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Short communication

The α_2-adrenoceptor antagonist, (+)-efaroxan, enhances acetylcholine release in the rat cortex in vivo

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Abstract

Noradrenergic modulation of the cortical cholinergic system in vivo was studied by examining the effect of the selective α_2-adrenoceptor antagonist (+)-efaroxan on cortical acetylcholine outflow in the conscious rat, using the microdialysis technique. (+)-Efaroxan produced a dose-dependent increase in acetylcholine outflow (up to 300% at 0.63 mg/kg) which persisted for up to 3 h and which was stereospecific. The results demonstrate that rat cortical acetylcholine release can be augmented by (+)-efaroxan and that α_2-adrenoceptors may be involved. (+)-Efaroxan may have therapeutic potential in disorders in which cortical acetylcholine release is deficient.

Keywords: Efaroxan; α_2-Adrenoceptor antagonist; Microdialysis; Acetylcholine release; Cortex, rat

1. Introduction

A diminution of brain cholinergic markers is well correlated with the cognitive and memory impairments observed in senile dementia of the Alzheimer's type. Nevertheless, the limited clinical efficacy of direct or indirect cholinergic drugs has led to suggestions that other neurotransmitter systems affected in the disorder might be important targets for pharmacological interventions. For example, the degeneration of the locus coeruleus-noradrenergic system, which occurs in several neurodegenerative diseases, has been hypothesized to be a factor in the evolution and the progression of these brain disorders (Colpaert, 1994). Evidence for a noradrenergic modulation of the cortical cholinergic system in vivo exists, yet the results of these studies are not entirely consistent. The release of acetylcholine was found to be reduced by noradrenaline, using the cortical epidural cup technique in the guinea pig (Beani et al., 1978). In contrast, acetylcholine turnover studies in the rat have demonstrated a positive influence of the noradrenergic system (Robinson et al., 1978; Wood and McQuade, 1986). An inhibitory role of α_2-adrenoceptors is suggested by some studies. Clonidine, an α_2-adrenoceptor agonist, diminished acetylcholine release in the guinea pig cortex in vivo (Moroni et al., 1983).

To characterize further the noradrenergic influence on the cortical cholinergic system in vivo, the present study examined the effect of an α_2-adrenoceptor antagonist on cortical acetylcholine release in the rat, using the microdialysis technique. This class of compound was of particular interest since α_2 antagonists such as idazoxan are capable of increasing the release of endogenous noradrenaline in the rat cortex in vivo (Dennis et al., 1987). The α_2-adrenoceptor antagonist efaroxan (Chapleo et al., 1984) was used in the present study. A portion of this work was presented at the 6th International Conference on In Vivo Methods (Tellez et al., 1994).

2. Materials and methods

All drugs and reagents were obtained from commercial sources, except for the stereoisomers of efaroxan, which were prepared by P. Mayer and J.-L. Maurel (CRPF). Drugs were dissolved in 0.9% NaCl and injected by the intraperitoneal route in a volume of 10 ml/kg. Doses refer to the free base equivalent of drug.

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Male Sprague-Dawley rats (Ico:OFA-SD[IOPS. Caw], Charles River, Saint-Aubin-lès-Elbeuf, France; 300–360 g) were anesthetized (0.5–3% halothane/O_2) and implanted with a microdialysis probe (CMA/12, 3 mm membrane length; Carnegie-Medicin) terminating in the medial prefrontal cortex at the following coordinates: 3 mm anterior to bregma, 1.5 mm lateral from the midline suture, and 4.5 mm ventral from the dura, at a medially directed vertical angle of 14°. The probe was fixed to the skull with three stainless steel jeweller's screws and dental acrylic cement. Microdialysis experiments were carried out 24 h after probe implantation. The probe was perfused at a flow rate of 2 μl/min with Ringer's solution (NaCl, 147 mM; KCl, 4 mM; $CaCl_2$, 1.3 mM) containing 0.5 μM neostigmine bromide, an acetylcholinesterase inhibitor. Dialysate samples were collected every 20 min. After a 4 h equilibration period, the drugs were administered by intraperitoneal injection. Dialysates were analysed by high-performance liquid chromatography with electrochemical detection, using the methodology described in detail by Damsma et al. (1987). Briefly, a mobile phase containing potassium phosphate (0.1 M), EDTA (0.2 mM) and tetramethylammonium chloride (2 mM) was used to separate acetylcholine and choline on a cation exchange column (Chromspher C18, Chrompack, Netherlands) loaded with laurylsulphate. Hydrogen peroxide was subsequently generated by passage of the column effluent through an enzyme reactor containing Lichrosorb-NH_2 activated with glutaraldehyde to which choline oxidase and acetylcholinesterase were covalently bound. The hydrogen peroxide was detected at a platinum electrode set at an oxidation potential of 500 mV (Waters model M460 detector). The limit of detection of the assay for acetylcholine was approximately 150 fmol/20 μl injection volume. Levels of acetylcholine and choline were calculated as fmol/20 μl dialysate volume. Levels were not corrected for dialysis probe recovery. Acetylcholine outflow in response to drug treatments was expressed as a percentage of baseline (average level of acetylcholine in 3 dialysate samples collected immediately before drug administration).

3. Results

After a 4 h equilibration period, the baseline level of acetylcholine in frontocortical dialysate samples averaged 600 fmol/20 μl, when neostigmine (0.5 μM) was present in the perfusion fluid. Under the conditions used in the present study, acetylcholine was not quantifiable in the absence of the cholinesterase inhibitor. Initial experiments were undertaken to validate the methodology, by examining the effects of certain conditions or drug treatments which are known to

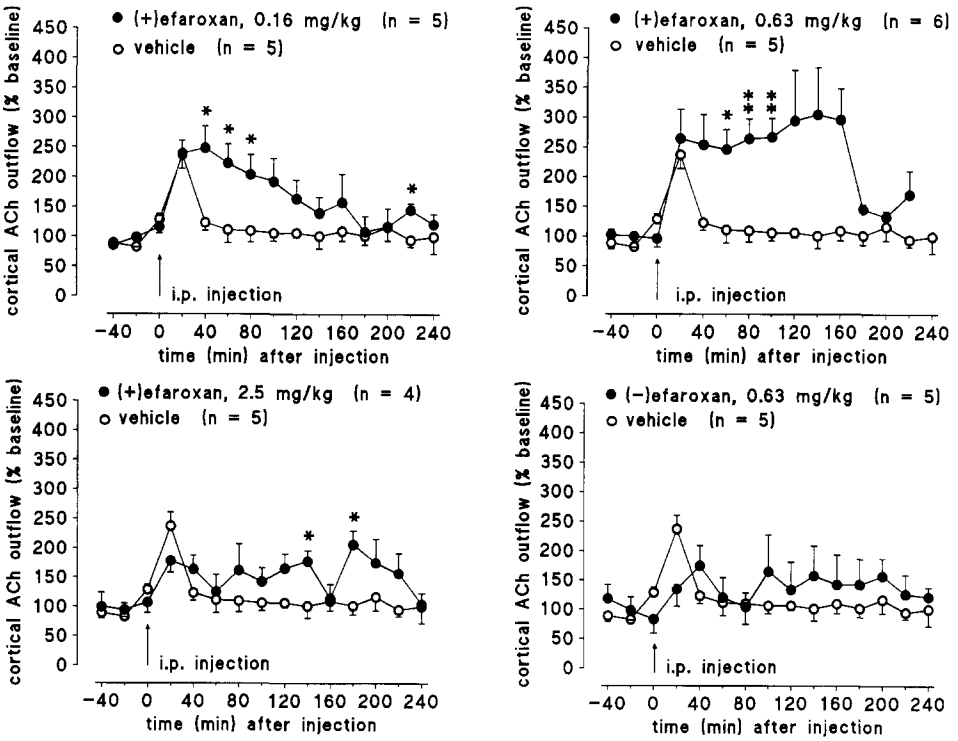

Fig. 1. Effect of the (+) and (−) isomers of efaroxan on acetylcholine outflow in the medial prefrontal cortex of conscious freely moving rats, as measured by microdialysis. Values are means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, Student's t-test for grouped data, compared to the corresponding samples for vehicle- (saline) treated animals. Abbreviations: i.p., intraperitoneal; n, number of rats per group.

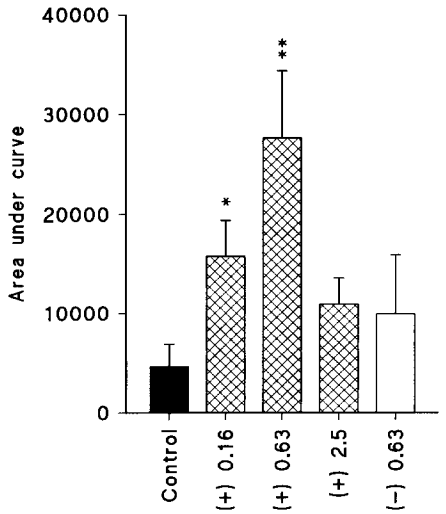

Fig. 2. Increase in acetylcholine outflow as an area-under-the-curve measurement from data shown in Fig. 1, calculated as the sum of the percentage increase over baseline in the 200 min period following the i.p. injection of saline vehicle (solid bar), (+)-efaroxan (hatched bars, doses indicated in mg/kg), and (−)-efaroxan (open bar). Values are means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to vehicle controls, Kruskal-Wallis + Mann-Whitney U-test.

affect cortical acetylcholine release in vivo. Thus, perfusion with Ringer's solution lacking calcium decreased acetylcholine outflow by more than 70%, while intraperitoneal injection of amphetamine (2 mg/kg) or tacrine (5 mg/kg) produced 4- to 5-fold increases in acetylcholine outflow (data not shown). The intraperitoneal injection of saline vehicle (NaCl, 0.9%) produced an immediate 2.5-fold increase of acetylcholine outflow which returned to baseline values within 40 min (Fig. 1). (+)-Efaroxan (0.16, 0.63 and 2.5 mg/kg i.p.) elicited an increase in acetylcholine outflow with a bell-shaped dose-response curve (Figs. 1 and 2). The greatest effect was observed with the 0.63 mg/kg dose: an immediate 2.5- to 3-fold augmentation, similar to that seen with vehicle injection alone, persisted for up to 3 h after injection. By comparison, a 0.63 mg/kg dose of the (−) isomer of efaroxan failed to change acetylcholine outflow significantly (Figs. 1 and 2). When expressed as a cumulative 'area-under-the-curve' measurement (Fig. 2), acetylcholine outflow in the 200 min period following the 0.16 and 0.63 mg/kg doses of (+)-efaroxan was 336% and 590% respectively of that induced by vehicle injection alone, while the effects of the 2.5 mg dose and the (−) isomer (232% and 211%) were not statistically significant.

4. Discussion

The prefrontal cortex in the rat is believed to be involved in arousal, vigilance and motivational responses, and interconnections with the hippocampus may underlie a role in cognition and memory (see references in Kolb and Tees, 1990). This region receives cholinergic and noradrenergic afferents originating from the nucleus basalo-magnocellularis and the locus coeruleus respectively, nuclei which in the human brain undergo significant cell loss in Alzheimer's disease (see references in Colpaert, 1994). For these reasons the cortical model was of interest for examining the possible noradrenergic modulation of acetylcholine release in vivo, using the microdialysis technique in freely moving conscious rats. Changes in acetylcholine outflow produced by calcium-free Ringer's and by systemic injection of amphetamine, tacrine and saline vehicle were entirely consistent with the results of other microdialysis studies (e.g. Messamore et al., 1993; Day et al., 1994) and validate the methodology used here.

The key finding of the present study was that the systemic administration of (+)-efaroxan, a selective α_2-adrenoceptor antagonist, was capable of producing a robust, dose-dependent and sustained increase in endogenous acetylcholine outflow in the medial prefrontal cortex of the rat in vivo. The stereoselectivity of the drug effect is strong support for its mediation by α_2-adrenoceptors, since (+)-efaroxan has a higher potency for these sites than the (−) isomer (Chan et al., 1993). Whether the receptor targets (presumably α_2) represent autoreceptors, or heteroreceptors on non-adrenergic neurons, remains an open question. It is known that α_2-adrenoceptor antagonists such as idazoxan augment noradrenaline release in the rat cortex in vivo, presumably by disinhibition, i.e. blockade of presynaptic inhibitory α_2-autoreceptors (Dennis et al., 1987). Thus, considering previous in vivo studies which have proposed a positive tonic influence of noradrenergic mechanisms on cortical acetylcholine dynamics in the rat (e.g. Wood and McQuade, 1986), noradrenergic activation might in part underlie the efaroxan-induced increase in acetylcholine release. The bell shape of the dose-response curve is not unlike that observed with other putative α_2-adrenoceptor antagonists (e.g. idazoxan and yohimbine) in behavioral (Colpaert, 1986) and EEG studies (Yavich et al., 1994) in the rat, and has been attributed to partial agonist actions at α_2-adrenoceptors (Colpaert, 1986) and to actions at non-α_2 receptors (Yavich et al., 1994). Which action underlies the present results is not known. Additional experiments are in progress to substantiate a role for α_2-adrenoceptors, and their localization, in the in vivo microdialysis model. The results of the present study indicate that, in the rat, cortical acetylcholine release can be augmented by (+)-efaroxan, an effect which may involve α_2-adrenoceptors. In addition, (+)-efaroxan may have therapeutic potential in the treatment of neurological disorders involving deficits in cortical acetylcholine release.

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