Journal of Neuroscience Research 18:621-625 (1987)

Chronic Nicotine Administration Increases Binding of [^{3}H]Domperidone in Rat Nucleus Accumbens

M.A. Reilly, E.P. Lapin, H.S. Maker, and A. Lajtha
Department of Neurology, Mount Sinai School of Medicine (E.P.L., H.S.M.), Department of Neurology, Beth Israel Medical Center (H.S.M.), Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island (A.L.), New York, and Nathan S. Kline Institute for Psychiatric Research, Orangeburg (M.A.R.), New York

An apparent inverse relationship between smoking and Parkinson's disease prompted an investigation of the effect of chronic nicotine administration on dopaminergic and serotonergic receptors in rat brain. Nicotine, 0.8 mg/kg, was injected once daily, five times per week, for 6 weeks. In nucleus accumbens the K_d for [^{3}H]domperidone was increased 2-4-fold, and the B_{max} was increased 1.5-2-fold. No changes were observed in the binding of [^{3}H]domperidone in caudate-putamen or in that of [^{3}H]ketanserin in frontal cortex. It is concluded that chronic nicotine administration may have a suppressant effect on central nervous system release of dopamine that in pre-parkinsonian persons causes an aversion to the effects of smoking.

Key words: Parkinson's disease, nicotine, dopamine receptors

INTRODUCTION

Several studies have shown that cigarette smokers are fewer in number among Parkinson's disease (PD) patients than among groups of persons who do not have PD, and that these differences in preference for smoking predate the appearance of PD symptoms [Godwin-Austen et al, 1982; Kahn, 1966; Kessler, 1978; Marttila and Rinne, 1980; Nefzger et al, 1968]. Earlier age at diagnosis and more rapidly progressive symptoms were correlated with an aversion to smoking in PD patients [Golbe and Duvoisin, 1982]. One of the most prominent neurochemical alterations characteristic of PD is a loss of dopaminergic neurons in the nigrostriatal [Hornykiewicz, 1966] and in the mesolimbic [Javoy-Agid et al, 1981] pathways. PD symptoms do not appear until 80% or more of the normal number of dopaminergic cells are lost [Riederer and Wuketich, 1976]. It is possible that persons who develop PD may have earlier subclinical loss of neurons that sensitizes them to the negative effects of cigarette smoking or reduces their perception of its pleasurable aspects.

Several reports have linked nicotine to dopamine receptor responses (see references in Discussion). We [Lapin et al, 1985, 1987] and others [Clarke and Kumer, 1983] have noted that nicotine given subcutaneously to rats has a dopamine-like effect on motor activity. We previously reported preliminary evidence [Reilly et al, 1986] and now present additional data indicating an increase in the maximum number of binding sites accompanied by a decrease in affinity for [^{3}H]domperidone in nucleus accumbens of rats chronically injected with nicotine at a dose of 0.8 mg/kg. No change occurred in [3H]domperidone binding in caudate-putamen or in [^{3}H]ketanserin binding in frontal cortex.

MATERIALS

[^{3}H]Domperidone (specific activity 30.6 Ci/mmol) and [^{3}H]ketanserin (specific activity 95.0 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Cinanserin was donated by E.R. Squibb and Sons (Princeton, NJ). Haloperidol was donated by McNeil Laboratories (Fort Washington, PA); it was prepared in 0.01 N HCl, then diluted 25-fold with buffer before addition to incubation mixtures. Tubes for total binding received an equal amount of "haloperidol vehicle," a mixture of 0.01 N HCl and buffer in the same proportions. Nicotine bitartrate was purchased from ICN Biomedicals (Plainview, NY); it was dissolved in saline, and the pH was adjusted to 7.4. Reagents for protein determination were purchased from Bio-Rad (Richmond,CA).

Received April 22, 1987; revised July 28, 1987; accepted July 29, 1987.

Address reprint requests to Margaret A. Reilly, Ph.D., Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY 10962.

METHODS

Animals

Male Sprague-Dowley rats (purchased at 140–150 g body weight from Taconic Farms, Germantown, NY) were housed under standard conditions with a 12-hr light-dark cycle and unlimited access to laboratory chow and water. After 1 week for acclimatization the rats were injected 5 days per week for 6 weeks with nicotine bitartrate 0.8 mg free base per kg body weight. Control animals received 1 ml/kg normal saline. Approximately 24 hr after the final injection, animals were decapitated and brains were rapidly removed and chilled. Kept on ice throughout dissection, brains were sliced in a cutting apparatus similar to that described by Heffner et al [1980]. Caudate-putamen, nucleus accumbens, and frontal cortex were excised from the slices with the aid of a dissecting microscope. Regions from two rats were pooled and stored frozen at −70°C until assay, usually within 6 weeks.

Binding of [^{3}H]Domperidone

The binding of [^{3}H]domperidone in caudate-putamen and nucleus accumbens was assayed by a modification of the method of Lazareno and Nahorski [1982]. Brain regions were homogenized in approximately 50 volumes of ice-cold buffer (Tris-HCl 50 mM, pH 7.6) with a Brinkmann Polytron (setting five, 20 secs) and centrifuged at 44,000g for 10 min at 4°C. The pellet was washed once with ice-cold buffer, recentrifuged, and resuspended for incubation in approximately 150 volumes (caudate-putamen) or 200 volumes (nucleus accumbens) of buffer. This homogenate was preincubated at 37°C for 5 min, then returned to an ice bath.

Incubations were performed under reduced light at room temperature (22°C ± 2°C) in triplicate in polystyrene tubes in a final volume of 1.0 ml (0.5 ml for the second series of nucleus accumbens assays). [^{3}H]Domperidone (0.03–2.5 nm final concentrations) was prepared in buffer containing 0.01% bovine serum albumin (BSA) to reduce binding to tubes and filters. The incubation was terminated after 45 min (Lazareno and Nahorski, 1982, report equilibrium is obtained within this time interval) by rapid filtration under vacuum through Whatman GF/B glass fiber filters moistened with buffer containing 0.01% BSA to reduce binding of ligand to filters. The filters were washed twice with 5 ml of ice-cold buffer and placed in vials with 3 ml of scintillation fluid (Liquiscint, National Diagnostics). After 18–24 hr, radioactivity bound to the filters was determined in a Packard Tri-Carb 4640 with 48% efficiency. Nonspecific binding of ligand to membranes, determined in the presence of 4 μM haloperidol, ranged from 10% of total binding at lower concentrations to 30% at higher concentrations. This was subtracted from total binding to yield specific binding. Free ligand was measured from tubes incubated and centrifuged at 400g; aliquots of supernatant were added to scintillation fluid. Protein concentration of the homogenate, determined by a protein-dye binding method [Bradford, 1976], was 0.07–0.1 mg per assay for caudate-putamen and 0.04–0.06 mg per assay for nucleus accumbens.

Binding of [^{3}H]Ketanserin

The binding of [^{3}H]ketanserin in frontal cortex was assayed by a modification of the method of Gandolfi et al [1985]. Membrane from brain regions was prepared as for [^{3}H]domperidone binding described above, with the exception that the homogenate was not preincubated. The final resuspension of the pellet was in 150 volumes of ice-cold buffer (Tris-HCl, 50 mM, pH 7.6). Incubations were performed under reduced light at 37°C in triplicate in polystyrene tubes in a final volume of 0.5 ml. The incubation was terminated after 30 min by rapid filtration in the same manner as for [^{3}H]domperidone, described above. Nonspecific binding of ligand to membranes was determined in the presence of 1 μM cinanserin [Peroutka and Snyder, 1981] and was subtracted from total binding. Free ligand and protein concentrations of the homogenate were determined in the same manner as above. Protein content was 0.08–0.1 mg per assay.

Analysis of Data

The affinity constant (K_d) and the maximum number of binding sites (B_{max}) were estimated by plotting the reciprocal of the free ligand (in nM concentration) against that of the specific binding (expressed as fmol/mg protein). Lines of best fit determined by linear regression yielded values in good agreement with those obtained using the LIGAND computer program [Munson and Rodbard, 1980]. Statistical comparisons utilized Student's t-test for unpaired data.

RESULTS

[^{3}H]Domperidone Binding

Chronic administration of nicotine influenced neither the K_d nor the B_{max} of [^{3}H]domperidone binding in caudate-putamen (Table I). However, the binding of this ligand in nucleus accumbens was significantly altered. In one series of assays (Table I, group A) the K_d increased four-fold from controls to nicotine-treated animals ($P < 0.001$) while the B_{max} doubled ($P < 0.001$). Because of the small amounts of tissue available, these values were based on three ligand concentrations per assay. To confirm these data, a second series of assays was performed in which incubation volumes were reduced by one-half, so that five to six ligand concentrations could be tested. In this series (Table I, group B), the K_d in the treated animals increased by 75% over the controls ($P < 0.05$)

TABLE I. Effect of Chronic Nicotine Administration on Binding in Rat Brain Regions†

Ligand	Region	K_d (nM)		B_{max} (fmol/mg protein)	
		Control	Nicotine	Control	Nicotine
[^{3}H]Domperidone	Nucleus accumbens				
	A	0.14 ± 0.015 (N = 4)	0.57 ± 0.067* (N = 5)	455 ± 44	844 ± 50*
	B	1.34 ± 0.31 (N = 4)	2.35 ± 0.09** (N = 4)	808 ± 119	1,250 ± 61***
	Caudate-putamen	0.25 ± 0.059 (N = 4)	0.16 ± 0.023 (N = 5)	890 ± 146	979 ± 167
[^{3}H]Ketanserin	Frontal cortex	0.86 ± 0.14 (N = 5)	0.97 ± 0.058 (N = 6)	1,721 ± 206	1,612 ± 76

†Assays were performed as described in Methods. Values are means ± SEM. Values not marked with an asterisk were not significantly different from controls.
*Differs from control, $P < 0.001$.
**Differs from control, $P < 0.05$.
***Differs from control, $P < 0.02$.

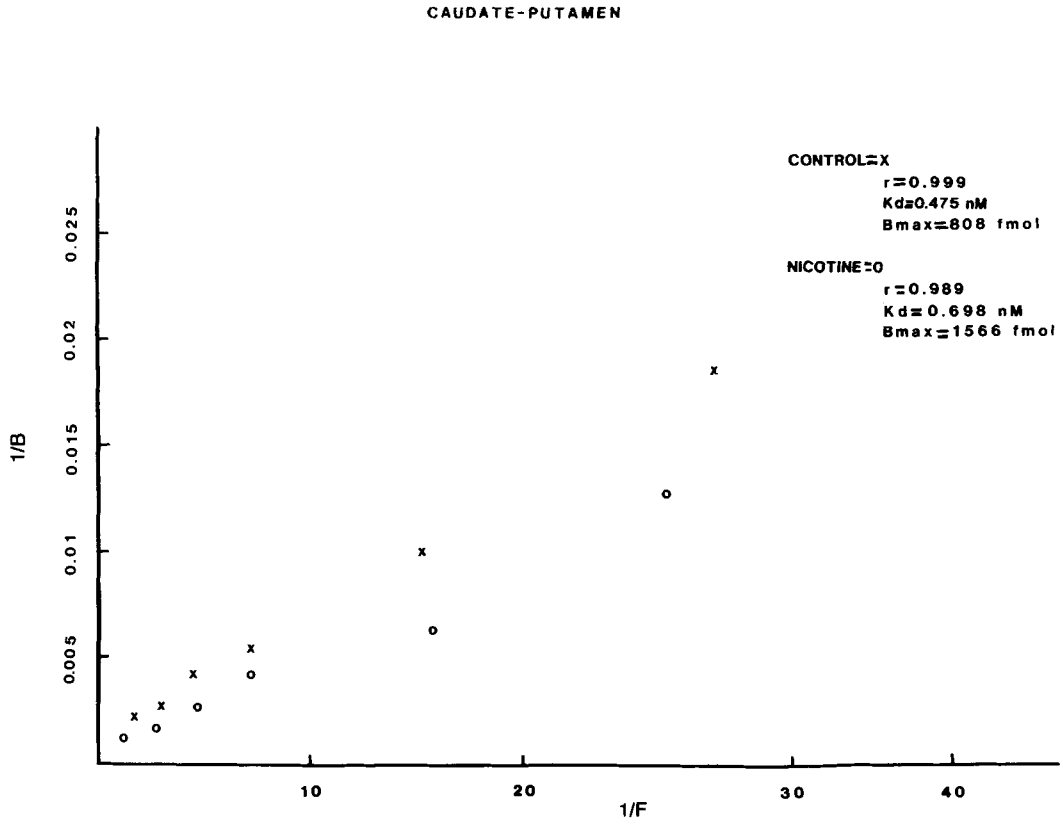

Fig. 1. Representative double-reciprocal plots used in the determination of the mean K_d and B_{max} in Table I. The experimental procedure is described under Methods. B is specific binding, fmol/mg protein; F is nM concentration of unbound ligand. There is no indication of more than one binding site at the ligand concentrations used.

and the B_{max} was 50% greater ($P < 0.02$). The concentration of nicotine in nucleus accumbens at 10 min after administration of nicotine 0.8 mg/kg was 2.4 pmol/mg tissue (unpublished observation).

[^{3}H]Ketanserin Binding

Chronic administration of nicotine did not alter [^{3}H]ketanserin binding in rat frontal cortex (Table I).

DISCUSSION

Nicotine has been shown in several studies to interact with dopamine in the central nervous system. In rats with unilateral lesions of the substantia nigra, nicotine administration induced ipsilateral rotation that was blocked by the nicotinic receptor blocker mecamylamine [Lapin et al, 1985] and also by the dopamine receptor antagonist haloperidol (Lapin et al, unpublished observation). Nicotine stimulated release of dopamine from slices of rat striatum [Giorguieff-Chesselet et al, 1979] and from striatal synaptosomes and micropunches [Sakurai et al, 1982]. Relatively large doses of nicotine (8 mg/kg over 2 hr) were reported to increase central nervous system turnover of dopamine and norepinephrine in rats [Andersson et al, 1984]. In contrast, we did not find any changes in the levels of dopamine or of 3,4 dihydroxyphenylacetic acid (DOPAC), homovanillic acid, or 3-methoxytyramine in several brain regions of our chronically treated rats either at the time of maximal locomotor response after nicotine administration or 24 hr after the last nicotine injection [Lapin et al, 1987].

These observed relationships between nicotine and central dopamine systems together with the reported inverse correlation between smoking and PD prompted this study of the effect of chronic nicotine administration on dopamine receptor binding. The ligand [^{3}H]domperidone was used because it is reported to be relatively selective for dopamine receptors [Baudry et al, 1979; Laduron and Leysen, 1979]. In rat striatum, Hamblin et al [1984] reported a K_d of 0.25 nM and Baudry et al [1979] found a K_d of 0.7 nM. In rat pituitary a K_d of 0.3 nM was observed by Bression et al [1983]. Because serotonin in human frontal cortex is reduced in PD [Scatton et al, 1983], the binding of [^{3}H]ketanserin was determined in rat frontal cortex after chronic nicotine treatment.

The only observed alteration in binding induced by nicotine was that in nucleus accumbens, which suggests that dopamine receptors in this brain region are different from those in caudate-putamen. Pharmacological and functional differences between the nigrostriatal and the mesolimbic dopaminergic systems have been reported by other investigators. Missale et al [1985] observed that active uptake of [^{3}H]dopamine into rat striatum was much more strongly inhibited by cocaine than was uptake in nucleus accumbens. Trifluoperazine markedly reduced the action of d-amphetamine in nucleus accumbens but was much less inhibitory in striatum [Jackson et al, 1975]. Dopaminergic activity in nucleus accumbens is reported to have greater importance in locomotor activity [Jackson et al, 1975; Kalivas and Miller, 1985; Kelly et al, 1975], while that in the striatum is concerned with sterotyped behavior [Jackson et al, 1975; Kelly et al, 1975]. Nicotine administration produced increases in locomotion in rats [Lapin et al, 1985; Clarke and Kumar, 1983]; this is consistent with our observation of an effect of nicotine in nucleus accumbens but not in striatum.

However, increases in receptor binding sites are more frequently found after prolonged *reduction* in neurotransmitter activity. Destruction of dopaminergic neurons induced marked increases in the binding but no change in the affinity of ligands for the dopamine receptor in striatum [Schultz, 1982]. Also in striatum, chronic administration of haloperidol and fluphenazine, which block dopamine receptors, and of reserpine, which depletes stored dopamine, produced an increase in [^{3}H]haloperidol binding sites [Burt et al, 1977].

Alterations in binding to other central nervous system receptors have been induced by chronic nicotine administration. An increase in the number of binding sites for [^{3}H]prazosin in rat cerebral cortex and hypothalamus/thalamus and for [^{3}H]clonidine in cerebral cortex, and an increase in the binding affinity of [^{3}H]quinuclidinyl benzilate were observed by Yamanaka et al [1985]. In mice, continuous infusion of nicotine increased specific binding of [^{3}H]nicotine and of [^{125}I]bungarotoxin in several brain areas [Marks et al, 1983].

Price et al [1978] suggested that PD akinesia might occur via degeneration of dopaminergic innervation of the nucleus accumbens. An early subclinical loss of neurons could alter the perception of the "rewarding" aspects of cigarette smoking. Or individual differences in dopamine neurons might influence the response to nicotine so that smoking to some persons is pleasurable and to others it is disagreeable. Nicotine-induced changes in dopamine receptors could be involved in the habitual use of tobacco. The observed alteration in [^{3}H]domperidone binding suggests that chronic administration of nicotine has a negative effect on dopamine release in the nucleus accumbens. In the presence of a malfunctioning dopaminergic system, this additional anti-dopaminergic effect might not be tolerated by preclinical PD patients. A deficit in dopaminergic neurotransmission may exist many years before clinical symptoms of PD become apparent.

ACKNOWLEDGMENTS

This research was supported by the Council for Tobacco Research Grant No. 1615, and by NY State BRS Grant No. 905-EO43R.

REFERENCES

Andersson K, Fuxe K, Eneroth P, Agnati LF, (1984): Differential effects of mecamylamine on the nicotine induced changes in amine levels and turnover in hypothalamic dopamine and noradrenaline nerve terminal systems and in the secretion of adenohypophyseal hormones in the castrated female rat. Evidence for involvement of cholinergic nicotine-like receptors. Acta Physiol Scand 120:489–498.

Baudry M, Martres MP, Schwartz JC (1979): [^{3}H]Domperidone: A selective ligand for dopamine receptors. Naunyn Schmiedeberg's Arch Pharmacol 308:231–237.

Bradford MM (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Bression D, Brandi AM, LeDafniet M, Cesselin F, Hamon M, Martinet M, Kerdelhue B, Piellon F (1983): Modifications of the high and low affinity pituitary domperidone-binding sites in chronic estrogenized rats. Endocrinology 113:1799–1805.

Burt DR, Creese I, Snyder SH (1977): Antischizophrenic drugs: Chronic treatment elevates dopamine receptor binding in brain. Science 196:326–328.

Clarke PBS, Kumar R (1983): Characterization of the locomotor stimulant action of nicotine in tolerant rats. Br J Pharmacol 80:587–594.

Gandolfi O, Barbaccia ML, Costa E (1985): Different effects of serotonin antagonists on ^{3}H-mianserin and ^{3}H-ketanserin recognition sites. Life Sci 36:713–721.

Giorguieff-Chesselet MF, Kemel ML, Wandscheer D, Glowinski J (1979): Regulation of dopamine release by presynaptic nicotinic receptors in rat striatal slices: Effect of nicotine in a low concentration. Life Sci 25:1257–1261.

Godwin-Austen RB, Lee PN, Marmot MG, Stern GM (1982): Smoking and Parkinson's disease. J Neurol Neurosurg Psychiatry 45:577–581.

Golbe LI, Duvoisin RC (1985): Smoking and Parkinson's disease. Neurology 35 (Suppl 1):275 (abstract).

Hamblin MW, Leff SE, Creese I (1984): Interactions of agonists with D-2 dopamine receptors: Evidence for a single receptor population exisiting in multiple agonist affinity-states in rat striatal membranes. Biochem Pharmacol 33:877–887.

Heffner TG, Hartman JA, Seiden LS (1980): A rapid method for the regional dissection of the rat brain. Pharmacol Biochem Behav 13:453–456.

Hornykiewicz O (1966): Dopamine (3-hydroxytyramine) and brain function. Pharmacol Rev 18:925–964.

Jackson DM, Anden NE, Dahlstrom A (1975): A functional effect of dopamine in the nucleus accumbens and in some other dopamine-rich parts of the rat brain. Psychopharmacologia 45:139–149.

Javoy-Agid F, Ploska A, Agid Y (1981): Microtopography of tyrosine hydroxylase, glutamic acid decarboxylase, and choline acetyltransferase in the substantia nigra and ventral tegmental area of control and Parkinsonian brains. J Neurochem 37:1218–1227.

Kahn HA (1966): The Dorn study of smoking and mortality among U.S. veterans. Report on eight and one-half years of observation. In "Epidemiological Approaches to the Study of Cancer and Other Chronic Diseases," NCI Monograph No. 19. Washington, DC: U.S. Government Printing Office, pp 1–125.

Kalivas PW, Miller JS (1985): Dopamine microinjection into the nucleus accumbens: Correlation between metabolism and behavior. Biochem Pharmacol 34:284–286.

Kelly PH, Seviour PW, Iversen SD (1975): Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res 94:507–522.

Kessler II (1978): Parkinson's disease in epidemiologic perspective. Adv Neurol 19:355–384.

Laduron PM, Leysen JE (1979): Domperidone, a specific in vitro dopamine antagonist, devoid of in vivo central dopaminergic activity. Biochem Pharmacol 28:2161–2165.

Lapin EP, Maker HS, Sershen H, Hurd Y, Lajtha A (1987): Dopamine-like action of nicotine: Lack of tolerance and reverse tolerance. Brain Res 407:351–363.

Lapin EP, Maker HS, Sershen H, Hurd Y, Lajtha A (1987): Dopamine-like action of nicotine: Lack of tolerance and reverse tolerance. Brain Res 407:351.

Lazareno S, Nahorski SR (1982): Selective labelling of dopamine (D_2) receptors in rat striatum by [^{3}H]domperidone but not by [^{3}H]spiperone. Eur J Pharmacol 81:273–285.

Marks MJ, Burch JB, Collins AC (1983): Effects of chronic nicotine infusion on tolerance development and nicotinic receptors. J Pharmacol Exp Ther 226:817–825.

Marttila RJ, Rinne UK (1980): Smoking and Parkinson's disease. Acta Neurol Scand 62:322–325.

Missale C, Castelletti L, Govoni S, Spano PF, Trabucci M, Hanbauer I (1985): Dopamine uptake is differentially regulated in rat striatum and nucleus accumbens. J Neurochem 45:51–56.

Munson PJ, Rodbard D (1980): LIGAND: A versatile computerized approach to characterization of ligand-binding systems. Anal Biochem 107:220–239.

Nefzger MD, Quadfasel FA, Karl VC (1968): A retrospective study of smoking in Parkinson's disease. Am J Epidemiol 88:149–158.

Peroutka SJ, Snyder SH (1981): Two distinct serotonin receptors: Regional variations in receptor binding in mammalian brain. Brain Res 208:339–347.

Price KS, Farley IJ, Hornykiewicz O (1978): Neurochemistry of Parkinson's disease: Relation between striatal and limbic dopamine. In Roberts PJ, Woodruff GN, Iversen LL (eds): "Advances in Biochemical Psychopharmacology," Vol 19. New York: Raven Press, pp 293–300.

Reilly MA, Lapin EP, Lajtha A, Maker HS (1986): Does chronic nicotine alter neurotransmitter receptors involved in Parkinson's disease? Fed Proc 45:805.

Riederer P, Wuketich S (1976): Time course of nigrostriatal degeneration in Parkinson's disease. J Neural Transm 38:277–301.

Sakurai Y, Takana Y, Kohjimoto Y, Honda K, Kamiya H-O (1982): Enhancement of [^{3}H]dopamine release and its [^{3}H]metabolites in rat striatum by nicotinic drugs. Brain Res 242:99–106.

Scatton B, Javoy-Agid F, Rouquier L, Dubois B, Agid Y (1983): Reduction of cortical dopamine, noradrenalin, serotonin and their metabolites in Parkinson's disease. Brain Res 275:321–328.

Schultz W (1982): Depletion of dopamine in the striatum as an experimental model of Parkinsonism: Direct effects and adaptive mechanisms. Prog Neurobiol 18:121–166.

Yamanaka K, Oshita M, Muramatsu I (1985): Alteration of alpha and muscarinic receptors in rat brain and heart following chronic nicotine treatment. Brain Res 348:241–248.