptide called the amyloid β-protein (Aβ) in the form of amyloid fibrils is one of the most prominent neuropathologic features of Alzheimer's disease. Numerous studies suggest that Aβ is toxic to neurons by free radical-mediated mechanisms. We have previously reported that melatonin prevents oxidative stress and death of neurons exposed to Aβ. In the process of screening indole compounds for neuroprotection against Aβ, potent neuroprotective properties were uncovered for an endogenous related species, indole-3-propionic acid (IPA). This compound has previously been identified in the plasma and cerebrospinal fluid of humans, but its functions are not known. IPA completely protected primary neurons and neuroblastoma cells against oxidative damage and death caused by exposure to Aβ, by inhibition of superoxide dismutase, or by treatment with hydrogen peroxide. In kinetic competition experiments using free radical-trapping agents, the capacity of IPA to scavenge hydroxyl radicals exceeded that of melatonin, an indoleamine considered to be the most potent naturally occurring scavenger of free radicals. In contrast with other antioxidants, IPA was not converted to reactive intermediates with pro-oxidant activity. These findings may have therapeutic applications in a broad range of clinical situations.

Brains of patients afflicted with Alzheimer's disease show abnormal expression of numerous oxidative stress indicators (1–5) as well as extensive evidence of oxidative damage to proteins (6) and nucleic acids (7, 8). A prominent feature of the Alzheimer's disease brain is the widespread cerebral deposition of a 40–43-amino acid peptide called the amyloid β-protein (Aβ)[1] in the form of amyloid fibrils within senile plaques and in cerebral and meningeal blood vessels (9, 10). A large body of data suggests that Aβ causes neuronal degeneration and death by mechanisms that involve reactive oxygen species (Refs. 11–14; reviewed in Ref. 15).

Since the severity of the dementia in Alzheimer's disease has been correlated best with the extent of synaptic loss and the degree of neuronal death (16, 17), enhancing neuronal survival has been a primary objective of many therapeutic strategies. We have recently reported that melatonin prevents oxidative stress and death of neurons exposed to the amyloid peptide (18, 19). In the process of screening indole compounds as neuroprotective agents, new properties were uncovered for an endogenous species, indole-3-propionic acid (IPA). IPA has previously been identified in the plasma and cerebrospinal fluid of humans, but its functions are not known (20, 21). IPA has, like melatonin, a heterocyclic aromatic ring structure with high resonance stability, which led us to suspect similar neuroprotective and antioxidant properties. Here, we report that IPA prevented oxidative stress and death of primary neurons and neuroblastoma cells exposed to Aβ. In addition, IPA also showed a strong level of neuroprotection in two other paradigms of oxidative stress. We found that the radical-scavenging efficiency of IPA surpassed the activity of several previously reported antioxidants, including melatonin, and that in contrast to many other free radical scavengers, IPA did not generate pro-oxidant intermediates.

These findings may be relevant to a number of disorders of aging that are associated with increased oxidative stress. They also raise the possibility that IPA may be a component of the body's natural defense against free radical-mediated injury.

EXPERIMENTAL PROCEDURES

Reagents

All chemicals used in scavenging activity assays were purchased from Sigma. The indolic acids were dissolved in 0.1 M NaOH; melatonin was dissolved in 0.1 M HCl for the scavenging assay or prepared as 0.1 M stock as described (19) for the cytoprotection assays. The solutions were diluted with ultrapure distilled water, and the pH was adjusted to 7.8 in the scavenging assay. SK-N-SH human neuroblastoma cells and PC12 rat pheochromocytoma cells were purchased from the American Tissue Culture Collection (Manassas, VA) and were maintained in RPMI 1640 medium (Fisher) supplemented with 10% fetal bovine serum (Life Technologies, Inc.) or 10% horse serum and 5% fetal bovine serum, respectively. E-18 fetal rat primary hippocampal neurons were obtained from Dr. G. Brewer and grown as described by Brewer *et al.* (22). Cultures were maintained in a humidified 5% CO_2 incubator. Aβ-(1–42) was synthesized at the W. M. Keck Laboratories (Yale University, New Haven, CT); its purity was evaluated by amino acid sequence and laser desorption mass spectrometry; and its concentration was calculated by amino acid analysis (23). A 1 mg/ml stock solution of Aβ-(1–42) was prepared in 50 mM $NaHCO_3$ (pH 9.6), aliquoted, lyophilized, and stored at −80 °C until used. Immediately before the experiments, aliquots of Aβ-(1–42) were solubilized in distilled deionized water and diluted to the final concentration in culture medium. Under

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[1] The abbreviations used are: Aβ, amyloid β-protein; IPA, indole-3-propionic acid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; HPLC-ECD, high performance liquid chromatography coupled to an electrochemical detector; DDTC, diethyldithiocarbamate; DHBA, dihydroxybenzoic acid.

these experimental conditions, Aβ-(1–42) consisted of a mixture of soluble monomeric/dimeric species, as judged by polyacrylamide electrophoresis and gel filtration chromatography, exhibiting a predominant β-structure (2% α-helix, 88% β-sheet, 10% random coil), as determined by circular dichroism analysis as described (23).

Cell Viability Assays

β-Amyloid Neurotoxicity—The effect of IPA on Aβ-mediated neurotoxicity and neuroprotection was tested in E-18 fetal rat primary hippocampal neurons and in SK-N-SH human neuroblastoma cells. For the experiments using primary neurons, viability was assessed with the vital fluorescent probe bodipy green (Molecular Probes, Inc., Eugene, OR) (19) and by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction method (24). For the experiments involving neuroblastoma cells, viability was determined by the MTT reduction method and by the trypan blue exclusion method (19). Different methods were used on different cell types to ensure that the consistency and reproducibility of the results were completely independent of the technique and cell type employed.

For the experiments with bodipy green, control cells (without the addition of Aβ) and cells exposed to Aβ or Aβ plus IPA or melatonin were incubated with 2 μM bodipy green for 30 min. The cells were scanned for maximum fluorescence via a section series by scanning laser confocal microscopy (Molecular Dynamics, Inc., Sunnyvale, CA). The images were subjected, as described (19), to three-dimensional FishNet modeling to obtain relative intensity measurements and section line "cutting" for histogram determination of relative fluorescence intensity levels using Silicon Graphics software. Assessment of cell viability with the MTT reduction assay was measured by two independent techniques consisting of (*a*) direct visualization of staining under a light microscope (25) and (*b*) measurement of the optical absorbance of cell lysates using a microplate reader (Bio-Rad) as described (24). For the trypan blue exclusion method, a minimum of 300 cells/well were counted. All experiments (for each cell type and for each method) were reproduced independently in duplicate. Primary hippocampal neurons were allowed to differentiate for 7–10 days and then treated for 24 h either with 1 μM Aβ-(1–42) alone or with Aβ plus either melatonin or IPA. 5-Methoxytryptamine, a related indole compound with weak hydroxyl radical-scavenging activity (26–28), was used as a negative control. Neuroblastoma cells were plated in 24-well tissue culture plates (Nunc, Roskild, Denmark) to an initial 50% density and allowed to reach 80% confluence prior to treatment.

Lipid Peroxidation Assay—Using measurements of malondialdehyde levels, we also examined the effects of IPA on Aβ-induced lipid peroxidation, an established indicator of oxidative toxicity. Lipid peroxidation was measured on PC12 cells exposed to 10 μM Aβ-(1–42) or 5 mM diethyldithiocarbamate (DDTC; an inhibitor of superoxide dismutase-1 (29)), alone or in combination with 50 μM melatonin or indole-3-propionic acid. Cell lysates from two independent triplicate experiments were collected after the mentioned treatments and used to measure malondialdehyde levels as described (30).

Oxidative Stress Experiments—We examined the properties of IPA in two paradigms of oxidative stress. SK-N-SH human neuroblastoma cells were exposed to 1 mM DDTC or 50 μM hydrogen peroxide for 24 h (31), alone or in combination with different concentrations of melatonin or IPA. The cells were grown as indicated above for the amyloid toxicity experiments. Cell viability was assessed by the trypan blue exclusion method. For each form of oxidative stress, three independent duplicate sets of experiments were conducted.

Hydroxyl Radical Scavenging Assays

Antioxidant Activity—Hydroxyl radicals were generated *in vitro* by hydrogen peroxide exposed to UV light (UV lamp, Model UV G-11, short-wave UV-254.) The solutions were irradiated immediately after preparation for 5 min at a distance of 5 cm to generate hydroxyl radicals by photolysis of hydrogen peroxide (26). This specific hydroxyl radical-generating system previously allowed the quantification of the hydroxyl radical-scavenging abilities of melatonin (26). Melatonin was dissolved as the hydrochloride salt, and IPA was dissolved as the sodium salt. All incubations were carried out in 0.1 M glycylglycine buffer (pH 7.8). Using photolysis of hydrogen peroxide, we reduced the probability of side reactions and were able to assure that only hydroxyl radical-scavenging activity (not other nonspecific effects such as metal chelation) was tested here (26). The spin-trapping agent 5,5-dimethyl-1-pyrroline *N*-oxide was used to measure hydroxyl radical adduct formation by high performance liquid chromatography coupled to an electrochemical detector (HPLC-ECD) (26). The results were validated by ESR (26). The fractions containing the hydroxyl radical adduct with the specific 1:2:2:1 ESR spectrum characteristic for the hydroxyl radical were quantitated as described previously (26). The hydroxyl radical-scavenging activity of melatonin and IPA was also measured using the specific hydroxyl radical-trapping reagent ABTS (2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)) (27). In the presence of highly reactive hydroxyl radicals, ABTS is oxidized to the stable ABTS cation radical, which can be measured photometrically as described by Poeggeler *et al.* (27). The kinetic parameters and second-order reaction constants were determined as described by Matuszak *et al.* (32) and Bors *et al.* (33).

Pro-oxidant Activity—To assay for pro-oxidant activity, a hydroxyl radical-generating system consisting of hydrogen peroxide, iron, and EDTA was employed. To this end, we incubated the test compounds and salicylate at a concentration of 0.1 mM in the presence of 1 mM hydrogen peroxide, 0.1 mM $FeCl_3$, and 1 mM EDTA. The pro-oxidant activity of compounds depends on the reduction of ferric iron to ferrous iron, which in turn catalyzes hydroxyl radical generation from hydrogen peroxide by the Fenton reaction (34). The hydroxyl radical adducts of salicylate, 2,3-dihydroxybenzoic acid (DHBA) and 2,5-DHBA, were measured by HPLC-ECD (35).

Oxidation Products of IPA—We performed pulse radiolysis with spectrophotometric detection using a 4-MeV van de Graaf accelerator as described previously (36) to determine the chemical nature of the 1-electron oxidation product of IPA. The radical-mediated oxidation of IPA (0.2 mmol dm^{-3}) was monitored at pH 7.8 in 0.1 M phosphate buffer by irradiation of potassium bromide solutions (0.05 mol dm^{-3}) saturated with oxygen-free nitrous oxide (N_2O; 0.02 mol dm^{-3}). To test for the metabolite generated upon the oxidation, IPA was oxidized by incubation of the indole (100 μM) in the presence of hydrogen peroxide (100 μM) and iron sulfate (10 μM) for 30 min on ice in darkness. The formation of a kynuric acid was measured using a Model LS50B fluorometer (Perkin-Elmer) at excitation and emission wavelengths of 360 and 450 nm, respectively. Loss of indole fluorescence was monitored at excitation and emission wavelengths of 285 and 345 nm, respectively.

RESULTS

IPA Is Neuroprotective against Aβ and Other Oxidative Insults—The addition of IPA prevented death of primary neurons and neuroblastoma cells exposed to Aβ (Figs. 1 and 2). The results obtained with IPA were extremely consistent in the two cell types used and as indicated by the different methods employed for assessment of cell survival. However, 5-methoxytryptamine, a related control indole with weak scavenging activity, offered no neuroprotection when measured in a series of parallel experiments (Fig. 1). In the primary rat hippocampal cultures evaluated by bodipy green fluorescence, 1 μM Aβ treatment resulted in almost complete reduction of cell fluorescence as compared with untreated cells (Fig. 1). In these experiments, similar concentrations (1 μM) of IPA or melatonin added to Aβ-containing media were sufficient to provide full protection from the neurotoxic effects of Aβ, as evidenced by levels of fluorescence comparable to control primary neurons. With the MTT reduction method, 1 μM Aβ was accompanied by a 50–60% reduction of cell viability, suggesting that this method is less sensitive than the fluorescence technique for detection of neuronal injury. In qualitative agreement with the results obtained by the bodipy green method, the MTT assay showed that the addition of IPA along with Aβ resulted in full protection from amyloid-mediated damage (Fig. 2). No measurable toxicity of IPA was observed when cells were exposed to IPA alone as evaluated by either method.

Similar results were obtained in SK-N-SH neuroblastoma cells. IPA fully protected these cells against Aβ-(1–42)-mediated neurotoxicity, as assessed by the trypan blue method (Fig. 3) and the MTT reduction assay (data not shown). In agreement with our previous studies (19), melatonin also demonstrated potent neuroprotective properties.

IPA was also effective in preventing lipid peroxidation induced either by Aβ or by DDTC. In these experiments, we used PC12 rat pheochromocytoma cells because we found the levels of lipid peroxidation induced in these cells to be more consistent than in other cell lines (19). As indicated in Fig. 4, when

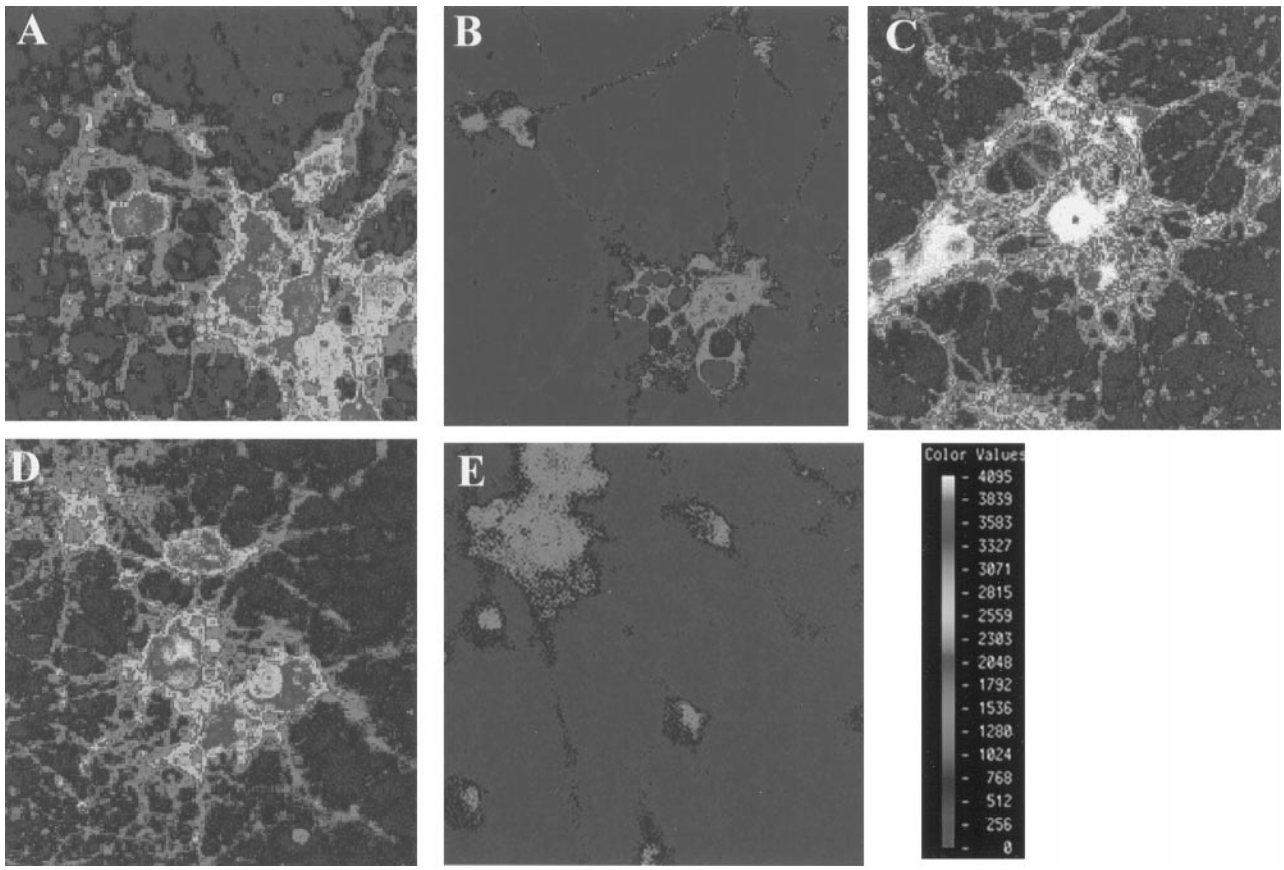

FIG. 1. **Cytoprotection against Aβ-(1–42) by IPA and melatonin using primary rat hippocampal neurons.** Shown are the results from bodipy green florescence and confocal laser microscopy. Representative control hippocampal neurons exhibited high fluorescence levels after incubation with bodipy green, as shown in *A*. Upon exposure to Aβ-(1–42), there was a dramatic decrease in bodipy green fluorescence throughout the cell population (*B*). The addition of melatonin (*C*) or IPA (*D*) along with Aβ-(1–42) totally prevented the changes induced by the peptide (magnification × 2000). 5-Methoxytryptamine, a control indole compound, showed no neuroprotective effects (*E*). Although a marked degree of neuroprotection was readily apparent, we individually measured the fluorescence of a minimum of 30 representative cells/well, and there were no significant differences in fluorescence intensity between the control and Aβ-(1–42) plus IPA or Aβ-(1–42) plus melatonin. *Pseudocolor scale bar* represents fluorescence intensity values.

IPA was added to the cells along with Aβ or DDTC, there was marked reduction of lipid peroxidation.

As shown in Fig. 5 (*A* and *B*), IPA also prevented death of neuroblastoma cells mediated either by exposure to DDTC or by treatment with H_2O_2. Neither DDTC nor H_2O_2 is a free radical, but both lead directly or indirectly to increased production of other reactive radicals (*i.e.* hydroxyl, peroxynitrite), causing oxidative damage within cells (reviewed in Ref. 37). Dose-response studies showed that the neuroprotective activity of IPA exceeded that of melatonin by ~10-fold when DDTC was used to induce oxidative stress and by ~5-fold when H_2O_2 was the oxidative agent, as measured by the trypan blue exclusion method (Fig. 5, *A* and *B*).

IPA Is a Potent Hydroxyl Radical Scavenger—In kinetic competition studies, melatonin and IPA reacted with hydroxyl radicals at a diffusion-controlled rate, yielding reaction constants of 4 and 8×10^{10} mol liter^{-1} s^{-1}, respectively, in both assay systems. As shown in Fig. 6 (*A* and *B*), IPA exhibited marked hydroxyl radical-scavenging efficiency, which exceeded the capacity of melatonin on an equimolar basis. In addition, the radical-scavenging capacity of IPA surpassed the efficiencies reported for other structurally related (*i.e.* indole-3-acetic acid) *and* unrelated (*i.e.* trolox) antioxidants by at least an order of magnitude (26, 27, 38).

IPA Does Not Form Pro-oxidant Intermediates—In contrast to other potent hydroxyl radical scavengers, including the structurally similar indole compounds indole-3-acetic acid and indole-3-pyruvic acid, we found that IPA was not converted to reactive pro-oxidant intermediates (Table I). On the other hand, ascorbate (vitamin C), trolox (a water-soluble analogue of vitamin E), and glutathione were highly pro-oxidant, as shown in Table I. These antioxidants and the indolic acids indole-3-acetic acid and indole-3-pyruvic acid *increased* hydroxyl radical generation in the presence of oxidized iron and thereby *promoted* hydroxyl radical adduct formation catalyzed by this transition metal (Table I). The measured pro-oxidant properties of the mentioned compounds are in agreement with previously published results (34, 39). Our results showed that 12 μs after pulse radiolysis of N_2O-saturated potassium bromide, solutions containing IPA generated the respective indolyl cation radical, characterized by an absorption band centered at 570 nm (ϵ = 2400 dm^3 mol^{-1} cm^{-1}) and another band at 360 nm (ϵ = 4800 dm^3 mol^{-1} cm^{-1}). Fluorometric measurements demonstrated a complete loss of indole fluorescence (excitation and emission wavelengths of 280 and 360 nm, respectively) with the formation of an oxidation product with excitation and emission maxima characteristic for a kynuric acid (excitation and emission wavelengths of 360 and 450 nm, respectively).

DISCUSSION

Two main lines of investigation have now converged to suggest that Aβ causes extensive degeneration and death of neurons by mechanisms that involve reactive oxygen species. One line of evidence is the identification of oxidative markers that co-localize within the amyloid deposits in Alzheimer's disease brain (1, 2) and in transgenic Alzheimer's disease mice (13, 14).

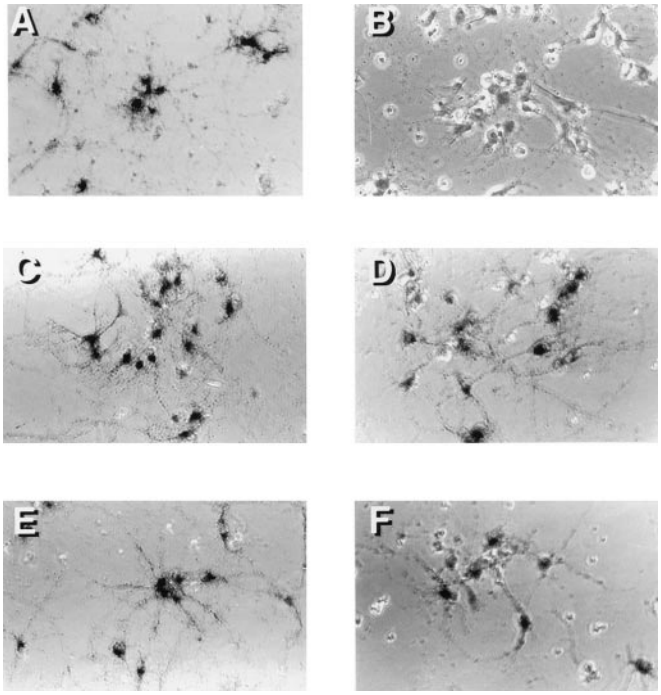

FIG. 2. **Cytoprotection against Aβ-(1–42) by IPA and melatonin using primary rat hippocampal neurons.** Shown are the results obtained with the MTT reduction method under light microscopy. Over 95% control hippocampal neurons stained dark after incubation with the formazan step of the MTT reduction method (*A*). Upon exposure to Aβ-(1–42), there was a striking decrease in staining in >50% of the neurons (*B*). The addition of melatonin (*D*) or IPA (*F*) along with Aβ-(1–42) totally protected the changes induced by the peptide alone, as demonstrated by a similar number of darkly staining cells and the degree of staining intensity. 5-Methoxytryptamine exhibited no neuroprotective effect (not shown). No cytotoxicity of melatonin or IPA was observed when the neurons were exposed to these compounds alone (*C* and *E*) (magnification × 400).

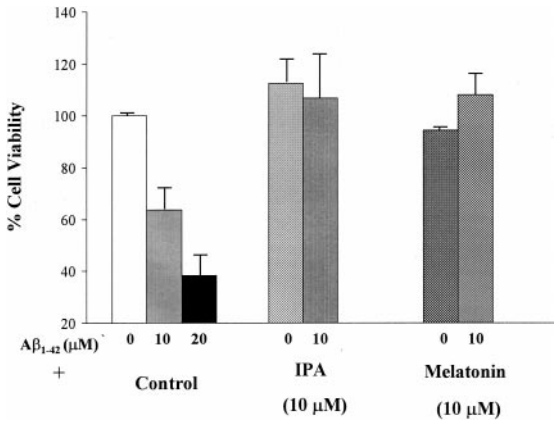

FIG. 3. **Confirmatory neuroprotection studies by IPA and melatonin against Aβ toxicity on SK-N-SH human neuroblastoma cells.** Shown are the results obtained with the trypan blue exclusion method. Results from these studies were extremely consistent and in agreement with the experiments on primary neurons. Aβ-(1–42) neurotoxicity was dose-dependent and completely prevented by IPA and melatonin. The *bar graph* and *lines* represent the means ± S.D., respectively, of three independent duplicate experiments. A minimum of 300 cells/well were counted.

A second, but equally important, body of data demonstrates that oxygen free radicals are produced upon exposure of cells to Aβ.

It has been recently shown that Aβ-mediated toxicity can be prevented by several endogenous free radical scavengers such as melatonin (18, 19) and α-tocopherol (43). The recently established neuroprotective and antioxidant properties of melatonin suggested to us that other similar compounds may share some of these features. In this study, we showed that IPA protected neuronal cells against oxidative stress and death mediated by Aβ, and these effects were consistent and reproducible in several paradigms of oxidation. The free radical-

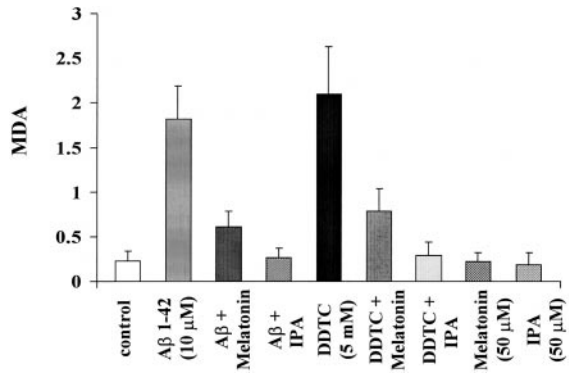

FIG. 4. **IPA and melatonin protect PC12 cells against oxidative stress induced by Aβ-(1–42) or DDTC.** Shown are the results obtained with the malondialdehyde assay for lipid peroxidation. The *bar graph* shows the means ± S.E. for each treatment, as indicated, of two independent triplicate experiments. Both IPA and melatonin were effective in preventing lipid peroxidation.

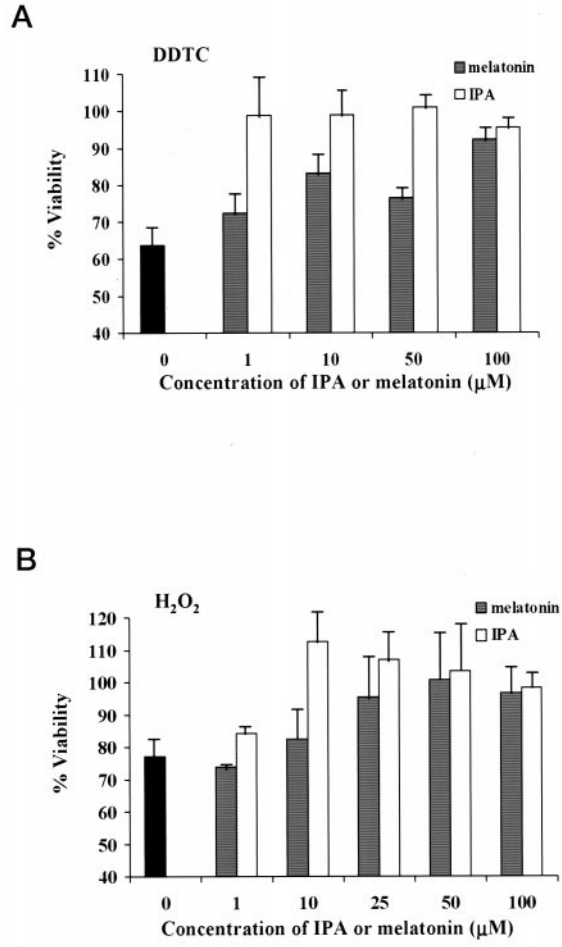

FIG. 5. **Dose-response studies of IPA and melatonin as a function of cell viability of SK-H-SH neuroblastoma cells exposed to DDTC (*A*) or hydrogen peroxide (*B*).** Shown are the results obtained with the trypan blue exclusion method. The *bar graphs* depict the means ± S.D. of three independent duplicate experiments. Note that with the concentrations used, a maximum level of neuroprotection with IPA was reached at 10 μM for either modality of injury. For melatonin, full neuroprotection was observed at 100 μM for DDTC and at 50 μM for hydrogen peroxide with this assay.

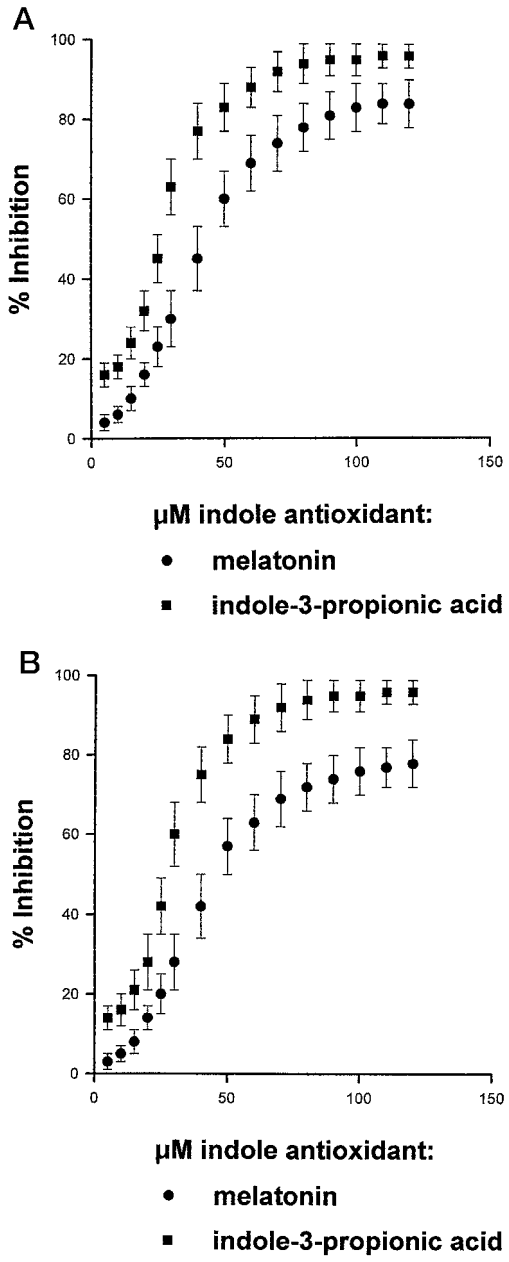

FIG. 6. **Hydroxyl radical-scavenging activity of IPA and melatonin.** Shown are the results obtained with the 5,5-dimethyl-1-pyrroline *N*-oxide method (*A*) and the ABTS method (*B*). Inhibition of 5,5-dimethyl-1-pyrroline *N*-oxide-hydroxyl radical adduct formation by IPA and melatonin is shown in *A* as the means ± S.E. of three independent triplicate experiments. Inhibition of ABTS cation radical formation by IPA and melatonin is shown in *B* (three independent triplicate experiments). ●, melatonin; ■, indole-3-propionic acid.

TABLE I

Pro-oxidant activities of several agents as evaluated by formation of 2,3- and 2,5-DHBA, an indicator of OH radical formation

The test compounds and salicylate were incubated at a concentration of 0.1 mM in the presence of 1 mM hydrogen peroxide, 0.1 mM $FeCl_3$ and 1 mM EDTA. The hydroxyl radical adducts of salicylate, 2,3-DHBA, and 2,5-DHBA were measured by HPLC-ECD. The measurements are expressed as a percentage of control and represent the means ± S.D. from six independent experiments.

Agent	2,3- and 2,5-DHBA
	% of control
Indole-3-propionic acid	4 ± 1
Indole-3-acetic acid	173 ± 14
Indole-3-pyruvic acid	152 ± 11
Melatonin	25 ± 6
Ascorbate	225 ± 18
Trolox	197 ± 16
Glutathione	165 ± 12

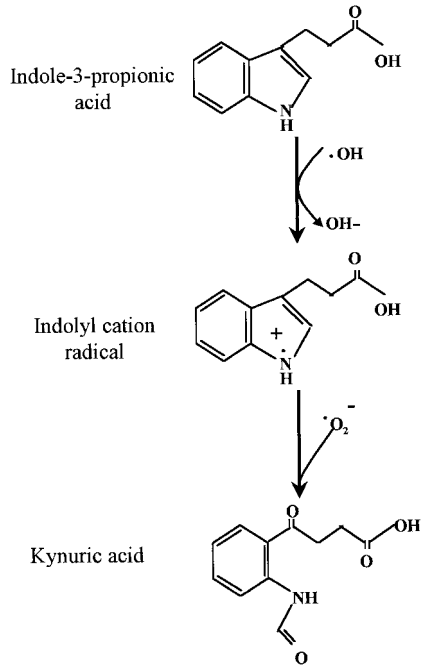

FIG. 7. **Hydroxyl radical-mediated oxidation of IPA.** Shown is the formation of a kynuric acid by the radical-mediated oxidation of IPA. IPA reacts with the hydroxyl radical, reducing this reactive oxygen species by electron donation to a hydroxyl anion. The indolyl cation radical in turn reacts with the superoxide anion radical and is oxidized to a kynuric acid.

scavenging properties of IPA surpassed those of melatonin, the most powerful known natural hydroxyl radical scavenger (29, 37, 38). Antioxidant activities were evident in various paradigms, which included measurements of lipid peroxidation (Fig. 4), superoxide dismutase inhibition (Fig. 5*A*), treatment with hydrogen peroxide (Fig. 5*B*), and kinetic competition studies (Fig. 6, *A* and *B*). These findings may have therapeutic relevance to Alzheimer's disease, a condition characterized by a pervasive level of oxidative stress.

The chemical structures and the scavenging mechanisms of melatonin and IPA show no similarities to the common chain-breaking phenolic antioxidants such as vitamin E (44). Vitamin E possesses a reactive hydroxyl group, which enables it to donate a hydrogen atom, thereby reducing free radicals that promote radical chain reactions such as peroxyl radicals. However, because of their high reactivity, chain-breaking antioxidants such as vitamin E autoxidize in the presence of transition metals and *increase* the formation of primary radicals such as hydroxyl radicals (34). In contrast, melatonin and IPA do not undergo autoxidation in the presence of transition metals and are endogenous electron donors that primarily detoxify hydroxyl radicals (which are the initiators of radical chain reactions.) Of all oxygen-derived free radicals, hydroxyl radicals are the most reactive.

Like melatonin, IPA is also found in plasma and cerebrospinal fluid under physiological conditions (20, 21). IPA is produced by deamination of L-tryptophan by commensal bacteria in human and animal intestines (45), although its production by other tissues has not been systematically investigated. The lack of pro-oxidant effects is perhaps the most important feature of IPA, which, as mentioned, is shared only by melatonin (28, 38). In contrast, significant enhancement of iron-mediated formation of hydroxyl radicals was found when we explored the

reactions catalyzed by glutathione, trolox, ascorbic acid, and selected indoles, in agreement with a previous study (34). It has also been demonstrated that under certain conditions, hydroxylated indoleamines such as *N*-acetylserotonin and 6-hydroxymelatonin or related indolyl acids such as indole-3-acetic acid can exhibit potent pro-oxidant properties (32, 39), although cytoprotective antioxidant activity has recently been claimed for these compounds (46). In the case of indole-3-acetic acid and related congeners, the formation of these pro-oxidant species appears to be dependent on the generation of reactive peroxyl radicals upon decarboxylation of the side chain and the addition of oxygen to a skatole (indole-3-methyl) radical (39). Although the presence of an electron-rich aromatic ring system in IPA allows the detoxification of highly reactive radicals by electron donation (Fig. 7), its side chain cannot be decarboxylated (39), and thus, unlike other indoles, it cannot be converted to a reactive pro-oxidant intermediate. Our results showed that IPA was completely devoid of any pro-oxidant activity (Table I). These properties make IPA and melatonin far superior to many other antioxidant compounds such vitamin E. In addition, these indoles are not cytotoxic, and in contrast to synthetic antioxidants, they are present in the body under physiological conditions.

Melatonin is a hydroxyl radical scavenger with a rate constant 3 orders of magnitude higher than that exhibited by chain-breaking antioxidants such as vitamin E. We demonstrate here that IPA is at least twice as potent as melatonin, making this compound the most effective hydroxyl radical scavenger detected to date. Because hydroxyl radicals cannot be enzymatically detoxified, radical-scavenging compounds have evolved early in life phylogeny to provide on-site protection against these reactive radicals. The physiological functions of IPA are not known, although it is intriguing to speculate on a protective role of this substance against oxidative stress. Additional studies must be conducted to explore this possibility. Interestingly, circulating serum levels of IPA also exceed those of melatonin. Future studies should also determine whether changes in endogenous IPA levels correlate with certain disorders in which free radicals may be implicated.

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REFERENCES

1. Pappolla, M. A., Omar, R. A., Kim, K. S., and Robakis, N. K. (1992) *Am. J. Pathol.* **140,** 621–628
2. Smith, M. A., Kutty, R. K., Richey, P. L., Yan, S.-D., Stern, D., Chader, G. J., Wiggert, B., Petersen, R. B., and Perry, G. (1994) *Am. J. Pathol.* **145,** 42–47
3. Aksenov, M. Y., Aksenova, M. V., Markesbery, W. R., and Butterfield, D. A. (1998) *J. Mol. Neurosci.* **10,** 181–192
4. Sayre, L. M., Zelasko, D. A., Harris, P. L., Perry, G., Salomon, R. G., and Smith, M. A. (1997) *J. Neurochem.* **68,** 2092–2097
5. Calingasan, N. Y., Uchida, K., and Gibson, G. E. (1999) *J. Neurochem.* **72,** 751–756
6. Smith, C. D., Carney J. M., Starke-Reed, P. E., Oliver, C. N., Stadtman, E. R., Floyd, R. A., and Markesberry, W. R. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 10540–10543
7. Lyras, L., Cairns, N. J., Jenner, A., Jenner, P., and Halliwell, B. (1997) *J. Neurochem.* **68,** 2061–2069
8. Mecocci, P., MacGarvey, U., and Beal, M. F. (1994) *Ann. Neurol.* **36,** 747–751
9. Glenner, G. G., and Wong, C. W. (1984) *Biochem. Biophys. Res. Commun.* **120,** 885–890
10. Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L., and Beyreuther, K. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82,** 4245–4249
11. Behl, C., Davis, J. B., Lesley, R., and Schubert, D. (1994) *Cell* **77,** 817–827
12. Goodman, Y., and Mattson, M. P. (1994) *Exp. Neurol.* **128**, 1–12
13. Pappolla, M. A., Chyan, Y.-J., Omar, R. A., Hsiao, K., Perry, G., Smith, M. A., and Bozner, P. (1998) *Am. J. Pathol.* **152,** 871–877
14. Smith, M. A., Hirai, K., Hsiao, K., Pappolla, M. A., Harris, P. L. R., Siedlak, S. L., Tabaton, M., and Perry, G. (1998) *J. Neurochem.* **70,** 2212–2215
15. Mattson, M. P. (1997) *Alzheimer's Dis. Rev.* **2,** 1–14
16. Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., Hansen, L. A., and Katzman, R. (1991) *Ann. Neurol.* **30,** 572–580
17. Hyman, B. T., West, H. L., Gomez-Isla, T., and Mui, S. (1995) in *Research Advances in Alzheimer's Disease and Related Disorders* (Iqbal, K., Mortimer, J. A., Winblad, B., and Wieniewski, H. M., eds) pp. 453–480, John Wiley & Sons, Inc., New York
18. Bozner, P., Grishko, V., LeDoux, S. P., Wilson, G. L., Chyan, Y.-C., and Pappolla, M. A. (1997) *J. Neuropathol. Exp. Neurol.* **56,** 1356–1362
19. Pappolla, M. A., Sos, M., Omar, R. A., Bick, R. J., Hickson-Bick, D. L. M., Reiter, R. J., Efthimiopoulos, S., and Robakis, N. K. (1997) *J. Neurosci.* **17,** 1683–1690
20. Young, S. N., Anderson, G. M., Gauthier, S., and Purdy, W. C. (1980) *J. Neurochem.* **34,** 1087–1092
21. Morita, I., Kawamoto, M., and Yoshiba, H. (1992) *J. Chromatogr.* **576,** 334–339
22. Brewer, G. J., Torricelli, J. R., Evege, E. K., and Price, P. J. (1993) *J. Neurosci. Res.* **35,** 567–576
23. Pappolla, M., Bozner, P., Soto, C., Shao, H., Robakis, N. K., Zagorski, M., Frangione, B., and Ghiso, J. (1998) *J. Biol. Chem.* **273,** 7185–7188
24. Hansen, M. B., Nielsen, S. E., and Berg, K. (1989) *J. Immunol. Methods* **119,** 203–210
25. Mattson, M. P., Cheng, B., Davis, D., Bryant, K., Lieberburg, I., and Rydel, R. E. (1992) *J. Neurosci.* **12,** 376–389
26. Tan, D.-X., Chen, L.-D., Poeggeler, B., Manchester, L. C., and Reiter, R. J. (1993) *Endocrinol. Jpn.* **1,** 57–60
27. Poeggeler, B., Reiter, R. J., Hardeland, R., Tan, D.-X., and Barlow-Walden, L. R. (1996) *Redox Rep.* **2,** 179–184
28. Chan, T.-Y., and Tang, P.-L. (1996) *J. Pineal Res.* **20,** 187–191
29. Frank, L., Wood, D., and Robert, R. (1987) *Biochem. Pharmacol.* **27,** 251–254
30. Omar, R. A., Yano, S., and Kikkawa, Y. (1987) *Cancer Res.* **47,** 3473–3476
31. Whittemore, E. R., Loo, D. T., and Cotman, C. W. (1994) *Neuroreport* **5,** 1485–1488
32. Matuszak, Z., Reszka, K. J., and Chignell, C. F. (1996) *Free Radical Biol. Med.* **23,** 367–372
33. Bors, W., Michel, C., and Saran, M. (1985) in *CRC Handbook of Methods for Oxygen Radical Research* (Greenwald, R. A., ed) pp. 181–188, CRC Press, Inc., Boca Raton, FL
34. Nappi, A. J., and Vass, E. (1997) *Biochim. Biophys. Acta* **1336,** 295–302
35. McCabe, D. R., Maher, T. J., and Acworth, I. N. (1997) *J. Chromatogr.* **691,** 23–32
36. Candeias, L. P., Everett, S. A., and Wardman, P. (1993) *Free Radical Biol. Med.* **15,** 385–394
37. Reiter, R. J. (1998) *Prog. Neurobiol.* **56,** 359–384
38. Poeggeler, B., Reiter, R. J., Tan, D.-X., Chen, L.-D., and Manchester, L. C. (1993) *J. Pineal Res.* **14,** 151–168
39. Candeias, L. P., Folkes, L. K., Rorssa, M., Parrick, P., and Wardman, P. (1995) *Free Radical Res.* **23,** 403–418
40. Deleted in proof
41. Deleted in proof
42. Deleted in proof
43. Behl, C., Davis, J., Cole, G. M., and Schubert, D. (1992) *Biochem. Biophys. Res. Commun.* **186,** 944–950
44. Poeggeler, B. (1998) in *Reactive Oxygen Species in Biology Systems: An Interdisciplinary Approach* (Gibbons, D. L., and Colton, C. D., eds) pp. 421–451, Plenum Press, New York
45. Jellet, J. J., Forrest, T. P., MacDonald, A., and Marrie, T. J. (1980) *Can. J. Microbiol.* **26,** 448–453
46. Moosmann, B., Uhr, M., and Behl, C. (1997) *FEBS Lett.* **413,** 467–472

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