0026-895X/03/6404-885–889$7.00
MOLECULAR PHARMACOLOGY
Copyright © 2003 The American Society for Pharmacology and Experimental Therapeutics
Mol Pharmacol 64:885–889, 2003

Vol. 64, No. 4
2598/1096078
Printed in U.S.A.

Functional Nicotinic Acetylcholine Receptors Are Expressed in B Lymphocyte-Derived Cell Lines

MARINA V. SKOK, ELENA N. KALASHNIK, LUDMILA N. KOVAL, VICTOR I. TSETLIN, YURIY N. UTKIN, JEAN-PIERRE CHANGEUX, and RÉGIS GRAILHE

Palladin Institute of Biochemistry, Kiev, Ukraine (M.V.S., E.N.K., L.N.K.); Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia (V.I.T., Y.N.U.); and Institut Pasteur, Paris, France (J.P.C., R.G.)

Received April 15, 2003; accepted July 3, 2003

This article is available online at http://molpharm.aspetjournals.org

ABSTRACT

Nicotine has been shown to affect B lymphocyte immune response. In this study, we have explored the presence of nicotinic receptors in B lymphocyte-derived cell lines, myeloma X63-Ag8 and hybridoma 1D6. We found that myeloma expressed on average 10,170 ± 1,100 [^{3}H]epibatidine and 6,730 ± 370 ^{125}I-α-bungarotoxin binding sites per cell, thus reflecting the presence of both homomeric and heteromeric nicotinic receptors. More specifically, the presence of α4- and α7-containing nicotinic receptor subunits was demonstrated in both myeloma and hybridoma cells with subunit-specific antibodies. It was significantly higher in dividing than in resting cells. Long-term exposure to nicotine, at physiological concentration found in smokers, resulted in up-regulation of both α4 and α7 subunits in hybridoma cells. Additionally, nicotine stimulated hybridoma cell proliferation, whereas it decreased antibody production. In contrast, α7-specific snake toxins inhibited cell proliferation but increased antibody production. It is concluded that myeloma and hybridoma cells express α4- and α7-containing nicotinic receptors, which participate in regulating cell proliferation and function.

Nicotine, the main bioactive component of cigarette smoke, affects the organism through nicotinic acetylcholine receptors (nAChRs), which are pentameric ligand-gated ion channels. In mammals, 17 different nAChR subunits (α1–α10, β1–β4, γ, δ, and ϵ) have been identified that form homo- and heteropentameric receptor subtypes. In the brain, the most abundantly expressed nAChR, composed of α4 and β2 subunits is considered to be a key player in nicotine addiction (Changeux et al., 1998). In addition to the central nervous system, nicotine affects respiratory tract, skin, vascular, and immune tissues. Consequently, neuronal nAChRs were found in keratinocytes (Grando, 1997), respiratory epithelial (Maus et al., 1998), vascular endothelial (Macklin et al., 1998), and ginginal and esophageal epithelial cells (Nguyen et al., 2000). They were also found in several tumor cell lines, such as cervical epithelial carcinoma, epidermoid skin carcinoma, lung adenocarcinoma, hepatoma, glioblastoma, and small cell lung carcinoma (Chini et al., 1992; Sciamanna et al., 1997). Interestingly, nAChRs were shown to be involved in promoting tumor growth (Codignola et al., 1994; Quik et al., 1994).

Of special interest is the presence of nAChRs in lymphocytes, because these cells produce acetylcholine, which may exert its effects through autocrine or paracrine transmission (Rinner et al., 1998). nAChR expression has been clearly demonstrated for both T lymphocytes and T lymphocyte-derived cell lines. In both cases, nicotine affected the ability of these cells to participate in the immune response (Sopori et al., 1998a; Sato et al., 1999). T lymphocytes are not the only class of immune cell affected by nicotine, because nicotine-treated animals were shown to have an altered B lymphocyte response to lipopolysaccharide, anti-IgM, and sheep erythrocytes (Savage et al., 1991). The expression of nAChRs in transformed B lymphocytes has also been reported (Sato et al., 1999; Lustig et al., 2001); however, their functional role is unknown.

The aim of our study was to explore nAChR expression in B lymphocyte-derived cell lines, myeloma and hybridoma, and to assess their sensitivity to nicotine and nAChR-specific antagonists. Herein, we describe a previously unrecognized presence of α4- and α7-containing nAChRs in these cells and show that they are involved in regulating hybridoma cell proliferation and antibody production.

Materials and Methods

Cell Cultures. Myeloma X63-Ag8 was obtained from permanent stocks of the Palladin Institute of Biochemistry (Kiev, Ukraine) and Pasteur Institute (Paris, France). Hybridoma 1D6 producing antibodies against nAChR peptide α3(191-182) was generated previously

This work was supported by European Molecular Biology Organization Short-Term Fellowships ASTF 9657 and 9953.

ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin; CTX, cobratoxin; WTX, weak toxin; NTII, neurotoxin II.

by us (Voitenko et al., 2001). The cells were grown in RPMI 1640 medium supplemented with 20 mM HEPES, 20 mM L-glutamine, 5×10^{-5} M β-mercaptoethanol, 50 μg/ml sodium pyruvate, 41 μg/ml insulin, 40 μg/ml gentamicin, and 10% fetal calf serum (Sigma-Aldrich, St. Louis, MO). Cell proliferation studies were performed in 96-well plates seeded initially with 5×10^3 cells/well. The cells were grown for 3 to 5 days in the presence of either nicotine or toxins and then the number of live cells per well was measured with the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (triazolyl blue) assay (Carmichael et al., 1987) and the cell doubling time ("2t") was calculated according to the equation $2t = t \times \ln 2/(\ln X - \ln X_0)$, where t is the period of cell incubation (hours); X_0 is the initial number of cells seeded; and X is the final number of cells obtained.

[^{3}H]Epibatidine and ^{125}I-α-Bungarotoxin (α-Bgt) Binding Studies. Myeloma X63-Ag8 cells (2–2.5 $\times$ 10^5/sample) were incubated on ice with either 20 nM ^{125}I-α-Bgt or 20 nM [^{3}H]epibatidine (Amersham Biosciences UK, Ltd., Little Chalfont, Buckinghamshire, UK) either in the presence (nonspecific binding) or in the absence (total binding) of 2 mM nicotine for 1 h. The saturating ligand concentrations were established in preliminary experiments. The cells were washed with cold PBS on glass microfiber filters (GF-C; Whatman, Clifton, NJ), and the filters were counted with either scintillation beta-counter (for epibatidine) or gamma-counter (for α-Bgt). The number of binding sites per cell was calculated using the equation $n = B \times N/c$, where n is the number of binding sites, B is the number of ligand moles bound, N is the Avogadro number, and c is the number of cells per sample. The number of ligand moles bound specifically was calculated as the difference of total and nonspecific binding using the measured radioactivity of a standard ligand sample.

ELISA and Cell-ELISA Assays. The amounts of antibody secreted by hybridomas were measured by conventional ELISA using corresponding peptide-BSA conjugate as a coating antigen and peroxidase-conjugated mouse IgG-specific antibodies, followed by a substrate solution containing *o*-phenylendiamine (Skok et al., 1999).

The numbers of antibody-secreting cells were calculated by ELISA-Spot assay. Hybridoma cells were grown on nitrocellulose filters (Bio-Rad, Hercules, CA) placed in the wells of 96-well tissue culture plate, 5×10^3 cells/well, in the presence or absence of nicotine for 3 days. The filters were then treated with peroxidase-conjugated goat anti-mouse IgG antibodies and the spots of antibody-secreting cells developed with a solution of 4-chloro-1-naphthol-containing substrate solution.

For Cell-ELISA, hybridoma cells were suspended in PBS and fixed to the wells of 96-well ELISA plate, 1×10^5 cells/well, by evaporation overnight at 37°C. The wells were blocked with 1% BSA and rat IgG (50 μg/ml). Biotinylated affinity-purified antibodies to nAChR subunits were added to the cells at a concentration of 10 to 20 μg/ml for 2 h at 37°C. After two washes with PBS/1% BSA, the wells were treated with avidin-peroxidase (1 h at 37°C; Sigma-Aldrich). After three additional washes, peroxidase activity was developed with *o*-phenylendiamine–containing substrate solution and the absorbance was registered with a MicroELISA autoreader (Dynatech Labs, Chantilly, VA).

Statistical Analysis. All results are expressed as mean ± standard error, and data were analyzed using Student's t test. Values at $p < 0.05$ were considered significant.

Results

The cell models used in this study were the mouse myeloma X63-Ag8 and hybridoma 1D6 (Skok et al., 2001). Both myeloma and hybridoma cells were used to study nAChR expression, whereas antibody-producing hybridoma was used to check both cell proliferation and antibody production upon nicotine/toxin treatment.

The presence of nAChRs was first studied by [^{3}H]epibatidine and ^{125}I-α-bungarotoxin binding. As shown in Fig. 1, myeloma X63-Ag8 cells demonstrated specific binding for both compounds, the calculated number of binding sites per cell being 10,170 ± 1,100 for epibatidine and 6,730 ± 370 for α-bungarotoxin (means ± S.E. of three independent experiments).

The presence of receptor protein on the cell surface was further investigated by Cell-ELISA using α subunit-specific antibodies generated by us previously (Skok et al., 1999, 2001; Voitenko et al., 2001). As shown in Fig. 2, both cell lines tested bound nAChR α4- and α7-specific antibodies, but not α3- and α5-specific antibodies.

Hybridoma 1D6 cells adhere to tissue culture plastics, whereas in the course of cultivation some of them are floating. When 1D6 cells were separated into floating and adherent ones, the floating cells bound significantly more nAChR-specific antibodies than the adherent ones (Fig. 3).

To determine whether nAChRs expressed were sensitive to nicotine, 1D6 cells were incubated with various doses of nicotine for 5 to 6 days before being tested by Cell-ELISA. As shown in Fig. 4, α4- and α7-specific antibody binding increased in 1D6 hybridoma cells after nicotine treatment.

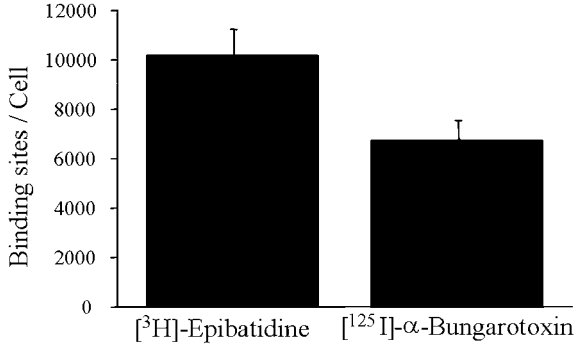

Fig. 1. [^{3}H]Epibatidine (20 nM) and ^{125}I-α-bungarotoxin (10 nM) binding sites per X63-Ag8 myeloma cells (2×10^5/sample). A mean and S.E. of three independent experiments is presented.

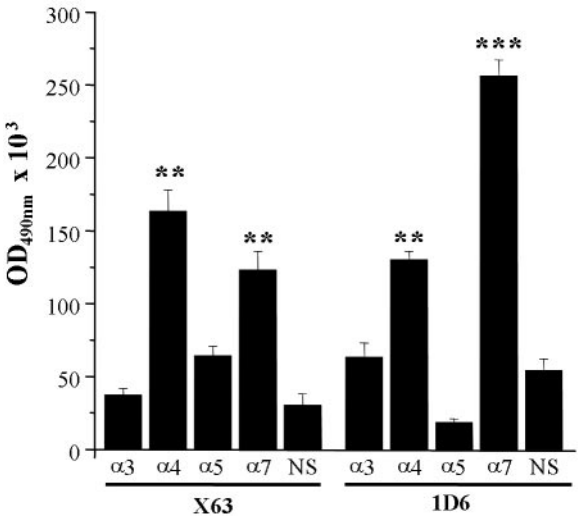

Fig. 2. Cell surface nAChR receptor expression studied by Cell-ELISA with α3, α4, α5, and α7 subunit-specific antibodies in myeloma X63-Ag8 and hybridoma 1D6 cells. Each column represents a mean and S.E. of the data for three independent measurements. NS, nonspecific binding measured with irrelevant rabbit immunoglobulins. **, $p < 0.005$; ***, $p < 0.0005$; the significance of difference compared with NS values).

To investigate the functional implication of nAChR expression in B cells, the rate of proliferation and antibody production of hybridoma cells were measured after treatment with either agonist (nicotine) or antagonist (nAChR-specific snake toxins). As illustrated in Fig. 5A, long-term nicotine exposure resulted in a dose-dependent stimulation of hybridoma cell proliferation; the calculated cell doubling time decreased from 24.3 ± 0.79 to 21.2 ± 0.13 h (means ± S.E. of three independent experiments; $p = 0.02$) in the presence of 10 μM nicotine. Accelerated proliferation was accompanied with a decrease in the number of antibody-producing cells (Fig. 5B). In contrast, the presence of either cobratoxin (CTX) or "weak toxin" (WTX), but not that of neurotoxin II (NT II) slowed hybridoma proliferation (Fig. 6A); the calculated cell doubling time increased from 15.3 ± 0.47 to 18.6 ± 0.93 h for 1 μM CTX ($p = 0.036$; $n = 3$) and to 21.5 ± 1.6 h for 1 μM WTX ($p = 0.02$; $n = 3$); the effect of NT II was nonsignificant. The difference in the initial rates of cell proliferation in two sets of experiments was caused by the use of different batches of fetal bovine sera in the culture media. The changes in cell numbers resulting from the incubation with these toxins were accompanied with an increase in the antibody amount secreted by the hybridoma (Fig. 6B). In this test, WTX exerted a maximal effect, whereas CTX and NT II were less effective.

Discussion

To demonstrate the presence of nicotinic receptors in myeloma X63-Ag8 cells, binding assays were performed with the selective radioligands [^{3}H]epibatidine and ^{125}I-α-Bgt (Fig. 1). Epibatidine is a potent agonist of heteromeric neuronal nAChRs (Sihver et al., 1999), whereas α-Bgt binds to both muscle-type nAChRs and homomeric neuronal-type nAChRs (Lindstrom et al., 1987). Our binding assays revealed that myeloma cells express both heteromeric and homomeric (or muscle-type) neuronal nAChRs.

The respective numbers of binding sites per cell (10,170 ± 1,100 for epibatidine and 6,730 ± 370 for α-Bgt) differed substantially from those previously reported for other nonexitable cell types. These numbers were lower than those in mature skin keratinocytes (Grando, 1997), but higher than those of respiratory endothelial (Maus et al., 1998), vascular endothelial (Macklin et al., 1998), and lymph node T cells (Maslinski et al., 1992). B lymphocyte-derived cell lines were previously found to lack nicotine-binding sites (Maslinski et al., 1992).

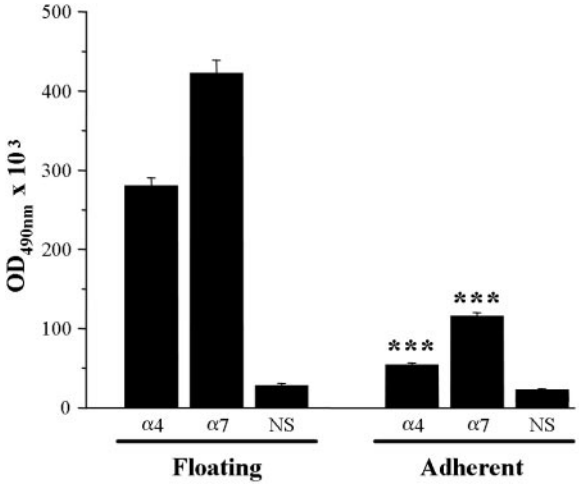

Fig. 3. Cell surface expression of α4 and α7 nAChR subunits in either floating or adherent 1D6 hybridoma cells. The data are presented similarly to those in Fig. 2, the significance of difference between floating and adherent cells for each subunit is shown (***, $p < 0.0005$).

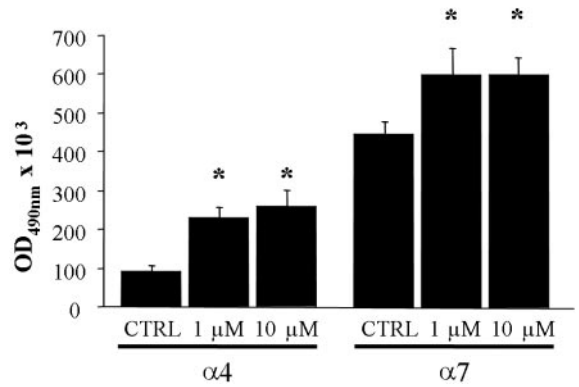

Fig. 4. Long-term nicotine (4–6-day) exposure effect on α4- and α7-specific antibody binding to hybridoma 1D6 studied by Cell ELISA. *, $p < 0.05$, the significance of difference compared with control values is indicated. A mean and S.E. of three independent experiments is presented.

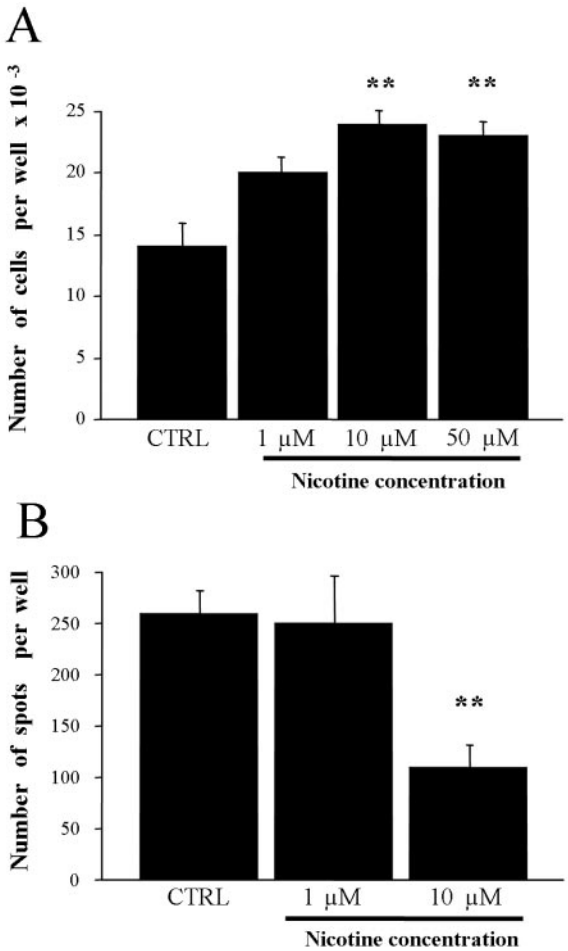

Fig. 5. Effect of chronic nicotine treatment on the number of live hybridoma 1D6 cells (A) and on the number of antibody-producing cells per well (studied by ELISA-Spot assay) (B) found after 4 days of incubation; the initial number of cells seeded was 5×10^3/well. Mean values and S.E. of six repeats are presented for each point. **, $p < 0.005$.

To determine the subtypes of nAChRs expressed in myeloma and hybridoma cells, we used various α subunit-specific antibodies previously generated by us (Skok et al., 1999, 2001; Voitenko et al., 2001), which provided specific immunolabeling of nAChR subtypes in the autonomic ganglia (Skok et al., 1999; Voitenko et al., 2001). In both myeloma and hybridoma cell lines, specific immunolabeling was obtained only with α4- and α7-specific antibodies (Fig. 2). These results strongly suggest that myeloma and hybridoma cells express α7 homomeric and α4-containing heteromeric nAChRs.

Neuronal-type nAChR expression was previously found in various non-neuronal tumor tissues: α-Bgt-binding nAChRs and RNA transcripts for α5 subunits were both detected in cervical epithelial carcinoma, epidermoid skin carcinoma, lung adenocarcinoma, hepatoma, and glioblastoma (Chini et al., 1992), whereas transcripts for α7 subunits were found in a small cell lung carcinoma (Sciamanna et al., 1997). α3 mRNA and corresponding receptor molecules were found in human T lymphocyte-derived cell lines, but not in myeloid cell lines (Battaglioli et al., 1998), whereas α2, α5, α6, and α7 nAChR subunit mRNAs were detected in both B and T lymphocyte-derived leukemic cell lines (Sato et al., 1999). Ours is the first demonstration of the presence of α7- and α4-containing nAChRs in murine B cell-derived myeloma and hybridoma cell lines.

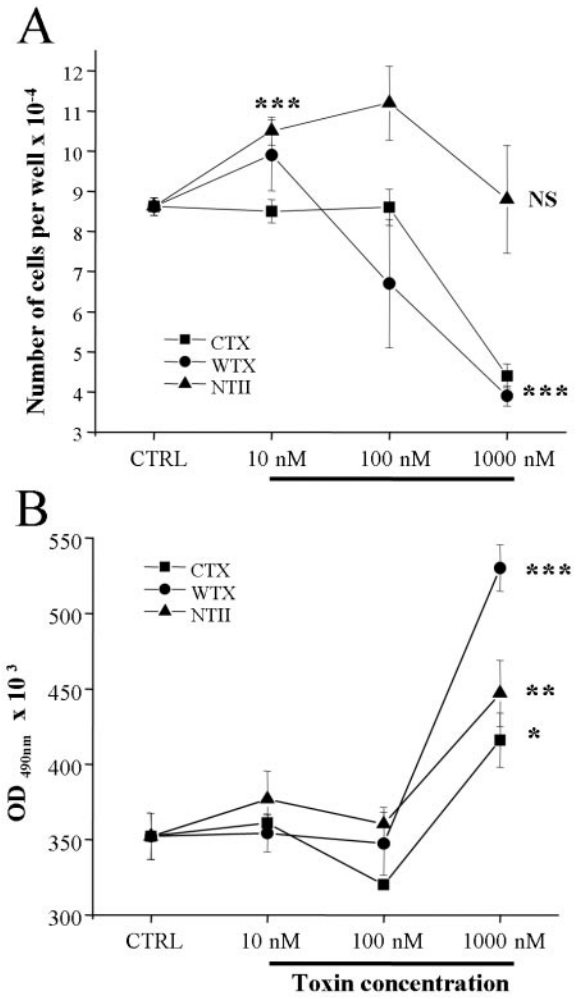

Fig. 6. Effect of CTX, WTX, and NT II on the number of live hybridoma 1D6 cells (A) and on the antibody amount found in the supernatant (expressed in absorption units of ELISA) (B), after 3 days of incubation with toxins; the initial number of cells seeded was 2.5×10^3/well. Each point represents a mean and SE for three to six independent measurements. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$).

We then set out to examine whether long-term exposure to nicotine would modulate the levels of $\alpha 4\beta$x and α7 nAChRs measured on the surface of these cells. This phenomenon, generally observed as an up-regulation, has been widely reported in the central nervous system (Marks et al., 1992; Yates et al., 1995; Peng et al., 1997), and more recently in respiratory epithelial cells (Maus et al., 1998). Interestingly, the nicotine- and epibatidine-binding sites were found to be up-regulated in polymorphonuclear blood cells of smokers (Benhammou et al., 2000). Some reports, however, have indicated that nicotine down-regulates α7-containing nAChRs in autonomic neurons (Kawai and Berg, 2001).

We found that long-term nicotine treatment resulted in the increase of α4- and α7-specific antibody binding in 1D6 hybridoma (Fig. 4). These data indicated that nAChRs expressed in hybridoma cells were sensitive to nicotine.

Hybridoma 1D6 produces antibodies to α3 nAChR peptide. It was obtained by us previously using SP-2/0 cells as a fusion partner, whereas SP-2/0 cells are themselves a hybrid of X63-Ag8 and normal mouse B lymphocytes (Harlow and Lane, 1988). However, because both myeloma X63-Ag8 cells (Fig. 2) and normal mouse B lymphocytes (our unpublished observation) were shown not to bind α3-specific antibody, we could not expect the effect of the antibody produced on the nicotine sensitivity of 1D6 hybridoma.

To study the nAChR function, we have examined hybridoma cell proliferation and antibody production upon nicotine or nAChR-specific cobra venom toxin treatments. α-CTX, similarly to α-Bgt, belongs to the group of long-chain α-neurotoxins and blocks the α7 and muscle-type nAChRs (Tsetlin, 1999). These receptors are also the targets for the recently characterized WTX, although its blocking of acetylcholine currents was observed at much higher concentrations than that of CTX (Utkin et al., 2001). NT II, similarly to other short-chain α-neurotoxins, acts predominantly on the muscle-type nAChRs (Tsetlin, 1999). Our data indicate that activating nAChRs by nicotine favored cell proliferation and prevented antibody production (Fig. 5), whereas blocking nAChRs by toxins prevented proliferation and favored antibody production (Fig. 6). The effect of CTX and WTX but not NT II supports the view that α7 nAChR, rather than muscle-type nAChRs contribute to the regulation of hybridoma proliferation. Earlier observations have shown almost identical irreversibility of acetylcholine current-blocking effects of CTX and WTX for rat α7 AChRs expressed in *Xenopus* oocytes (Utkin et al., 2001) or *Lymneae stagnalis* (Vulfius et al., 2001).

The connection of nAChRs with hybridoma cell proliferation was further supported by the experiments where 1D6 cells were separated into floating and adherent ones. This is a classical "mitotic shake-off" approach to separate dividing (floating) from resting (adherent) cells (Alberts et al., 1989). We found that floating cells expressed much more α4 and α7 nAChRs than adherent ones (Fig. 3); therefore, the number of surface receptors increased in dividing cells. This is consistent with the observed nAChR up-regulation upon nicotine

treatment (Fig. 4), which also stimulated hybridoma cell proliferation (Fig. 5A).

The involvement of α7 nAChRs in promoting tumor cell proliferation was previously documented (Codignola et al., 1994; Quik et al., 1994). Our data support this finding by indicating that α7 nAChRs are involved in hybridoma cell proliferation. Because hybridoma cells have the alternative either to enter the cell cycle (proliferate) or to produce antibodies, stimulating proliferation automatically decreases the antibody production. In contrast, blocking proliferation with CTX or WTX favors antibody production. These data suggest that α7 nAChRs expressed in hybridoma modulate cell division together with antibody production.

The blocking effect of toxins on cell proliferation found in our experiments suggests that nAChRs in hybridoma are endogenously activated. One of the explanations might be the presence of endogenous acetylcholine in the cell culture medium. Although not verified by us, this is consistent with the reported evidence for acetylcholine production by lymphocytes (Rinner et al., 1998).

Our results are in accordance with the reported lymphocytosis (Hughes et al., 1985; Loembe et al., 2001), the lower levels of serum immunoglobulins in cigarette smokers (Andersen et al., 1982), and the loss of antibody-producing cells in nicotine-treated rats (Sopori et al., 1998b).

Acknowledgments

We thank Dr. N. Mechawar for critically reading the manuscript and insightful discussions.

References

Alberts B, Bray D, Lewis J, Raff M, Roberts K, and Watson JD (1989) *Molecular Biology of the Cell*, 2nd ed, Garland Publishing, Inc., New York.
Andersen P, Pedersen OF, Bach B, and Bonde GJ (1982) Serum antibodies and immunoglobulins in smokers and nonsmokers. *Clin Exp Immunol* **47:**467–473.
Battaglioli E, Gotti C, Terzano S, Flora A, Clementi F, and Fornasari D (1998) Expression and transcriptional regulation of the human alpha3 neuronal nicotinic receptor subunit in T lymphocyte cell lines. *J Neurochem* **71:**1261–1270.
Benhammou K, Lee M, Strook M, Sullivan B, Logel J, Raschen K, Gotti C, and Leonard S (2000) [(3)H]Nicotine binding in peripheral blood cells of smokers is correlated with the number of cigarettes smoked per day. *Neuropharmacology* **39:**2818–2829.
Carmichael J, DeGraff WG, Gazdar AF, Minna JD, and Mitchell JB (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of radiosensitivity. *Cancer Res* **47:**943–946.
Changeux JP, Bertrand D, Corringer PJ, Dehaene S, Edelstein S, Lena C, Le Novère N, Marubio L, Picciotto M, and Zoli M (1998) Brain nicotinic receptors: structure and regulation, role in learning and reinforcement. *Brain Res Brain Res Rev* **26:**198–216.
Chini B, Clementi F, Hukovic N, and Sher E (1992) Neuronal-type α-bungarotoxin receptors and the α5-nicotinic receptor subunit gene are expressed in neuronal and nonneuronal human cell lines. *Proc Natl Acad Sci USA* **89:**1572–1576.
Codignola A, Tarroni P, Cattaneo MG, Vicentini LM, Clementi F, and Sher E (1994) Serotonin release and cell proliferation are under the control of alpha-bungarotoxin-sensitive nicotinic receptors in small-cell lung carcinoma cell lines. *FEBS Lett* **342:**286–290.
Grando SA (1997) Biological functions of keratinocyte cholinergic receptors. *J Investig Dermatol Symp Proc* **2:**41–48.
Harlow E and Lane D (1988) *Antibodies. A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
Hughes DA, Haslam PL, Townsend PJ, and Turner-Warwick M (1985) Numerical and functional alterations in circulatory lymphocytes in cigarette smokers. *Clin Exp Immunol* **61:**459–466.
Kawai H and Berg DK (2001) Nicotinic acetylcholine receptors containing A7 subunits on rat cortical neurons do not undergo long-lasting inactivation even when up-regulated by chronic nicotine exposure. *J Neurochem* **78:**1367–1378.
Lindstrom J, Schoepfer R, and Whiting P (1987) Molecular studies of the neuronal nicotinic acetylcholine receptor family. *Mol Neurobiol* **1:**281–337.
Loembe MM, Lamoureux J, Deslauriers N, Darveau A, and Delage R (2001) Lack of CD40-dependent B-cell proliferation in B lymphocytes isolated from patients with persistent polyclonal B-cell lymphocytosis. *Br J Haematol* **113:**699–705.
Lustig LR, Peng H, Hiel H, Yamamoto T, and Fuchs PA (2001) Molecular cloning and mapping of the human nicotinic acetylcholine receptor alpha10 (CHRNA10). *Genomics* **73:**272–283.
Macklin KD, Maus AD, Pereira EF, Albuquerque EX, and Conti-Fine BM (1998) Human vascular endothelial cells express functional nicotinic acetylcholine receptors. *J Pharmacol Exp Ther* **287:**435–439.
Marks MJ, Pauly JR, Gross SD, Deneris ES, Hermans-Borgmeyer I, Heinemann SF, and Collins AC (1992) Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment. *J Neurosci* **9:**2765–2784.
Maslinski W, Laskowska-Bozek H, and Ryzewski J (1992) Nicotinic receptors of rat lymphocytes during adjuvant polyarthritis. *J Neurosci Res* **31:**336–340.
Maus AD, Pereira EF, Karachunski PI, Horton RM, Navaneetham D, Macklin K, Cortes WS, Albuquerque EX, and Conti-Fine BM (1998) Human and rodent bronchial epithelial cells express functional nicotinic acetylcholine receptors. *Mol Pharmacol* **54:**779–788.
Nguyen VT, Hall LL, Gallacher G, Ndoye A, Jolkovsky DL, Webber RJ, Buchli R, and Grando SA (2000) Choline acetyltransferase, acetylcholinesterase and nicotinic acetylcholine receptors of human gingival and esophageal epithelia. *J Dent Res* **79:**939–949.
Peng X, Gerzaniich V, Anand R, and Wang F (1997) Chronic nicotine treatment up-regulates 3 and 7 acetylcholine receptor subtypes expressed by the human neuroblastoma cell line SH-SY5Y. *Mol Pharmacol* **51:**776–784.
Quik M, Chan J, and Patrick J (1994) alpha-Bungarotoxin blocks the nicotinic receptor mediated increase in cell number in a neuroendocrine cell line. *Brain Res* **655:**161–167.
Rinner I, Kawashima K, and Schauenstein K (1998) Rat lymphocytes produce and secrete acetylcholine in dependence of differentiation and activation. *J Neuroimmunol* **81:**31–37.
Sato KZ, Fujii T, Watanabe Y, Yamada S, Ando T, Kazuko F, and Kawashima K (1999) Diversity of mRNA expression for muscarinic acetylcholine receptor subtypes and neuronal nicotinic acetylcholine receptor subunits in human mononuclear leukocytes and leukemic cell lines. *Neurosci Lett* **266:**17–20.
Savage SM, Donaldson LA, Cherian S, Chilukuri R, White VA, and Sopori ML (1991) Effects of cigarette smoke on the immune response. II. Chronic exposure to cigarette smoke inhibits surface immunoglobulin-mediated responses in B cells. *Toxicol App Pharmacol* **111:**523–529.
Sciamanna MA, Griesmann GE, Williams CL, and Lennon VA (1997) Nicotinic acetylcholine receptors of muscle and neuronal (alpha7) types coexpressed in a small cell lung carcinoma. *J Neurochem* **69:**2302–2311.
Sihver W, Gillberg PG, Svensson AL, and Nordberg A (1999) Autoradiographic comparison of [^{3}H](−)nicotine, [^{3}H]cytisine and [^{3}H]epibatidine binding in relation to vesicular acetylcholine transport sites in the temporal cortex in Alzheimer's disease. *Neuroscience* **94:**685–696.
Skok M, Lykhmus E, Bobrovnik S, Tzartos S, Tsouloufis T, Vanderesse R, Coutrot F, Thong Cung M, Marraud M, Krikorian D, et al. (2001) Structure of epitopes recognized by the antibodies to alpha(181–192) peptides of neuronal nicotinic acetylcholine receptors: extrapolation to the structure of acetylcholine-binding domain. *J Neuroimmunol* **121:**59–66.
Skok MV, Voitenko LP, Voitenko SV, Lykhmus EY, Kalashnik EN, Litvin TI, Tzartos SJ, and Skok VI (1999) Alpha subunit composition of nicotinic acetylcholine receptors in the rat autonomic ganglia neurons as determined with subunit-specific anti-alpha(181–192) peptide antibodies. *Neuroscience* **93:**1427–1436.
Sopori ML, Kozak W, Savage SM, Geng Y, and Kluger MJ (1998a) Nicotine-induced modulation of T Cell function. Implications for inflammation and infection. *Adv Exp Med Biol* **437:**279–289.
Sopori ML, Kozak W, Savage SM, Geng Y, Soszynski D, Kluger MJ, Perryman EK, and Snow GE (1998b) Effect of nicotine on the immune system: possible regulation of immune responses by central and peripheral mechanisms. *Psychoneuroendocrinology* **23:**189–204.
Tsetlin V (1999) Snake venom alpha-neurotoxins and other 'three-finger' proteins. *Eur J Biochem* **264:**281–286.
Utkin YN, Kukhtina VV, Kryukova EV, Chiodini F, Bertrand D, Methfessel C, and Tsetlin VI (2001) "Weak toxin" from *Naja kaouthia* is a nontoxic antagonist of α7 and muscle-type nicotinic acetylcholine receptors. *J Biol Chem* **276:**15810–15815.
Voitenko LP, Voitenko SV, Skok MV, Purnyn HE, and Skok VI (2001) Nicotinic acetylcholine receptor subtypes in rat superior cervical ganglion neurons as studied by sequential application of two alpha-subunit-specific antibodies. *Neurosci Lett* **303:**37–40.
Vulfius CA, Krasts IV, Utkin YN, and Tsetlin VI (2001) Nicotinic receptors in *Lymnaea stagnalis* neurons are blocked by alpha-neurotoxins from cobra venoms. *Neurosci Lett* **309:**189–192.
Yates SL, Bencherif M, Fluhler EN, and Lippiello PM (1995) Up-regulation of nicotinic acetylcholine receptors following chronic exposure of rats to mainstream cigarette smoke or alpha 4 beta 2 receptors to nicotine. *Biochem Pharmacol* **50:**2001–2008.

Address correspondence to: Dr. Marina V. Skok, Palladin Institute of Biochemistry, 9 Leontovicha Str., 01030 Kiev, Ukraine. E-mail: skok@biochem.kiev.ua