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Subject: Progress Report

The Behavioral Pharmacology Program

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Major objectives of the Behavioral Pharmacology Program are (1) To develop a better understanding of the reinforcing actions of nicotine and nicotine analogues, (2) To gain insight into the neurobehavioral actions of nicotine, and (3) To develop and use animal behavior techniques to screen nicotine analogues for their nicotine eliciting properties.

NICOTINE SELF-ADMINISTRATION

A major objective of the behavioral research program is to evaluate the reinforcing properties of nicotine and nicotine analogues. This is being accomplished by utilizing intravenous and intracerebral (IVT) self-administration techniques. Intravenous self-administration has proven to be one of the most useful and powerful techniques devised for compound evaluation and comparison. This is a critical test for the nicotine analogues because it has been previously shown that reinforcing efficacy does not always correlate with interoceptive generalization (discriminative).

Two reports (Hanson, et al. 1977 and Lang, et al. 1977) have shown that rats can be trained to lever press for intravenously delivered nicotine. However, neither report used appropriate control measures to clearly show that nicotine was functioning as a reinforcer. A first step in our laboratory was to demonstrate clearly that nicotine can function as an intravenously delivered reinforcer.

Rats were prepared with an indwelling venous catheter made of siliconized rubber. The catheter was anchored in the external jugular vein and passed subcutaneously until it exited through the animals back. This was connected via protective tubing and swivel joints to a remote injection pump. Responding on one arbitrarily selected response lever was automatically programmed to activate the injection pump for 6 seconds, delivering an injection of 0.1376mℓ of solution directly into the animals blood stream. Responses on the control lever were recorded but had no programmed consequence.

CONTROL STUDIES

Nicotine self-administration was initially established at 32ug/kg/injection. Nicotine was available 24 hours per day, under a fixed ratio 1 schedule. Generally 10-14 sessions are necessary for responding to stabilize. After stable behavior is obtained, changes are made in the nicotine delivery procedure to determine if lever pressing is maintained by the contingency established between lever pressing and nicotine delivery. Changes include substitution of saline for nicotine, reversal of nicotine-lever and control (action of) lever functions, and automatic non-contingent nicotine injections. The results from a small number of animals show that nicotine self-administration by rats is maintained by the response-nicotine contingency, rather than by other behavioral effects of the drug. Substitution of saline for nicotine failed to maintain responding. When nicotine (32ug/kg/injection) was reintroduced, the number of injections rose to previous levels. During the self-administration, responding occurred almost entirely on the lever delivering nicotine. Control lever responses were less than 10% of all total number of responses. When nicotine injections were delivered non-contingently responding decreased as a function of the frequency of the non-contingent injection.

These results show clearly that nicotine can function as a positive reinforcer for rats.

INJECTION DOSE

Nicotine self-administration was established during 24 hr/day access sessions at 32*ug/kg*/injection under FR 1 conditions. After stabilization, the effects of injection dose was determined on response rate and nicotine intake (*mg/kg*/day). Injection doses were presented in descending order (64,32,16,8.0, 4.0, and 2.0*ug/kg*/injection). Rats were tested for a minimum of 7 days at each dose. The result shows that as the dose of nicotine was decreased from 64.0 to 4.0*ug/kg*/injection, the number of injections increased. At 2.0*ug/kg*/injection, the response rate decreased. The *mg/kg*/intake was highest at 64.0*ug/kg*/injection (2.5*mg/kg*/day) and decreased with decreasing concentrations.

Future studies:

1) Examine a dose response curve under various schedules.
2) Examine the effects of cholinergic antagonist and ~~antagonist~~ AGONISTS of self-administration behavior.
3) Attempt direct substitution of nicotine analogues.
4) Demonstrate that nicotine self-administration does not interfere with ongoing behavior.
5) Show that termination of self-administration does not produce behavioral impairments.
6) Nicotine self-administration does not alter self-administration of other reinforcers (food, water, saccharine, etc.).

NON NUTRITIVE

PROSTRATION SYNDROME

The laboratory has been able to demonstrate the prostration syndrome reliably with both (-) nicotine and (+)-nicotine, with the latter being about 1/10 to 1/20 as active. Having gained a reliable data base with both (-)-nicotine and (+)-nicotine, it is now essential to examine relative potency of the nicotine analogues, most importantly analogues that have shown to be nicotine-like in the discrimination tests, yet have a different structural configuration. In addition, studies to locate sites of action and determine the extent of the behavioral prostration are now in progress. Even without detailed knowledge of the underlying physiological mechanisms, the use of behavioral measures will provide important information about the sites of action in the brain. We are currently using scheduled controlled behavior to evaluate the effects of intraventricular injections of nicotine. Operant performance has been shown to be more sensitive than activity rating scales, and provides a more stable baseline from which comparisons can be made.

Although the prostration syndrome is a reliable screen for behaviorally active nicotine analogues, the rating scale developed by Dr. Abood provides only a descriptive interpretation of the compounds' effects, and does not permit a determination of possible prolonged changes in CNS activity. However, previous investigations (DeNoble & Begleiter, 1976, DeNoble & Caplan, 1977, Bowman, 1980, Mele and Caplan, 1980) have demonstrated that schedule-controlled behavior is sensitive to CNS changes.

Schedule-controlled behavior is a research technique that is based upon principles of operant conditioning. This technique produces a highly stable and reproduceable baseline of behavior which has been shown to be

dependent on the integrity of the CNS. Therefore, this technique was used to measure CNS recovery times in nicotine-infused rats.

Twelve male albino rats weighing between 190 and 230 grams and 120 days old were used. They were gradually reduced to 80% of their free feeding body weight. The animals were then trained to press a lever in a standard operant chamber for a single delivery of milk. Subsequently, the contingency for reinforcement was increased to a fixed ratio 16 (FR 16). (Under an FR schedule, reinforcement is contingent upon completing "x" number of responses.) Animals were trained daily (Monday-Friday) during two successive 15-minute sessions with a 5-minute time out period after the first 15 minutes. After a stable baseline of behavior was obtained, the animals were anesthetized and implanted with a stainless steel cannula into the left lateral ventricle of the brain. Following two or three days of recovery from surgery, rats were retested under the FR16 schedule. Intraventricular injections of saline or nicotine were administered when there was less than 10% variance in daily response rate for 5 days. The animals were first tested with saline. Testing began at the 5-minute time out period during their daily session. Animals were infused with 5$\mu\ell$ of 0.9% saline solution and then placed immediately back in the box. The animals were tested in repetitive 15-minute segments until (1) baseline behavior was recaptured or (2) until their response rate decreased (satiation). Having established a baseline with the saline infusion, the animals were then infused with 5μg of (ℓ)-nicotine (free base) in 5$\mu\ell$. Figure 1 shows that the infusion of saline produced no major change in response rate. However, the same animals infused with 5μg of (ℓ)-nicotine in 5$\mu\ell$ showed a suppression in response rate.

Using schedule-controlled behavior as a measure, rats continued to display behavioral disruptions 10-12 minutes post infustions. Observations of these animals via a one-way mirror revealed that typical locomoting and grooming behaviors were displayed 5 to 7 minutes before recovery under the FR schedule. The duration of suppression in response rate was approximately 110% longer than that observed with Dr. Abood's scale where recovery from the prostration effects occurs 3-5 minutes post infusion.

Recent electroencephalographic recordings taken by Dr. Abood after intraventricular injections of nicotine into rats have demonstrated that recovery of baseline hippocampal activity occurs 10-12 minutes post infusion. These latencies in conjunction with the latencies found in the schedule-controlled behavioral task demonstrate that prolonged CNS changes are taking place.

As a continuation of that study, these animals were then tested with twice their original dose of ℓ-nicotine (5ug to 10ug in 5uℓ). The time between infusions for all animals was no less than 7 days and daily response rate showed less than a 15% variance from day to day. Surprisingly, the duration of the suppression in response rate produced by the infusion of 10ug of ℓ-nicotine was shorter than that produced by 5ug of ℓ-nicotine. There are several possible explanations of this result.

First, it is possible that the rat developed a metabolic tolerance. Metabolic tolerance can be divided into two major categories; peripheral and central. Some of the peripheral mechanisms include alteration in enzymatic degradation, changes in absorption, or changes in lipid solubility. Central mechanisms refer to the neural adaptation to the presence of the substance such that "normal" function occurs with the substance present. We can rule out

the peripheral mechanism because we are delivering the nicotine intravenously and therefore bypassing the peripheral system.

While the presence of central cellular adaptation cannot be fully ruled out as a possible contributing factor in the change in behavioral response, it is unlikely since the infusions were given 7 days apart.

The other explanation of the data is that the rats rapidly developed behavioral tolerance. Behavioral tolerance is a diminished effect (behavioral) of a compound with repeated exposure to that compound without the induction of metabolic tolerance. In order to examine the development of tolerance, we increased the requirement for reinforcement from 16 to 32. Once the rats were stabilized under the FR-32 schedule, they were infused during the daily session with 5ug of nicotine on two occasions separated by a mean of 5 days. The second infusion of nicotine had a diminished effect. Presently we are designing a series of studies which will more accurately characterize the development of tolerance. In addition, we are selectively manipulating neurotransmitter systems to better understand the central action of nicotine.

We demonstrated that 2'-methylnicotine was behaviorally active in discrimination and prostration tests and is equally as potent as (-)-nicotine. Using Dr. Abood's rating scale, 2'methylnicotine did not produce some of the peripheral signs of prostration. (Less motor control loss, no hyperventilation, no piloerection, no excess urination or defecation). In view of these findings, we tested 2'-methylnicotine using scheduled controlled behavior. Under FR 32, the rats were tested first with 5ug of ℓ-nicotine in Sml, then 5 days later with 5ug of 2'-methylnicotine in Sml. The data shows that the 2'-methylnicotine produced less suppression of response rate under the FR 32 schedule. This result

could reflect 2'-methylnicotine's cross tolerance to l -nicotine and/or a diminished peripheral effect. In the next series of studies we will separate these events.

In the future we will be conducting studies in which the selective blockade of neural structures will be evaluated on the behavioral components of prostration. We think that this will provide evidence for the sites of action of nicotine and nicotine analogues.

DISCRIMINATION STUDIES

As part of the ongoing nicotine discrimination program, we have completed initial testing on a series of additional compounds. These compounds included various dialkylaminomethylpyridines, metanicotine, its dihydro derivative, and several isomeric nicotines.

Addition of the dimethylaminomethyl substituent to the pyridine ring in the 3 position produced "nicotine-like" responses from the animals whereas the isomeric 2- and 4- substituted compounds gave no indication of activity. The 3-pyridyl derivatives yielded "nicotine" responses in 55% of the animals at a 4.0 mg/kg dose. (This level represents 10x that of the training dose used in the nicotine discrimination task.) When the dose was increased to 8.0 mg/kg, 4 animals responded on the nicotine correct lever and 3 animals gave incomplete tests.

Metanicotine, unlike its dihydro derivative, showed nicotinic activity in the animals tested. Apparently, the unsaturation in the side chain, in this compound, is necessary to produce activity, since metanicotine at a dose of 4.0 mg/kg produced nicotine cues in approximately half of the animals. When the dose was increased to 8.0 mg/kg, 100% of the animals responded on the nicotine correct lever.

Of the isonicotine derivatives, the 3,3'-substituted N-methylpyrrolidine showed activity while the 2,3'-isonicotine did not. At a dose only 5x that of the normal discrimination training dose of ℓ-nicotine (2.0 mg/kg), the 3,3'-isonicotine was active in 9 out of 11 animals. When the dose was reduced to 1.0 mg/kg, 63% of the animals tested responded with the nicotine correct lever.

A series of open-chain nicotines were synthesized. A methylethyl-aminomethyl substituent or a diethylaminoethyl substituent was added to the pyridine ring in the third position. Animals injected with a wide range of doses of each compound did not respond on the nicotine correct lever. However, one open-chain compound, 3-dimethylaminomethyl pyridine, did produce behavioral activity. At a 4.0 mg/kg/body weight dose, 75% of the animals responded on the nicotine correct lever. Only 40% of the animals tested at a dose of 2.0 mg/kg/body weight emitted a nicotine response. Note that these doses are 5 to 10 times higher than the daily dose of ℓ-nicotine used in the discrimination task.

The most interesting finding was with the 2'-methylnicotine. At a 0.4 mg/kg/body weight dose, 100% of the animals tested responded on the nicotine correct lever. This is the same dose used during the daily nicotine training sessions. Doses of the 2'-methylnicotine higher than 0.4 mg/kg/body weight produced incomplete responding.

Presently, we are preparing to do a dose response curve using the 2'-methylnicotine. A dose response curve will allow us to access 2'-methyl-nicotine's relative potency to dℓ-nicotine and ℓ-nicotine. The effectiveness of 2'methylnicotine in the discrimination task is now being tested with

preinjections of mecamylamine and hexamethonium. These results should indicate whether the discrimination of the 2'-methylnicotine is centrally or peripherally mediated.

Major Strengths of the Behavioral Pharmacology Section

1) The use of sensitive and reliable behavioral measures for the evaluation of compounds.

2) To use behavioral events as indicators of nervous system activity.

3) To directly correlate behavioral and nervous system changes induced by nicotine in the intact organism.

4) To provide empirical evidence that nicotine is a positive reinforcer that does not produce dependence.

Major Weakness of the Behavioral Pharmacology Program

The rate of expansion of this program has been great and two types of additional personnel are necessary. First, it is important to obtain a high level assistant (MS or BS) and second, to increase our technician staff by an additional person. The depth of difficulty of the present research requires the Assistant (L. Carron) to continously monitor our technician, in addition to running her own studies. My time is divided as follows: 50% running animals; 40% data analysis and overseeing other studies; and 10% literature reading. We find that the lack of personnel is a major failure of this program.

Vet. of Pharmacology

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