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In vitro and in vivo studies investigating possible antioxidant actions of nicotine: relevance to Parkinson's and Alzheimer's diseases

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Abstract

An inverse relationship appears to exist between cigarette smoking and the risk of Parkinson's and Alzheimer's diseases. Since both diseases are characterized by enhanced oxidative stress, we investigated the antioxidant potential of nicotine, a primary component of cigarette smoke. Initial chromatographic studies suggest that nicotine can affect the formation of the neurotoxin 6-hydroxydopamine resulting from the addition of dopamine to Fenton's reagent (i.e., Fe^{2+} and H_2O_2). Thus, under certain circumstances, nicotine can strongly affect the course of the Fenton reaction. In in vivo studies, adult male rats being treated with nicotine showed greater memory retention than controls in a water maze task. However, neurochemical analysis of neocortex, hippocampus, and neostriatum from these same animals revealed that nicotine treatment had no effect on the formation of reactive oxygen species or on lipid peroxidation for any brain region studied. In an in vitro study, addition of various concentrations of nicotine to rat neocortical homogenates had no effect on lipid peroxidation compared to saline controls. The results of these studies suggest that the beneficial/protective effects of nicotine in both Parkinson's disease and Alzheimer's disease may be, at least partly, due to antioxidant mechanisms.

Keywords: Nicotine; Iron binding; Antioxidant; Free radical; Cognition

1. Introduction

Numerous epidemiological studies have demonstrated an inverse relationship between cigarette smoking and the risk of developing idiopathic Parkinson's disease (PD) [1–4]. One of the compounds in cigarette smoke thought to be responsible for this effect might be nicotine. Studies have demonstrated that not only cigarette smoke, but also nicotine gum and nicotine patches, can decrease the tremors and bradykinesia of PD [5,6]. In addition to PD, cigarette smoking/nicotine appears to have an inverse relationship with Alzheimer's disease (AD) [7,8]. Consistent with such a relationship, nicotine administration to AD patients enhances their attention and information processing [9–12]; in addition, it has been found that nicotine treatment improves the cognitive function of both young and aged rats [13–15].

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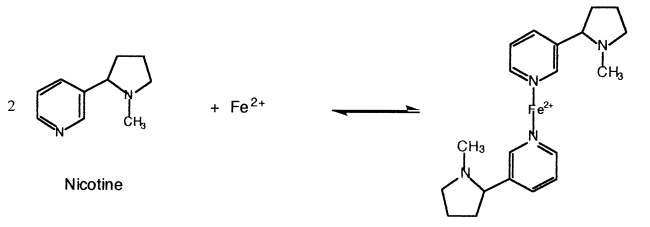
There is increasing evidence for the involvement of free radicals and iron (Fe)-induced oxidative stress in the pathogenesis of Parkinson's and Alzheimer's diseases [16]. In PD brains, a specific increase in Fe levels occurs within the substantia nigra. This could be a cause of the degeneration of nigrostriatal dopaminergic (NS-DA) neurons that is such a characteristic feature of PD. Most NS-DA neurons contain neuromelanin deposits [17], which bind higher amounts of Fe in PD [18] and increase the vulnerability of NS-DA neurons to degeneration in PD [17]. Given this evidence for the involvement of Fe in PD, we have recently published a scheme of reactions which could lead to an initiation and continuation of PD pathogenesis [19]. The basic principle is the fact that neurotoxic 6-hydroxydopamine (6-OHDA) is formed from the oxidation of DA in the presence of free (solvated) Fe(II) ions and H_2O_2 (i.e., Fenton's reagent). In normal brains, almost all Fe should be stored or bound in 'storage compounds' like the protein ferritin, so that it should be Fenton-inactive. However, it has been shown that 6-OHDA is able to free Fe(II) from storage in ferritin [20], thus facilitating the Fenton reaction which in turn leads to further production of 6-OHDA. In NS-DA neurons, we propose that this cycle is continuous, forming strongly oxidizing radicals which destroy cellular components such as the cell membrane.

If nicotine in cigarette smoke has a preventative effect towards PD, there are several possible mechanisms involving the above reactions that could protect NS-DA neurons. One very plausible mechanism of nicotine action in PD involves the known ability of nicotine to form complexes with Fe(II), probably via the pyridine nitrogen



(1)

However, no detailed structural investigations have been done and coordination might also occur via the pyrrolidine nitrogen. Consistent with the ability of nicotine to bind Fe^{2+} are studies reporting that: (1) nicotine binds directly to the iron ions of thromboxane synthase, thus inhibiting that enzyme [23], and (2) nicotine reduces transferrin-mediated Fe uptake by reticulocytes and placental cells [24,25]. Based on the above findings, we hypothesized that nicotine may be protective against PD through complex formation with Fe(II), thus yielding Fenton-inactive Fe(II) and less oxidative stress.

In the present study, we first investigated the 'antioxidant' potential of nicotine as an inhibitor of the Fenton reaction. In follow-up in vivo and in vitro animal studies, we then sought to determine whether nicotine's mechanism of cognitive enhancement in rats is associated with measurable antioxidant actions in several brain areas, as indexed by reactive oxygen species (ROS) and thiobarbituric acid reactive product (TBAR) formation.

2. Materials and methods

2.1. Chromatographic study

2.1.1. Chromatography

All experiments were performed using an HP 1090M LC with a UV/VIS detector connected to an HP 79995A workstation (HP 9000 Computer, Series 300). Samples were introduced using a 25 μl injection loop. Separations were carried out using a 250×4.6 mm ID ODS Hypersil 5μ column, operated at a flow rate of 1.2 ml/min and at a temperature of 40°C. The mobile phase consisted of methanol-aqueous acetic acid buffer (1:1, v/v). The buffer was prepared by adding 240 mg sodium acetate crystals (p.a. grade) to 1000 ml of water (HPLC grade) and then adjusting the pH to 3.55 (±0.02) with glacial acetic acid (100%). Peak identifications were performed by admixture with authentic standards.

2.1.2. Chemicals

Dopamine (DA), 6-OHDA, and 5-OHDA were obtained as the hydrochlorides from Sigma and used without further purification. Nicotine (from Aldrich) was purified via the dihydroiodide according to Pictet and Genequand [26], followed by an extraction with ether after neutralization in aqueous solution.

2.1.3. Sample preparation

To phosphate-buffered solutions of DA (pH 7.5), iron(II) and hydrogen peroxide were added and the resulting mixture was made acidic (pH 1.0) after 1 min to slow down the reaction. Then the samples were analyzed by HPLC as described above.

2.2. In vivo animal study

Twenty 2 month old male Sprague-Dawley rats were pretreated for 4 days with twice daily intraperitoneal (i.p.) injections of nicotine (0.8 mg/kg, as the hydrogen tartrate salt; $n=10$) or vehicle (isotonic saline; $n=10$). On day 5 of treatment, all rats began 4 days of acquisitional training (days 5–8) in the Morris water maze. Three hours prior to behavioral testing, rats received their first of two daily injections of nicotine or saline. For all 4 days of acquisition, mean latency to reach a submerged platform in quadrant II was determined from four daily trials, with each trial initiated in a different quadrant. On day 9 of treatment, animals were given a 60 s memory retention trial at 3 h after their first of two daily treatments with nicotine or saline. For this probe trial, the platform was removed and animals were placed in the quadrant opposite to the former platform quadrant. The percentage of time spent in each of the four quadrants was recorded. A preference for the former platform quadrant, as indicated by a greater amount of time spent in that quadrant, suggests superior memory retention. Acquisitional data were analyzed using two-way ANOVA for repeated measures and memory retention data were analyzed with a one-way ANOVA and Student's *t*-test. Post-hoc analysis involved Fischer's LSD tests. A *P*-value less than 0.05 was considered significant.

On day 10, a final treatment with nicotine or saline was given and animals were euthanized within several hours thereafter. Following brain removal, the neocortex, hippocampus, and neostriatum were dissected out bilaterally and weighed. Samples were then quick frozen and stored at −80°C until neurochemical analysis. Reactive oxygen species (ROS) formation was determined in the right tissue samples via 2′,7′-dichlorofluorescein (DCF) fluorescence. Tissues from the left side were assayed for thiobarbituric acid reactive product (TBAR) formation, an index of lipid peroxidation.

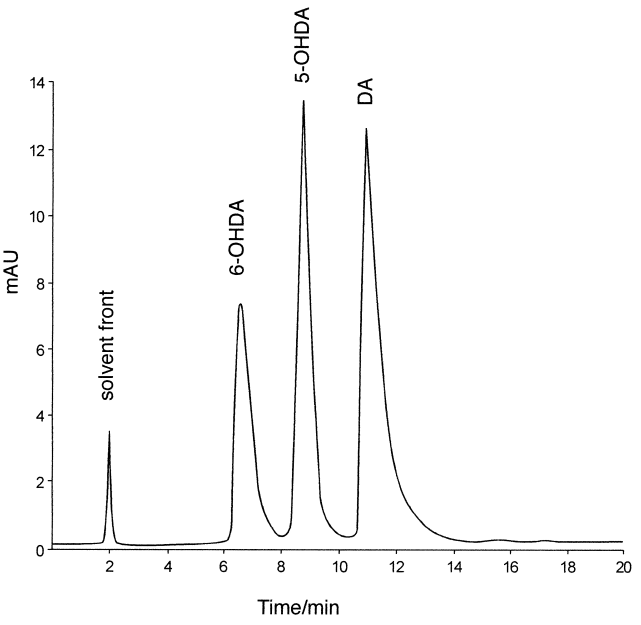


Fig. 1. Chromatogram of the optimized separation of the standard solution containing equal amounts of dopamine, 6-OHDA, and 5-OHDA. The peaks labeled as 6-OHDA and 5-OHDA are in actual fact their oxidation products resulting from oxidation on the column during the separation (detection at 265 nm).

The DCF fluorescence assay for ROS detects primarily the levels of hydrogen peroxide, superoxide radical, and hydroxyl radical. Right side tissue samples were prepared for DCF fluorescence according to the basic methodology of LeBel et al. [27]. A 0.25% homogenate was prepared for each sample using cold buffer (40 mM Tris, pH 7.4). Samples were incubated at 37°C with 1.25 mM of 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) in methanol for 15 min at 37°C. Formation of the fluorescent probe DCF was measured using a fluorometer with excitation and emission wavelengths set at 488 nm and 525 nm, respectively. Readings were taken immediately after incubation (time 0) and at 30 min intervals for 1.5 h thereafter. DCF formation is reported in μM/μg protein. Results were analyzed using Student's *t*-test, with $P<0.05$ being considered significant.

Left side tissue samples were prepared for TBAR formation analysis according to the basic methodology of Socci et al. [28]. In brief, 5% tissue homogenates were prepared in a solution of isotonic saline and Desferol (0.029 mM final concentration in ho-

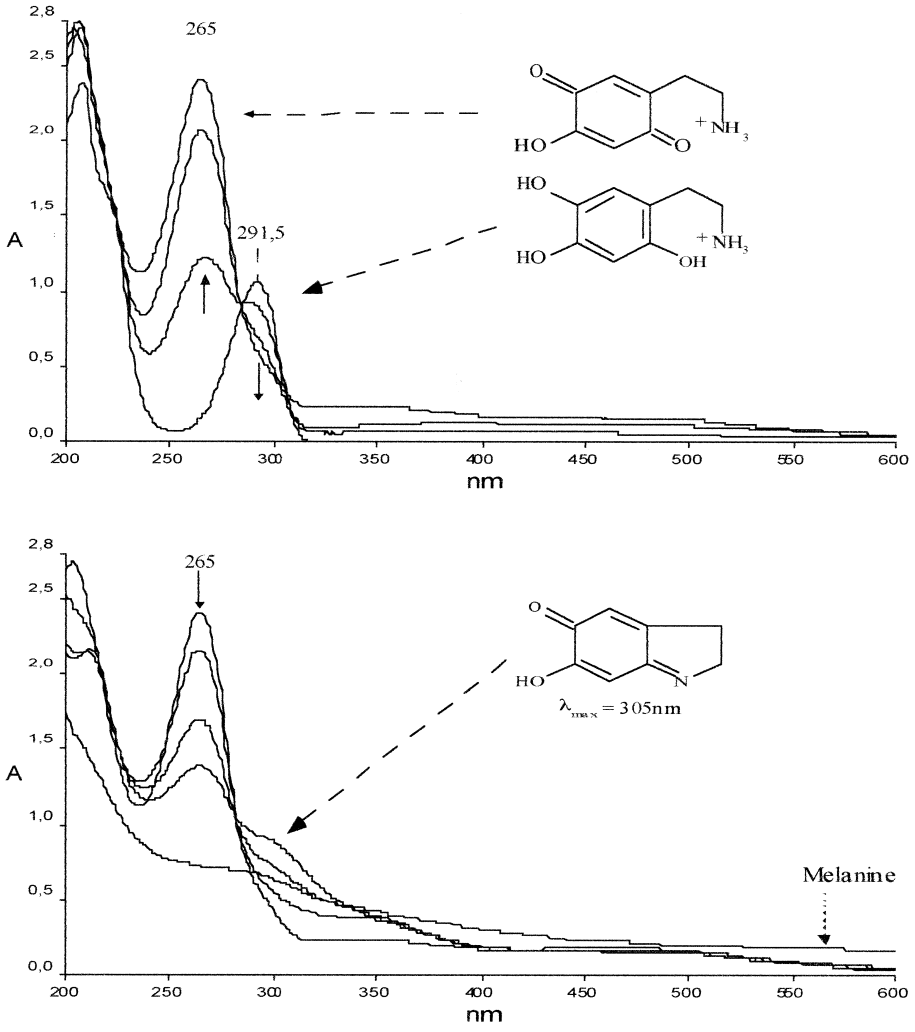


Fig. 2. Oxidation of 6-OHDA in the presence of Fe(II,III) ions (UV/VIS spectra).

mogenate). To these homogenates, 300 μl of 25% trichloroacetic acid, 150 μl of 1% thiobarbituric acid, and 10 μl 5% butylated hydroxytoluene (BHT) were added. (Desferol (an iron chelator) and BHT were included in the homogenization solution to eliminate TBAR formation resulting from tissue iron released during homogenization and from breakdown of lipid hydroperoxides, respectively – this allowed a true determination of TBAR formation in each tissue.) Tissue solutions were incubated for 45 min at 90°C and then centrifuged for 10 min at $15\,000 \times g$. The change in supernatant color was read spectrophotometrically at 535 nm. TBAR formation is reported in pmol/μg protein. Results were analyzed using Student's *t*-test, with $P < 0.05$ considered significant.

2.3. In vitro animal study

Neocortical tissue from five male Sprague-Dawley rats (5 months old) was dissected out and used to assess in vitro TBAR formation in the presence of nicotine or Desferol. Tissue preparation for the in vitro experiment followed the same TBAR formation procedure as above, except at the step where Desferol was added prior to homogenizing. Six aliquots were taken from each 5% tissue homogenate and either Desferol (4.76 mM final homogenate concentration), nicotine (0.71, 1.43, 2.38, or 4.76 mM final homogenate concentration), or saline was added. Thus, at this step, the process of chelating freed tissue Fe during homogenization depended on either Desferol (traditional chelator), nicotine (possible che-

lator), or saline (no chelator). This allowed testing of varying concentrations of nicotine for ability to suppress TBAR formation. Results are expressed as percent change from the Desferol-containing homogenate, as this homogenate represents 'basal' TBAR formation (i.e., presumably no tissue iron was available to induce TBAR formation during the assay). Statistical analysis between saline and either Desferol standard or nicotine concentrations was done with Student's *t*-test, while comparisons between nicotine concentrations were done with a repeated measures one-way ANOVA (significance at $P < 0.05$).

3. Results

3.1. Chromatographic study

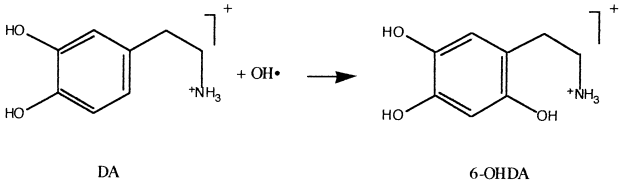
3.1.1. Chromatographic data

In Fig. 1, the optimized separation of the standard solution containing DA, 6- and 5-OHDA is shown. 5-OHDA and the neurotoxin 6-OHDA are possible oxidation products of the investigated oxidation reaction of DA with H_2O_2 in the presence of iron(II) [29–31]. The peaks labeled as 6-OHDA and 5-OHDA, respectively, are in actual fact their oxidation products resulting from oxidation on the column during the separation. As both 5-OHDA and 6-OHDA are oxidized in the presence of dioxygen in very short times under the given conditions, via their semiquinones, to relatively stable quinones [19,31,32], the oxidation products detected by HPLC refer, therefore, to their respective quinones. As no other oxidation products are found within this time scale, quantification is possible on the basis of the peak areas. This was verified not only for 6-OHDA (see Fig. 2), but also for 5-OHDA by measurements on a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer. We could observe a shift of the absorption maximum, in the case of 6-OHDA from 291.5 nm to 265 nm and of 5-OHDA from 270 nm to 235 nm. Therefore, we used different wavelengths for UV/VIS detection on the HPLC; for DA 280 (±2) nm, for 6-OHDA 265 (±2), for 5-OHDA 235 (±2) nm, and as reference 375–425 nm.

The occurrence of strong peak tailing is typical for separations of basic compounds in acidic medium on a reversed phase column, as we used it [33], and seems to have its origin in Fe traces remaining on the column. However, they do not influence the argument given by the HPLC results. The acidic medium is needed to keep the oxidation process as minimal as possible.

Fig. 3 shows that 6-OHDA is produced during the oxidation of dopamine with H_2O_2 in the presence of iron(II). For peak identification of the *p*-quinone of 6-OHDA, a reference spectrum at the beginning of the peak (identified as dopaminochrome) was subtracted from that of the peak maximum. The amount of 6-OHDA built in the solution is, as expected, dependent on both the iron(II) and H_2O_2 concentration (see Figs. 4 and 5). In a two step reaction, about 1% of the original available amount of DA reacts to form neurotoxic 6-OHDA. However, this absolute amount is not conclusive because of the occurrence of both the autoxidation and oxidation of DA due to the presence of hydrogen peroxide, yielding the respective semiquinones followed by quinone and melanin formation [19]. $OH^{\bullet}$ radicals (or equally ferryl species), might well act as oxidizing or hydroxylizing species (reaction 2). Relative peak heights (or areas) between the 6-OHDA and the DA peaks may serve as quantitative tools for comparison.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\bullet$$



DA 6-OHDA

(2)

Without going into a detailed kinetic analysis, the results can be interpreted as an approximately first order dependence of 6-OHDA production on Fe(II) and an approach of a steady state concentration of 6-OHDA with increasing H_2O_2 concentration, assuming that 6-OHDA is further oxidized by H_2O_2 to finally form melanin (Figs. 4 and 5).

To determine whether nicotine's ability to form complexes with iron(II) is capable of making iron(II) Fenton-inactive, the analogous analysis was made with nicotine added to the reaction mixture. Fig. 6 shows the production of 6-OHDA by addition of a

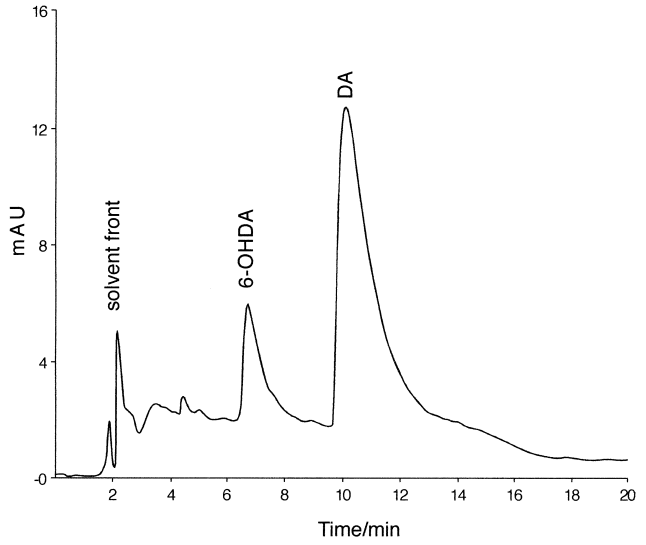


Fig. 3. Separation of a reaction mixture containing 5 mmol/l DA, 2 mmol/l $FeCl_2$, and 5 mmol/l H_2O_2 (detection at 265 nm).

5-fold molar excess of nicotine to the reaction mixture. The amounts of 6-OHDA formed appear to be reduced by a factor of 2 when compared to the amount formed without nicotine. Adding nicotine to an analogous solution of iron(III), the amount of 6-OHDA produced was again reduced. However, an equal amount of 5-OHDA appears to be formed (Fig. 7). The sum of the areas below the 6-OHDA and the 5-OHDA peaks in the presence of Fe(III) equals the area below the 6-OHDA peak found in the presence of Fe(II).

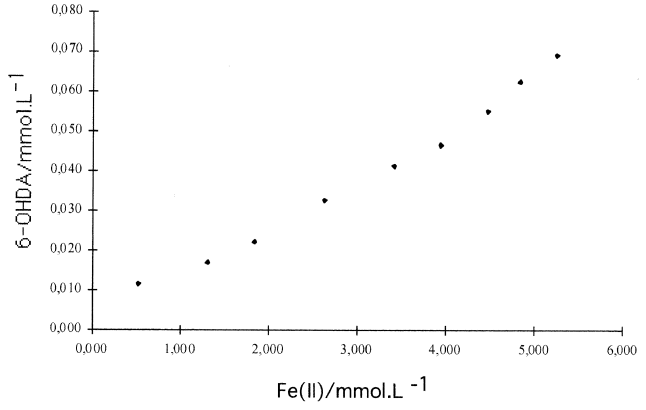


Fig. 4. Variation of 6-OHDA production with Fe(II) concentration with 5 mmol/l DA and 5 mmol/l H_2O_2.

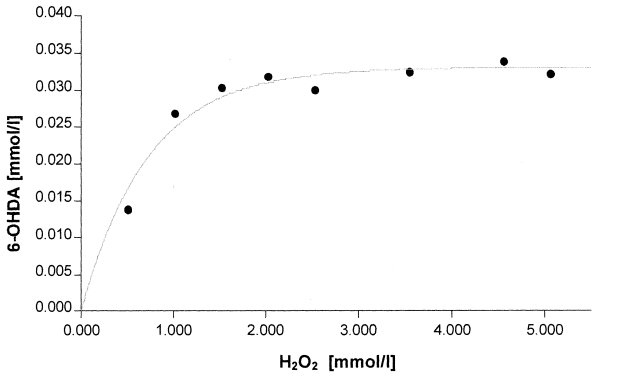


Fig. 5. Variation of 6-OHDA production with H_2O_2 concentration in a reaction mixture with 5 mmol/l DA, 2.5 mmol/l Fe(II). The fitted line refers to a steady state assumption.

3.2. In vivo animal study

In water maze acquisition (Fig. 8A), there was no significant difference between saline-treated and nicotine-treated rats in overall learning or in the rate of learning ($P > 0.05$). Both groups improved their acquisition performance, as seen by a decrease in latencies over the 4 day test period ($P < 0.001$). In the follow-up memory retention trial, however, only the nicotine-treated group showed significant memory retention (Fig. 8B). This enhanced memory is indicated by the greater amount of time spent by nicotine-treated rats in the quadrant (Q2) formerly containing

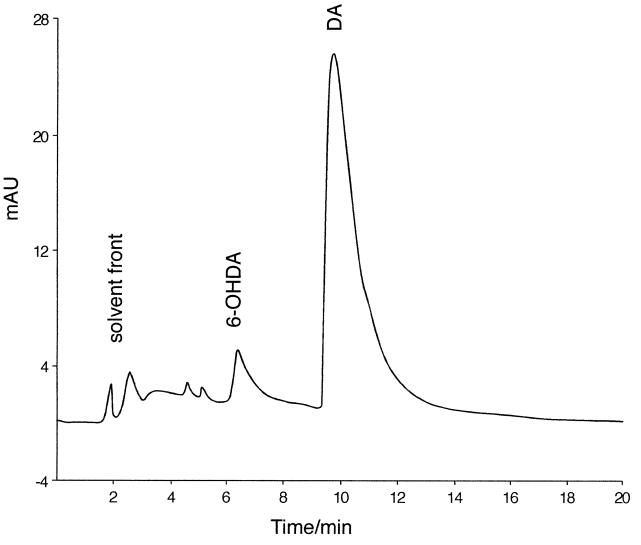


Fig. 6. Formation of 6-OHDA in a mixture of 5 mmol/l DA, 1 mmol/l Fe(II), and 1 mmol/l H_2O_2 in the presence of 5 mmol/l nicotine (detection at 265 nm).

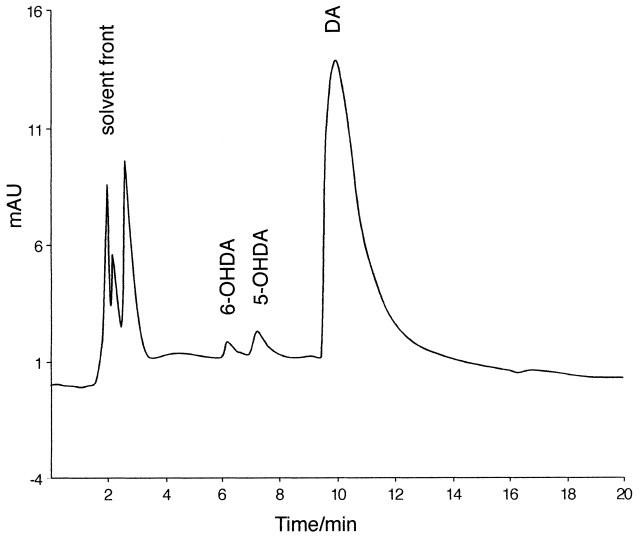


Fig. 7. Formation of 6-OHDA in a mixture of 5 mmol/l DA, 1 mmol/l Fe(III), and 1 mmol/l H_2O_2 in the presence of 5 mmol/l nicotine (detection at 265 nm).

the platform than in any other quadrant ($P < 0.002$). Although the amount of time spent in the former platform quadrant by both groups was not significantly different, the saline-treated group exhibited no specific preference for the former platform quadrant over the other three quadrants ($P > 0.05$). Saline-treated animals had a preference for both the former platform quadrant and quadrant 3, as indicated by no significant difference in the percentage time spent in these quadrants ($P > 0.05$).

ROS formation in the neocortex, hippocampus, and neostriatum from behaviorally tested animals was not significantly affected by nicotine, either overall or across time (Fig. 9). For all three brain areas, however, ROS formation did increase over time ($P < 0.05$). TBAR formation in the neocortex, hippocampus, and neostriatum was similarly unaffected by nicotine treatment in comparison to saline-treated controls (Fig. 10A).

3.3. In vitro animal study

As shown in Fig. 10B, analysis of TBAR formation in neocortical homogenates revealed a 20-fold increase in TBAR formation in saline homogenates compared to Desferol-containing control homogenates ($P < 0.001$). The 20-fold increase in TBAR formation in saline homogenates was stimulated by release of endogenous iron during tissue homogenization. This effect of iron was dramatically decreased by Desferol, an iron chelator. By contrast, nicotine had no such effect on the elevation in TBAR formation induced by endogenous iron since there was no significant difference in TBAR formation between saline homogenates and the different nicotine homogenates ($P > 0.05$ for all concentrations). There also were no significant differences between the various nicotine homogenates in TBAR formation.

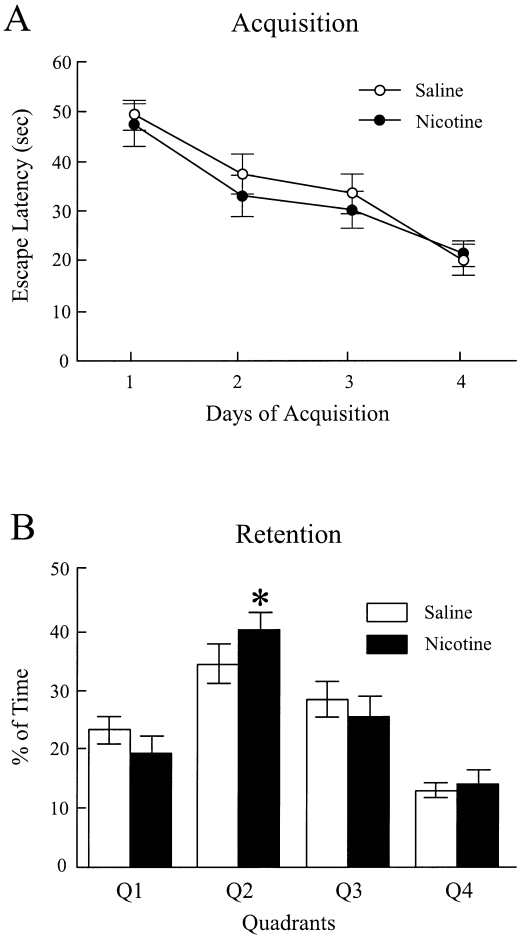


Fig. 8. Water maze acquisition (A) and memory retention (B) for young adult rats being treated with nicotine or saline solution. In B, Q1–Q4 represent the pool's four quadrants, with Q2 being the quadrant that formerly contained the escape platform. Nicotine-treated animals, but not saline-treated animals, showed a quadrant preference for Q2 in percent of swim time, indicative of superior memory retention. The asterisk indicates a significant difference ($P < 0.05$) from the other three quadrants.

4. Discussion

Given the inverse relationship between cigarette smoking and both Parkinson's and Alzheimer's disease, it is possible that nicotine has certain beneficial/protective effects resulting in a preservation of motor abilities and cognition, respectively. Indeed, nicotine administration has been shown to decrease motor disabilities in PD and improve cognitive performance in AD patients [1–6,9–12]. The present study sought to determine whether one possible mechanism of nicotine's therapeutic/preventative actions could be through antioxidant properties (i.e., via reduction in oxidative stress reactions). With in vivo experiments, we found no evidence that nicotine exerts antioxidant capacities. However, in vitro experiments appear to suggest a reduction of the Fenton activity towards dopamine. Due to the experimental conditions, quantitative results showing the dependence of educt concentrations are not available and no detailed kinetic or chemical mechanistic interpretation is possible at the moment beside assuming that the complex formation between nicotine and Fe(II) plays a role, probably involving the oxidation of the nicotine.

A further possible explanation of the results might be due to reaction 3, reported as a possible way to prepare nicotine-pyrrolidinyloxide [21,22].

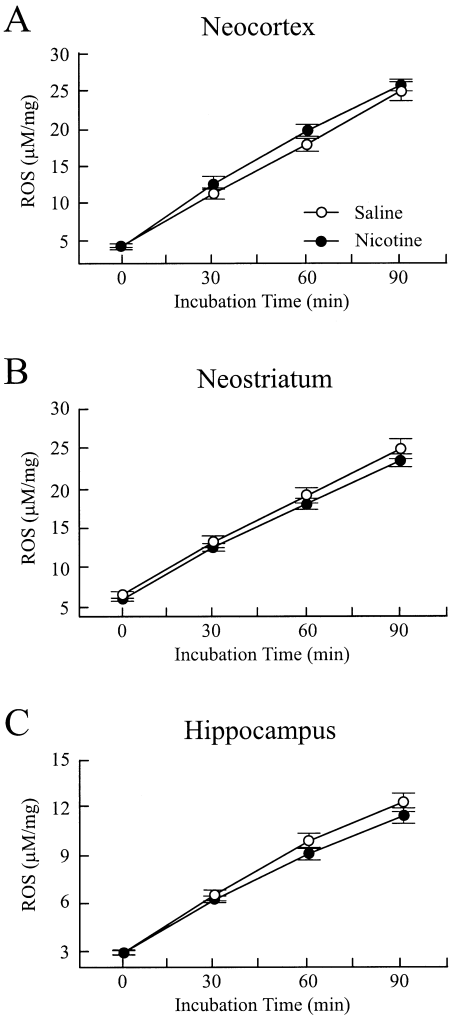


Fig. 9. Time course of ROS formation in neocortex (A), neostriatum (B), and hippocampus (C) from nicotine-treated and saline-treated adult rats. Nicotine had no effect on ROS formation in any of the tissues analyzed.

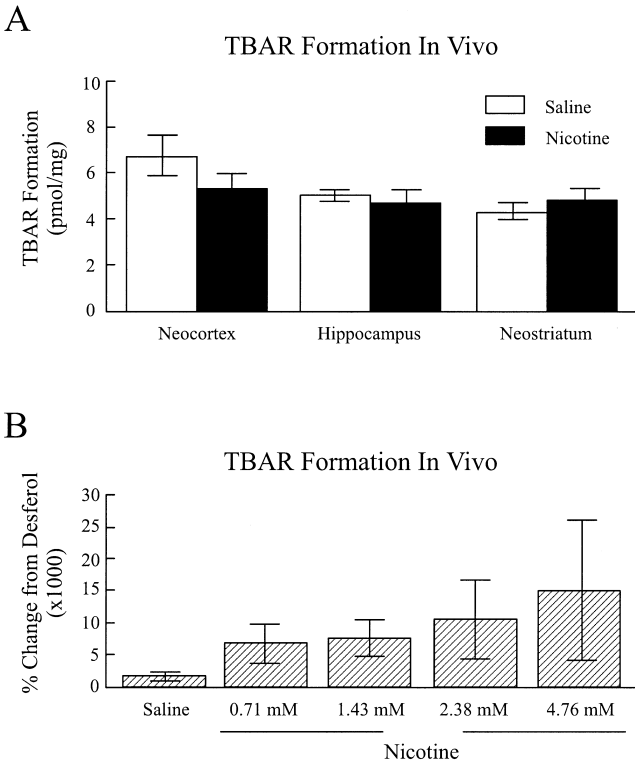
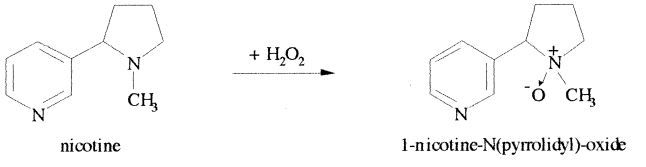


Fig. 10. (A) In vivo TBAR formation in neocortex, hippocampus, and neostriatum from nicotine-treated and saline-treated adult rats. (B) Effects of various nicotine concentrations on neocortical TBAR formation in vitro. In both in vivo and in vitro determinations, nicotine did not affect TBAR formation.



(3)

Attempts were made to follow this reaction by UV absorption spectroscopy. An absorption maximum of nicotine is found at 258 nm; its retention times under the given HPLC conditions are larger than 20 min, excluding interference with the HPLC results given above. The found kinetics indicate that this reaction is much too slow to have any physiological significance.

However, the results from the present study need considerable further work (which indeed is currently going on in our laboratory), to elucidate the exact nature of the effect of nicotine on the reaction between dopamine and Fenton's reagent. It is entirely possible that, if nicotine really does inhibit Parkinsonism, the mechanism involved could be totally different, i.e. not by preventing Fe(II) from producing neurotoxic 6-OHDA. Along this line, nicotine has recently been shown to increase the concentration of dopamine in the brain [34]. In view of the inverse relationship between cigarette smoking and PD [1–4], it is also possible that other active substances in cigarette smoke (i.e., CO and CN^-) are inhibitory to the Fenton reaction. Since both CO and CN^- form strong, low spin complexes with Fe(II), their Fenton activity is presently being investigated in our laboratories.

Nicotine has a well-established cognition-enhancing effect in both normal humans and those with AD [9,12,35]. Numerous animal studies, including our own studies in rats [13,14], have likewise indicated a beneficial effect of nicotine treatment on learning/memory processes [35]. Because we have also shown that treatment with antioxidants (both synthetic and naturally occurring) can enhance cognitive performance in aged rats [28,36], it was reasonable to postulate that nicotine may be exerting some of its cognition-enhancing effects through antioxidant mechanisms. However, despite the ability of nicotine in the present study to improve memory retention in adult rats, there was no reduction in either ROS formation or TBAR formation in brains from these same behaviorally tested animals. Thus, nicotine did not appear to affect either reactive oxygen species production or lipid peroxidation within cognitively important brain areas (i.e., neocortex, hippocampus, and neostriatum) in animals showing enhanced cognition. Similarly, our follow-up in vitro experiment involving neocortical homogenates indicated no suppression of TBAR formation with various concentrations of nicotine included in the incubation mixture. No evidence for nicotine exerting anti-oxidant properties in brain tissue was, therefore, found. In earlier studies involving white blood cells, evidence was provided for an antioxidant action of nicotine [37,38]. Results from these two studies and the present one suggest that antioxidant actions of nicotine could be tissue-specific (i.e., present in blood cells, but not in brain tissue). Alternatively, nicotine may have had antioxidant actions in the brains of animals in our study, with these effects being manifested in oxidative markers not measured (i.e., protein oxidation, DNA oxidation, hydroxyl radical formation, etc.).

Finally, it is important to note that the beneficial/preventative effects of nicotine on motor and cognitive skills could involve a variety of non-antioxidant mechanisms acting separately or in concert with one another. First, nicotine's effects may involve activation of postsynaptic or presynaptic nicotinic receptors on neurons, with the latter resulting in release of acetylcholine and non-cholinergic neurotransmitters [39,40]. Second, nicotine has the ability to increase glucose uptake in the brain [41,42]. In this context, it is noteworthy that glucose administration to AD patients improves their cognitive performance [43]. Third, nicotine administration increases regional cerebral blood flow [44,45], thus having potentially beneficial effects on neuronal function/survivability.

In summary, our chromatographic experiments suggest that nicotine might provide some of its therapeutic actions in PD and AD through antioxidant mechanisms. Our animal experiments, however, suggest that non-antioxidant mechanisms are primarily responsible for nicotine's multiplicity of motor/cognitive actions.

Acknowledgements

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References

[1] D.M. Morens, A. Grandinetti, D. Reed, L.R. White, Lancet 343 (1994) 356.
[2] D.M. Morens, D. Grandinett, D. Reed, L.R. White, G.W. Ross, Neurology 45 (1995) 1041.
[3] A. Grandinetti, D.M. Morens, D. Reed, D. Maceachern, Am. J. Epidemiol. 139 (1994) 1129.
[4] J.M. Gorell, B.A. Rybicki, C.C. Johnson, E.L. Peterson, Neurology 52 (1999) 115.
[5] A. Ishikawa, T. Miyatke, J. Neurol. Sci. 117 (1993) 28.
[6] K.O. Fagerstrom, O. Pomerleau, B. Giordani, F. Stelson, Psychopharmacology 116 (1994) 117.
[7] C.M. Van Duijn, A. Hofman, Br. Med. J. 302 (1991) 1491.
[8] D.E. Brenner, W.A. Kukull, G. van Belle, J.D. Bowen, W.C. McCormick, L. Teri, E.B. Larson, Neurology 43 (1993) 293.
[9] G.M. Jones, B.J. Sahakian, R. Levy, D.M. Warburton, J.A. Gray, Psychopharmacology 108 (1992) 485.
[10] D.M. Warburton, Prog. Neuropsychopharmacol. Biol. Psychiatry 16 (1992) 181.
[11] P. Newhouse, A. Potter, J. Corwin, R. Lenox, Med. Chem. Res. 2 (1993) 628.
[12] B.J. Sahakian, J.T. Coull, Drug Dev. Res. 31 (1994) 80.
[13] G.W. Arendash, P.R. Sanberg, G.J. Sengstock, Pharmacol. Biochem. Behav. 52 (1995) 517.
[14] D.J. Socci, P.R. Sanberg, G.W. Arendash, Neurobiol. Aging 16 (1995) 857.
[15] R. Maggio, M. Riva, F. Vaglini, F. Fornai, R. Molteni, M. Armogida, G. Racagni, G.U. Corsini, J. Neurochem. 71 (1998) 2439.
[16] C.W. Olanow, G.W. Arendash, Curr. Opin. Neurol. 7 (1994) 548.
[17] E. Hirsch, A.M. Graybiel, Y.A. Agid, Nature 334 (1988) 345.
[18] K. Jellinger, E. Kienzl, G. Rumpelmair, P. Riederer, H. Stachelberger, D. Ben-Shachar, M. Youdim, J. Neurochem. 59 (1992) 1168.
[19] W. Linert, E. Herlinger, R.F. Jameson, E. Kienzl, K. Jellinger, M.B.H. Youdim, Biochim. Biophys. Acta 1316 (1996) 160.
[20] H.P. Monterio, C.C. Winterbourn, Biochem. Pharmacol. 38 (1989) 4177.
[21] A. Pinner, R. Wolffenstein, Ber. Dtsch. Chem. Ges. 24 (1891) 63.
[22] A. Pinner, R. Wolffenstein, Ber. Dtsch. Chem. Ges. 34 (1901) 2411.
[23] M. Goerig, V. Ullrich, G. Schettler, C. Foltis, A. Habenicht, Clin. Invest. 70 (1992) 239.
[24] H. McArdle, J. Tysoe, J. Cell. Physiol. 134 (1988) 509.
[25] T.T. Loh, Clin. Exp. Pharmacol. Physiol. 9 (1982) 11.
[26] A. Pictet, P. Genequand, Ber. Dtsch. Chem. Ges. 30 (1897) 2117.
[27] C.P. LeBel, I.M. Odunze, J.D. Adams, S.C. Bondy, Biochem. Biophys. Res. Commun. 163 (1989) 860.
[28] D.J. Socci, B.M. Crandall, G.W. Arendash, Brain Res. 693 (1995) 88.
[29] A. Slivka, G. Cohen, J. Biol. Chem. 260 (1985) 15466.
[30] H.W. Richter, W.H. Waddell, J. Am. Chem. Soc. 105 (1983) 5434.
[31] E. Herlinger, R.F. Jameson, W. Linert, J. Chem. Soc. Perkin Trans. II 259 (1995) 121.
[32] E. Herlinger, R.F. Jameson, W. Linert, J. Inorg. Biochem. 59 (1995) 280.
[33] B. Law, P.F. Chan, J. Chromatogr. 552 (1991) 429.
[34] J.S. Fowler, N.D. Volkow, G.J. Wang, N. Pappas, J. Logan, R. MacGregor, D. Alexoff, C. Shea, D. Schleyer, A.P. Wolf, D. Warner, I. Zezulkova, R. Cilento, Nature 379 (1996) 733.
[35] E.D. Levin, Psychopharmacology 108 (1992) 417.
[36] C.A. Sack, D.J. Socci, B.M. Crandall, G.W. Arendash, Neurosci. Lett. 205 (1996) 181.
[37] D. Nowak, U. Ruta, Exp. Pathol. 38 (1990) 249.
[38] K. Aoshiba, A. Nagai, K. Konno, Am. J. Respir. Crit. Care Med. 150 (1994) 1101.
[39] P.P. Rowell, D.L. Winkler, J. Neurochem. 43 (1984) 1593.
[40] S. Wonnacott, J. Irons, C. Rapier, B. Thorne, G. Lunt, Prog. Brain Res. 79 (1989) 157.
[41] D. McNamara, D.M. Larson, S.I. Rapoport, T.T. Soncrant, J. Cereb. Blood Flow Metab. 10 (1990) 48.
[42] S. Shibata, K. Kodama, Y. Koga, S. Ueki, S. Watanabe, Brain Res. 603 (1993) 248.
[43] C.A. Manning, M.E. Ragozzino, P.E. Gold, Neurobiol. Aging 14 (1993) 523.
[44] F. Grunwald, H. Schrock, H. Kuschinsky, Neurosci. Lett. 124 (1991) 108.
[45] D.G. Linville, S. Williams, J.L. Raszkiewicz, S.P. Arneric, J. Pharmacol. Exp. Ther. 267 (1993) 440.