PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

(E) Nicotine Program

CONFIDENTIAL

To: W. L. Dunn

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From: V. DeNoble and L. Carron

Subject: Research Progress Concerning Discrimination and Prostration Studies

A series of additional compounds have been tested in the nicotine-discrimination task. These compounds were tested in order to access their behavioral activity. Dialkyaminoalkyl pyridines as well as some isomeric nicotines and a 2'-alkyl of nicotine were tested at various dose levels. See Table 1.

The isonicotine derivatives were basically inactive. Seven rats were tested with the 4-isonicotine at the 8.0 mg/kg/body weight dose; and, 71% of the animals responded on the saline correct lever. The 2-isonicotine and the 4,3'-isonicotine showed no behavioral activity. The 3,3'-isonicotine has been the only compound out of the isonicotine derivatives to show significant behavioral activity (See memo to V. J. DeNoble from L. Carron, C. Levy and A. Allen). INJECTION WITH

A series of open-chain nicotines were synthesized. A methylethylaminomethyl 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 (-)-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'-methylnicotine's relative potency to d,ℓ-nicotine and ℓ-nicotine. The effectiveness of 2'-methylnicotine in the discrimination task is now being tested with pre-injections of mecamylamine and hexamethonium. These results should indicate whether the discrimination of the 2'-methylnicotine is centrally or peripherally mediated.

PROSTRATION

Data gathered from both the discrimination and prostration studies have proven to be highly correlated with respect to behavioral activity. Therefore, we tested the 2'methylnicotine for its ability to produce the prostration syndrome.

The data presented in Tables 1 and 2 indicate that the 2'-methylnicotine is behaviorally active in both tests, and appears to be at least equally as potent as (-)-nicotine.

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 (FR16). (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 µl 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 5 µg of (ℓ)-nicotine(free base) in 5 µl. Figure 1 shows that the infusion of saline produced no major change in response rate. However, the same animals infused with 5 µg of (ℓ)-nicotine in 5 µl showed a suppression in response rate.

Using schedule-controlled behavior as a measure, rats continued to display behavioral disruptions 10-12 minutes post infusions. 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.

Jh

cc: R. Seligman
T. Osdene
T. Sanders
J. Seeman

Lisa Carron

Table I

BEHAVIORAL ACTIVITY OF NICOTINE ANALOGUES IN NICOTINE-DISCRIMINATION TASK

	Dose (mg/kg)	Number of Rats Tested	% of Rats Emitting Nicotine Responses	% of Rats Emitting Saline Responses	% of Rats Emitting Incomplete Responses
l,ℓ 2-Isonicotine	8.0	7	0	100	0
	4.0	5	0	100	0
l,ℓ-4-Isonicotine	8.0	6	0	100	0
	4.0	7	29	71	0
l,ℓ4,3'-Isonicotine	8.0	4	0	100	0
l,ℓ3-Methylethylamino-methylpyridine	0.8	4	25	75	0
	0.4	4	20	80	0
	0.2	4	25	75	0
l,ℓ3-Diethylaminoethyl-pyridine	4.0	4	0	100	0
	2.0	4	0	100	0
l,ℓ3-Dimethylaminomethyl-pyridine	4.0	4	25	75	0
	2.0	5	40	60	0
l,ℓ2'-Methylnicotine	1.6	5	0	0	100
	0.8	3	0	0	100
	0.4	4	100	0	0
	0.2	4	25	75	0

Table 2

A COMPARISON OF DOSE RESPONSE FUNCTIONS FROM INTRAVENTRICULAR INFUSIONS OF ℓ-NICOTINE AND d,ℓ2'-METHYLNICOTINE

	(ℓ)-Nicotine		d,ℓ 2'-Methylnicotine
5 µg		Retest	
$\bar{X}$	2.25	1.57	1.00
S.E.	0.36	0.46	0.36
10 µg			
$\bar{X}$	1.29		2.00
S.E.	0.45		0.33
20 µg			
$\bar{X}$	2.71		2.29
S.E.	0.45		0.20

FIG. 1 - The effect of intraventricular infusion of either ℓ-nicotine or saline on fixed-ratio responding for milk reinforcement. Latency to respond (mean time in minutes) is shown as a function of either saline or ℓ-nicotine infusions. Each bar represents a mean of 12 animals ± S.E. The line across the graph represents the mean latency for behavioral recovery ±S.E. using Dr. Abood's scale (for 15 animals).

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