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Evidence for functional nicotinic receptors on pancreatic β cells

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Abstract

Epidemiological studies associate smoking with reduced insulin secretion. We hypothesized that nicotine could negatively affect pancreatic β-cell function. Acute or 48-hour exposures to nicotine (10^{-4} to 10^{-6} mol/L) moderately inhibited insulin release at basal (3.3 mmol/L) and/or elevated (27 mmol/L) glucose in rat and human islets. Acute exposure to nicotine (10^{-6} mol/L) inhibited tolbutamide (200 μmol/L)-induced insulin release by 41% ($P < .05$), but did not affect secretion induced by KCl (20 mmol/L) or 3-isobutyl-1-methylxanthine (1 mmol/L) (tested in rat islets). Specific binding of [^{3}H]nicotine was demonstrated in rat islets and in a β-cell line of rat origin, INS-1. Such binding was enhanced by 48 hours of coculture with nicotine (10^{-7} mol/L). Expression of mRNA for the nicotinic receptor subunits α2, α3, α4, α5, α7, and β2 was detected in INS-1 cells by reverse transcriptase polymerase chain reaction. Acute exposure to cytisine (10^{-6} mol/L), an agonist of α4, β2 subunits, partially inhibited tolbutamide-induced insulin release. Specific binding of α bungarotoxin (10^{-10} mol/L), an antagonist of the α7 subunit, could be demonstrated in INS-1 cells, and culture with α bungarotoxin modestly increased insulin release in postculture incubations at basal and elevated glucose, $P < .05$. Our data indicate that functional nicotinic receptors are present in pancreatic islets and β cells.

1. Introduction

Cigarette smoking is a risk factor for type 2 diabetes [1,2]. An epidemiological study in Stockholm, Sweden, in addition, showed that snuffing was associated with a risk for type 2 diabetes [3]. Nicotine is an obvious common constituent of smoking and snuffing. Nicotine would therefore be a prime "suspect" for the diabetogenic effect of tobacco use.

Previous evidence links a diabetogenic effect of smoking to insulin resistance [4-6], and in vivo studies provide evidence that nicotine can induce insulin resistance [7,8]. Such effects may be secondary to increased sympathetic tone and/or to release of insulin-antagonistic hormones such as cortisol [9]. However, recent evidence suggests that the risk associated with nicotine exposure may also involve insulin secretion. Smoking and snuffing were thus associated with a low insulin response in the Stockholm study [3]. Similar results were obtained for smoking in another population-based study [10].

Long-term administration of nicotine in rats results in reduction in plasma insulin levels [11,12]. However, because nicotine administration reduces body weight and plasma glucose [11,12], one cannot deduce from these studies a direct effect of nicotine on β cells. A previous study of in vitro effects of nicotine on β-cell function in rat pancreatic islets tested for acute effects in rat pancreatic islets of nicotine as well as of the nicotinic antagonist α bungarotoxin [13]. There was no effect of nicotine but a stimulatory one by α bungarotoxin, and the latter effect was antagonized by nicotine. Although suggestive of functional nicotinic receptors, the previous study did not directly test for presence of nicotinic receptors or for longer term effects of nicotine. Furthermore, effects of nicotine in vitro on human β cells have, to our knowledge, not been tested. Against this background, we here tested for functional effects by acute and longer term exposure to nicotine in rat and human pancreatic islets of Langerhans as well as for the presence of nicotinic receptors in rat islets and in a clonal β-cell line of rat origin (INS-1).

2. Materials and methods

2.1. Materials

Nicotine (free base), tolbutamide, potassium chloride (KCl), and 3-isobutyl-1-methylxanthine (IBMX) were from

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Sigma Co (St Louis, Mo). Cytisine was from Tocris Cookson Inc (Missouri). Collagenase was from Boehringer Mannheim (Mannheim, Germany). [^{3}H]nicotine (specific activity, 85 or 50 Ci/mmol) (3-[^{125}I]iodotyrosyl) α bungarotoxin (specific activity, 2000 Ci/mmol) were from American Radiolabeled Chemicals, Inc (St Louis).

2.2. Isolation and culture of pancreatic islets

2.2.1. Rat islets

Male Sprague-Dawley rats were obtained from Scanbur Co (Stockholm, Sweden). At the time of experiments, the rats weighed between 250 and 350 g. They had free access to tap water and a standard pellet diet. They were exposed to a 12-hour light (6:00 AM to 6:00 PM) and 12-hour dark cycle. The local ethical committee for research on animals approved the experimental protocols. Pancreatic islets were isolated by collagenase digestion as described [14]. Islets visibly free of exocrine tissue were selected under a stereo microscope. Some islets were used for acute experiments. Other islets were cultured in RPMI-1640 medium containing glucose (11 mmol/L) supplemented with 10% heat-inactivated fetal calf serum and 100 U/mL penicillin. Culture was carried out at 37°C in a humidified (5% CO_2, 95% air) atmosphere for 48 hours with the additions dictated by the experimental protocols.

2.2.2. Human islets

Pancreases were obtained from cadaver donors after consent from an organ donor registry or from relatives. The age of donors varied between 41 and 62 years and body mass index between 20 and 42 kg/m^2. The islets were isolated at the Division of Clinical Immunology at the University of Uppsala as previously described in detail [15]. The purity of the islet preparations in this study varied from 40% to 80%. The islets were sent to our laboratory in culture medium CMRL 1066, arriving on the same day. Islets were then cultured in RPMI-1640 medium containing glucose (5.5 mmol/L) supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 U/mL), and streptomycin (0.1 mg/mL).

2.3. Culture of INS-1 cells

INS-1 cells (passage number between 50 and 60) were a kind gift from Dr Claes Wollheim, Geneva, Switzerland. The cells were grown in monolayer cultures as described previously [16]. The medium used was RPMI-1640, supplemented with HEPES (10 mmol/L), 10% heat-inactivated fetal calf serum, glutamine (2 mmol/L), sodium pyruvate (1 mmol/L), 2-mercaptoethanol (50 μmol/L), penicillin (100 IU/mL), and streptomycin (100 μg/mL). Cells were cultured at 37°C in a humidified (5% CO_2, 95% air) atmosphere. The cells were seeded 7 days before use in 75 cm^2 flasks at a density of 2.0×10^6 cells per flask. The cells were grown to 60% to 80% confluence during 4 to 5 days before being used in experimental protocols.

2.4. Insulin secretion and islet insulin contents

Islets were preincubated in Krebs-Ringer bicarbonate (KRB) medium for 30 minutes. The KRB medium had the following composition (in mmol/L): Na^+ 143, K^+ 5.8, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 124.1, PO_4^{3-} 1.2, SO_4^{3-} 1.2, CO_3^{2-} 25. The medium was supplemented with HEPES (10 mmol/L), 0.2% bovine serum albumin, and glucose (3.3 mmol/L). The pH was 7.4. Final 60 minutes of batch-type incubations were carried out in triplicates, each tube containing 3 islets in 300 μL KRB and 3.3 or 27 mmol of glucose per liter. Aliquots of the incubation media were removed for assay of insulin. Islets that had been exposed to basal glucose (3.3 mmol/L) in final incubation were retrieved and transferred into 200 μL of acid-ethanol (0.18 mmol of HCl per liter in 95% ethanol) for the determination of islet insulin content. Insulin was measured by radioimmunoassay as described [17] using dextran 170–coated charcoal to separate antibody-bound insulin from free insulin. INS-1 cells were preincubated with KRB medium with basal glucose for 4 hours. Final batch type incubations were carried out for 60 minutes in 300 μL KRB and 3.3 or 27 mmol of glucose per liter.

2.5. MTT assay

The viability of INS-1 cells was measured by of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described [18].

2.6. ATP content

Batches of 10 islets or 10^5 INS-1 cells were transferred to Eppendorf tubes containing 40 μL of NaOH solution (0.04 mol of NaOH per liter, 2 mmol of EDTA per liter) and stored at −80°C. Before assay, 60 μL of lysis reagent was added to islets or cells. The lysate was passed through a 23-gauge needle and vortexed. Adenosine triphosphate (ATP) was assessed luminometrically by the luciferin-lucifarese reaction, using a commercially available assay (Boehringer).

2.7. Binding of [^{3}H]nicotine and [^{121}I]α bungarotoxin

Fifty microliters of cold KRB containing [^{3}H]nicotine (0.5 nmol/L) were added to microcentrifugation tubes, each containing 150 μL of a mixture of dibutyl- and dinonylphthalate (10:3 v/v) layered above 50 μL of urea (6 mol/L) as previously described [19]. Then, 10 rat islets or 1.0×10^5 INS-1 cells in 50 μL of cold KRB were added to the nicotine-containing KRB of each tube. If not otherwise mentioned, the tubes were incubated without shaking on ice for 20 minutes, using air as gas phase. Incubations were terminated by centrifugation for 30 seconds in a microcentrifuge. The tips of the tubes, containing islets or cells, were cut off through the oil layer, 5 mL of scintillation fluid added and the ^{3}H radioactivity counted. Total binding was calculated as the radioactivity remaining with the islets or cells. Unspecific binding of [^{3}H]nicotine was assessed from the ^{3}H radioactivity

Table 1
Primers used for amplification of rat nAChRs

Subunit	Nucleotide sequence	T_m (°C)	Size (bp)	Accession number
α2	5′ -AAAGGGAGGAGACAGAGGAAGAGGCAGACGGTCAGCAATGTAGTGTA-3′	55	222	L10077
α3	5′ -AACCTGCTCCCCAGGGTCATGTTTCACTTTGGATGGCTTCTTTGATTT-3′	55	300	L31621
α4	5′ -GTCAAAGACAACTGCCGGAGACTTTGATGAGCATTGGAGCCCCACTGC-3′	60	300	L31620
α5	5′ -AGTGGGGCTGGACCTAAATCTCGCAAAAAGCCCTAAAGTCCCAATGA-3′	60	196	J05231
α6	5′ -TGCCGCCACTGCCATAATTCTACCTCCTTGTTCATTGTG-3′	55	172	NM057184
α7	5′ -AAGAGGCCCGGAGAGGACAACGCCACATACGACCCCAGAG-3′	60	190	S53897
β2	5′ -AGGGCGAGGCGGTTTTCTTGCGTACGCCATCCACTGCT-3′	55	180	L31622
β3	5′ -TGGGTGAAGAGGCTGTTATCGCTGGCGGGAGTCTGTT-3′	60	157	J04636
β4	5′ -CATGGCATCCTGGGTCAAGCTGGGGAGGCCTGCTGTGT-3′	60	258	U42976

The table shows nucleotide sequences, annealing temperature, size of amplified fragments and accession number obtained from GenBank database.

remaining in islets or INS-1 cells, which had been incubated in the copresence of unlabeled nicotine (10^{-4} mol/L).

Binding of [^{121}I]α bungarotoxin (5.0 mmol/L) was measured by the same procedure as for [^{3}H]nicotine, except for a longer time of exposure (overnight). Unspecific binding was assessed from the radioactivity remaining in INS-1 cells incubated in the copresence of unlabeled α bungarotoxin (10^{-5} mol/L).

2.8. RNA extraction and RT-PCR

Total RNA from INS-1 cells and rat brain was isolated by using Micro-to-Midi Total RNA isolation kit (Invitrogen). Traces of contaminating genomic DNA were removed in all RNA samples by treatment with DNase. Reverse transcription was performed from 1 μg total RNA together with $MgCl_2$ (5 mmol/L), 1 × polymerase chain reaction (PCR) buffer, nucleotide triphosphate binding protein (NTP) (2.5 mmol/L), 20 U RNase inhibitor, random hexamers (2.5 μmol/L), 50 U Moloney-murine leukemia virus (M-MLV) reverse transcriptase, and sterile water in a total volume of 20 μL at 42°C for 30 minutes, 95°C for 5 minutes, and then cooled. The PCR was carried out in a reaction volume of 50 μL using a master mix containing $MgCl_2$ (2 mmol/L) (1 mmol/L for b2), 1 × PCR buffer, dNTPs (0.2 mmol/L), 0.5 μmol/L each of the forward and reverse primers, 0.5 μL Taq DNA polymerase (Promega), and 4 μL of reverse transcriptase (RT) reaction mixture. After an initial denaturation at 94°C for 3 minutes, 30 cycles were performed consisting of 94°C for 30 seconds, 55°C (α2 α3 α4, α6, β2 primers) or 60°C (α5, α7, β3, and β4 primers) for 30 seconds, and 72°C for 30 seconds followed by a final extension at 72°C for 5 minutes. The nucleotide sequence of primers and sizes of PCR product are shown in Table 1. Aliquots of the DNA samples were examined by electrophoresis on 1.5% agarose gels containing 0.5 μg/mL ethidium bromide. Sizes of the PCR products were estimated from the migration of a DNA size marker [100 base pair (bp) DNA ladder, Invitrogen]. Control reactions were carried out with samples without reverse transcription to cDNA to ensure that the detected product was not the result of genomic DNA contamination. Products from RT-PCR with total RNA extracted from rat brain were used as positive controls.

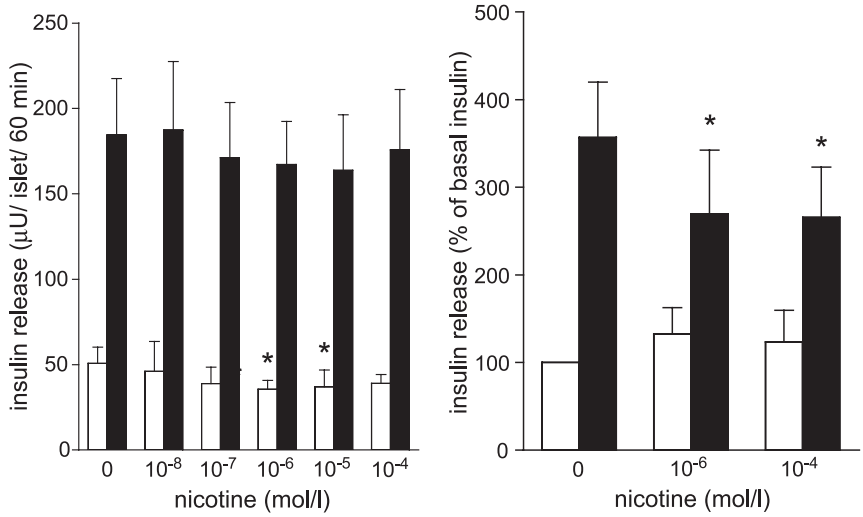

Fig. 1. Effects of acute exposure to nicotine on insulin release in the presence of 3.3 (unfilled bars) or 27 mmol/L (filled bars) of glucose. Acute effects were tested in 60-minute incubations on rat (left graph) and human (right graph) islets. Results are means ± SEM of 6 to 8 experiments. Asterisk indicates $P < .05$ vs no nicotine.

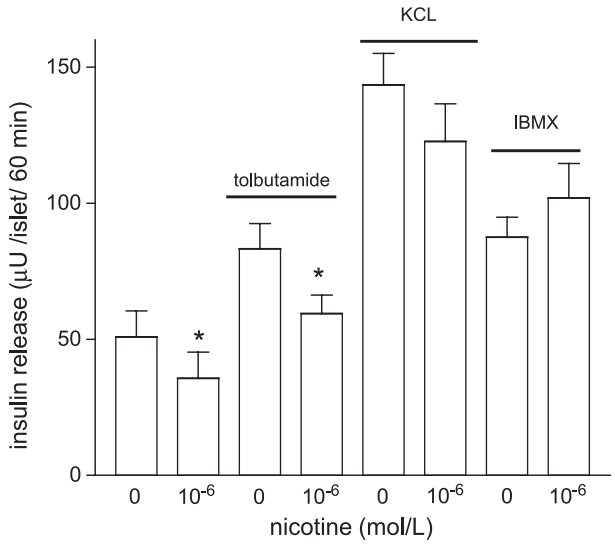

Fig. 2. Effects of nicotine on tolbutamide-, on KCl-, and on IBMX-induced insulin release from rat pancreatic islets. Acute effects were tested in 60-minute incubations in the presence of glucose (3.3 mmol/L). Results are means ± SEM of 6 to 7 experiments. Asterisk indicates $P < .05$ vs no nicotine.

2.9. Statistical analysis

All results are expressed as means ± SEM of the number of experiments indicated in table and figure legends. Insulin data from human pancreatic islets were expressed as percent changes because of interindividual differences between pancreas preparations. Thus, the percent release at glucose (27 mmol/L) was calculated from the basal release obtained for each of the concentrations of nicotine. Significance testing was carried out by paired Student *t* test. A *P* value of <.05 was considered significant.

3. Results

3.1. Acute exposure to nicotine exerts inhibitory effects on insulin secretion

In rat islets, a 60-minute exposure to nicotine exerted moderate inhibitory effects, which were, during some conditions, significant. Thus, 10^{-7} to 10^{-4} mol/L concentration of nicotine in the presence of 3.3 mmol/L concentration of glucose either tended to inhibit ($P < .1$) or significantly ($P < .05$) inhibited insulin release (Fig. 1). In human islets, 10^{-6} and 10^{-4} mol/L concentrations of nicotine significantly inhibited the insulin response to 27 mmol/L concentration of glucose (Fig. 1).

In INS-1 cells, exposure to nicotine (10^{-7} mol/L) inhibited insulin release in the presence of basal glucose (3.3 mmol/L) (no nicotine: 75 ± 5 μU/10^5 cells; nicotine: 56 ± 5 μU/10^5 cell, n = 9, $P < .05$).

Acute exposure of rat islets to nicotine (10^{-6} mol/L) inhibited insulin release stimulated by tolbutamide (200 μmol/L) by 41% ($P < .05$). Secretion induced by KCl (20 mmol/L) or IBMX (1 mmol/L) was not affected (Fig. 2).

3.2. Effects of a 48-hour exposure to nicotine on insulin secretion and islet insulin contents

In rat pancreatic islets, coculture with nicotine (10^{-4} mol/L) for 48 hours inhibited insulin release in final incubations (performed in the absence of nicotine) during the presence of glucose (3.3 or 27 mmol/L) (Fig. 3). Islet contents of insulin were slightly decreased (−18 ± 6%, n = 7, $P < .05$). In human islets, coculture with nicotine (10^{-6} mol/L or 10^{-4} mol/L) significantly diminished glucose-induced insulin release (Fig. 3). Islet contents of insulin were not significantly affected after culture with nicotine (10^{-4} mol/L) (−14 ± 9%, n = 4).

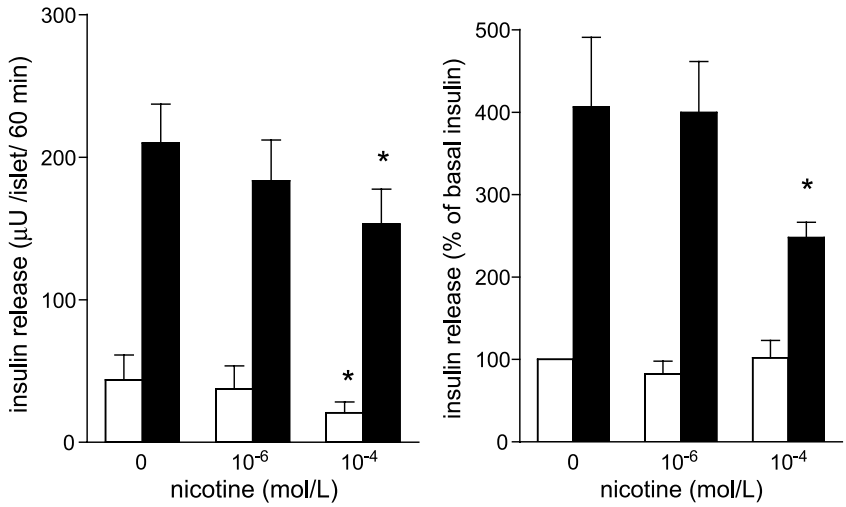

Fig. 3. Effects of a 48-hour exposure to nicotine on insulin secretion from rat (left graph) or human (right graph) islets. Islets were cultured in the presence or absence of nicotine after which 60-minute final incubations were performed (without nicotine) in the presence of 3.3 mmol/L (unfilled bars) or 27 mmol/L concentration of glucose (filled bars). Results are means ± SEM of 5 to 8 experiments. Asterisk indicates $P < .05$ vs no nicotine.

3.3. Nicotine during culture does not affect ATP contents or cell viability

Coculture with 10^{-7}, 10^{-6}, or 10^{-4} mol/L concentration of nicotine for 48 hours did not influence ATP content in islets (no addition: 17 ± 3 pmol/islet, exposure to 10^{-7} mol/L; 15 ± 4 pmol/islet, to 10^{-6} mol/L; 14 ± 3 pmol/islet, to 10^{-4} mol/L; 16 ± 3 pmol/islet, n = 4). Nicotine at a concentration of 10^{-7}, 10^{-6}, or 10^{-4} mol/L was without effect on ATP contents also in INS-1 cells (no addition: 7.9 ± 0.2 nmol/10^5 cells, exposure to 10^{-7} mol/L; 7.5 ± 0.3 nmol/10^5 cell, to 10^{-6} mol/L; 8.0 ± 0.3 nmol/10^5 cell, to 10^{-4} mol/L; 7.6 ± 0.2 nmol/10^5 cell, n = 4). Culture with nicotine in concentrations varying from 10^{-8} to 10^{-4} mol/L did not affect viability of INS-1 cells as tested by the MTT assay (data not shown).

3.4. [^{3}H]nicotine binds specifically to rat islets and INS-1 cells

To test for the presence of nicotinic receptors in islets, we used [^{3}H]nicotine as ligand in a concentration 0.5 nmol/L. Unspecific binding was assessed from the ^{3}H radioactivity remaining in the copresence of unlabeled nicotine (10^{-4} mol/L).

Specific binding in rat islets was detected [48 ± 3 disintegrations per minute (dpm)/islet, n = 4, $P < .05$]. To investigate binding separately on β cells, we tested for binding of [^{3}H]nicotine in INS-1 cells. Twenty-minute incubations of INS-1 cell performed on ice were found to be optimal for these binding studies (data not shown). Specific binding was detected (79 ± 33 dpm/islet, n = 6, $P < .05$).

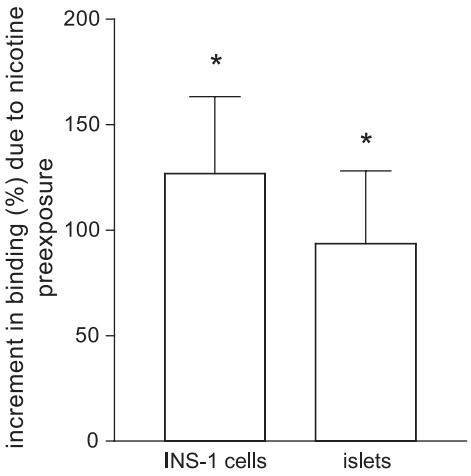

Fig. 4. Effects of nicotine preexposure on subsequent binding of [^{3}H]nicotine. Rat islets or INS-1 cells were cultured for 48 hours in the presence of nicotine (10^{-7} mol/L). Subsequent binding was assessed after washing (INS-1 cells, left bar) or after 2 hours of incubation without nicotine (islets, right bar). Results are expressed as percent of results obtained without preexposure to nicotine. Means ± SEM of 4 to 5 experiments. Asterisk indicates $P < .05$ vs no preexposure to nicotine.

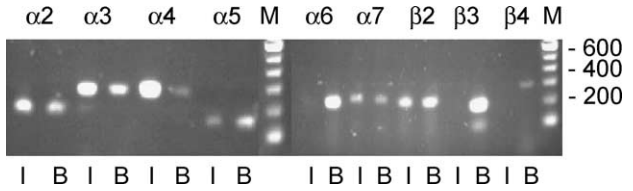

Fig. 5. Ethidium bromide stained agarose gel showing PCR products amplified from INS-1 cells (I) and rat brain (B) with primers specific for 9 neuronal acetylcholine receptor (nAChR) subunits. One microgram of total RNA from rat brain and INS-1 cells were used as template for cDNA synthesis and 4 μL cDNA was then amplified for 30 cycles by PCR. A DNA size marker (100 bp DNA ladder) is shown in lane 9 and 20. mRNA for α2 was amplified as 222 bp, α3 and α4 as 300 bp, α5 as 196 bp, α6 as 172 bp, α7 as 190 bp, β2 as 180 bp, β3 as 157 bp, and β4 as 257 bp.

3.5. Previous exposure to nicotine (10^{-7} mol/L) enhances binding of nicotine

We exposed INS-1 cells to nicotine (10^{-7} mol/L) for 48 hours during culture conditions after which [^{3}H]nicotine binding was assessed. The concentration of nicotine chosen during culture is similar to circulating concentrations in heavy smokers [20]. The preexposure to nicotine significantly increased specific binding as tested with [^{3}H]nicotine (0.1-0.5 nmol/L). In the presence of [^{3}H]nicotine (0.5 nmol/L), the radioactivity associated with specific binding increased more than 2-fold (Fig. 4).

Similar experiments were carried out in rat islets, except that islets precultured with or without nicotine were further cultured without nicotine for 2 hours (to ensure complete wash-out of intra-islet nicotine) before the binding of [^{3}H]nicotine was tested. Under these conditions, coculture of islets with nicotine (10^{-7} mol/L) for 48 hours increased specific binding of [^{3}H]nicotine (0.5 nmol/L) added post-culture by 2-fold (Fig. 4).

3.6. Subunits of nAChR mRNA are expressed in INS-1 cells

Reverse transcriptase polymerase chain reaction experiments were performed to determine which subunits of the nAChRs were expressed in INS-1 cells. The effectiveness of RT-PCR was investigated using total RNA from rat brain as positive controls. Transcripts for all 9 investigated nAChR subunits were detected in rat brain ensuring that the primers were working successfully (Fig. 5). Experiments using total RNA from INS-1 cells showed the presence of α2, α3, α4, α5, α7, and β2 nAChR subunits, whereas the β3 and β4 subunits could not be detected in INS-1 cells under conditions in which these transcripts were observed in rat brain (Fig. 5). The actual size of the PCR products was found to be close to the size expected on the basis of primer positions. Control reactions were carried out where samples not reverse transcribed to cDNA were amplified to ensure that the detected product was not the result of genomic DNA contamination. No products were detected from these negative controls.

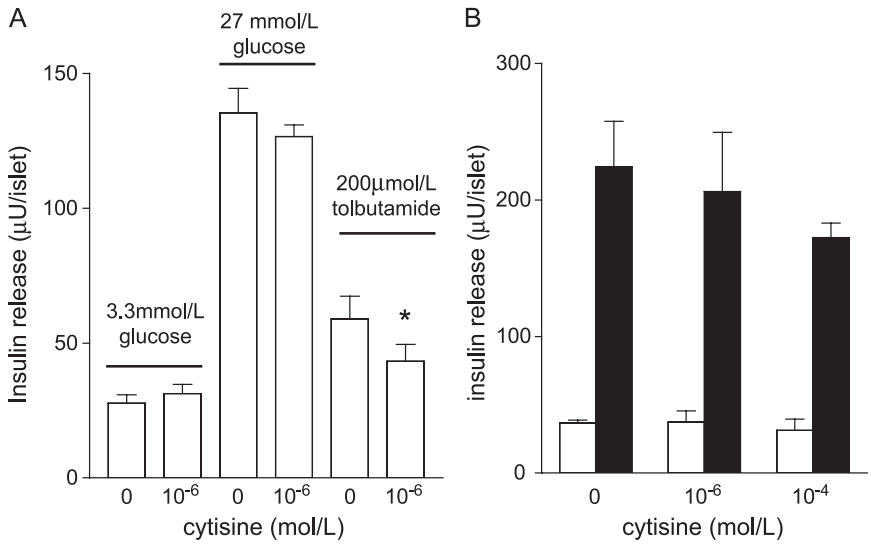

Fig. 6. Effects of cytisine on insulin release. A, Left graph depicts results obtained during acute exposure to cytisine in the presence of 3.3 or 27 mmol/L concentration of glucose or 3.3 mmol/L concentration of glucose together with 200 μmol/L concentration of tolbutamide. B, Right graph depicts effects of a 48-hour exposure to cytisine on postculture insulin secretion from rat islets in the presence of 3.3 (unfilled bar) or 27 mmol/L (filled bar) concentration of glucose. Results are means ± SEM of 3 to 5 experiments. Asterisk indicates $P < .05$ vs no cytisine.

3.7. Effect of cytisine on insulin secretion in rat islets

Cytisine is an agonist of α4, β2 subunits of nicotinic receptors [20]. In light of our findings on mRNA for nicotinic receptor subunits, it was therefore of interest to test for functional effects of cytisine. A 60-minute exposure to 10^{-6} cytisine moderately inhibited tolbutamide (200 μmol/L) -induced insulin secretion in rat islets (Fig. 6A). Exposure during culture to cytisine (10^{-4} or 10^{-6} mol/L) for 48 hours on the other hand did not significantly affect postculture insulin release (Fig. 6B) or insulin contents (results not shown). Acute or 48-hour exposures to cytisine failed to affect insulin secretion from INS-1 cells (results not shown).

3.8. Specific binding of α bungarotoxin in INS-1 cells

α Bungarotoxin is an antagonist of nicotinic α7 subunits [21]. Because we detected mRNA for this subunit in INS-1 cells, it was of interest to test for binding of α bungarotoxin. Unspecific binding was assessed from the radioactivity remaining in INS-1 cells incubated in the copresence of unlabeled α bungarotoxin (10^{-5} mol/L).

Specific binding was detected (21 ± 4 dpm/10^5 cell, n = 5). Binding tended to increase after culture with nicotine (10^{-7} mol/L) for 48 hours (91 ± 36 dpm/10^5 cell, n = 5, $P = .11$).

3.9. Effect of coculture with α bungarotoxin for 48 hours on insulin secretion

INS-1 cells were cultured with or without α bungarotoxin (10^{-10} mol/L) for 48 hours. Culture with α bungarotoxin modestly but significantly increased insulin release in postculture incubations in the presence of glucose with a concentration of 3.3 mmol/L (16 ± 2%, n = 4, $P < .05$) and glucose with a concentration of 27 mmol/L (20 ± 5%, n = 4, $P < .05$). Culturing islets during the same conditions failed to affect postculture insulin secretion (results not shown).

4. Discussion

This study demonstrates that nicotine can exert negative effects on insulin secretion both acutely and as a result of longer time exposure to the alkaloid. The effects observed were generally modest or moderate. However, they could still be of clinical significance.

Thus, the concentrations of nicotine used appear relevant in relation to tobacco use. It has been reported that the mean plasma nicotine level in average smokers varies from 7.4×10^{-9} to 1.7×10^{-7} mol/L and that the peak plasma concentration of nicotine after smoking could range from 3.0×10^{-8} to 6.2×10^{-7} mol/L [20]. (The concentration of nicotine in pancreas is, to our knowledge, not known.) Thus, the concentrations of nicotine that produce effects in our experiments are not vastly above those that can be encountered in plasma at least in heavy smokers. Also, it is reasonable to suggest that lower concentrations of nicotine than those having effects during a 48-hour exposure time could produce effects during longer exposure times (decades or more in smokers). Furthermore, the enhanced binding to nicotinic receptors that we observe as a result of previous nicotine exposure (discussed further below) could potentially alter the sensitivity for inhibition by nicotine, although the present study did not provide evidence to this point.

Intra-islet ganglionic activation of postganglionic adrenergic signals could possibly relay negative effects on β-cell function. However, our results indicate that the effects that we observe are due to nicotine interacting with

receptors on β cells. Thus, our binding data provide direct evidence, to our knowledge for the first time, for the presence of nicotinic receptors both in pancreatic islets and INS-1 cells. In addition, mRNAs for a number of subunits known to be present in nicotinic acetylcholine receptors of neuronal type (review Ref. [21]) could be demonstrated in INS-1 cells. Whether all these mRNA expressions correspond to the presence of receptor proteins is not clear. However, binding of α bungarotoxin to INS-1 cells indicates the presence of the α7 subunit protein in these cells, and the enhancing effect of this nicotinic receptor antagonist on insulin secretion, albeit modest, indicates functionality. Furthermore, the effects in rat islets on tolbutamide-induced insulin secretion by cytisine, which is a ligand of the α4, β2 subunits, indicate functionality of these subunits. (Such effects could not be tested in INS-1 cells due to poor stimulation by tolbutamide in these cells, data not shown).

Our results disagree with conventional knowledge, which limits the presence of nicotinic receptors to innervation [22]. However, also other non-neuronal cell types have in recent years been found to possess nicotinic receptors, such as keratinocytes [23] and cells of the immune system [24].

The increased receptor binding by long-term nicotine that we find is similar to characteristics of other nicotinic receptors. The same effect of previous exposure to nicotine has been demonstrated in keratinocytes [23] and may underlie increased binding of nicotine to peripheral blood cells in smokers vs nonsmokers [25]. Nicotine exposure also increases nicotine binding in neurons possessing "classical" nicotinic receptors [26]. It should be stressed that enhancement of nicotine binding does not necessarily translate into alteration of functional responses.

In general, nicotinic receptors provide channels by which ionic fluxes in the cell membrane can be regulated. The nicotine effects on tolbutamide as well as on basal and glucose-stimulated insulin release are at least compatible with effects on ion fluxes in β cells. Further studies are obviously needed to directly test for nicotine interactions with ion fluxes in β cells.

Incidentally, our data suggest effects on glucose-induced insulin secretion in human islets that are not present in rat islets. Further studies are underway to investigate this possible difference between species.

Our measurements of ATP and MTT did not indicate nonspecific toxic effects of nicotine in the present experiments. However, our results do not rule out non–receptor-mediated effects of tobacco use on β cells. Nicotine could thus produce oxidative stress that may inhibit β-cell function, and Wetsher et al [27] reported that nicotine induced oxidative stress in rat pancreatic islets. As to diabetogenicity, one should also acknowledge the likelihood of the insulin resistance effects that were previously mentioned.

In summary, our in vitro study demonstrates that acute and longer term exposure to nicotine can inhibit some aspects of insulin release from β cells. Such effects may be mediated by interaction with nicotinic receptors on β cells, the presence of which is evidenced here for the first time.

Acknowledgments

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