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Nicotine administration stimulates the *in vivo* N-methyl-D-aspartate receptor/nitric oxide/cyclic GMP pathway in rat hippocampus through glutamate release

[1]Ernesto Fedele, [1]Giorgia Varnier, [1]Maria Antonia Ansaldo & [1,2]Maurizio Raiteri

[1]Dipartimento di Medicina Sperimentale, Sezione di Farmacologia e Tossicologia, Università di Genova, Viale Cembrano 4 - 16148 Genova, Italy

1 The *in vivo* effects of nicotine on the nitric oxide (NO) synthase/cyclic GMP pathway of the adult rat hippocampus have been investigated by monitoring the levels of extracellular cyclic GMP during microdialysis in conscious unrestrained animals.

2 Intraperitoneal (i.p.) administration of nicotine caused elevation of cyclic GMP levels which was prevented by mecamylamine. The effect of nicotine was abolished by local infusion of the NO synthase inhibitor N^G-nitro-L-arginine (L-NOARG) or by the soluble guanylyl cyclase blocker 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (ODQ).

3 Local administration of the NMDA receptor antagonists *cis*-4-(phosphonomethyl)-2-piperidinecarboxylic acid (CGS19755) and dizocilpine (MK-801) inhibited by about 60% the nicotine-induced elevation of cyclic GMP. Nicotine was able to stimulate cyclic GMP outflow also when administered directly into the hippocampus; the effect was sensitive to mecamylamine, L-NOARG, ODQ or MK-801.

4 Nicotine, either administered i.p. or infused locally, produced augmentation of glutamate and aspartate extracellular levels, whereas the outflows of γ-aminobutyric acid (GABA) and glycine remained unaffected. Following local administration of high concentrations of nicotine, animals displayed symptoms of mild excitation (sniffing, increased motor and exploratory activity) during the first 20–40 min of infusion, followed by wet dog shake episodes; these behavioural effects were prevented by mecamylamine or MK-801, but not by L-NOARG or by ODQ.

5 It is concluded that (a) nicotine stimulates the production of NO and cyclic GMP in the hippocampus; (b) this occurs, at least in part, through release of glutamate/aspartate and activation of NMDA receptors. Modulation of the NMDA receptor/NO synthase/cyclic GMP pathway may be involved in the cognitive activities of nicotine.

Keywords: Nicotine; nitric oxide; cyclic GMP; endogenous amino acids; microdialysis

Introduction

Nicotine has been found to improve memory performance in a variety of species, including human, and in various behavioural paradigms. Nicotine was shown to be effective in attenuating memory deficits resulting from lesions of the septohippocampal pathway or aging in experimental animals. Some aspects of the cognitive impairment of Alzheimer's patients can apparently be attenuated by nicotine (Levin, 1992; Warburton, 1992; Lawrence & Sahakian, 1995).

One likely target for these effects is the hippocampus, an area of the CNS primarily involved in processing cognitive functions that receives a rich cholinergic innervation and displays a dense nicotinic receptor expression especially of the α_7 subtype (Woolf, 1991; Seguela *et al*., 1993). The density of neuronal nicotinic receptors in the hippocampus is severely reduced in Alzheimer's disease and in other pathologies characterized by cognitive deficits although it would seem that α_4 β_2 nicotinic ACh receptors are principally lost (Nordberg, 1994; Maelicke & Albuquerque, 1996).

The mechanisms underlying the cognitive effects of nicotine are poorly understood. It has to be noted that there is little evidence for synaptic transmission mediated by nicotinic receptors in the hippocampus. Nicotine is instead known to act on presynaptic receptors sited on axon terminals of different brain areas, including the hippocampus, to enhance neurotransmitter release, as shown in several studies with synaptosomes (see Wonnacott, 1997 and references therein). These findings, together with recent results from electrophysiological experiments (McGehee *et al*., 1995; Gray *et al*., 1996) have led to the idea that activation of pre- rather than postsynaptic nicotinic receptors may underlie the behavioural effects of nicotine and that CNS nicotinic receptors may essentially modulate rather than mediate transmission.

Excitatory synaptic transmission is a likely target for modulation by nicotinic receptors and nicotine was in fact able to enhance glutamatergic synaptic transmission by activation of presynaptic nicotinic receptors followed by entry of Ca^{2+} ions into glutamate-releasing nerve terminals (McGehee *et al*., 1995; Gray *et al*., 1996).

The released glutamate can activate multiple postsynaptic receptors of which the NMDA type is known to be intimately involved in cognitive processes (Morris *et al*., 1986; Hawkins *et al*., 1993). The influx of Ca^{2+} into postsynaptic cells through the NMDA channel activates calmodulin which, in turn, stimulates nitric oxide (NO) synthase to produce NO. Finally, NO will activate soluble guanylyl cyclase thus producing cyclic GMP (Garthwaite, 1991; Snyder & Bredt, 1991; Murad, 1994), a classical intracellular second messenger whose cytosolic levels are then reduced back to basal values by selective phosphodiesterases (Nicholson *et al*., 1991; Beavo, 1995).

Evidence has been provided that neuronally generated NO is involved in memory formation. Blocking the production of NO by inhibiting NO synthase has been found to significantly impair learning in various tasks and in several species of

[2]Author for correspondence.

laboratory animals (Chapman *et al*., 1992; Estall *et al*., 1993; Papa *et al*., 1994; Kendrick *et al*., 1997; Meyer *et al*., 1998). In as much as inhibition of guanylyl cyclase prevented the formation of the olfactory memory in sheep (Kendrick *et al*., 1997), cyclic GMP also appears to play an important role in cognitive processes.

Previous studies showed that monitoring extracellular cyclic GMP during *in vivo* microdialysis of the hippocampus permits the study of the glutamate receptor/NO/cyclic GMP pathway in freely-moving rats (Vallebuona & Raiteri, 1994, 1995; Fedele *et al*., 1996, 1997a,b). In the present work we investigated the nicotine-glutamate interaction by monitoring the effects of nicotine on hippocampal extracellular levels of cyclic GMP.

Methods

Neurosurgery and dialysis procedure

Male Sprague-Dawley rats (250–300 g, CD-COBS, Charles River, Calco, Italy) were anaesthetized with Equitesin 3 ml kg^{-1} (composition in g l^{-1}: 9.6 pentobarbital; 42.4 chloral hydrate; 21.2 $MgSO_4$; 396 propylene glycol and 100 ethanol), placed in a stereotaxic frame (David Kopf Instruments, West Hempstead, NY, U.S.A.) and implanted with a microdialysis probe which was transversely positioned into the dorsal hippocampi according to the following co-ordinates: AP = +3.8, H = +6.5 from the interaural line (Vallebuona & Raiteri, 1994; Paxinos & Watson, 1986). A piece of dialysis fibre made of a co-polymer of acrylonitrile sodium methallyl sulphonate (AN69HF Hospal S.p.A., Bologna, Italy; 0.3 mm outer diameter with more than 40,000 mol. wt cut-off) was covered with epoxy glue to confine dialysis to the area of interest (10 mm glue-free zone). The skull was exposed and two holes were drilled on the lateral surface at the level of the dorsal hippocampi. One dialysis probe, held straight by a tungsten wire inside, was inserted transversely into the brain so that the glue-free zone was exactly located in the target area. The tungsten wire was withdrawn and stainless steel cannulae (22-gauge diameter, about 15 mm long) were glued to the ends of the fibre, which were bent up and fixed vertically to the skull with dental cement and modified Eppendorf tips. After a 24 h recovery period, rats were placed into perspex cages and the probes infused at a flow rate of 5 μl min^{-1} (CMA/100 microinjection pump, CMA Microdialysis Stockholm, Sweden) with modified Ringer's medium containing (in mM): NaCl 145, KCl 3, $CaCl_2$ 1.26, $MgCl_2$ l, buffered at pH 7.4 with 2 mM phosphate buffer. Consecutive samples were collected every 20 min following a stabilization period of 1 h. At the end of the experiment, rats were killed and the correct position of the probe was verified by histological examination of the fibre tract. The *in vitro* recovery for cyclic GMP, under these experimental conditions, was 35 ± 4.1 ($n = 3$ different probes).

Cyclic GMP and endogenous amino acids determination

Cyclic GMP content in the dialysates (90 μl) was assayed by a commercially available radioimmunoassay kit (Amersham dual range, Amersham Radiochemical Centre, Buckinghamshire, U.K.) using the acetylation protocol. Under these experimental conditions, the sensitivity of the assay is 2 fmol 100 μl^{-1} (standard curve range 128–2 fmol 100 μl^{-1}).

Endogenous amino acids were determined by high performance liquid chromatography (h.p.l.c.) analysis following automatic derivatization (Waters 715 Ultrawisp; Milford, MA, U.S.A.) of the samples (50 μl) with *o*-phthalaldehyde (808 mg sodium tetraborate, 50 mg *o*-phthalaldehyde, 1 ml methanol, 50 μl 2-mercaptoethanol in 10 ml of h.p.l.c. grade water) for 4 min and resolution through a C_{18}-reverse phase chromatographic column (10×4.6 mm, 3 μm; Chrompack, Middleburg, The Netherlands) which was kept at constant temperature (30°C); the column was connected to a fluorimetric detector set at 350 and 450 nm for excitation and emission, respectively. Buffers and gradients programme were as follows: solvent A, 0.1 M sodium acetate (pH 5.8)/methanol, 80 : 20; solvent B, 0.1 M sodium acetate (pH 5.8)/methanol, 20 : 80; solvent C, 0.1 M sodium acetate (pH 6.0)/methanol, 80 : 20; gradient programme, 100% C for 4 min from the initiation of the programme; 90% A and 10% B in 1 min; isocratic step for 2 min; 78% A and 22% B in 2 min; isocratic step for 6.50 min; 66% A and 34% B in 1.10 min; isocratic step for 1.50 min; 42% A and 58% B in 1.10 min; isocratic step for 3.50 min; flow rate 0.9 ml min^{-1} (Waters 600MS gradient system). Homoserine was used as internal standard. Under these conditions, the detection limit was 100 fmol 100 μl^{-1}.

Statistics and expression of results

The data presented (cyclic GMP and endogenous amino acids) are expressed as percentages of the mean basal value, which was determined by averaging the content of the first 2–3 samples, collected before drug treatments. Percentage differences between controls and drug-treated animals were analysed by two-way ANOVA with repeated measures over time followed by Mann-Whitney *U*-test. Differences were considered significant at the level of $P < 0.05$.

Materials

Nicotine hydrogen tartrate, mecamylamine hydrochloride, L-N^G-nitroarginine were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.); 1H-[1,2,4]oxadiazolo[4,3-a] quinoxaline-1-one (ODQ) was obtained from Tocris Cookson (Bristol, U.K.). Dizocilpine (MK-801) and *cis*-4-(phosphonomethyl)-2-piperidinecarboxylic acid (CGS19755) were generous gifts from Merck Sharp & Dohme (Harlow, Essex, U.K.) and Ciba-Geigy Corporation (Summit, NJ, U.S.A.), respectively.

ODQ (10 mM) was dissolved in dimethyl sulphoxide (DMSO) and diluted to the desired concentration in modified Ringer's medium; 1% DMSO did not modify cyclic GMP basal levels (data not shown) and was not included in the modified Ringer's medium used for controls. All the final solutions were buffered at pH 7.4. For i.p. injections, nicotine was dissolved in 0.9% NaCl buffered at pH 7.4 with phosphate buffer.

The experimental procedures *in vivo* were approved by the Ethic Committee of the Department of Experimental Medicine, Pharmacology and Toxicology Section, University of Genoa, according to European legislation on the use and care of laboratory animals (CEE 86/609).

Results

Intraperitoneal administration of nicotine increases cyclic GMP extracellular levels in the hippocampus of freely moving rats: involvement of the NMDA/NOS/guanylyl cyclase system

Under control conditions, the extracellular levels of cyclic GMP in the dorsal hippocampi of awake rats amounted to

16.33 ± 1.28 fmol 100 μl^{-1} ($n = 60$; not corrected for the *in vitro* recovery) and were stable and detectable for the whole time-course of the experiment.

As shown in Figure 1, i.p. administration of nicotine hydrogen tartrate dose-dependently (2 and 4 mg kg^{-1} corresponding to 0.701 and 1.403 mg of free base, respectively) enhanced the dialysate levels of the cyclic nucleotide which peaked in the fraction following the injection and returned to basal values within 20 min; at the highest dose tested, nicotine caused a 3 fold increase of cyclic GMP. These effects were not due to stress induced by the manipulation of the animals during the injection since i.p. administration of saline did not cause any effect on extracellular cyclic GMP.

In order to assess whether the nicotine-induced elevation was mediated by nicotinic ACh receptors, we investigated the effect of nicotine in the presence of the nicotinic antagonist mecamylamine, which was delivered locally in the dorsal hippocampi by retrodialysis one fraction before, together, after the i.p. injection of the agonist and till the end of the experiment. At the concentration of 500 μM, mecamylamine almost completely abolished the potentiation caused by nicotine (Figure 1); in preliminary experiments, we found that local perfusion of mecamylamine alone, up to 2 mM, did not change the baseline levels of cyclic GMP (data not shown).

Since it is generally accepted that most of the cyclic GMP present in the central nervous system is produced upon the activation of soluble guanylyl cyclase by NO, it was of interest to determine whether the increase caused by nicotine was mediated by the activation of this multistep biochemical pathway. Hippocampal infusion of the NO-synthase inhibitor, L-NOARG, at the concentration of 100 μM, completely prevented the increase of cyclic GMP by systemic nicotine (4 mg kg^{-1}; Figure 2); similar results were obtained when ODQ, a selective blocker of soluble guanylyl cyclase, was present in the dialysis stream (100 μM; Figure 2). The drop of hippocampal cyclic GMP levels below control levels during NOS or guanylyl cyclase inhibition is in accordance with previous reports (Vallebuona & Raiteri, 1994; Fedele *et al.*, 1996, 1997a, b).

Finally, it would seem that NMDA glutamatergic receptors are involved in the phenomenon observed since local infusion of CGS19755 (100 μM) or MK-801 (30 μM) was able to inhibit the nicotine (4 mg kg^{-1})-mediated cyclic GMP increase by approximately sixty per cent (Figure 2). The two antagonists did not modify cyclic GMP basal levels on their own (data not shown).

At the highest dose, nicotine caused convulsions which lasted approximately 10 min; local infusion of mecamylamine in the hippocampus, before, during and after nicotine i.p. administration, did not attenuate this behaviour despite its efficacy in preventing cyclic GMP elevation. Similar results have been obtained when L-NOARG, ODQ, MK-801 or CGS19755 were present in the dialysis stream.

Effects of intrahippocampal administration of nicotine on cyclic GMP extracellular levels in freely moving rats

To determine whether intrahippocampal circuits were involved in the action of nicotine, we studied the effects of nicotine directly administered in the hippocampus by mean of retrodialysis infusion. As can be seen in Figure 3, continuous presence of nicotine in the dialysis fluid (5 and 20 mM) was

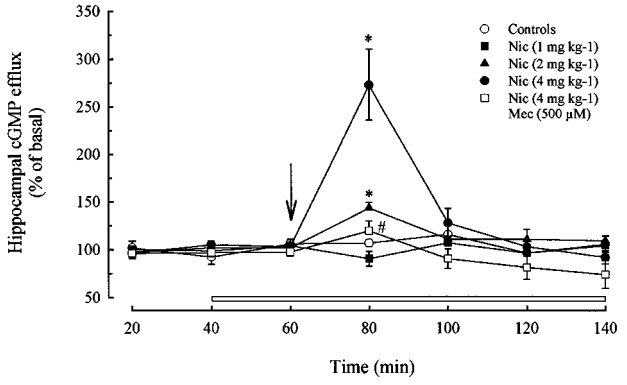

Figure 1 Effects of systemically administered nicotine on cyclic GMP extracellular levels in the hippocampus of freely-moving rats. Nicotine hydrogen tartrate was injected intraperitoneally after the third fraction had been collected (arrow); when present, mecamylamine was infused locally in the dorsal hippocampi by retrodialysis one fraction before the injection of nicotine and for the time indicated by the horizontal empty bar. Control animals were injected with vehicle. Each point represents the means ± s.e.mean of four to six different experiments. $*P < 0.05$ at least vs controls; $\#P < 0.05$ at least vs nicotine 4 mg kg^{-1}. For further technical details see Methods.

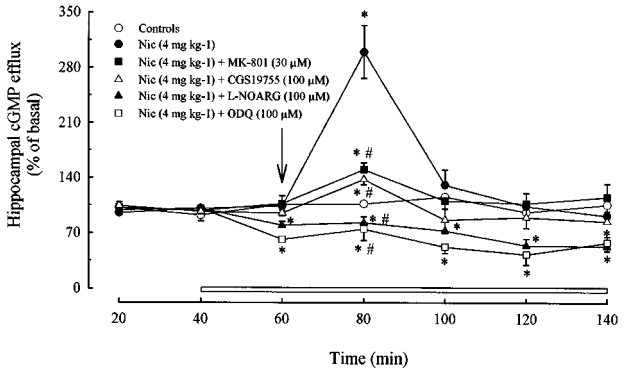

Figure 2 Systemic nicotine-induced cyclic GMP elevation is prevented by local perfusion of MK-801, CGS19755, L-NOARG or ODQ. Nicotine (4 mg kg^{-1}) was injected i.p. at the end of the third fraction as indicated by the arrow; MK-801, CGS19755, L-NOARG or ODQ, when present, were infused locally in the dorsal hippocampi one fraction before and together with nicotine (horizontal empty bar). Each point represents means ± s.e.mean of four different experiments. $*P < 0.05$ at least vs controls. $\#P < 0.05$ at least vs nicotine 4 mg kg^{-1}.

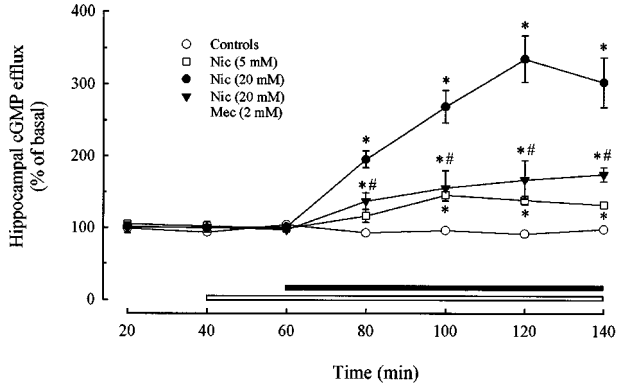

Figure 3 Effects of local administration of nicotine on cyclic GMP extracellular levels in the hippocampus of freely-moving rats. Nicotine was administered in the dorsal hippocampi by retrodialysis for the time indicated by the horizontal solid bar whereas mecamylamine, when present, was infused one fraction before and together with nicotine (horizontal empty bar). Each point represents the means ± s.e.mean of three to six different experiments. $*P < 0.05$ at least vs controls; $\#P < 0.05$ at least vs nicotine 20 mM.

able to enhance the production of the cyclic nucleotide, the maximal increase (200% over control values) peaking after 60 min of infusion with 20 mM nicotine; at 1 mM nicotine failed to affect cyclic GMP extracellular levels (data not shown). Also in this case, the nicotine-mediated enhancement of cyclic GMP was sensitive to mecamylamine (2 mM), L-NOARG (100 μM), ODQ (100 μM) or MK-801 (30 μM; Figures 3 and 4).

Following nicotine local administration (5–20 mM), animals displayed clear symptoms of mild excitation (sniffing, increased motor and exploratory activity) during the first 20–40 min of infusion. At the highest concentration, however, wet dog shakes were evident after 40 min and continued till the end of the experiment; these behavioural effects were completely prevented when either mecamylamine or MK-801, but not L-NOARG or ODQ, were co-infused with nicotine (Table 1).

Hippocampal endogenous amino acids: effects of nicotine

Since the cyclic GMP response to nicotine was reduced by NMDA receptor antagonists, we checked whether nicotine was capable of eliciting the release of endogenous excitatory amino acids. Under our experimental conditions, the extracellular basal levels of endogenous aspartate, glutamate, glycine and GABA were 18.96 ± 2.57, 245.08 ± 33.49, 550.51 ± 57.56 and 12.20 ± 1.04 pmol 100 μl^{-1} (mean $\pm$ s.e.mean; $n = 8$), respectively. Intraperitoneal injection of nicotine (4 mg kg^{-1}) caused a rapid and transient augmentation of both aspartate and glutamate extracellular levels but did not influence those of glycine and GABA (Figure 5A).

Also the continuous intrahippocampal infusion of the drug (20 mM) enhanced aspartate and glutamate efflux, the effect being stable as long as nicotine was present in the dialysis stream; glycine and GABA did not change significantly (Figure 5B).

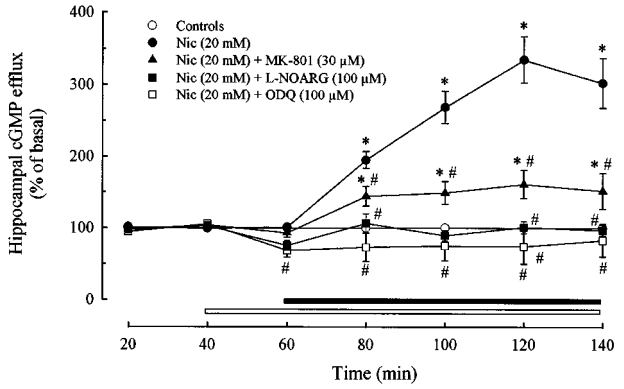

Figure 4 Blockade of NO-synthase, guanylyl cyclase or NMDA receptors prevents cyclic GMP increase by local nicotine administration. Rats were infused locally in the dorsal hippocampi with nicotine for the time indicated by the horizontal solid bar; MK-801, L-NOARG or ODQ, when present, were infused one fraction before and together with nicotine (horizontal empty bar). Each point represents the means $\pm$ s.e.mean of three to six different experiments. $^*P < 0.05$ at least vs controls; $\#P < 0.05$ at least vs nicotine 20 mM.

Table 1 Behavioural changes induced by nicotine perfusion in the dorsal hippocampi of freely-moving rats

Drugs (mM)	*WDS*	n
Nicotine (20)	97.22 ± 12.30*	6
Nicotine (20) Mecamylamine (2)	11.17 ± 3.46#	6
Nicotine (20) MK-801 (0.03)	15.83 ± 4.32#	3
Nicotine (20) L-NOARG (0.1)	118.60 ± 10.11*	5
Nicotine (20) ODQ (0.1)	90.35 ± 7.88*	5

Nicotine was perfused in the dorsal hippocampi by retrodialysis for 80 min during which animals' behaviour was recorded. WDS, wet dog shake episodes; each value represents means $\pm$ s.e.mean of n different experiments. $^*P < 0.001$ vs controls, $\#P < 0.001$ vs nicotine.

Discussion

Nicotine, administered peripherally, was able to elicit significant augmentation of the extracellular levels of cyclic GMP in the hippocampus. This effect was dependent on the activation of CNS nicotinic receptors since it was prevented when the nicotinic receptor antagonist mecamylamine was infused directly into the hippocampus. The lack of activity of mecamylamine on cyclic GMP basal levels when infused alone into the hippocampus is indicative of a phasic rather than a tonic control of the NO/cyclic GMP pathway by nicotinic receptors.

The concentration of nicotine reached at brain nicotinic receptors is unknown. However, assuming a 150 ml of body volume for a rat weighing 250 g, a complete absorption of the

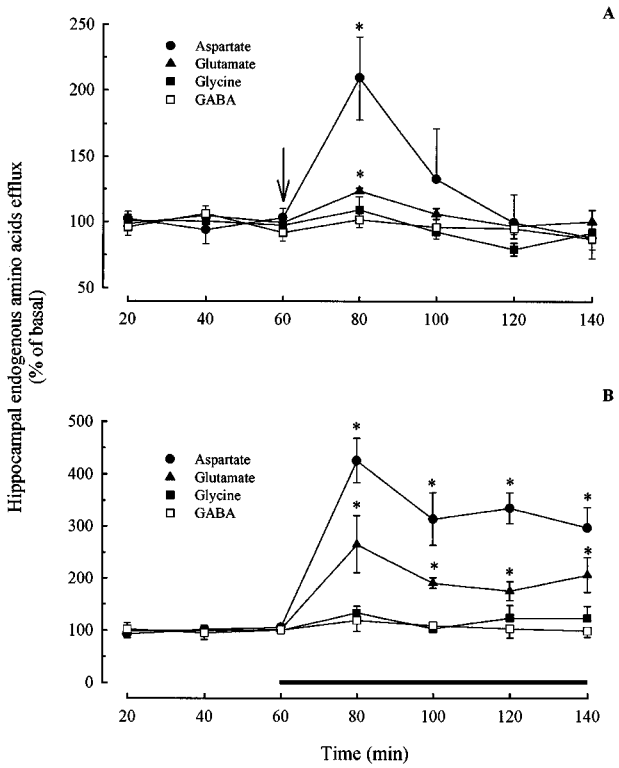

Figure 5 Administration of nicotine in the hippocampus causes elevation of endogenous glutamate and aspartate. (A) Nicotine (4 mg kg^{-1}) was injected i.p. at the end of the third fraction (arrow). (B) Nicotine (20 mM) was infused through the probe for the time indicated by the horizontal solid bar. Each point represents the means $\pm$ s.e.mean of three to four different experiments. For the sake of clarity, basal control values for each amino acid have not been reported but were always stable and detectable for the whole time-course of the experiment. $^*P < 0.05$ at least vs controls.

drug, no metabolic degradation or excretion and a homogeneous distribution throughout the body, the i.p. injection of 2 mg kg^{-1} of nicotine would lead to a concentration of $\sim 7\ \mu M$. Such a concentration, clearly overestimated, is compatible with the low micromolar concentrations of nicotine achieved with smoking (Henningfield *et al*., 1993).

Blocking NO synthase activity with L-NOARG prevented the nicotine elevation of extracellular cyclic GMP indicating the involvement of NO in the nicotine effect. Furthermore, the cyclic GMP response to nicotine, either administered intraperitoneally or directly infused into the hippocampus, originates from activation of soluble guanylyl cyclase since it was prevented by ODQ, a compound shown to selectively inhibit the soluble form of guanylyl cyclase both *in vitro* (Garthwaite *et al*., 1995) and *in vivo* (Fedele *et al*., 1996).

The findings that CGS19755 and MK-801, selective competitive and non-competitive NMDA receptor antagonists, respectively, both reduced the nicotine-induced cyclic GMP elevations indicate a functional interaction with the glutamatergic system. Indeed, recent reports describe the ability of nicotine to enhance excitatory synaptic transmission (McGehee *et al*., 1995; Gray *et al*., 1996). This most likely occurs because nicotine can evoke release of glutamate/aspartate, as previously observed (Toth, 1996) and now confirmed in our experiments of *in vivo* microdialysis showing that systemic and local nicotine can elevate the extracellular levels of the two excitatory amino acids, but not those of GABA or glycine. In the case of GABA, our results are at variance with the recent data showing that α_7-type nicotinic ACh receptors are present on the cell bodies of hippocampal GABAergic interneurons (Jones & Yakel, 1997; Frazier *et al*., 1998). However it should be remembered that subtle changes of GABA synaptic levels induced by nicotine might have been lost by microdialysis sampling of extracellular fluids. In addition, small variations might have been masked by the very efficient reuptake mechanisms shown to greatly influence *in vivo* GABA levels (Fink-Jensen *et al*., 1992; Waldmeier *et al*., 1992; Rowley *et al*., 1995).

Altogether these effects of nicotine on glutamatergic transmission suggest that nicotine activates the NO synthase/guanylyl cyclase system indirectly. In other words nicotine, by acting at the presynaptic level, would enhance the release of glutamate and aspartate which, in turn, stimulate NMDA receptors thus triggering the cascade of events leading to cyclic GMP production. It should be noted, however, that a significant portion of the cyclic GMP response to nicotine was not sensitive to NMDA receptor antagonists, suggesting that other receptors may play a role. Receptors of the AMPA type stimulated by the glutamate released by nicotine may well contribute to cyclic GMP production; unfortunately, testing this hypothesis is precluded by the fact that, under basal conditions, infusion of AMPA/kainate receptor antagonists alone into the hippocampus results in a robust increase of extracellular cyclic GMP (Fedele *et al*., 1997a,b) which would mask their possible antagonistic activity on the nicotine-induced effect. Another possibility is that postsynaptic nicotinic receptors present on hippocampal neurons and able to mediate increases of cytosolic Ca^{2+} levels (Albuquerque *et al*., 1997) are directly involved in the activation of NO synthase and the subsequent production of cyclic GMP.

The effect of systemically administered nicotine might have involved activation of hippocampal afferents mediated by nicotinic receptors located outside the hippocampus. This is unlikely, however, since nicotine produced elevation of cyclic GMP levels also when directly administered in the hippocampus by mean of retrodialysis infusion. The high concentrations of nicotine needed to elicit a cyclic GMP response during local infusion may appear surprising. However, in recent *in vivo* studies with nicotine infused through the dialysis probe at millimolar concentrations (Toth, 1996; Marshall *et al*., 1997), it was clearly shown that, for unknown reasons, the drug crosses the dialysis membrane in very low amounts ($\sim 0.5\%$ of the original concentration).

Rats administered with 5 mM of nicotine locally into the hippocampus displayed signs of moderate excitation (increased locomotor and exploratory activity); however, when the hippocampus was continuously infused with 20 mM of nicotine, the mild behavioural changes observed during the first 20–40 min were followed by wet dog shake episodes in keeping with the well known convulsive effects produced by relatively large doses of nicotine (Silvette *et al*., 1962). Interestingly, correlation between density of α_7-nicotinic hippocampal receptors and susceptibility to convulsions has been reported in the literature (Miner *et al*., 1986). Since these effects were prevented by mecamylamine, they are mediated by nicotine receptor activation; MK-801 also blocked the behavioural effects of nicotine indicating the involvement of the glutamatergic system. Interestingly, inhibition of neither NO synthase nor of guanylyl cyclase affected the number of wet dog shakes (Table 1). Similar results had been obtained in previous studies where we found that direct activation of hippocampal glutamate receptors with exogenous NMDA, AMPA or cyclothiazide elicited either biochemical and behavioural responses which, as expected, were prevented by co-infusion of the selective antagonists (Vallebuona & Raiteri, 1994; Fedele *et al*., 1997a). As in the present case, however, co-infusion of L-NOARG or ODQ did not alter the behavioural episodes despite their efficacy in completely preventing the elevation of cyclic GMP extracellular levels. Thus, it would seem that excessive, direct or indirect, stimulation of glutamatergic neurotransmission might result in abnormal excitatory hippocampal output which, by recruiting other limbic circuits, lead to the preconvulsive/convulsive state whereas activation of hippocampal NOS and guanylyl cyclase would represent only a 'secondary effect' and would not be instrumental in triggering the behavioural consequences. This view is further supported by the findings that hippocampal infusion of the NO-donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP) or the phosphodiesterase inhibitor 3-isobutyl-1-methylxantin (IBMX) were able to increase cyclic GMP levels by 3 and 8 fold, respectively, without affecting the animals' normal behaviour. On the other hand, convulsions elicited by nicotine i.p administration were not attenuated by hippocampal infusion of mecamylamine, MK-801, CGS19755, L-NOARG or ODQ indicating that, in this case, stimulation of hippocampal nicotinic receptors triggering glutamate release and the consequent activation of the NMDA/NOS/cyclic GMP hippocampal pathway do not play a major role in initiating the cascade of events leading to epileptic behaviour. Activation of nicotinic receptors localized in other brain regions thus appears to be responsible of the behavioural alterations observed following systemic administration of nicotine.

Although the present results provide no information on the possible relations between nicotine activation of the hippocampal glutamate receptor/NO/cyclic GMP pathway and cognitive activities of the drug, several lines of investigation have highlighted the pivotal role of this biochemical cascade in synaptic plasticity phenomena involved in memory formation. In fact, it has been extensively reported that hippocampal long term potentiation (LTP) can be prevented by NO-synthase inhibitors, at least under certain stimulation conditions

(Böhme *et al*., 1991; O'Dell *et al*., 1991; Schuman & Madison, 1991; Bon *et al*., 1992; Haley *et al*., 1992) and exogenous NO is able to induce an enduring enhancement of hippocampal synaptic potentials (Böhme *et al*., 1991; Zhuo *et al*., 1993, 1994a). Similarly, blockade of soluble guanylyl cyclase has been shown to reduce LTP (Zhuo *et al*., 1994b; Boulton *et al*., 1995), whereas bath application of membrane-permeable analogues of cyclic GMP produce long-lasting potentiation of synaptic strength (Zhuo *et al*., 1994a,b). Finally, recent work by Kendrick and coworkers (1997) suggests that the whole pathway may be involved in memory formation. In fact, the ability of sheep to learn to recognize the odours of their lambs was found to be inhibited following blockade of ionotropic glutamate receptors, NO synthase or guanylyl cyclase in the olfactory bulb. Monitoring cyclic GMP extracellular levels in the CNS of freely moving rats during learning tasks appears as a promising approach to a better understanding of cognitive processes.

Nicotine has been found to elicit release of dopamine in *in vivo* studies using microdialysis (Imperato *et al*., 1986; Pontieri *et al*., 1996) and this effect in the nucleus accumbens has been linked to the dependence liability for the drug (Fibiger & Phillips, 1987; Corrigall *et al*., 1992; Pontieri *et al*., 1996). The nicotinic receptors involved in the release of dopamine in the mesolimbic system and of glutamate in the hippocampus may well be receptor subtypes having different subunit composition (McGehee *et al*., 1995; Gray *et al*., 1996; Kaiser *et al*., 1998). In this case nicotinic receptor agonists able to selectively activate the glutamate/NO/cyclic GMP pathway in the hippocampus could ameliorate cognition without dangerously affecting the dopaminergic reward system.

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