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Sustained Nicotine Release Comparisons in Six Inbred Rat Strains

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ERICKSON, C. K. AND K. I. BYERS. *Sustained nicotine release comparisons in six inbred rat strains.* PHARMACOL BIOCHEM BEHAV **33**(1) 63–67, 1989.—The utility of an implantable nicotine reservoir for rats (INR_r) in both sexes of several rat strains is described. INR_rs with similar nicotine release rates produced higher blood nicotine levels in small female rats compared to larger male Sprague-Dawley rats. Blood nicotine levels declined significantly over a 32-day exposure to the INR_r in female Sprague-Dawley rats. In several inbred rat strains (Sprague-Dawley, Fischer, Buffalo, Marshall, Irish, Maudsley), 15-day INR_r exposure produced characteristic body weight changes and blood nicotine level changes. Blood nicotine levels in both sexes of various strains are primarily dependent upon body weight characteristics. We conclude that the INR_r can be an important tool for the study of the chronic effects of nicotine in rats.

Nicotine Rats Inbred strains Sustained release Implant Blood levels

WE have previously reported on a useful subcutaneously-implanted glass reservoir for the prolonged release of nicotine in rats (4). The device itself is nontoxic, easily and inexpensively produced in the laboratory, and adaptable for behavioral, pharmacological, and toxicological studies of the effects of nicotine in the rat. The initial studies of the release characteristics of the Implantable Nicotine Reservoir for Rats (INR_r) were carried out in female Sprague-Dawley rats weighing 200–250 grams (6). [An Implantable Nicotine Reservoir for mice (INR_m) is also available (5)]. In our earlier report, we noted that the INR is much less expensive than the Alzet minipump, the INR releases nicotine continuously for 4–5 times longer than the minipump, and INR can hold a relatively large volume of nicotine alkaloid for toxicological studies (6).

A review of the literature shows that both mice and rats of various sizes and strains have been used for nicotine studies. For example, studies on the oral administration of nicotine in female Swiss-Webster mice, 18–22 grams (9); and on the genetics of the nicotine response in male and female BALB, C57BL, DBA and C3H mice of various weights (8), are only two of many reports using different strains of mice to investigate the effects of nicotine. Reports on rats include studies on the effects of nicotine on locomotor activity in male and female Simonsen Sprague-Dawley rats, weight range 220–540 grams (3); nicotine effects in pregnant Charles River CD (Sprague-Dawley) rats, 60 days old (10); nicotine-elicited catecholamine release in male Wistar rats, 180–250 grams (7), and nicotine and ethanol interactions in male Fischer 344 rats, 160–224 grams (1). Thus, investigators are using different rat strains of various sizes for many different reasons, perhaps because a strain is readily available or for comparative studies. The significance of using different strains in nicotine studies is unclear; however, ethanol clearance in various rat strains is significant (4), and we may assume differences in nicotine pharmacokinetics in various rat strains may also be significant.

It is not clear whether the sustained release device for rats will be useful for rat strains of different ages and gender. The purpose of the present study was to describe the effects of a fixed-release INR_r on several rat strains of both sexes and different body weights, to determine the overall usefulness of this device. We now report that INR_rs releasing a fixed dose of nicotine over time produce various blood nicotine levels that are related to body weight, strain, and gender. However, blood nicotine levels are sufficiently high to be useful in chronic nicotine studies.

GENERAL METHOD

Materials

The drug and chemical sources were as follows: nicotine alkaloid, Sigma Chemical Co. (St. Louis, MO); Silastic® elastomer, 382 Medical Grade Elastomer mixed with catalyst, Catalyst M Stannous Octoate, Dow Corning Co. (Midland, MI); N-ethyl nornicotine, a gift from Dr. P. Jacobs, University of California at San Francisco.

Animals

Male and female rats of different strains were obtained from

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several sources; BUF/N (Buffalo)—Charles River Co., Wilmington, MA; F344 (Fischer)—Charles River Co., Wilmington, MA; ACI/N (Irish)—Charles River Co., Wilmington, MA; M520/N (Marshall)—Gibco Labs., Madison, WI; MR/N (Maudsley)—Animal Breeding Colony, National Institutes of Health; and SD/ARC (Sprague-Dawley)—derived from Sprague-Dawley stock originally obtained from Charles River and bred at the University of Texas.

Upon delivery, the rats were acclimated to the vivarium for 7–14 days. They were housed in groups of 4–5 in wire bottomed stainless steel cages on a 12/12 light/dark cycle at 25°C. Food (Lab Blox, Purina) and water were available ad lib. The rats were unfortunately of various ages and weights, as described in the Results section, because of the difficulty in obtaining animals from limited colonies from the sources listed above.

Implants

The method for making INR_rs in our laboratory has recently been described (6). Briefly, the INR_r is a 22-mm long glass cylinder (5 mm i.d.), closed on one end by flame-sealing. The other end is flame-polished and sealed, after filling with 0.2 ml nicotine alkaloid, with Silastic® elastomer mixed with catalyst. After curing, each glass "cup" is tested for rate of nicotine release in vitro in a rotating tube filled with deionized water. After 24 hours, samples of the deionized water are taken and analyzed spectrophotometrically for nicotine. INR_rs releasing between 2.95–4.42 mg nicotine/24 hours were chosen for these studies.

Each INR_r is implanted subcutaneously along the back of the rat, under light ether anesthesia, so that the surface area of the device is in contact with tissue, as previously described (6). INR_rs are implanted 8 days after manufacture to allow for stabilization of nicotine release.

Measurement of Blood Nicotine Levels

The in vivo measurement of nicotine has previously been described (6). Briefly, daily tail-tip blood samples were collected and nicotine was measured by a gas chromatographic method, using an internal standard N-ethylnornicotine bis-oxalate.

Statistical Analyses

Single and multiple-factor Analyses of Variance were used for the data within each experiment. The paired *t*-test was used to test for significant changes in body weights and blood nicotine levels. The two-sample (or pooled) *t*-test (using the differences obtained between the initial and final values) was used to compare males and females in each strain and also to compare between the strains, in body weights and blood nicotine levels.

EXPERIMENTS

1. INR Release Vs. Body Weight Study

The purpose of this experiment was to determine whether blood nicotine levels were affected by body weight changes over the course of a 17-day study. Nine female Sprague-Dawley rats were implanted with devices averaging 3.18 mg nicotine released/24 hours.

2. Male Vs. Female Study

The purpose of this experiment was to compare blood nicotine levels obtained in male and female rats of the same age, using INR_r devices with similar release rates. Five female and five male Sprague-Dawley rats (70–80 days old) were implanted with INR_rs of almost identical release rates (3.99 and 4.02 mg/24 hours).

3. Long-Term Study

The purpose of this experiment was to examine the stability of blood nicotine levels over time. Eight female Sprague-Dawley rats, 232–294 grams, were implanted with INR_rs releasing 3.16 mg/24 hours. Blood nicotine levels and body weights were followed for 32 days.

4. Strain Study

The purpose of this experiment was to observe the differences in blood nicotine levels in various rat strains to INR_r devices releasing similar amounts of nicotine. Up to 10 rats of each sex of several rat strains were implanted with INR_rs releasing nicotine at in vitro rates ranging from 3.09 to 3.53 mg/24 hours.

RESULTS

Experiment 1. INR Release Vs. Body Weight Study

The relationship between calculation of the amount of nicotine in the blood versus calculation based upon body weight is shown in Fig. 1. There were no significant differences among points in the two sets of data [$F = 1.01$ (*df*: 8,49); 0.87 (*df*: 8,49); Single-factor repeated measures ANOVA].

Experiment 2. Male Vs. Female Study

The effects of nicotine release in the two sexes are shown in Fig. 2. The females averaged 265.4 ± 15.0 grams, while the males averaged 399.0 ± 10.1 grams in weight. When calculated on the basis of amount of nicotine released per unit of body weight, female levels were approximately 15 mg/kg/day, while the male levels were approximately 10 mg/kg/day, averaged over the 9-day period (data not shown). Average body weights increased over the 9-day experiment (females, 6.6 grams; males, 9.6 grams). There was a sex difference, $F(1) = 17.3$, $p<0.05$, but no day-to-day difference, $F(4) = 5.6$, using a two-factor ANOVA.

Experiment 3. Long-Term Study

Figure 3 illustrates that blood nicotine levels gradually, but insignificantly, decline during this time. Also during this time, body weights increased from an average of 262.3 ± 8.04 grams on Day 1 to 278.1 ± 6.66 grams on Day 32. There was no significant difference across days, $F(13,81) = 2.79$, using a single-factor repeated measures ANOVA.

Experiment 4. Strain Study

The effects of the sustained release of nicotine on blood levels of nicotine are shown in Table 1. Although blood levels of the drug were measured at least 8 times during each of the 15-day studies, only the blood levels on Days 5 and 15 are presented, as representative of blood level changes occurring early versus later in the study of each strain. According to a three-factor repeated measures ANOVA, there were significant strain differences, $F(4) = 7.06$, $p<0.01$, sex differences, $F(1) = 33.8$, $p<0.01$, and interaction differences, $F(4) = 3.65$, $p<0.05$. In some strains, blood levels remained stable (as in male and female Sprague-Dawley, male Fischer, male and female Buffalo, male and female Marshall, and female Irish). In others, they decreased (female Fischer, male Irish, male Maudsley) over time. In several strains, we saw an unusually high blood nicotine level on Day 3 (as depicted in Fig. 3 with Sprague-Dawley rats).

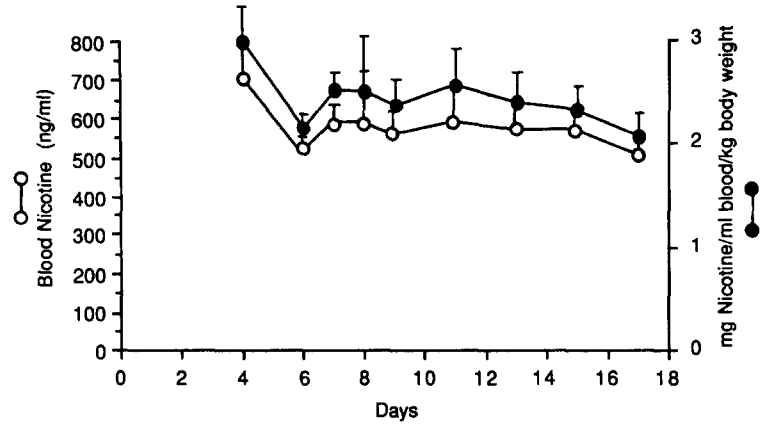

FIG. 1. Nicotine release in relation to body weight in female Sprague-Dawley rats. INR_rs were releasing an average of 3.18 mg/24 hours (range=2.95–3.38). n=3–9, including samples lost during the assay procedure. Each point is the mean ± S.E. No data were collected for the first 3 days because of assay problems. There are no significant differences between points on any day.

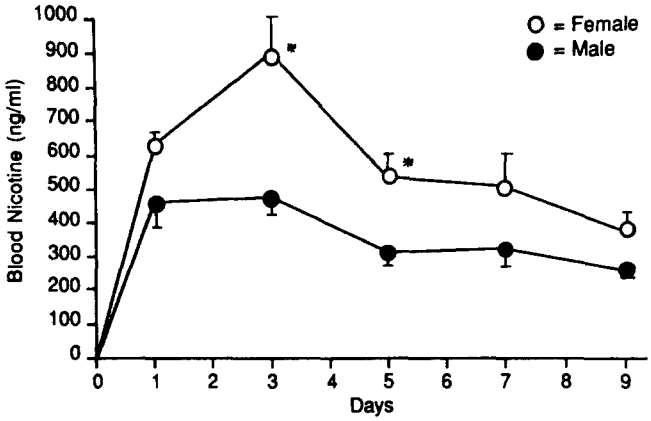

FIG. 2. Nicotine release in male and female Sprague-Dawley rats. Average INR_r release in female rats was 3.99 (range=3.63–4.27) mg/24 hours; in male rats, 4.02 (range 3.81–4.42) mg/24 hours. n=5, each point is the mean ± S.E. *$p<0.05$, difference from males.

GENERAL DISCUSSION

A previous study showed that the INR was useful in young female Sprague-Dawley rats (6). The present study further defines the INR_r release characteristics and extends our earlier results to other strains. These strains have also been used for a study on their characteristic responses to ethanol (4).

The data in Fig. 1 indicate that calculation of blood nicotine in relation to body weight does not (in female rats) significantly change the characteristics of the graph. In other words, the difference in blood nicotine levels on Day 6 compared to Day 4, for example, is not an artifact of a major change in body weights between the two days. In fact, body weights over the 17-day period remained quite stable (average body weight, Day 1, 264.1 ± 15.0 g; on Day 17, 250.2 ± 14.3 g). This is probably the reason for the close relationship between the two graphs. The day-to-day variability in blood nicotine (alone or adjusted for body weight) is probably due to alterations in diet, hydration, and/or blood flow around the implant. Because there is little difference when blood nicotine levels are adjusted for body weight, all

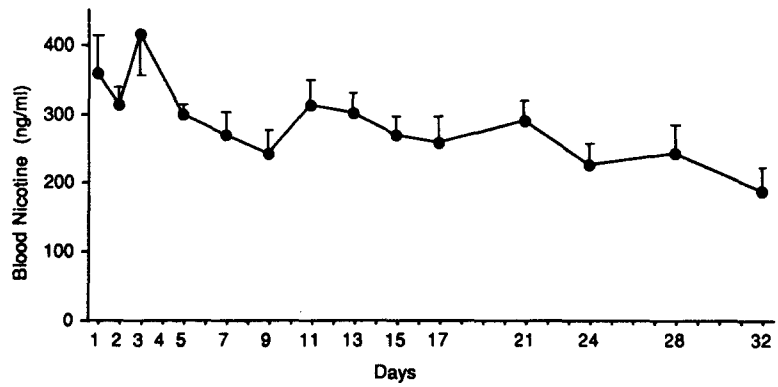

FIG. 3. Long-term effects of sustained release of nicotine in female Sprague-Dawley rats. The release rate of the INR_r averaged 3.16 (range 3.00–3.45) mg nicotine/24 hours. n=8, each point is the mean ± S.E. There was no significant decline of blood nicotine levels over days.

TABLE 1

NICOTINE RELEASE IN SEVERAL RAT STRAINS

Strain	Sex	Initial Weight (g)	Final Weight (g)	n	INR Release (mg/24 hr)*	Blood Levels (mg/ml) Day 5	Day 15
Sprague-	M	370.2 ± 9.9	387.3 ± 11.5[a]	10	3.37	127.1 ± 17.9	79.8 ± 7.3
Dawley	F	242.7 ± 7.5	242.3 ± 7.1	10	3.53	455.4 ± 25.4	455.3 ± 25.7
Fischer	M	189.6 ± 9.6	203.2 ± 9.4[c]	10	3.52	227.5 ± 35.8	244.3 ± 17.9
(F344/N)	F	137.7 ± 4.6	137.7 ± 4.0	10	3.28	418.0 ± 52.7	267.2 ± 21.1[d]
Buffalo	M	302.3 ± 4.1	276.5 ± 5.4[c]	6	3.48	191.2 ± 45.8	364.6 ± 25.3
(BUF/N)	F	214.8 ± 6.5	174.4 ± 12.9[a]	7	3.50	470.0 ± 27.2	518.4 ± 84.4
Marshall	M	281.8 ± 15.5	267.4 ± 15.3[b]	10	3.47	261.1 ± 37.0	316.2 ± 44.7
(M520/N)	F	204.0 ± 8.9	182.2 ± 9.0[c]	6	3.09	346.1 ± 50.2	344.9 ± 73.8
Irish	M	243.8 ± 4.7	249.8 ± 8.1	6	3.26	124.1 ± 8.6	95.9 ± 2.0[d]
(ACI/N)	F	158.7 ± 4.9	151.3 ± 2.0	3	3.11	250.9 ± 42.8	148.8 ± 8.0
Maudsley	M	270.7 ± 11.5	256.5 ± 13.8[c]	10	3.47	349.5 ± 34.5	261.5 ± 21.1[f]
(MR/N)	F			Not available			

* ± S.E. of INR Release was always 0.13 or less.
[a] $p<0.05$, compared to initial weight; [b] $p<0.01$; [c] $p<0.001$; paired t-test.
[d] $p<0.05$, compared to Day 5; [e] $p<0.01$; [f] $p<0.001$; paired t-test.

succeeding experiments are reported in terms of blood nicotine levels only.

Figure 2 illustrates that INR_rs with similar nicotine release rates produce markedly different blood nicotine levels in male and female Sprague-Dawley rats. It appears that body weight markedly affects blood nicotine levels,when release of nicotine is constant. Although factors other than body weight may have contributed to the differences shown in Fig. 2, we have no data which would identify those factors.

In the long-term study shown in Fig. 3, it is interesting that blood nicotine levels fall slowly over the study. Part of this decrease is caused by an overall increase in body weight (about 16 grams in 32 days), but this cannot account for the approximately 50% decrease in blood nicotine levels from Day 1 to Day 32. Our previous study showed that the INR_r release rate decreases around 40% from its initial release rate over 40 days. This is a likely second reason for the decrease in blood nicotine levels in this study.

In Fig. 3 the unusual increase in blood nicotine levels on Day 3 is depicted very clearly. This increase was seen in our previous study (6) and in all the strains in the present study (data not shown). We have no data to explain this phenomenon, but we speculate that the increase may be due to a buildup and then a release from lipid stores [nicotine distributes in fat, (9)]. Another possibility is that the increase may be related to a liver insult after two days of high blood nicotine levels, followed by an adaptation of the liver to resume normal metabolism of nicotine.

In Table 1 we see that the rat sexes and strains are affected differently by the INR_r release of nicotine. Previous studies have shown that male and female rats respond differently to nicotine (2). Again, larger males have lower blood nicotine levels than females. In some strains, blood nicotine levels declined when body weights remained stable (e.g., female Fischer, male and female Irish). In another strain, blood nicotine levels remained stable with increased body weight (male Sprague-Dawley).Two strains showed decrements in body weight with no change (male and female Buffalo; female Marshall) in blood nicotine levels.

From this complex picture evolves these important generalizations:

1) Female rats show higher and more stable and predictable blood nicotine levels over 15 days of INR_r exposure than males.

2) In two-thirds of the strains, blood nicotine levels are stable after 15 days of INR_r exposure in males, despite significant changes in body weight over time.

The results reported in this paper provide the foundation for later studies on the disposition and metabolic tolerance characteristics of nicotine.

If the INR_r is to be used in strains other than Sprague-Dawley, further studies are needed to confirm the present observations and to more fully characterize the blood nicotine changes in studies designed for longer than 15 days. It appears that all strains can tolerate high levels of nicotine produced by the INR_r without major signs of toxicity. Therefore, the INR_r should be a useful tool for pharmacological and toxicological studies requiring long-term nicotine exposure.

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