Pharmacology Biochemistry & Behavior, Vol. 31, pp. 713–715. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

BRIEF COMMUNICATION

A Device for the Sustained Release of Nicotine in the Mouse[1]

CARLTON K. ERICKSON[2] AND KATHE I. BYERS

Alcohol and Drug Abuse Research Program, Institute for Neurological Sciences Research College of Pharmacy, The University of Texas, Austin, TX 78712

Received 4 April 1988

ERICKSON, C. K. AND K. I. BYERS. *A device for the sustained release of nicotine in the mouse*. PHARMACOL BIOCHEM BEHAV 31(3) 713–715, 1988.—A subcutaneously-implantable reservoir for the sustained release of nicotine in mice is described. The device, dubbed INR_m to differentiate it from an earlier Implantable Nicotine Reservoir for Rats (INR_r), is a small glass cup sealed with Silastic® polymer. Three sizes are described, which release 0.75–2.05 mg of nicotine per 24 hours. When implanted into mature CD-1 female mice, the largest device produces blood nicotine levels of 445 ng/ml, which remain relatively stable for at least 19 days. These blood nicotine levels produce no weight loss and minimal body temperature reduction over the time period of testing. INR_ms, like the INR_r, are nontoxic, reproducible, inexpensive, and adaptable for behavioral, pharmacological, and toxicological studies of nicotine in mice.

Nicotine Mice Body temperature Blood levels Sustained release Implant

IN an earlier publication, we described a subcutaneously-implantable glass reservoir for the sustained release of nicotine in rats (3). That device, dubbed INR_r for Implantable Nicotine Reservoir for Rats, has been utilized in at least two studies for its ability to maintain high blood nicotine levels in rats (1,6).

Chronic administration of nicotine has been used to study the long-term pharmacological and toxicological effects of this drug. Methods of chronic nicotine administration in such studies have generally involved oral administration in the drinking water and intravenous infusion. In an oral administration study, a steady-state plasma level of nicotine of 34.4 ng/ml was maintained for 5–6 weeks (7). This apparently produced no toxicity (fluid intake and weight gain were normal), and allowed a demonstration of the distribution of nicotine in the body. In the intravenous infusion studies, blood nicotine levels were not measured, but intravenous doses ranged from 1.0–7.0 mg/kg/hr up to 12 days, allowing studies on neurotransmitter receptor changes in brain (4), and tolerance and receptor changes (5).

We have developed an INR which may be useful for investigators who wish to study the chronic effects of nicotine in mice with the ease of a single surgical manipulation, producing high sustained blood nicotine levels for at least 19 days.

METHOD

Materials

The drug and chemical sources have previously been described (3): 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

Eleven young female CD-1 mice, initial weight 16–18 grams, were used for studies with the smallest INR_m (Prototype I). Thirty-six mature female CD-1 mice, 30–35 grams, were used for studies with two larger prototypes (II and III). The mice were obtained from the University of Texas breeding colony, and housed in groups of 6–10 in suspended plastic cages, on a 12/12 light/dark cycle at 25°C. Food (Lab Blox, Purina) and water were available ad lib.

INR_m Development

Previous studies in our laboratory established that liquid nicotine alkaloid is best for these release studies using

[1]This work was supported by grant no. 1208 from The Council for Tobacco Research-U.S.A., Inc.

[2]Requests for reprints should be addressed to Carlton K. Erickson, College of Pharmacy, The University of Texas, Austin, TX 78712-1074.

TABLE 1
CHARACTERISTICS OF THREE INR_m PROTOTYPES

Prototype	Internal Diameter (mm)	Surface Release Area (mm^2)	In Vitro Release (mg/24 hr)
I	2.25	3.97	0.75
II	3.00	7.07	1.65
III	4.00	12.57	2.05

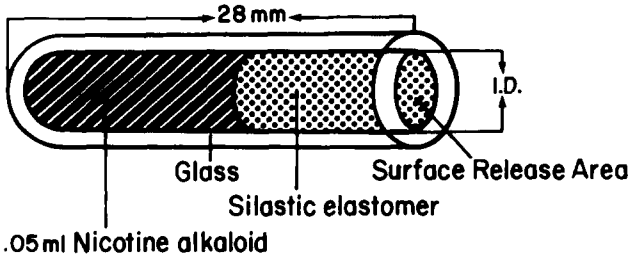

FIG. 1. The Implantable Nicotine Reservoir for Mouse (INR_m). I.D. = internal diameter.

permeable Silastic® elastomer (3). As with the INR_r, we designed a glass reservoir in the form of a glass cup (Fig. 1). The characteristics of the three prototypes are described in Table 1. The INR_m is slightly longer than the INR_r, but smaller in diameter. INR_ms are sealed with Silastic® elastomer and allowed to cure. The manufacture of the devices has been previously described (3), with smaller diameter glass tubing used for the INR_m compared to the INR_r.

The reservoir is subcutaneously implantable along the back of a mouse, using light ether anesthesia, as previously described (3). The surface release area of the tube is placed so that it is in contact with subcutaneous tissue. The implant has been found to release nicotine at a rate proportional to the surface release area of the device.

Preimplantation In Vitro Release Studies

We determined the in vitro release of nicotine at constant temperature by incubating the reservoirs in 67-ml screw-capped tubes filled with deionized water. These were inverted at 25 RPM in a temperature-controlled water bath at 37°C for 9 days, with daily nicotine sampling and replacement of water. Analysis of nicotine in these studies was by ultraviolet spectrophotometry at λ_{max} 261 nm.

Measurement of Blood Nicotine Levels

The measurement of blood nicotine levels, using gas chromatography and an internal standard of N-ethyl nornicotine bis-oxalate, has been previously described (3). Retroorbital blood samples (100 μl) were collected under light ether anesthesia.

RESULTS

In Vitro Release Studies

Figure 2 shows the release of nicotine into deionized water at 37°C for each of the 3 prototypes for 9 days. The

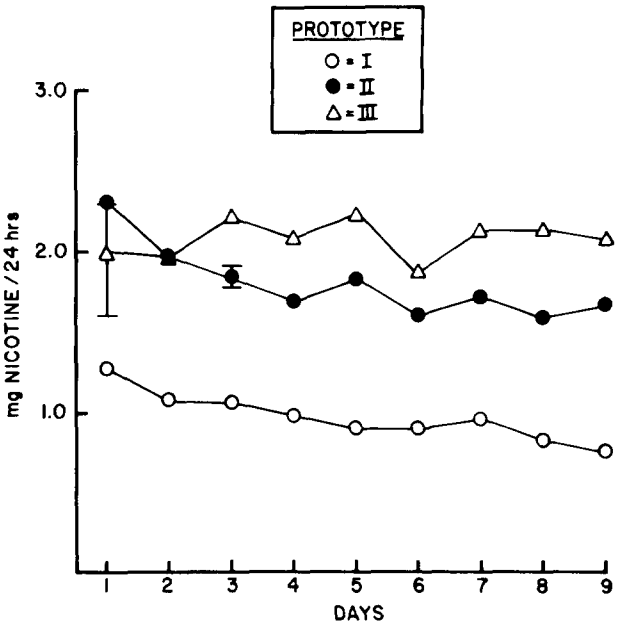

FIG. 2. In vitro release of nicotine from three prototype INR_ms, incubated in deionized water in sealed glass tubes in a constant temperature water bath. Analysis of released nicotine was by spectrophotometry. Each point is the average release of 5 reservoirs, measured separately. ±S.E. are indicated for the largest and smallest variances seen in the study.

mean release rates indicated in Table 1 were calculated by averaging the release rates of 5 tubes of each prototype on Day 9 of release.

In Vivo Release Studies

Prototype I INR_ms and sham implants (without nicotine) were implanted in 11 female mice, and body weight and rectal temperatures (Yellow Springs Instruments monitor) were measured for 19 days. These mice continued to grow, with no signs of nicotine toxicity, and a sham-implanted group (INR_m without nicotine) grew at the same rate (Table 2).

Prototypes II, III, and sham implants were implanted in 5 female mice each. Table 2 also shows that the higher nicotine levels had no significant effect on body weight or temperatures measured initially and after 18 days. However, with Prototype III, the largest device, there was a significant drop in body temperature on Day 2 after implantation to 31.0±1.18°C ($p<0.05$).This was probably caused by the effects of the large nicotine levels on the animals before tolerance developed.

TABLE 2

BODY WEIGHT AND TEMPERATURE CHANGES IN INR_m IMPLANTED MICE

Prototype	N	Body Weights (g)		Temperature (°C)		Blood Nicotine (ng/ml, mean ± S.E.)
		Initial	Final*	Initial	Final*	
I (Young mice)	5	16.6 ± 0.71	27.0 ± 5.0	36.1 ± 0.21	36.9 ± 0.11	63.1 ± 8.3
I—Sham	6	17.0 ± 0.54	27.8 ± 0.31	36.5 ± 0.15	37.6 ± 0.13	—
II (Mature mice)	5	33.0 ± 0.57	33.1 ± 0.64	37.0 ± 0.25	35.7 ± 0.41	296.6 ± 30.6
III (Mature mice)	5	31.0 ± 1.64	31.5 ± 1.33	35.4 ± 0.14	36.3 ± 0.24	445.4 ± 62.8
III—Sham	5	31.2 ± 1.19	31.8 ± 1.09	36.5 ± 0.38	37.0 ± 0.22	—

*After 19 days in young mice, after 18 days in mature mice.
There were no statistically significant differences when calculated by Student's *t*-test, between initial and final values.

DISCUSSION

The INR_m, from the results presented here, produces much higher blood nicotine levels than the oral administration in drinking water method (7), and is simpler than the intravenous infusion method (4,5), which requires a detailed surgical procedure, and connection of the animal to a pump for the duration of the experiment.

Even at high blood nicotine levels of almost 450 ng/ml, there was no effect on body weight in young and mature mice, and only a suggestion of an effect on body temperature (on Day 2) using the largest protype. This suggests that mice, like rats (3), can tolerate high nicotine levels, unlike humans who become nauseated at blood levels of around 50 ng/ml, produced by the forced smoking of 3 cigarettes per hour for 7 hr (8).

Comparing the INR_m with the Alzet minipump, we believe that the INR_m is less expensive, it releases nicotine 4–5 times longer than the minipump, and it produces relatively stable blood nicotine levels (3). The INR_m can easily be customized for large or small mice, simply by changing the internal diameter of the glass cups.

The thickness of the Silastic® plug, as discussed in our previous report on the INR_r, does not appear to affect the rate of release of nicotine. We have no data on the release characteristics of this device, although previous Silastic implants (for ethanol) in our laboratory showed zero order release kinetics (2).

The INR_m is inexpensive to produce, and should be valuable for studies on the pharmacologic, behavioral, and toxicological properties of nicotine.

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