Nicotine-Induced Inhibition of Neuronal Phospholipase A2\(^1\)

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ABSTRACT
A protective effect of nicotine against glutamate-induced neurotoxicity has previously been reported in cultured striatal and cortical neurons. The aim of this study was to investigate whether nicotine also inhibits glutamate-evoked arachidonic acid release from cultured striatal neurons. (\(-\))-Nicotine selectively inhibited the release of \(^{3}\)H-arachidonic acid induced by the joint stimulation of \(\alpha\)-amino-3-isoxazol-5-propionic acid and metabotropic receptors, whereas the response evoked by the sole activation of \(N\)-methyl-\(\alpha\)-aspartate receptors remained unchanged. The inhibitory effect of (\(-\))-nicotine was not mediated by nicotinic receptors because it was neither reproduced by acetylcholine (in the presence of atropine) or 1,1-dimethyl-4-phenyl piperazinium, nor reversed by dihydro-\(\beta\)-erythroidine or hexamethonium, two central nicotinic receptor antagonists.

Using primary cultures of neurons originating from either the mouse striatum or the rat cerebral cortex, we and others have recently demonstrated a protective effect of (\(-\))-nicotine and other nicotinic receptor agonists against glutamate neurotoxicity (Marin et al., 1994; Akaike et al., 1994). Although the mechanism involved in the glutamate-induced neuronal death is still debated, a role of nitric oxide and superoxide anions, formed under NMDA receptor stimulation, has been suggested (Lafon-Cazal et al., 1993; Hewett et al., 1994; for review see Coyle and Puttfarcken, 1993). Superoxide anions could originate from the \(\text{Ca}^{2+}\)-dependent uncoupling of neuronal mitochondrial electron transport (Reynolds and Hastings, 1995; Dugan et al., 1995) and the metabolism of arachidonic acid (Kukreja et al., 1986; Chan et al., 1988; Lafon-Cazal et al., 1993). In addition, arachidonic acid could contribute directly to glutamate-induced neuronal death by inhibiting the uptake of this excitatory aminoacid into neurons or neighboring astrocytes (Chan et al., 1983; Barbour et al., 1989; Volterra et al., 1992). Therefore, the aim of our study was to investigate whether the neuroprotective effects of nicotinic receptor agonists result from an inhibition of the glutamate-induced release of arachidonic acid.

In mouse cultured striatal neurons, arachidonic acid is released after glutamate exposure by two distinct and additive mechanisms: the first one involves the activation of NMDA receptors, although the second results from the joint activation of AMPA and metabotropic receptors (Dumuis et al., 1988, 1990, 1993). Therefore, we have examined the effects of (\(-\))-nicotine and its congeners on the release of this unsaturated fatty acid mediated by either NMDA receptors or the coactivation of AMPA and metabotropic receptors. As it will be shown, (\(-\))-nicotine only decreases the response resulting from the joint stimulation of AMPA and metabotropic receptors. However, nicotinic receptors did not appear to be involved in this response. Complementary experiments were thus performed to look for a possible modulation by (\(-\))-nicotine of the AMPA-evoked currents and the formation of inositol phosphates mediated by metabotropic receptors. The effect of (\(-\))-nicotine on the activity of cytosolic and membrane-bound PLA2 in striatal neurons was also investigated.

ABBREVIATIONS: trans-ACPD, (1S-3R)-(1-amino)cyclopentane-1,3-dicarboxylic acid; AMPA, \(\alpha\)-amino-3-isoxazol-5-propionic acid; DHBE, dihydro-\(\beta\)-erythroidine; DMPP, 1,1-dimethyl-4-phenyl piperazinium; GPT, glutamate-pyruvate transaminase; MK-801, (\(+\))-5-methyl-10,11-dihydro-5H-dibenzo[\(a\,d\)]-cyclopehten-5,10-imine hydrogen maleate; NMDA, \(N\)-methyl-\(\alpha\)-aspartate; p-BPB, \(p\)-bromophenacyl bromide; PLA2, phospholipase A2; PPC, 1,2-bis-(1-pyrenecanoyl)-sn-glycero-3-phosphocholine.
Methods

Chemicals. Swiss mice were obtained from Iffa Credo (Lyon, France), culture media from Gibco (Paris, France) and fetal calf serum from Dartch (Brumath, France). [5,6,8,9,11,12,14,15-3H]-arachidonic acid (209 Ci/mmole) and myo-[2-3H]-inositol (17.1 Ci/mmole) were purchased from Amersham (Les Ulis, France), PPC from Molecular Probe (Eugene, OR). DHBE was kindly provided by Dr. C. Vidal (Institut Pasteur, Paris, France), Quiescal and transy-ACPD were purchased from Toxis, Neuramin (Bristol, UK). All other chemicals and reagents were purchased from Sigma (Saint Quentin Fallavier, France).

Primary culture of striatal neurons. Primary neuronal cultures were prepared as previously described (Weiss et al., 1986). Briefly, striata were removed from 14- to 15-day old Swiss mouse embryos and dissociated cells were seeded on either 12-well (106 cells/well containing 1 ml of culture medium) or 90-mm culture dishes (2 × 105 cells per dish containing 15 ml of culture medium). Culture dishes were previously coated successively with poly-L-ornithine (15 μg/ml, MW = 40,000) and culture medium containing 10% fetal calf serum. The culture medium included a 1:1 mixture of Dulbecco’s modified Eagle’s medium and F12 nutrient, supplemented with 1 g-glucose, 2 mM NaHCO3, 13 mM HEPES buffer (5 mM, pH 7.4), penicillin-streptomycin (5 IU/ml), 1 mM Na2SeO3 (30 nM). Cells were then main-

tained for 11 to 13 days at 37°C in a humidified atmosphere containing 8% CO2 without medium change. Under these conditions, the cultures were shown to be highly enriched in neurons thanks to immunocytochemistry experiments using anti-microtubule-associated protein 2 monoclonal antibodies (IgG1, Biomakor, Rehovot, Israel). In addition, fewer than 7% of the cells exhibited immunoreactivity with a rabbit antibody raised against glial fibrillary acid protein (Dakopatts, Glostrup, Denmark) (data not shown).

For patch clamp recordings, cells were seeded on 35-mm culture dishes (1.25 × 105 cells per dish) previously coated successively with 7.5 μg/ml poly-L-ornithine and 1 μg/ml laminin, in 1.5 ml culture medium composed of a 1:1 mixture of minimal essential medium and F12 nutrient, supplemented with 33 mM d-glucose, 2 mM L-glutamine, 1 mM Na pyruvate, 25 μg/ml insulin and 5% fetal calf serum. After 4 days in vitro, 0.5 μM cytisine-aranibofuransoside was added to the culture medium to prevent the proliferation of glial cells.

Measurement of [3H]-arachidonic acid release. Neurons, grown for 11 to 13 days in 12-well culture dishes were incubated for 18 hr in the presence of [3H]-arachidonic acid (1 μCi/ml) added directly to the culture medium. Under these conditions, cells incorporated 85 to 90% of the radioactivity. To remove nonincorporated [3H]-arachidonic acid, cells were subjected to three brief washes with 1 ml Krebs bicarbonate buffer containing 124 mM NaCl, 3.5 mM KCl, 1.25 mM KH2PO4, 26.3 mM NaHCO3, 1.2 mM CaCl2, 10 mM glucose, 1 mg/ml fatty acid-free bovine serum albumin, previously equilibrated with 95% O2/5% CO2 and pressurred at 37°C. After a 10-min preincubation period, cultures were exposed to drugs for 15 min in this medium. Experiments were performed in the absence of external Mg++ to eliminate the voltage-dependent Mg++ block of NMDA receptors (Nowak et al., 1984). At the end of the incubation period, the medium was collected and samples were centrifuged at 100,000 g for 10 min to remove dislodged cells and radioactivity in the supernatants was measured by β-scintillation counting. Released radioactivity, representing 1 to 3% of total incorporated radioactivity, was assumed to be essentially [3H]-arachidonic acid since this unsaturated fatty acid was previously shown to be poorly metabo-

lized in striatal neurons cultured in the same conditions (Oomagari et al., 1991).

Determination of phospholipase A2 activity. Spontaneous PLA2 activity was estimated in subcellular fractions prepared from striatal neurons using the fluorogenic substrate PPC according to the method described by Piomelli and Greenard (1991). Neurons, grown in 90-mm culture dishes were washed twice in phosphate buffered saline supplemented with 33 mM glucose (PBS glucose) and homogenized in 0.5 ml of an ice-cold hypotonic buffer containing 1 mM EDTA, 1 mM EGTA, 10 mM sodium pyrophosphate, 10 mM HEPES (pH 7.4) and 100 μM phenylmethylsulfonyl fluoride. Soluble and membrane fractions were obtained by centrifuging the cell lysate for 15 min at 40,000 × g. PLA2 assay was performed in a stirred quartz cuvette maintained at 37°C using a F2,000 Hitachi fluorim-

eter (excitation wavelength, 340 nm; emission wavelength, 380 nm). PPC (2 μM) was added to 1.5 ml of incubation medium consisting of 100 mM Tris-HCl (pH 9), 0.1 mM dithiothreitol and, unless other-

wise indicated, 2 mM CaCl2. After 2 min (fluorescence stabilization), soluble or particulate fractions (containing 0.1 mg proteins) were transferred into the cuvette and the rate of increase in fluorescence due to the release of pyreneedanoic acid was monitored every 10 sec over a 10-mn period. Changes in fluorescence due to the liberation of pyreneedanoate were calibrated with known concentrations of this fatty acid.

Measurement of [3H]-inositol phosphate formation. Neurons, grown in 12-well culture dishes, were incubated overnight in the presence of myo-[2-3H]-inositol (2 μCi/ml) added directly to the culture medium. Nonincorporated radioactivity was removed by three brief washes of cells with 1 ml Krebs bicarbonate buffer. Neurons were then exposed to drugs for 15 min at 37°C in the same medium supplemented with 10 mM LiCl. The incubation was stopped by lysing the cells with successive additions of 0.1% Triton X-100 in 0.1 M NaOH (0.4 ml) and of 0.1% Triton X-100 in 0.1 M HCl (0.4 ml). [3H]-inositol phosphates contained in the cell lysate were then isolated and estimated as described previously (El-Etr et al., 1989).

Whole-cell patch clamp recording. After 9 to 13 days in cul-

ture, cells were recorded using a patch-clamp amplifier (Axopatch-1D, Axon Instruments, CA). Current recordings were obtained in whole-cell configuration at −60 mV holding potential. They were filtered at 5 kHz, digitized and analyzed off-line with an ACQUIIS1 program. All experiments were performed at room temperature. Internal (electrode) solution contained 145 mM K+ -glucosate, 1 mM MgCl2, 0.1 mM CaCl2, 1 mM EGTA and 10 mM HEPES buffer (pH 7.2). The external solution contained 145 mM NaCl, 2.5 mM KCl, 1.5 mM MgCl2, 1.5 mM CaCl2, 10 mM d-glucose, 10 mM HEPES buffer (pH 7.3) and 1 μM tetrodotoxin. Under these experimental condi-

tions, resistance of the pipettes ranged from 2 to 4 MΩ. Cells were continuously superfused at a rate of 1.5 ml/min. AMPA and/or (-)-nicotine were dissolved in the external solution and ejected by gravity from separate reservoir syringes connected to a U-tube micropere-

fusing system.

Results

Nicotine inhibits the release of [3H]-arachidonic acid mediated by the coactivation of AMPA and metabotropic receptors in striatal neurons. As described previously in several neuronal populations (Lazare-

wicz et al., 1990; Stella et al., 1995), the maximally effective concentration of glutamate (100 μM) stimulated the release of [3H]-arachidonic acid from striatal neurons (311 ± 11% of basal release, mean ± S.E.M. of three independent experiments (n = 3) performed in triplicate). The exposure of neu-

ronal cells to 1 mM (-)-nicotine, a concentration providing the maximal protection against NMDA receptor-mediated neurotoxicity (Marin et al., 1994), did not alter the sponta-

neous release of [3H]-arachidonic acid, but decreased by 37 ± 5% (n = 3) the response evoked by glutamate (fig. 1). Further experiments were performed to identify which component of
the glutamate response (that evoked by the activation NMDA receptors or the costimulation of AMPA and metabotropic receptors) was inhibited by nicotine.

(-)-Nicotine decreased the liberation of [3H]-arachidonic acid induced by 200 μM NMDA (53 ± 4% of the NMDA response measured in the presence of (-)-nicotine, n = 3, fig. 1). However, we have recently shown that in particular experimental conditions, NMDA induces the release of glutamate from striatal neurons that in turn activates AMPA and metabotropic receptors (Williams et al., 1995). Therefore, to test whether nicotine inhibits the release of [3H]-arachidonic acid induced by the sole activation of NMDA receptors, endogenous glutamate was eliminated enzymatically, thanks to the use of GPT, which converts glutamate into α-ketoglutarate and alanine in the presence of 1 mM pyruvate (Williams et al., 1995). Under this experimental condition, the basal release of [3H]-arachidonic acid remained unchanged whereas the NMDA response was markedly reduced, indicating that part of the NMDA-evoked release of [3H]-arachidonic acid can be attributed to the NMDA-evoked release of glutamate (fig. 1). Interestingly, the remaining NMDA-induced release of [3H]-arachidonic acid measured in the presence of GPT was not inhibited by 1 mM (-)-nicotine (fig. 1). This result indicates that nicotine does not inhibit the response solely mediated by NMDA receptors. A similar indirect stimulation of [3H]-arachidonic acid release was induced by veratridine (10 μM). Indeed, the veratridine-evoked response was strongly reduced when endogenous glutamate was eliminated enzymatically by GPT, and the remaining release of [3H]-arachidonic acid was insensitive to (-)-nicotine (1 mM, fig. 1). As expected, the veratridine-evoked responses were totally prevented by tetrodotoxin (1 μM, data not shown).

Taken together, these results suggest that nicotine selectively inhibits the release of arachidonic acid mediated by the co-activation of AMPA and metabotropic receptors. Accordingly, (-)-nicotine almost totally prevented the release of [3H]-arachidonic acid induced by 1) the coapplication of maximally effective concentrations of AMPA (30 μM) and trans-ACPD (300 μM) (Williams et al., 1995), 2) quisqualate (100 μM), a nonselective agonist of AMPA and metabotropic receptors and 3) glutamate (100 μM, in the presence of 1 μM MK-801, a selective noncompetitive NMDA receptor antagonist) (fig. 1). It should be noted that GPT did not alter the release of [3H]-arachidonic acid induced by quisqualate or the coapplication of AMPA and trans-ACPD (fig. 1). This indicates that the costimulation of AMPA and metabotropic receptors does not trigger glutamate release from striatal neurons, contrasting to what was observed with NMDA.

Nicotinic receptors are not involved in the nicotine response. The nicotine-induced inhibition of [3H]-arachidonic acid release resulting from the costimulation of AMPA and metabotropic receptors does not appear to be mediated by nicotinic receptors. Indeed, neither the agonists of central nicotinic receptors acetylcholine (1 mM, in the presence of 1 μM atropine) or DMPP (1 mM), nor the antagonists, DHBE or hexamethonium (each at 100 μM) reproduced or blocked the inhibitory effect of (-)-nicotine (1 mM), respectively (table 1).

Nicotine neither alters AMPA-evoked currents nor inhibits the glutamate-induced inositol phosphate accumulation in striatal neurons. As described above, nicotine only inhibited the release of [3H]-arachidonic acid resulting from the joint stimulation of AMPA and metabotropic receptors. This observation led us to investigate separately the effect of nicotine on the AMPA-evoked currents and the formation of inositol phosphates resulting from the stimulation of metabotropic glutamate receptors.

In 17% of the cells tested (n = 23 cells tested), (-)-nicotine (1 mM) induced an inward current (42 ± 19 pA, n = 4) in striatal neurons (clamped at −60 mV), which rapidly desensitized (in less than 10 sec, experiments not illustrated), as expected for a nicotinic receptor-mediated response (McGehee and Role, 1995). In all neurons tested, AMPA (30 μM) induced a typical biphasic response, consisting of a peak (880 ± 330 pA, n = 4) followed by a slowly decreasing current (650 ± 180 pA, measured 5 sec after the onset of AMPA application, n = 4) (fig. 2a). The amplitude of the AMPA-induced current remained unchanged in striatal neurons (n = 11) exposed to 1 mM (-)-nicotine 20 min before and
The slow desensitization process observed during prolonged exposure to AMPA (8 min) was not altered by successive local applications of (-)-nicotine performed for increasing times (0.5, 1, 2 min, fig. 2b).

It has been reported recently that nicotine stimulates inositol phospholipid breakdown in frog pituitary melanotrophs (Garnier et al., 1994). However, (-)-nicotine (1 mM) failed to increase the accumulation of [3H]-inositol phosphates in striatal neurons (table 2). In addition, (-)-nicotine did not inhibit the accumulation of [3H]-inositol phosphates triggered by either glutamate (100 μM), quisqualate (100 μM) or trans-ACPD (300 μM) (table 2).

Nicotine inhibits soluble and membrane-bound phospholipase A2 activities. Phospholipase A2 activity is likely responsible for the release of [3H]-arachidonic acid mediated by NMDA receptors and the joint stimulation of AMPA and metabotropic receptors in striatal neurons. Indeed, mepacrine (100 μM) and the histidine reagent p-BPB (100 μM) (Volwerk et al., 1974), two nonspecific inhibitors of phospholipase A2, strongly depressed the release of [3H]-arachidonic acid induced by glutamate (100 μM), quisqualate (100 μM), the coapplication of AMPA (30 μM) and trans-ACPD (300 μM) or NMDA (200 μM, in the presence or absence of GPT) (table 3).

The inhibitory effect of (-)-nicotine on glutamate-induced [3H]-arachidonic acid release did not result from a direct interaction with either AMPA or metabotropic receptors, since this compound did not alter both AMPA-evoked currents and the generation of inositol phosphate derivatives induced by agonists of metabotropic receptors. Moreover, no evidence indicated that (-)-nicotine decreased glutamate-evoked [3H]-arachidonic acid release by interacting with nicotinic receptors. Therefore, we have examined whether this compound inhibits directly neuronal PLA2 activities. Using a fluorimetric method allowing the quantification of PLA2 activity in cell-free systems (Piomelli and Greengard, 1991), we were able to detect spontaneous PLA2 activities in both particulate and soluble fractions from striatal neurons (27.9 ± 1.5 and 14.8 ± 1.5 pmol of pyrene decanoate released/min/mg proteins, mean ± S.E.M. of four determinations performed on different sets of cultured neurons, respectively). Increasing concentrations of (-)-nicotine inhibited progressively PLA2 activity in the particulate fraction, the maximal effect (25 ±
TABLE 3
Effects of mepacrine and p-BPB on the release of [3H]-arachidonic acid evoked by glutamatergic receptor agonists

<table>
<thead>
<tr>
<th>[3H]-Arachidonic Acid Release (% of Basal)</th>
<th>None</th>
<th>Mepacrine</th>
<th>p-BPB</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100 ± 1</td>
<td>80 ± 15</td>
<td>105 ± 5</td>
</tr>
<tr>
<td>Glutamate</td>
<td>310 ± 20b</td>
<td>202 ± 7ab</td>
<td>166 ± 1ab</td>
</tr>
<tr>
<td>AMPA + trans-ACPD</td>
<td>190 ± 7b</td>
<td>112 ± 6b</td>
<td>124 ± 1b</td>
</tr>
<tr>
<td>Quisqualate</td>
<td>178 ± 11a</td>
<td>117 ± 7b</td>
<td>117 ± 2b</td>
</tr>
<tr>
<td>NMDA</td>
<td>265 ± 6a</td>
<td>145 ± 6ab</td>
<td>160 ± 7ab</td>
</tr>
<tr>
<td>NMDA + GPT</td>
<td>156 ± 4a</td>
<td>107 ± 2a</td>
<td>115 ± 2a</td>
</tr>
</tbody>
</table>

Striatal neurons were exposed for 15 min to drugs used at the following concentrations: glutamate (100 μM), AMPA (30 μM), trans-ACPD (300 μM), quisqualate (100 μM), NMDA (200 μM), GPT (4 IU/ml, in the presence of 1 mM pyruvate), mepacrine (100 μM) and p-BPB (100 μM). Results, expressed in % of the release of [3H]-arachidonic acid measured in the absence of any treatment (basal, 3570 ± 60 dpm/well, n = 3) are the means ± S.E.M. of data obtained in three independent experiments, each being performed in triplicate. *Significantly different (P < .01) from the corresponding value obtained in the absence of glutamatergic receptor agonist. **Significantly different (P < .01) from the corresponding value obtained in absence of PL2 inhibitor (analysis of variance, followed by Student Newman Keuls’ test).

4% inhibition, n = 4) being reached with 30 μM (-)-nicotine (fig. 3). The inhibitory effect of nicotine was more pronounced in the soluble fraction (51 ± 4% inhibition induced by the maximally effective concentration of (-)-nicotine, n = 4) and the concentration-dependency for (-)-nicotine was biphasic, consisting of a relatively high affinity component, with an efficacy similar to that observed in the particulate fraction, and an additional low potency component (fig. 3). Interestingly, a similar biphasic inhibition was observed on the release of [3H]-arachidonic acid from living cells stimulated by glutamate or the combined addition of AMPA and trans-ACPD (fig. 4). In the soluble fraction as well as in living neurons, the first phase of nicotine inhibition (high affinity component) was achieved with about 30 μM (-)-nicotine although the second phase (low affinity component) required 1 mM (-)-nicotine (figs. 3 and 4). As for the nicotine effect in intact neurons, the (-)-nicotine-evoked inhibition of soluble (table 1) and particulate (data not shown) PLA2 activities was neither reproduced by 1 mM acetylcholine (in the presence of 1 mM atropine), nor reversed by DHBE or hexamethonium (each at 100 μM). However, DMPP (1 mM) was found to inhibit soluble and particulate PLA2 activities by 43 ± 3 and 29 ± 6% (n = 4), respectively.

The different PLA2 isoforms expressed in neuronal cells have not yet been identified. Nevertheless, a previous report indicated that synaptosomes from rat cerebral cortex express both Ca2+-dependent and independent PLA2 activities (Piomelli and Greengard, 1991). The Ca2+-dependent isoform was entirely associated with membranes and fully inhibited by the histidine reagent p-BPB. The Ca2+-independent enzyme was distributed approximately equally in soluble and particulate fractions and was less sensitive to p-BPB (Piomelli and Greengard, 1991). Striatal neurons were found to express only Ca2+-independent PLA2 activities. Indeed, the removal of Ca2+ from the incubation medium did not significantly change PLA2 activities estimated in both the particulate and the soluble fraction (data not shown). However, particulate and soluble PLA2 could be distinguished by their sensitivity to p-BPB. Indeed, this compound, used at 100 μM, induced a more pronounced inhibition of the particulate PLA2 activity than that measured in the soluble fraction (68 ± 1 and 51 ± 3% inhibition, n = 4, respectively, fig. 5). Moreover, in the presence of p-BPB, the inhibitory effect induced by the low concentration of (-)-nicotine (30 μM) was suppressed in both the particulate and the soluble fractions.
whereas the high concentration (1 mM) still decreased PLA2 activity in the soluble fraction (fig. 5).

**Discussion**

In our study, we have demonstrated that (-)-nicotine inhibits selectively the release of [3H]-arachidonic acid evoked by the joint stimulation of AMPA and metabotropic glutamate receptors in striatal neurons although the response mediated by NMDA receptors remains unchanged. This inhibition of arachidonic acid release is probably not involved in the protective effect of nicotine against the neuronal death induced by glutamate. Indeed, the glutamate-induced neurotoxicity in striatal neurons was mainly mediated by NMDA receptors (Marin et al., 1994). Moreover, the neuroprotective effect of nicotine involved nicotinic receptors (Akaike et al., 1994, Marin et al., 1994). This differs from the nicotine-evoked inhibition of arachidonic acid release described in this study because it was neither prevented by nicotinic receptor antagonists, nor reproduced by acetylcholine or DMPP.

We provide several observations suggesting that (-)-nicotine decreases the release of arachidonic acid evoked by the costimulation of AMPA and metabotropic glutamate receptors by inhibiting PLA2: 1) [3H]-arachidonic acid release induced by glutamate receptor stimulation was probably mediated by PLA2. Indeed, mepacrine and p-BPB, two inhibitors of PLA2 that act through distinct mechanisms, strongly depressed the stimulated release of [3H]-arachidonic acid in intact neurons; 2) (-)-nicotine inhibited both soluble and particulate PLA2 activities; 3) the inhibition of [3H]-arachidonic acid release and the decrease in PLA2 activities evoked by (-)-nicotine were neither reproduced by acetylcholine nor antagonized by DHBE and hexamethonium. Only DMPP inhibited soluble and particulate PLA2 activities whereas it did not alter the release of [3H]-arachidonic acid induced by the coactivation of AMPA and metabotropic receptors. The lack of effect of DMPP on [3H]-arachidonic acid release from living neurons could be due to the low membrane permeability of this compound. Finally, increasing concentrations of (-)-nicotine produced a biphasic inhibition of both [3H]-arachidonic acid release from living neurons and PLA2 activity in the soluble fraction. It is interesting to note that only the first phase (high potency component) was observed in the particulate fraction. These data suggest that striatal neurons possess at least two PLA2 isoforms differing in both their nicotine sensitivity and their subcellular location: the isoform with a high potency for nicotine being present in both soluble and particulate fractions, and another isoform having a lower potency for nicotine and only found in the soluble fraction. Further supporting the expression of two PLA2 isoforms differing in their nicotine-sensitivity in striatal neurons, p-BPB (used at 100 μM) selectively suppressed the PLA2 activity displaying the highest potency for nicotine and present in both particulate and soluble fractions. Accordingly, two distinct Ca2+-independent PLA2 isoforms that differ by their molecular weight, pH optima and substrate preferences, have been already described in bovine brain (Hirashima et al., 1992, for review see Ackerman and Dennis, 1995). However, a direct interaction of nicotine with two PLA2 isoforms must be confirmed by experiments using purified enzymes, in particular to rule out a possible interaction of nicotine with PLA2 regulatory proteins.

(-)-Nicotine decreased neither the AMPA-evoked currents nor the accumulation of inositol phosphate derivatives induced by metabotropic receptor agonists in striatal neurons. These results demonstrate that the inhibitory effect of (-)-nicotine on the release of arachidonic acid does not result from an antagonistic action on glutamate receptors and support further the link between the reduction in [3H]-arachidonic acid release and the inhibition of PLA2 activity. They also indicate that, despite its hydrophobic nature, (-)-nicotine does not inhibit phospholipase C, ruling out a nonspecific inhibitory effect on all phospholipase activities.

Surprisingly, our results suggest that the release of arachidonic acid from neurons evoked by glutamate, which is dependent on the presence of extracellular Ca2+ (Dumuis et al., 1988; Lazarewicz et al., 1990), is mediated by Ca2+-independent PLA2. Indeed, similarly to what was described in bovine brain (Hirashima et al., 1992), PLA2 activities were found to be Ca2+-independent in both soluble and particulate fractions obtained from cultured striatal neurons. This raises the question of the link between glutamate and PLA2 activation in intact neurons. By analogy to what was shown for cytosolic PLA2 from macrophages (for review see Clark et al., 1995), the increase in cytosolic Ca2+ concentration after glutamate receptor stimulation could trigger the transloca-

**Fig. 5.** Effect of p-BPB on (-)-nicotine-induced inhibition of soluble and particulate PLA2 activities. PLA2 activity was estimated in both the soluble and particulate fractions from striatal neurons, in the absence (- p-BPB) or presence (+ p-BPB) of 100 μM p-BPB and, when indicated, 30 μM or 1 mM (-)-nicotine (NIC). Results, expressed as described in the legend to figure 3, are the means ± S.E.M. of four determinations performed on subcellular preparations obtained from four different cultures. *, **Significantly different (P < .05 and P < .01) from the corresponding control PLA2 activity (CONT); †Significantly different (P < .05) from the corresponding PLA2 activity measured in the presence of 30 μM (-)-nicotine (analysis of variance, followed by Student Newman Keuls’ test).

![Diagram](image-url)
tion of neuronal PLA2 from the cytosol to the membrane, where it can interact with its phospholipid substrate. Alternatively, activation of Ca\(^{2+}\)-independent PLA2 activity could result from changes in intracellular pH evoked by glutamate in neurons, a phenomenon that depends on the presence of extracellular Ca\(^{2+}\) (Hartley and Dubinsky, 1993).

The concentrations of (−)-nicotine required to inhibit PLA2 activities are known to induce a strong and rapid desensitization of nicotinic receptors, varying usually between the millisecond and second time ranges (for review see McGehee and Role, 1995). Therefore, it is not surprising that the nicotine effects described here using biochemical methods do not involve nicotinic receptors. Similarly, one can suspect that some if not all behavioral modifications after nicotine treatments do not involve the stimulation of nicotinic receptors, but rather result from their rapid desensitization. This could explain, for instance, why wild-type mice treated with nicotine display performances on the passive avoidance test (a test of associative memory) similar to those measured with untreated homozygous mutant mice lacking functional nicotinic receptors (Picciotto et al., 1995). Therefore, in addition to the stimulation and desensitization of nicotinic receptors, our findings indicate that nicotine can also modify other neuronal properties that could account for some long-term effects of nicotine.

References


