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5-Hydroxytryptamine-mediated effects of nicotine on endogenous GABA efflux from guinea-pig cortical slices

[1]Clementina Bianchi, Luca Ferraro, Sergio Tanganelli, Michele Morari, Gianpiero Spalluto, Michele Simonato & Lorenzo Beani

Department of Pharmacology, University of Ferrera, Via Fossato di Mortara 17-19, 44100 Ferrera, Italy

1 The effect of nicotine on endogenous basal GABA outflow was studied in guinea-pig cerebral cortex slices.

2 Nicotine 1.86-18.6 μmol l^{-1} significantly decreased the basal, tetrodotoxin-sensitive GABA efflux, whereas at higher concentrations (186-620 μmol l^{-1}) nicotine increased it. The inhibition was prevented by mecamylamine while the facilitation was blocked by mecamylamine, (+)-tubocurarine and tetrodotoxin.

3 The effect of nicotine was due to an indirect 5-hydroxytryptaminergic action. In fact, MDL 72222 (1 μmol l^{-1}) completely prevented the alkaloid inhibition and methysergide (1 μmol l^{-1}) reversed the facilitation into inhibition; concomitant treatment with methysergide and MDL 72222 antagonized the effect of nicotine at 186 μmol l^{-1}

4 Lower concentrations of 5-HT (3-10 μmol l^{-1}) decreased, whereas higher concentrations (30-100 μmol l^{-1}) increased, spontaneous GABA outflow. The inhibition of GABA efflux was prevented by MDL 72222 whereas the facilitation was reversed by methysergide (1 μmol l^{-1}) into inhibition, and prevented by MDL 72222 1 μmol l^{-1}.

5 These results suggest that, by activating nicotinic receptors present on 5-hydroxytryptaminergic terminals, nicotine releases 5-HT which, in turn, inhibits or increases the secretory activity of cortical GABA interneurones via 5-HT_3 and methysergide-sensitive receptors, respectively.

Keywords: Nicotine; GABA release; 5-HT receptors; cerebral cortex.

Introduction

It is well known that nicotine improves alertness, attention and memory function. These effects are most likely due to its interaction with specific brain receptors, which increase the firing rate and secretion of many neurones (Balfour, 1982). Nicotine increases 5-hydroxytryptamine (5-HT) and dopamine release (Westfall *et al.*, 1983; Imperato *et al.*, 1986; Balfour, 1989; Carboni *et al.*, 1989; Riberio *et al.*, 1993; Nisell *et al.*, 1994) as well as acetylcholine release (Beani *et al.*, 1985; Nordberg *et al.*, 1989) both *in vivo* and *in vitro* preparations. Moreover, the drug increases excitatory amino acid outflow *in vivo* (Garza De La *et al.*, 1989; Beani *et al.*, 1991; Toth *et al.*, 1993) and *in vitro* (Perez de la Mora *et al.*, 1991). As regards GABA release, different responses have been reported depending on the brain area. Nicotine appears to increase amino acid efflux in unstimulated substantia nigra and globus pallidus slices. This effect is partly mediated by dopamine (Kayadjanian *et al.*, 1994). Conversely, the drug decreases GABA release in the cerebral cortex through 5-hydroxytryptaminergic and enkephalinergic neurones (Beani *et al.*, 1991).

With the aim of further analysis of the 5-hydroxytryptaminergic mechanism(s) through which nicotine affects GABA cells, an *in vitro* study was performed on GABA efflux in guinea-pig cerebral cortex slices, a relatively simple model containing spontaneously firing GABA interneurones (Beani *et al.*, 1986; Ferraro *et al.*, 1993). A preliminary account of this investigation has been published (Beani *et al.*, 1995).

Method

Guinea-pigs of either sex (average weight of 350-400g) were kept under standard conditions (12 h dark/12 h light cycle, free access to food and water). The animals were decapitated under light anaesthesia, the fronto-parietal cortices were rapidly excised, transferred into an oxygenated Krebs solution at room temperature and sliced (400 μm thick slices) with a vibratome-like apparatus (Beani *et al.*, 1978). After 30 min to allow re-equilibration of the tissue, sets of 2-3 slices (average wet weight 50-60 mg each) were separately transferred into four 0.9 ml chambers and perfused at 0.5 ml min^{-1} at 37°C with Krebs solution (composition in mM: NaCl 118.5, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11) bubbled with 95% O_2 and 5% CO_2. After 20 min, 6-7 samples were collected every 5 min from each chamber: 3 prior to and 3-4 after drug treatment. When used, antagonists were added to the Krebs solution at the start of superfusion. The GABA content of the samples was measured by mass-fragmentographic analysis with a Finningan 4510 mass spectrometer (Bertilsson & Costa, 1976).

Statistical analysis

Statistical differences were checked by ANOVA analysis followed by Newman -Keuls multiple range test as specified in the figure legends.

Drugs

Freshly prepared solution of the following drugs were used: (-)-nicotine bitartrate, 5-hydroxytryptamine creatine sulphate, mecamylamine hydrochloride, methysergide maleate, (+)-tubocurarine chloride (Sigma Chemical Co., St. Louis, MO, U.S.A.), naloxone (Salars, Italy), 1αH, 3α, 5αH-tropan-3yl-3,5-dichlorobenzoate (MDL 72222, Merrel Dow, France), prazosin hydrochloride (Pfizer, New York, NY), ketanserin, (Janssen, Belgium). Tetrodotoxin (RBI, MA, U.S.A.).

[1] Author for correspondence.

Results

Spontaneous GABA efflux

The basal efflux of GABA from guinea-pig cerebral cortex slices remained steady for more than 30 min and was 255±12.8 pmol g^{-1} min^{-1} (mean±s.e.mean of 100 determinations). If the slices were perfused with a medium containing Ca^{2+} 0.1 mmol l^{-1}, or tetrodotoxin (TTX) 0.5 μmol l^{-1}, the GABA efflux was significantly reduced (after 30 min) to 54±6% and to 53±3% respectively (means±s.e.mean, 6 expts., $P<0.05$). Thus, about one half of the spontaneous GABA efflux was Ca^{2+}-and Na^{+}-dependent and appeared to be linked to the neuronal activity, as previously reported (Beani *et al.*, 1986; Ferraro *et al.*, 1993).

Effect of nicotine on GABA efflux

Nicotine 1.86–620 μmol l^{-1} was added to the superfusion medium after three collection periods and was maintained until the end of the experiment. As shown in Figure 1a, at low concentrations (1.86–18.6 μmol l^{-1}) nicotine consistently decreased GABA efflux, whereas at higher concentrations (186–620 μmol l^{-1}) nicotine transiently increased it to 30–35% but only in the first 5–10 min (Figure 1a). This suggests that high doses showed a ceiling effect and caused rapid desensitization. At 62 μmol l^{-1} the drug was apparently ineffective. Thus, the alkaloid displayed a concentration-dependent, biphasic action quite evident when the maximal inhibitory and facilitatory effects were considered (Figure 1b).

Effects of nicotine antagonists on GABA efflux.

None of the antagonists tested modified spontaneous GABA efflux, thus ruling out any endogenous nicotinic tonic control in the slices kept at rest. However, the decrease in GABA outflow induced by low nicotine concentrations was completely prevented by mecamylamine 2.5 μmol l^{-1}, but not by (+)-tubocurarine up to 4.5 μmol l^{-1} (Figure 2a), while the

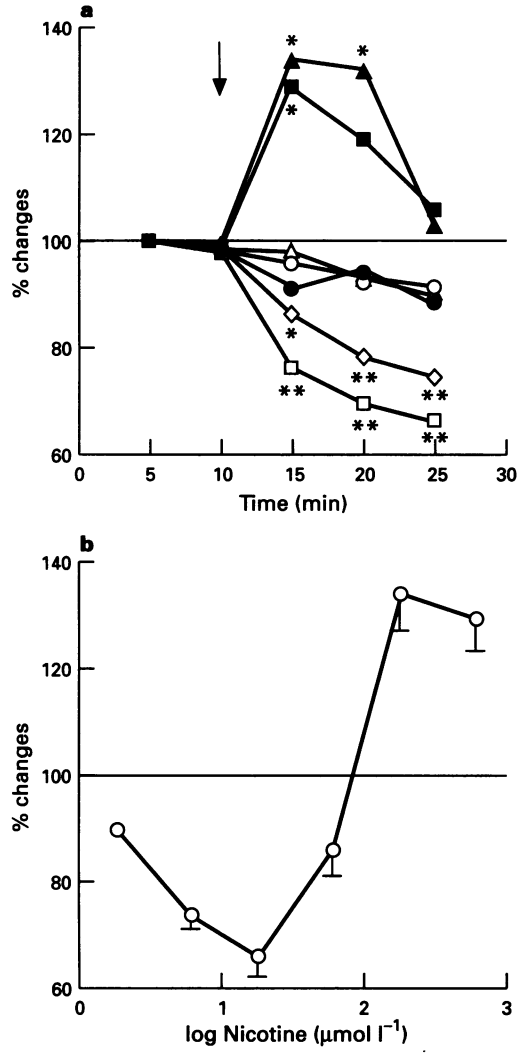

Figure 1 Effect of nicotine (at arrow) on endogenous GABA efflux from guinea-pig cerebral cortex slices. (a) Controls (○); nicotine μmol l^{-1}: 1.86 (△); 6.2 (◇); 18.6 (□); 62(●); 186 (▲); 620 (■). (b) Relationships between nicotine concentrations (maximal effect) and GABA efflux. Data represent the percentage changes±s.e.mean with respect to pretreatment value (at least 5–8 expts.). Average efflux of endogenous GABA before treatment was 276±22 pmol min^{-1} g^{-1}. The s.e.mean in (a) were less than 10%. Significantly different from pretreatment value according to ANOVA followed by Newman-Keuls multiple range test: * $P<0.05$; ** $P<0.01$.

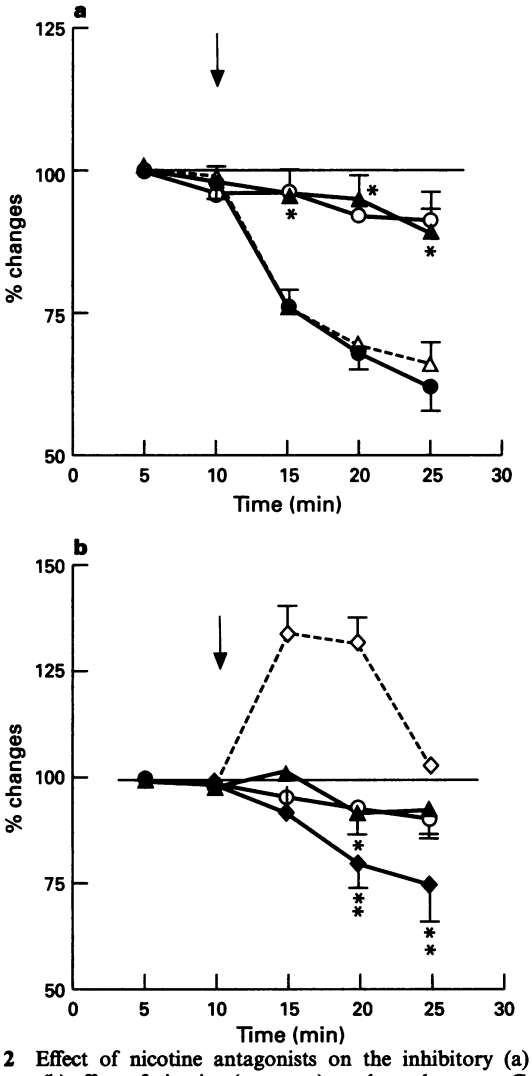

Figure 2 Effect of nicotine antagonists on the inhibitory (a) and facilitatory (b) effect of nicotine (at arrow) on the endogenous GABA efflux from guinea-pig cerebral cortex slices. (a) Controls (○); nicotine 18.6 μmol l^{-1} (△); nicotine plus mecamylamine 2.5 μmol l^{-1} (▲); nicotine plus (+)-tubocurarine 4.5 μmol l^{-1} (●). (b) Controls (○); nicotine 186 μmol l^{-1} (◇); nicotine plus mecamylamine 2.5 μmol l^{-1}(▲); nicotine plus (+)-tubocurarine 4.5 μmol l^{-1} (◆). Data represent the percent changes ±s.e.mean with respect to pretreatment value (at least 8 experiments). Average efflux of endogenous GABA before treatment was 270±10 pmol min^{-1} g^{-1}. Significantly different from nicotine alone according to ANOVA followed by Newman-Keuls multiple range test: *$P<0.05$, **$P<0.01$.

increase in GABA efflux induced by high nicotine concentrations was antagonized both by mecamylamine 2.5 μmol l^{-1} and by (+)-tubocurarine 4.5 μmol l^{-1}. It is worth noting that nicotine 186 μmol l^{-1}, in the presence of (+)-tubocurarine, showed an inhibitory effect near to the limit of significance ($P=0.05$, Figure 2b), and less than that evoked by low alkaloid concentrations in normal slices. Again, rapid desensitization to high doses appeared to come into play. Interestingly, TTX 0.5 μmol l^{-1} completely abolished the effect of nicotine 186 μmol l^{-1} (nicotine 186 μmol l^{-1}, 134% ± 7; nicotine plus TTX, 99% ± 3; mean ± s.e.mean of 6 expts., $P< 0.05$).

Involvement of other transmitters in the nicotine effect

To check for the possible involvement of other neurotransmitters in the nicotine modulation of GABA release, the slices were pretreated with various antagonists which have been found to be, *per se*, without effect on spontaneous GABA efflux.

Nicotine inhibition was unaffected by naloxone 0.3 μmol l^{-1}, idazoxan 0.1 μmol l^{-1} and phaclofen 10 μmol l^{-1} (data not shown, $n=5$ for each drug). Figure 3a shows that methysergide 1 μmol l^{-1} and ketanserin 0.1 μmol l^{-1} also did not change the inhibition induced by nicotine 18.6 μmol l^{-1}, while MDL 72222 1 μmol l^{-1} completely prevented it. This 5-HT_3 antagonist at 1 μmol l^{-1} also prevented the inhibition of GABA efflux induced by nicotine 186 μmol l^{-1} in the presence of (+)-tubocurarine 4.5 μmol l^{-1} (data not shown, $n=4$).

On the other hand, the facilitation of GABA outflow induced by higher alkaloid concentrations (i.e. 186 μmol l^{-1}), was unmodified by prazosin (Figure 3b) and by MDL 72222 1 μmol l^{-1} ($n=3$). However, it was reversed into inhibition by methysergide 1 μmol l^{-1} and completely prevented by simultaneous pretreatment with methysergide and MDL 72222 1 μmol l^{-1} (Figure 3b).

Effect of 5-HT on spontaneous GABA efflux

Since the above findings proved that endogenous 5-HT is involved in the nicotine effects, exogenously applied 5-HT was checked for its ability to modulate GABA efflux. In the 3–100 μmol l^{-1} range, 5-HT caused inhibition or facilitation of GABA efflux, depending on the concentrations tested. At 3–10 μmol l^{-1} the amine steadily decreased, whereas at 30–100 μmol l^{-1} it transiently increased GABA efflux as nicotine did (Figure 4a). To ascertain what subtypes of 5-HT receptors were involved, the experiments were repeated with 5-HT receptor antagonists. MDL 72222 completely antagonized the effect of low (3–10 μmol l^{-1}) amine concentrations, whereas methysergide reversed into inhibition the increase in GABA caused by 5-HT 100 μmol l^{-1}. MDL 72222 1 μmol l^{-1} plus methysergide 1 μmol l^{-1} prevented any effect of high amine concentrations (Figure 4b).

Discussion

The measure of GABA efflux from cortical slices at rest can be considered a simple and convenient tool to study neurotransmitter or drug influences on the secretory activity of GABA interneurones, isolated from any intra and subcortical input. These inferences are based on: (i) the consistent [Ca^{2+}]- and TTX-sensitivity of the spontaneous efflux (see also Beani *et al.*, 1986; Ferraro *et al.*, 1993) and (ii) the lack of a primary effect of the receptor antagonists tested. Clearly the extracellular concentrations of a variety of endogenous agonists in the cortical slices are below the threshold required to modulate tonically GABA release.

The present findings demonstrate for the first time that nicotine influences in a complex fashion spontaneous GABA efflux: decreasing it at low concentrations yet increasing at higher ones (see Figure 1). Nicotine effects were already evident in the first 5 min of drug contact with the slices (Figure 1), indicating that the rate of ligand penetration into the tissue (Rice *et al.*, 1985) and the rate of GABA efflux were compatible with the time interval of sampling. The inhibition of GABA efflux elicited by nicotine 6.2–18.6 μmol l^{-1} was long-lasting and was prevented by mecamylamine. These findings agree with recent observations showing that nicotine inhibits GABA efflux from cerebral cortex of freely moving guinea-pigs (Beani *et al.*, 1991).

Conversely, the increase in GABA efflux displayed by nicotine at higher concentrations (186–620 μmol l^{-1}), known to undergo desensitization, was short lasting and already maximal at 186 μmol l^{-1}. In addition it was prevented by TTX, mecamylamine and (+) tubocurarine. Therefore nicotine appeared to activate transiently the TTX-dependent neurosecretory process of either GABA interneurones or their axons at the preterminal level through nicotinic receptors of both ganglionic and neuromuscular junction type (Lukas, 1989; Lena *et al.*, 1993). This excitatory action of nicotine confirms the results obtained by Limberger *et al.* (1986) in rat caudatal slices.

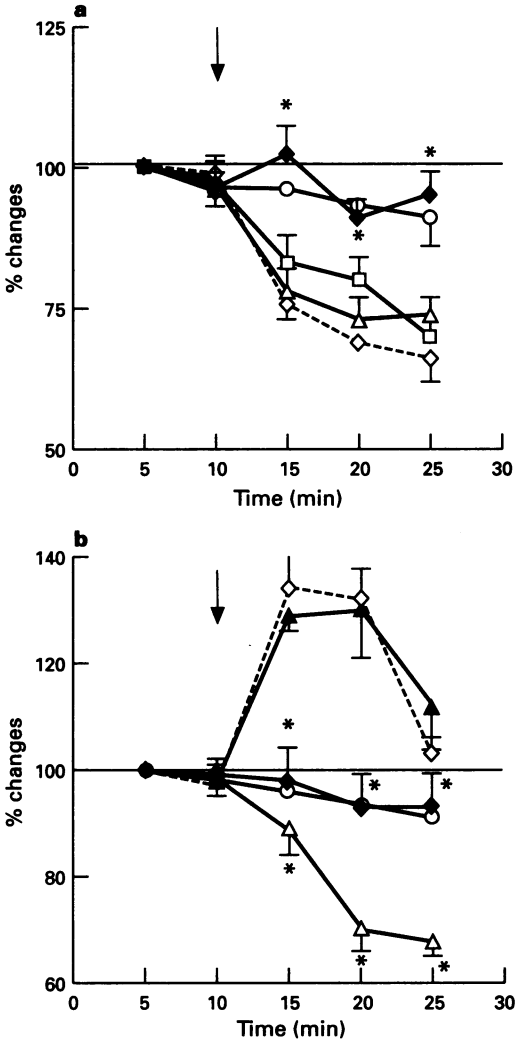

Figure 3 Effect of different antagonists on spontaneous GABA efflux from guinea-pig cerebral cortex slices. (a) Controls (○); at the arrow: nicotine 18.6 μmol l^{-1} (◇); nicotine after: methysergide 1 μmol l^{-1} (△); ketanserine 0.1 μmol l^{-1} (□); MDL 72222 1 μmol l^{-1} (◆). (b) Controls (○); at the arrow: nicotine 186 μmol l^{-1} (◇); nicotine after: prazosin 1 μmol l^{-1} (▲); methysergide 1 μmol l^{-1}(△); methysergide plus MDL 72222 1 μmol l^{-1} (◆). Data represent the percentage changes ± s.e.mean with respect to pretreatment value (at least 5–8 experiments). Average efflux of endogenous GABA before treatment was 271 ± 15 pmol min^{-1} g^{-1}. Significantly different from nicotine alone according to ANOVA followed by Newman-Keuls multiple range test: *$P<0.01$.

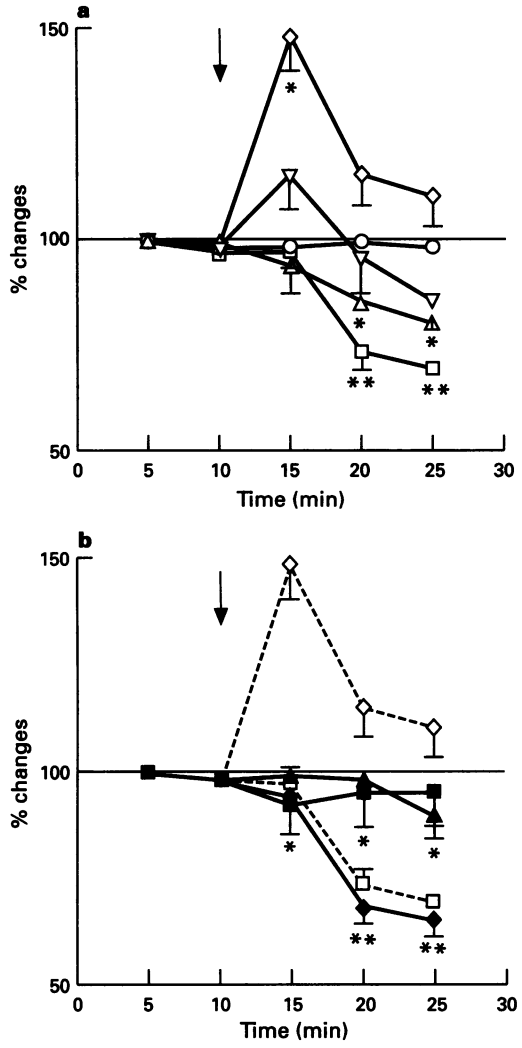

Figure 4 Effect of 5-HT antagonists on the inhibitory and facilitatory effect of 5-HT (at arrow) on the spontaneous GABA efflux from guinea-pig cerebral cortex slices. (a) Controls (○); 5-HT μmol l^{-1}: 3 (△); 10 (□); 30 (▽); 100 (◇). (b) 5-HT 10 μmol l^{-1} alone (□) and after MDL 72222 1 μmol l^{-1} (■); 5-HT 100 μmol l^{-1} alone (◇), after methysergide 1 μmol l^{-1} (◆) and after methysergide plus MDL 72222 (▲). Data represent the percentage changes ±s.e.mean with respect to pretreatment value (at least 5–6 experiments). Average efflux of endogenous GABA before treatment was 300±21 pmol l^{-1}. Significantly different from pretreatment value according to ANOVA followed by Newman-Keuls multiple range test: $*P<0.05$; $**P<0.01$.

The experiments performed to ascertain whether nicotine modified GABA spontaneous efflux directly or indirectly, favour an exclusively indirect 5-hydroxytryptaminergic action and demonstrate that GABA neurones can be controlled through different 5-HT receptors which can evoke opposite responses. This statement is further supported by the evidence that exogenous 5-HT faithfully mimics nicotine effects. In addition our results fit well with the histological studies of Mamounas *et al.* (1992) showing that rat cortical GABA interneurones are enveloped by a rich network of 5-HT terminals. Such arrangements can explain the preferential 5-hydroxytryptaminergic mechanism through which nicotine modulates GABA cells in the cortex of the rodents. This conclusion is at variance with the findings of Wonnacott *et al.* (1989), who reported a direct, nicotine-induced increase in [^{3}H]-GABA efflux in rat hippocampal synaptosomes. Differences in animal species, brain area and type of preparation may be the reason for these discrepancies.

GABA efflux inhibition by nicotine was fully prevented by MDL 72222, a 5-HT_3 antagonist (Hoyer *et al.*, 1994). Thus the alkaloid appeared to release 5-HT which, in turn, restrained the activity of cortical GABA interneurones. The inhibition of GABA release by nicotine through 5-HT_3 receptors agrees with the results of Clöez-Tayarami *et al.*, (1992) who demonstrated that 5-HT inhibits [^{3}H]-GABA efflux from hippocampal synaptosomes. Our recent findings proving that nicotine, injected s.c., reduces GABA outflow from the cerebral cortex of freely moving guinea-pigs by activating methysergide-sensitive receptors (Beani *et al.*, 1991) can be explained by assuming that nicotine, injected in the whole animal, increases 5-HT release from 5-hydroxytryptaminergic terminals inpinging upon corticopetal GABA cells, possibly provided with inhibitory methysergide-sensitive receptors.

Endogenous 5-HT seems also to play a crucial role in increasing GABA outflow. Indeed, the inversion of nicotine-mediated GABA facilitation into inhibition caused by methysergide, suggests that high nicotine concentrations could release so much 5-HT as to increase GABA efflux through excitatory methysergide-sensitive receptors, not involved in the *in vivo* effects. The lack of any facilitation by nicotine, even if given at high doses in the whole animal (Beani *et al.*, 1991), might depend on the relatively low concentrations attained in the brain (Yamamoto *et al.*, 1967; Rosencrans & Schechter, 1972). That the effects of nicotine *in vivo* differ from those seen in cortical slices is also confirmed by the lack of any influence of naloxone in nicotine-treated slices, whereas the inhibitory control operated by the endogenous opioids on GABA release is well documented in freely moving animals (Beani *et al.*, 1991). Most likely nicotine, injected in the whole animal, releases endogenous opioids from subcortical enkephalinergic neurones which, in turn, affect GABA structure projecting to the cerebral cortex, where GABA efflux has been measured with the epidural cup technique.

Other observations appear to restrict the *in vitro* effects of nicotine on cortical GABA efflux to its 5-HT-releasing properties: any role of α_2 and $GABA_B$ receptors in the inhibition has been ruled out since neither idazoxan nor phaclophen affected it. Similarly, also the involvement of noradrenaline (known to be released by nicotine, Balfour, 1982; 1989; and to enhance GABA efflux through α_1 adrenoceptors, Beani *et al.*, 1986; Ferraro *et al.*, 1993) can be ruled out since prazosin did not prevent the increase in GABA efflux. In conclusion, nicotine can negatively or positively modulate spontaneous GABA efflux in guinea-pig cerebral cortex slices via releasing endogenous 5-HT. While the increase in the GABA outflow, produced by high nicotine concentrations, appears to be of minor pharmacological significance, the inhibitory effect produced by low nicotine concentrations is reminiscent of the inhibition of GABA efflux produced *in vivo*, which can be tentatively related to the well-known stimulation of alertness and attention elicited by this drug through its interaction with many neuronal species.

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References

BALFOUR, D.J.K. (1982). The effect of nicotine on brain neurotransmitters systems. *Pharmacol. Ther.*, **16**, 269–282.

BALFOUR, D.J.K. (1989). Influence of nicotine on the release of monoamines in the brain. *Prog. Brain Res.*, **79**, 165–172.

BEANI, L., BIANCHI, C., GIACOMELLI, A. & TAMBERI, F. (1978). Noradrenaline inhibition of acetylcholine release from guinea-pig brain. *Eur. J. Pharmacol.*, **48**, 179–193.

BEANI, L., BIANCHI, C., NILSSON, L., NORDBERG, A., ROMANELLI, L. & SIVILOTTI, L. (1985). The effect of nicotine and cytisine on ^{3}H-acetylcholine release from cortical slices of guinea-pig brain. *Naunyn-Schmied Arch. Pharmacol.*, **331**, 293–296.

BEANI, L., TANGANELLI, S., ANTONELLI, T. & BIANCHI, C. (1986). Noradrenergic modulation of cortical acetylcholine release is both direct and γ-amino-butyric acid-mediated. *J. Pharmacol. Exp. Ther.*, **236**, 230–236.

BEANI, L., TANGANELLI, S., ANTONELLI, T., FERRARO, L., MORARI, M., SPALLUTO, P., NORDBERG, A. & BIANCHI, C. (1991). Effect of acute and subchronic nicotine treatment on cortical efflux of [^{3}H]-D-aspartate and endogenous GABA in freely moving guinea-pigs. *Br. J. Pharmacol.*, **104**, 15–20.

BEANI, L., TANGANELLI, S., FERRARO. L., MORARI, M. & BIANCHI, C. (1995). 5-HT mediated effects of nicotine on GABA release in guinea-pig cortical brain slices. *Br. J. Pharmacol.*, **114**, 391P.

BERTILSSON, L. & COSTA, E. (1976). Mass-fragmentographic quantitation of glumatic acid and GABA in cerebellar nuclei and sympathetic ganglia of the rat *J. Chromatogr.*, **118**, 395–399.

CARBONI, E., ACQUAS, E, FRAU, R. & DI CHIARA, G. (1989). Differential inhibitory effects of a 5-HT antagonist on drug-induced stimulation of dopamine release. *Eur. J. Pharmacol.*, **164**, 515–519.

CLOËZ-TAYARAMI, I., HAREL-DUPAS, C. & FILLION, G. (1992). Inhibition of [^{3}H]γ-aminobutyric acid release from guinea-pig hippocampal synaptosomes by serotonergic agents. *Fund. Clin. Pharmacol.*, **6**, 333–341.

FERRARO, L., TANGANELLI, S., CALO', G., ANTONELLI, T., FABRIZI, A., ACCIARRI, N., BIANCHI, C., BEANI, L. & SIMONATO, M. (1993). Noradrenergic modulation of γ-aminobutyric acid outflow from the human cerebral cortex. *Brain Res.*, **629**, 103–108.

GARZA DE LA, R. REEDMAN, R. & HOFFER, J. (1989) Nicotine-induced inhibition of cerebellar Purkinje neurons: specific actions of nicotine and selective blockade by mecamylamine. *Neuropharmacology*, **28**, 495–501.

HOYER, D., CLARK, D.E., FOZARD, J.R., HARTIG, P.R., MARTIN, G.R., MYLECHARANE, E.J., SAXENA, P.R. & HUMPHREY, P. (1994). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.*, **46**, 158–193.

IMPERATO, A., MULAS, A. & DI CHIARA, G. (1986). Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. *Eur. J. Pharmacol.*, **132**, 337–338.

KAYADIANIAN, N., RÉTEAU, S., MENÉTRY, A. & BESSON MARIE-JO (1994). Stimulation by nicotine of the spontaneous release of [^{3}H]γaminobutyric acid in the substantia nigra and in the globus pallidus of the rat. *Brain Res.*, **649**, 129–135.

LENA, L., CHANGEUX, J.-P. & MULE, C (1993). Evidence for 'preterminal' nicotinic receptors on GABAergic axons in the rat interpeduncular nucleus. *J. Neuroscience*, **13**, 2680–2688.

LIMBERGER, N., SPÄTH, L. & STARKE, K. (1986). A search for receptors modulating the release of γ-[^{3}H] amino-butyric acid in rabbit caudate nucleus slices. *J. Neurochem.*, **46**, 1109–1117.

LUKAS, R.J. (1989). Nicotinic acetylcholine receptor diversity: agonist binding and functional potency. *Prog. Brain Res.*, **79**, 117–127.

MAMOUNAS, L.A., WILSON, M.A., AXT, K.J. & MOLLIVER, M.E. (1992). Morphological aspects of serotonergic innervation. *Advances Biosci.*, **85**, 97–118.

NISELL, M., NOMIKOS, G.G. & SVENSSON, T.H. (1994). Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. *Synapse*, **16**, 36–44.

NORDBERG, A., ROMANELLI, L., SUNDWALL, A., BIANCHI, C. & BEANI, L. (1989). Effect of acute and subchronic nicotine treatment on cortical acetylcholine release and on nicotinic receptors in rats and guinea-pigs. *Br. J. Pharmacol.*, **98**, 71–78.

PEREZ DE LA MORA, M., MENDEZ-FRANCO, J., SALCEDA, R., AGUIRRE, J.A. & FUXE, K. (1991). Neurochemical effects of nicotine on glutamate and GABA mechanisms in the brain. *Acta Physiol. Scand.*, **141**, 241–250.

RIBERIO, E.B., BETTIKER, R.L., BOGDANOV, M. & WURTMAN, R.J. (1993). Effect of systemic nicotine on serotonin release in rat brain. *Brain Res.*, **621**, 311–318.

RICE, M.E., GERHARDT, G.A., HIERL, P.M., NAGY, G. & ADAMS, R.N. (1985). Diffusion coefficient of neurotransmitters and their metabolites in brain extracelluar fluid space. *Neuroscience*, **15**, 891–902.

ROSENCRANS, J.A. & SCHECHTER, M.D. (1972). Brain area nicotine levels in male and female rats of two strains. *Arch. Int. Pharmacodyn.*, **196**, 46–54.

TOTH, E., VIZI, S. & LAJTHA, A. (1993). Effect of nicotine on levels of extracelluar amino acids in regions of the rat brain in vivo. *Neuropharmacol.*, **32**, 827–832.

WESTFALL, T.C., GRANT, H. & PERRY, H. (1983). Release of dopamine and 5-hydroxytryptamine from rat striatal slices following activation of nicotinic receptors. *Gen. Pharmacol.*, **14**, 321–325.

WONNACOTT, S., IRONS, J., RAPIER, C., THORNE, B. & LUNT, G. (1989). Presynaptic modulation of transmitter release by nicotinic receptors. *Prog. Brain Res.*, **79**, 157–163.

YAMAMOTO, I., INOKI, R., TAMARI, Y. & IWATSUBO, K. (1967). Effect of reserpine on brain levels of ^{14}C-nicotine in relation to nicotine-induced convulsions. *Arch. Int. Pharmacodyn.*, **166**, 102–109.

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