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To: Dr. R. B. Seligman Date: November 3, 1980

From: T. S. Osdene

Subject: Status of Nicotine Program

The major goal of the Nicotine Program is to develop nicotine analogues which will have desirable effects on the central nervous system (CNS) without the undesirable effects of nicotine on the peripheral nervous system (PNS). We would also like to be able to understand at the molecular level where and how nicotine exerts its beneficial effects on the CNS. During the last year we have made excellent progress in the Nicotine Program. Investigations of the chemistry, pharmacology, behavioral pharmacology and psychological effects of nicotine and nicotine analogues are all making contributions toward the accomplishment of our goals. Toward this end we have developed a broad program which entails the synthesis and characterization of nicotine analogues which have a rational basis in the classical Structure-Activity Relationships (SAR) approach to understanding the pharmacological basis of the actions of nicotine. Pharmacological evaluation of these analogues has been carried out at INBIFO, at the University of Rochester under the direction of Dr. Leo Abood and at the Medical College of Virginia. Studies of behavioral pharmacology of the nicotine analogues was begun in Dr. Abood's laboratory with the prostration syndrome. This assay has been transferred to the Behavioral Research group at R&D where they have improved upon Abood's bioassay. Our Behavioral Research Group has instituted discrimination studies and self-administration studies for the purpose of evaluating the behavioral pharmacology of appropriate nicotine analogues.

Future studies will concentrate on nicotine-receptor binding studies and isolation of the nicotine receptor. The goal in the binding studies is to correlate receptor binding data on nicotine analogues with results from in vivo studies such as the prostration syndrome, nicotine discrimination assay and rat blood pressure. Isolation, identification, and characterization of the nicotine receptor is the ultimate challenge and the potential rewards for structure-activity relationships and understanding nicotine pharmacology justify this approach. Nicotine analogues with an electrophilic site will be prepared and specific nicotine receptor binding and covalent bonding will occur. Subsequently, the radiolabeled analogue can be used in receptor isolation studies. Another approach involves the use of affinity chromatography in which a nicotine analogue is covalently bound to a chromatographic support and the nicotine receptor will bind to the analogue. This will greatly facilitate the separation of the receptor from a myriad of other proteins present in the preparation. We will soon begin determining pK_a's of the analogues and their oil/water partition coefficients which quantitate basicity and lipophilicity.

Nicotine self-administration experiments in the Behavioral Research group have demonstrated that nicotine can function as a positive reinforcer in rats. Work will now be directed toward elaboration of these studies in which experiments will be designed to demonstrate that nicotine self-administration does not interfere with ongoing behavior or alter self-administration of other reinforcers such as food and water. Experiments will also determine that termination of self-administration does not produce behavioral impairments. The prostration syndrome will be used to study relative potency of nicotine analogues which have been shown to be nicotine-line in discrimination tests. Studies to locate sites of action of nicotine in the CNS and to determine the extent of behavioral prostration are also in progress. Operant performance using schedule controlled behavior will be used to investigate duration of CNS changes following nicotine and nicotine analogue infusions. Tolerance studies will be required to define precise mechanisms for observed behavioral disruptions following administration of analogues. Discrimination studies will be continued as a valuable screening tool for identifying nicotine-like properties of analogues.

A qualified pharmacologist has recently been hired at INBIFO and this should facilitate pharmacological screening studies at that site. In the interim we have been using the facilities at the University of Rochester and the Medical College of Virginia for rat blood pressure studies.

Dr. Berntson at Ohio State will be undertaking studies with animals to determine if nicotine effects on facilitated recall and learning are permanent or "state dependent". The possibility that these effects are mediated via nicotine release of vasopression will also be studied.

Please note that after our experiments are completed and if sufficient material remains it is sent to ICI for further pharmacological screening under our existing contract arrangements.

In summary, I believe the program has made considerable strides in our knowledge of the action of nicotine and should be continued with perhaps even greater emphasis. I believe the program holds great potential for the future and has been very cost effective.

Attached are specific accomplishments of the program.

TSO/mro

cc: Dr. W. L. Dunn
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SPECIFIC ACCOMPLISHMENTS FOR THE NICOTINE PROGRAM

I. SYNTHESIS AND CHEMISTRY OF NICOTINE ANALOGUES

A. Synthesis

1. A series of 6-alkylnicotines were prepared since 6-methylnicotine was found to be as active or more active than nicotine. In addition to 6-methyl, 6-ethyl, 6-isopropyl, 6-t-butyl, 6-cyclopropyl and 6-vinylnicotine were prepared.

2. A series of X, 3'-isonicotines were prepared which increased pyrolidine accessibility and increased N---N' distance.

3. A series of 2'-alkylnicotine were prepared which increased the basicity of the pyrrolidine ring and decreased its accessibility. 2'-Methylnicotine is of particular interest since it is equipotent with nicotine in behavioral studies and causes hypotension in rats injected via the jugular vein. This hypotensive response is being tested for confirmation.

4. Homo and bishomo nicotines were prepared where the C-3, C-2' bonding is intact but the rings are separated by one and two carbons respectively.

5. Metanicotine, dihydrometanicotine and dihydro-methylmetanicotine were prepared. In this series an acyclic moiety replaces nicotine's pyrrolidine ring. This yields structural variation with regard to unsaturation, degree of nitrogen substitution, chain flexibility and N---N' distance.

6. Optically active 4'-alkylnicotinoids such as cis and trans-4'-methylnicotines and related 3'-alkyl and 5'-alkylnicotinoids were prepared. These compounds will give information regarding substitution and spatial tolerances at the nicotinic receptor.

7. 2,3'-Bismethylenenicotine, a novel bridged nicotinoid was prepared. The importance of ring-ring orientation and molecular rigidity can be studied with bridged nicotines.

8. 6-Hydroxymethyl- and 6-(2-hydroxyethyl)nicotine were prepared for the first time. These compounds as the 6-halo alkylnicotines will be used for the recetor alkylation and isolation studies.

9. Many of the analogues in 1-8 are also available as myosmine and nornicotine derivatives because of the synthetic route used to prepare the nicotine derivatives.

10. A procedure for separating a d,ℓ-nornicotine analogue into its enantiomers is nearing completion.

11. Nicotine and 5-methylnicotine in optically active form have been prepared by asymmetric synthesis. This is the first time any nocotine compound has been synthesized asymmetrically.

B. Chemistry

1. The effect of substitution on conformational properties and motional processes of nicotine and some of its analogues in solution has been studied.

2. The nucleophilicity of the two nitrogens of nicotine are critical to its activity. We are monitoring this parameter by determining reactivity of nicotine and its analogues with a standard alkylating agent, iodomethane. We hypothesize that the factors which stabilize (or destabilize) the alkylation transition states similarly affect the noncovalent binding of nicotine with its receptor. Correlations have been made between alkylation rates and biological activity for nicotine and methyl substituted derivatives.

3. A wide range of 1-methyl-2-phenylpyrrolidines and the quaternary salts of these and many of the other nicotine analogues were prepared for the alkylation studies above.

II. BEHAVIORAL PHARMACOLOGY

A. Nicotine Self-Administration

1. We have demonstrated in our laboratory that nicotine can function as an intravenously delivered positive reinforcer in rats.

2. Nicotine self-administration for a minimum of 7 days at each dose has been shown to be a dose-dependent relationship for doses from 2.0-64.0 µg/kg/injection.

B. Prostration Syndrome

1. A reliable data base has been established with d-nicotine and ℓ-nicotine with the former 1/10 to 1/20 as active as the latter.

2. Using schedule-controlled behavior (operant conditioning) we have shown that rats continue to show behavioral disruptions 10-12 minutes after an infusion of nicotine. This represents a substantial improvement over Dr. Abood's activity scale where recovery is apparent in 3-5 minutes.

3. The duration of suppression in response rate (bar pressing) produced by infusion of 10 μg of ℓ-nicotine was shorter than that produded by 5 μg of ℓ-nicotine. This may be a result of behavioral tolerance and less likely it could be metabolic tolerance or neural adaptation. A series of studies are planned to more accurately characterize the development of tolerance.

4. 2'-Methylnicotine was behaviorally active in discrimination and prostration tests and is equipotent with ℓ-nicotine. 2'-Methylnicotine was also tested using schedule controlled behavior. 2'-Methylnicotine produced less supression of response than ℓ-nicotine and this may reflect either cross tolerance to ℓ-nicotine and/or a diminished peripheral effect. Experiments are planned to separate these effects.

C. Discrimination Studies

1. Addition of the dimethylaminomethyl substituent to the pyridine ring in the 3 position produced "nicotine-like" responses whereas isomeric 2- and 4-substituted compounds were inactive.

2. Metanicotine showed "nicotine-like" activity while the dihydro derivative did not.

3. Of the isonicotine derivatives, the 3,3'-substituted N-methylpyrrolidine showed activity while 2,3'-isonicotine did not.

4. 2'-Methylnicotine at 0.4 mg/kg resulted in 100% of the animals tested responding on the nicotine correct lever.

5. Experiments are planned with preinjection of mecamylamine and hexamethonium to determine if 2'-methylnicotine discrimination is centrally or peripherally mediated.

III. EXTERNAL PHARMACOLOGY AT UNIVERSITY OF ROCHESTER/DR. ABOOD

1. Dr. Abood has continued to consult with us and support the Nicotine Program.

2. A contract peripheral pharmacology testing program is in progress.

3. Nicotine analogues have been tested in Dr. Abood's laboratory for cross-laboratory comparisons in the prostration syndrome test.

4. Dr. Abood has tested analogues for binding to glass fiber filters, rat blood pressure and prostration syndrome.

5. Binding constants for nicotine analogues have been determined by competition of analogues with ^{3}H-acetylcholine for torpedo membranes.

6. Nicotine and nicotine analogue binding was determined on solubilized rat brain tissue.

7. Rat blood pressure studies have shown a number of analogues to be hypotensive agents at low doses.

8. 6-Methylnicotine was hypertensive as is nicotine.

9. A small number of compounds were hypotensive upon jugular vein infusion and hypertensive upon iliac vein infusion. These results are being subjected to confirmation at the Medical College of Virginia.