Nicotine and ACTH 4-10 Analog Org 2766 as Synergistic Protective/Growth Factors for Nigrostriatal Neurons

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AIM The principal aim of this study is to investigate the separate and combined neurotrophic effects of nicotine and a non-steroidogenic ACTH peptide fragment, Org 2766, on the regeneration of lesioned nigrostriatal neurons. In addition, an experiment using a nicotinic antagonist will validate the specificity of a neurotrophic action of nicotine. Previous studies by us and others (reviews [5,13]) have shown that both nicotine and ACTH peptides can act as growth factors on motoneurons, stimulating neurite sprouting and axonal regeneration. The effects of postnatal treatment with either nicotine or ACTH on serotonin high-affinity uptake are similar.[9] It is also known that nicotine is an effective stimulus for pituitary release of ACTH so that a synergistic action between these two agents may be involved as a result of smoking.

SIGNIFICANCE The nigrostriatal pathway is intimately related to areas of the brain, such as the corpus striatum, that are involved in extrapyramidal control of motor function. Degeneration of the nigrostriatal pathway, with extreme loss of dopamine (DA), results in disturbances in motor function, such as rigidity and tremor in Parkinson's disease. It is now widely accepted that there is a negative correlation between smoking and Parkinson's disease[3], so that a protective action of nicotine on nigrostriatal neurons may be inferred. Dopaminergic neurons of the nigrostriatal pathway possess nicotinic receptors[4]; nicotine causes DA secretion in the brain[2,15], and has been shown to provide some protection against degeneration of lesioned nigrostriatal DA neurons.[8] Org 2766, a potent ACTH 4-10 analog, also has been demonstrated to improve functional recovery from brain lesions.[7] Combined therapy with these two neurotrophic factors could result in some regeneration, or protection from degeneration, in neurodegenerative diseases such as Parkinson's.

BACKGROUND Animals with almost complete unilateral lesions of the DA nigrostriatal system are widely used as a model for certain aspects of human Parkinson's disease. Our study will use this model to assay the effectiveness of nicotine and/or Org 2766 on recovery of motor function. A major regulatory input into the striatum can be destroyed by the stereotaxic placement of the neurotoxin 6-hydroxydopamine (6-OHDA) into the nigrostriatal pathway. Pretreatment with desiprimine, a specific inhibitor of norepinephrine uptake, spares the norepinephrine neurons but does not affect serotinergic or dopaminergic neurons. This technique, together with the specific localization of the chemical lesion, permits a controlled and precise evaluation of nicotine and peptide effects on regeneration.

Unilateral destruction of the DA nigrostriatal system affects rotational behavior in rats, behavior that is readily quantifiable. Rotational testing is carried out in cylindrical chambers, 18" in diameter. Each animal is fitted with a tether harness, then attached to an optical rotational sensing device which feeds into a calibration unit adapted to allow computer analysis (Coulborn Instruments). The number of full and partial rotations is recorded, and also expressed as rotations per minute. This technique eliminates the subjective nature of many behavioral tests. Direct DA agonists such as apomorphine produce rotation away from the site of the lesion (contralateral) whereas amphetamine, an indirect DA agonist, produces rotation directed toward the side of the lesion (ipsilateral). Nicotine has been shown to provoke a mild degree of ipsilateral rotation.[10]

Presynaptic and postsynaptic changes at the DA synapse develop to compensate for the deficit in DA resulting from the 6-OHDA lesion. Presynaptically, the surviving DA neurons become hyperactive and DA metabolism accelerates as indicated by the increase in DA metabolites such as 3,4 dihydroxybenzylamine (DOPAC) and homovanillic acid (HVA).[6] This compensatory increase in DA release from surviving DA neurons has been reported from post-mortem studies of Parkinsonian brains.[11] Postsynaptic changes in DA receptor sensitivity may also compensate for the deficient DA transmission. On the other hand, the protective effect of chronic nicotine treatment may be the result of desensitization of excitatory nicotinic cholinergic receptors on surviving DA nerve terminals reducing DA release and metabolism.[8]

Postsynaptic receptor supersensitivity may be measured by behavioral and biochemical techniques. Our protocol incorporates both approaches: rotational and open field behavior will be analyzed, and receptor binding assays will provide information on changes in D_2 receptors. Determination of DA, DOPAC and HVA levels will provide further information concerning compensatory changes in DA metabolism.

In terms of peptide enhancement of neuronal regeneration, our laboratory and others have shown that ACTH fragments such as ACTH 4-10 and its analogs markedly improve peripheral nerve regeneration, accelerating the return of motor and sensory function, improving motor unit formation in the reinnervated muscles and enhancing the parameters of the motor endplate.[13,14] It has also been demonstrated that the ACTH 4-10 analog Org 2766 facilitates functional recovery after destruction of the mesolimbic DA system,[16] which is involved in the dependent behavior characteristic of smokers. Org 2766 also enhances behavioral recovery after unilateral transection of the fimbria fornix.[12] These results imply a central effect for ACTH peptides.

In a preliminary study we have shown that rats treated with Org 2766 following unilateral 6-OHDA lesions in the substantia nigra show an early, significantly higher total number of rotations than their saline-treated counterparts, indicating a possible neurotrophic action of Org 2766 in this model of central regeneration. Behavioral motor activity, such as rearing, grooming and exploratory behavior was affected by peptide treatment under the influence of amphetamine but not following apomorphine: this may indicate a compensatory effect in the mesolimbic DA system.[1]

EXPERIMENTAL PROTOCOL Male rats, anesthetized appropriately, will undergo unilateral destruction of the substantia nigra through stereotaxic placement of an infusion of 4 l 6-OHDA (8 µg), following pretreatment with desiprimine. Administration of nicotine and/or ACTH peptide will begin immediately after surgery. Nicotine will be chronically administered through implanted Alzet minipumps calculated to deliver 0.125 mg/kg per h during 2 weeks. Org 2766, the ACTH 4-10 analog, will be administered i.p. at a dosage of 10µg/kg/24 h. Another group of rats will receive both nicotine and Org 2766. A third group of rats will receive both nicotine and a nicotine antagonist dimethylaminoethyl benzoate monofumarate (25 mg/kg) kindly supplied by Dr. Leo Abood.

Rotational behavior will be tested for 15 days on a 48 h schedule, beginning 24 h after surgery and induced by the administration of apomorphine (0.5 mg/kg) or amphetamine (2 mg/kg). Behavioral motor activity (exploratory behavior and grooming) will be observed in an open field environment to examine any compensatory effects in the mesolimbic system. Following these determinations, the rats will be sacrificed and changes in striatal DA concentrations, activities of enzymes involved in DA synthesis, the formation of DA metabolites, and the drug-induced rotational behavior analyzed. Survival and/or morphological changes of DA cells, identified by immunocytochemistry, in the substantia nigra, striatum and nucleus accumbens will indicate whether both the nigrostriatal and mesolimbic pathways are affected. DA neurons in brain slices will be

labelled with antibodies against tyrosine hydroxylase, while cholinergic neurons will be labelled with monoclonal antibodies against CAT to determine any parallel changes in the cholinergic system. As rotational behavior is mediated by D_2 receptors, our binding assay will use tritiated benzamide raclopride, which is extremely selective for the dopamine D_2 receptor.

Statistics ANOVA will be used for appropriate post-hoc comparisons, followed by Mann-Whitney for non-parametric data. Student's t-test will be used for simple 2 paired tests. No animal subgroup will contain less than 6 rats.

Time Schedule

First Year: Effect of separate administration of nicotine or Org 2766 on rotational and open field behavior. Morphological and immunocytochemical study of the brains. Effect of nicotine antagonist.
Second Year: Effect of combined administration of nicotine and Org 2766 on rotational and open field behavior. Morphological and immunocytochemical study of the brains.
Third Year: Studies on DA receptor binding and concentrations of DA and its metabolites.

Budget. No salary is requested for either the Principal Investigator or the Co-Principal Investigator, each of whom will spend 20% of their time on this project.

First Year (includes 4 station rotometry system)	$65,000
Second Year (includes 5% COLA)	$55,000
Third Year (includes 5% COLA)	$55,000

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