J. Osborne (F) Nicotine Prog

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Date: February 18, 1980

From: • C. Levy and L. Carron Carl-Levy LCarron

Subject: • Prostration Syndrome

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Abood and his coworkers have reported (1978;1979) that (+)-nicotine is 1/100 as active as (-)-nicotine in producing a prostration syndrome when injected into the lateral ventricles of rats. This difference in potency between the two stereoisomers of nicotine is greater than other reported differences which vary from 1/7 to 1/50 depending upon the test used (Aceto et al., 1979). Since Abood suggests that the prostration syndrome is mediated by noncholinergic nicotinic receptors that show a high degree of stereospecificity, it seemed important for us to try to replicate his findings.

IN CONNECTION WITH

The subjects in the first study were six male albino rats weighing 170-210 g at the time of surgery. Each rat was anesthetized with barbiturate anesthesia and implanted with a stainless steel cannula aimed at the left lateral ventricle. During testing the severity of the prostration syndrome was rated by an observer using Abood's scale (previously described in memo dated Oct. 8, 1979). Beginning two to seven days following surgery each rat was tested with 5 µg (-)-nicotine, 5 µg (+)-nicotine and 50 µg (+)-nicotine, in that order.* All solutions were made from the free base, diluted with isotonic saline and infused in a volume of 5 µl.

The results are shown in Table 1 below. Infusions of 5 µg (-)-nicotine and 50 µg (+)-nicotine both produced a more severe prostration syndrome than did infusion of 5 µg (+)-nicotine ($p<.05$; Wilcoxon Matched-Pairs Signed-Ranks Test). Four out of six rats had higher prostration scores after infusion of 5 µg (-)-nicotine than after 50 µg (+)-nicotine, (this difference cannot be tested statistically due to the small N and two tied scores) suggesting that a higher dose of (+)-nicotine needed to be tested.

TABLE 1

Prostration Scores for Rats Infused with (-)- and (+)-Nicotine

Subject	5 µg (-)-nicotine	5 µg (+)-Nicotine	50 µg (+)-nicotine
S31	3	1	3
S32	4	0	3
S34	4	1	3
S35	3	0	3
S36	3	1	2
S37	3	1	2
$\bar{X}$	3.3	0.7	2.7

SUBJECT TO PROTECTIVE ORDER

*If the rat's prostration syndrome resulting from the 5 µg (-)-nicotine was not scored as either "3" or "4," the rat was retested with a longer (1 mm) infusion tube. If this did not increase the rat's score, he was dropped from the study. Tests were conducted on Tuesdays and Fridays.

The two rats that scored "2" when they were infused with 50 µg of (+)-nicotine were further tested with 100 µg (+)-nicotine and 10 µg (-)-nicotine, infused in a volume of 5 µl. An additional three rats weighing 200-210 g were implanted with cannulae and tested with 5 µg (-)-nicotine, 50 µg (+)-nicotine, 100 µg (+)-nicotine and 10 µg (-)-nicotine (in that order with 3-4 days between tests). These results are shown in Table 2 below. It can be seen that only the infusions of 50 µg (+)-nicotine resulted in prostration syndromes which differed in severity from the standard 5 µg (-)-nicotine. These data suggest that (+)-nicotine is ten to twenty times less potent than (-)-nicotine in producing a prostration syndrome.

TABLE 2

Prostration Scores for Rats Infused with (-)- and (+)-Nicotine

Subject	5 µg (-)-nic.	50 µg (+)-nic.	100 µg (+)-nic.	10 µg (-)-nic.
S36	3	3	3	3
S37	3	2	3	3
S38	3	3	3	3
S40	3	2	3	3
S41	3	3	3	3
$\bar{X}$	3.0	2.4	3.0	3.0

Since our data showed a ten to twenty-fold difference in potency between the (+)- and (-)-isomers of nicotine, rather than a 100-fold difference, it was of interest to determine the potency of the racemic mixture of the two isomers.

The subjects in this study were 6 male albino rats weighing 164 to 210 g at the time of surgery. The surgical and testing procedures were identical to the preceding ones. Each rat was tested with 5 µg (-)-nicotine, 2.5 µg (-)-nicotine, 2.5µg(±)-nicotine, 5 µg (±)-nicotine, and 5 µg (-)-nicotine, in that order.* All solutions consisted of the free base diluted with isotonic saline and infused in a volume of 5 µl. The results are shown in Table 3.

It can be seen that the doses of the racemic mixture are substantially more potent than similar doses of (+)-nicotine and almost as potent as doses of (-)-nicotine, suggesting that it is the (-)-nicotine that is primarily responsible for the observed prostration resulting from administration of the racemic mixture.

One surprising finding from this study was the rather strong prostration syndrome caused by the 2.5 µg (±)-nicotine. Based upon Abood's dose-response work (in which infusion volume varied with dose), we had assumed that such a low dose of (-)-nicotine (1.25 µg in 5 µl) would produce only a very weak prostration syndrome. At this point we decided that we should evaluate the importance of infusion volume by constructing our own dose-response curve, holding infused volume constant.

*The order of infusion for rat S42 was out of sequence from the rest, that is, he received the 2.5 µg of (-)-nicotine first then he received 2.5 µg of the (±) mixture.

The subjects for this study were six male albino rats weighing 160 to 209 g at the time of surgery. Again, the surgical and testing procedures were identical to the preceding ones. Each rat was tested with 5 µg (-)-nicotine, 0.60 µg (-)-nicotine, 1.25 µg (-)-nicotine, 2.50 µg (-)-nicotine, and 5 µg (-)-nicotine. All solutions were made from the free base diluted with isotonic saline and infused in a volume of 5 µl.

The results are shown in Table 4. Contrary to data from the literature, the (-)-nicotine shows a clear effect at lower doses. These data also show, once again, that the (-)-nicotine in the racemic mixture contributes almost solely to the observed response. The (-)-nicotine administered at 1.25 µg in 5 µl produced the same effect as the racemic mixture of nicotine given at twice the concentration. Hence, these data would suggest that at concentrations lower than 5 µg in 5 µl, (+)-nicotine contributes little, if nothing, to the observed response. (Data reported earlier in this memo showed a minimal effect from (+)-nicotine at a concentration of 5 µg in 5 µl.)

To summarize, our experiments in contrast to Abood's work, show that at high concentrations the (+)-nicotine is about 1/10 to 1/20 as active as the (-)-nicotine. We have also shown that the volume of infusion contributes significantly to the response. By keeping volume constant, we demonstrated that both the racemic mixture of nicotine and the (-)-nicotine solution produced a stronger response at lower concentrations.

TABLE 3

(±)-Nicotine and (-)-Nicotine in 5 µl

Subject	5 µg (-)-nic.	2.5 µg (-)-nic.	2.5 µg (±)-nic.	5 µg (±)-nic.	5 µg (-)-nic.
S43	3	3	1	2	2
S44	3	2	2	3	3
S46	3	2	2	2	2
S48	3	3	2	3	3
S50	3	3	2	3	3
S42	3	2	3	3	3
$\bar{X}$	3.00	2.50	2.00	2.66	2.66

TABLE 4

Subject	(-)-Nicotine in 5 ul 5 ug	0.6 ug	1.25 ug	2.50 ug	5 ug
S51	3	0	1	2	2
S52	4	1	3	3	3
S54	3	1	3	3	3
S57	3	0	1	2	2
S59	3	1	2	3	3
S61	4	1	2	3	3
$\bar{X}$	3.33	0.67	2.00	2.67	2.67

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PRODUCED IN TEXAS
IN CONNECTION WITH
LITIGATION 2/87

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