Chronic continuous infusion of nicotine increases the disappearance of choline acetyltransferase immunoreactivity in the cholinergic cell bodies of the medial septal nucleus following a partial unilateral transection of the fimbria fornix

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Abstract. Previous studies have demonstrated that chronic continuous nicotine treatment via minipumps partially protects against mechanically induced degeneration of the nigrostriatal dopamine neurons in the male Sprague-Dawley rat. In the present study we investigated how a 4-week continuous infusion with (-)-nicotine via minipumps implanted subcutaneously in the male Sprague-Dawley rat (0.125 mg/kg⁻¹ h⁻¹) influences the anterograde and retrograde changes occurring in the septohippocampal cholinergic neurons following a unilateral transection of the fimbria fornix. Choline acetyltransferase and acetylcholinesterase immunocytochemistry was performed in combination with computer-assisted morphometry and microdensitometry. Measurements of choline acetyltransferase enzyme activity was performed in the dorsal hippocampus. The chronic nicotine infusion significantly increased the disappearance of the choline acetyltransferase immunoreactive nerve cell area within the medial septal nucleus of the lesioned side. However, the disappearance of the acetylcholinesterase immunoreactive enzyme terminals within the dentate gyrus (molecular layer) and of choline acetyltransferase enzyme activity within the dorsal hippocampus was not found to be influenced by the chronic nicotine infusion. Thus, chronic infusion of (-)-nicotine does not appear to exert any protective activity on mechanically injured septohippocampal cholinergic neurons but may instead increase their dysfunction. In comparison with the dopaminergic neurons it may therefore be that the continuous chronic nicotine exposure does not lead to sufficient desensitization of the nicotinic cholinceptors of the cholinergic neurons to reduce the chronic influx of sodium and calcium ions via the nicotinic ion channels and thus intraneuronal calcium levels and energy demands. Interactions between the high-affinity tyrosine kinase receptors for the neurotrophins and other growth factors and the nicotinic receptors may also be different from those taking place within the nigral dopaminergic neurons. Thus, heterogeneities may exist among central neuronal systems with regard to their trophic responses to chronic continuous nicotine treatment.

Key words: Fimbria lesion – Acetylcholine – Degeneration – Medial septal nucleus – Dorsal hippocampus – Nicotinic receptors

Chronic nicotine treatment has previously been shown to counteract the disappearance of tyrosine hydroxylase immunoreactive (ir) nerve cell bodies and terminals in the nigrostriatal dopamine (DA) neurons of the male rat after partial hemitransection [10]. These effects are associated with increases in DA levels and reduced DA utilization in the substantia nigra and in the surviving forebrain DA nerve terminal system [5]. These protective actions on partially lesioned nigrostriatal DA neurons are also associated with a reduced burst-firing and increases in extracellular basal DA levels [8, 11]. These results can be of relevance for explaining the reduced incidence of Parkinson’s disease in smokers. They open the possibility of putative protective actions of chronic nicotine also in other neurodegenerative disorders such as Alzheimer’s disease.
In the present study we therefore analyzed whether chronic nicotine treatment can counteract the degeneration of the septohippocampal cholinergic neurons taking place after unilateral fimbria fornix transection [7], using mainly choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) immunocytochemistry in combination with imaging analysis.

Materials and methods

Animals

Male specific pathogen-free Sprague-Dawley rats (B&K Lab, Stockholm, Sweden; body weight 200 g) were used. They were kept under regular day and night conditions at a constant temperature (+23 °C) and given food pellets and water ad libitum.

Fimbria lesion

The unilateral fimbria fornix transection [7] was performed with the rat in chloral hydrate (35 mg/100 g b.wt.) anesthesia at the level immediately in front of the dorsal hippocampus (Bregma level, −1.3 mm). The lesion was made with a thin knife (2 mm). It was entered perpendicularly and with a 90° angle to the midline. The lateral border of the knife was located 2.2 mm lateral to the midline and was lowered to a level 6 mm below the pia mater. By means of morphological analysis using Cresyl violet staining this mechanical lesion was found to produce axotomy of large numbers of fibers within the fimbria.

Nicotine treatment protocol

Immediately following the lesion Alzet minipumps (model 2003, SMA, London, UK) were implanted subcutaneously in the neck. The minipumps contain nicotine hydrogen (+)-tartrate (BDH Chemicals, Boole, UK) in amounts calculated to produce a dose of 0.125 mg kg⁻¹ h⁻¹. This dose was chosen since in this laboratory previous experiments have shown that the serum nicotine levels obtained are similar to those found in smokers (50–70 ng/ml). Immediately after the fimbria lesion the rats were also injected with nicotine (0.5 mg/kg four times intraperitoneally) at 30-min intervals so that prompt nicotine concentrations could be obtained within the central nervous system. Three other groups were also analyzed: (a) animals with fimbria fornix lesions unilaterally receiving saline injections instead of nicotine as described above and implanted with minipumps containing saline instead of nicotine; (b) sham-operated rats (all neurosurgery except the making of the knife cut) receiving nicotine treatment as described above; (c) sham-operated rats receiving saline treatment as described above.

Body temperature and body weight

These two parameters were studied 2 weeks before and 4 weeks after the fimbria lesion. No significant changes were observed among the four groups analyzed.

Immunocytochemistry

The rats were killed 4 weeks after the fimbria lesion under chloral hydrate anaesthesia (350 mg/100 g b.w.t., intraperitoneally) by perfusion through a cannula inserted in the ascending aorta with 50 ml saline (37 °C) followed by 150 ml fixation fluid (4 °C) over 6 min. The fixative (modification of Zamboni and deMartino [21]) consisted of 4% (w/v) paraformaldehyde, 0.2% (w/v) picric acid in 0.1 M phosphate buffer, pH 6.9. The brains and spinal cords were rinsed in 10% sucrose solutions in 0.1 M phosphate-buffered saline. The sections of the medial septal nucleus and the dorsal hippocampus were made in a cryostat (Leitz 1720). Coronal 20-μm-thick sections were made, and the Paxinos and Watson Atlas was used [15]. The sections were mounted on object glasses coated with gelatin-chrome alum, and the incubations were performed in a humidified chamber. The primary antibodies were diluted with 0.3% Triton and 1% bovine serum albumin (Sigma; 0.1 M phosphate-buffered saline). The goat ChAT antiserum employed has previously been characterized by Tago et al. [17] and Bruce et al. [2] and was used in a dilution of 1/1000 (see also [18]). The rabbit AChE antiserum has been characterized by Marsh et al. [13] and was used in a dilution of 1/1500. The indirect immunoperoxidase technique was used to detect the primary antibody involving the avidin-biotin-peroxidase system (Vectastain, Vector, Burlingame, CA, USA) [9]. The sections were incubated overnight with the primary antibody in a humidified chamber. 3,3'-Diaminobenzidine tetrahydrochloride [0.05% (w/v) DAB, Sigma, St. Louis, MO, USA] was used as a chromogen. The hydrogen peroxide concentration was 0.05%.

ChAT enzyme activity

Four weeks after the fimbria lesion the rats were killed, and the dorsal hippocampus and the septal nucleus with the tractus diagonalis band nuclei