Pharmacology Biochemistry & Behavior, Vol. 17, pp. 183–185, 1982. Printed in the U.S.A.

A New Subcutaneously-Implantable Reservoir for Sustained Release of Nicotine in the Rat[1]

CARLTON K. ERICKSON,[2] SALOMON A. STAVCHANSKY, KATHE I. KOCH AND JAMES W. McGINITY

Drug Dynamics Institute, College of Pharmacy, The University of Texas, Austin, TX 78712

Received 19 December 1981

ERICKSON, C. K., S. A. STAVCHANSKY, K. I. KOCH AND J. W. McGINITY. *A new subcutaneously-implantable reservoir for sustained release of nicotine in the rat.* PHARMAC. BIOCHEM. BEHAV. 17(2) 183–185, 1982.—A subcutaneously-implantable reservoir for the sustained release of nicotine is described. The device, dubbed INR for Implantable Nicotine Reservoir, is a small glass cup sealed with Silastic® polymer. It releases 3.4 mg of nicotine per 24 hours. When implanted into moderately-sized female Sprague-Dawley rats it produces blood nicotine levels of 400–500 ng/ml which remain relatively stable over at least 18 days. INRs are nontoxic, reproducible, inexpensive, and adaptable for pharmacological and toxicological studies in rats and other small animals.

Nicotine Sustained release Rats Body temperature Blood levels Implant

NICOTINE, the predominant alkaloid in tobacco, has become a widely studied drug for its pharmacological and toxicological effects. It may induce or exacerbate several cardiovascular, respiratory, and neoplastic states in both humans and laboratory animals [7, 8, 10].

In humans, nicotine is normally administered by inhalation of cigarette smoke or buccally, through the lining of the mouth by absorption of solubilized nicotine from chewing tobacco. Blood nicotine levels often remain high as the result of chain-smoking or the continual chewing of tobacco. In animals, forced-administration methods for nicotine include parenteral injection [13–16], administration by inhalation of cigarette smoke [3], solubilization in drinking water [14] and injection in single doses [2] or chronically via Alzet minipump [1] into the ventricles of the brain. Nicotine has also been self-administered intravenously [17]. The most common route of forced administration in animals is by parenteral injection. Oftentimes, up to 6 daily doses are given, the duration of each dose being 15–120 minutes, depending upon the size of the dose [9,13].

Previous animal studies involving tolerance to nicotine or to the toxicological effects of continual exposure to nicotine have been hampered by the inability of investigators to administer nicotine conveniently over long periods of time. We felt that a sustained-release form of nicotine would be useful, in much the same manner as sustained release forms for morphine [11], ethanol [5,6], and amphetamine [4] have been useful for studying chronic toxicity and dependence in animals. This paper reports the successful development of a glass implant with a Silastic® cap through which nicotine is slowly released.

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-ethylnornicotine, a gift from Dr. P. Jacobs, University of California at San Francisco.

Animals

Sixteen female SD/ARC rats (derived from Sprague Dawley stock originally obtained from Charles River and bred at The University of Texas) were used in the experiments. They were housed in groups of 3–4 in wire bottomed stainless steel cages on a 12/12 light/dark cycle at 25°. Food (Lab Blox, Purina) and water were available ad lib. The rats weighed 200–250 g at the start of the experiment.

Implant Manufacture

Preliminary (unpublished) results in our laboratory indicated that the solid nicotine bitartrate did not release easily from a silastic matrix such as the one used for morphine [11]. Therefore, we chose the liquid nicotine alkaloid for our release studies. In further tests we found that the release of

[1]This work was supported by grant no. 1208 from The Council for Tobacco Research—U.S.A., Inc. Portions of this work were reported in *Fedn Proc.* **39:** 316, 1980.

[2]Send reprint requests to: Carlton K. Erickson, College of Pharmacy, The University of Texas, Austin, TX 78712.

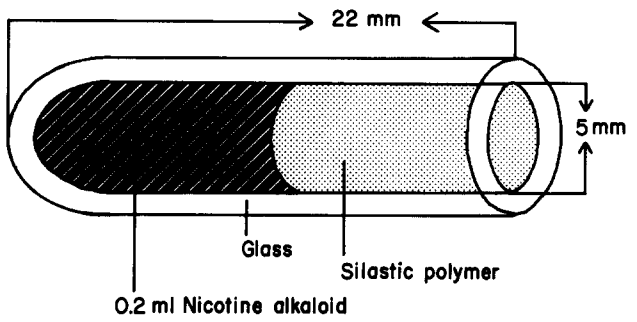

FIG. 1. The Implantable Nicotine Reservoir, INR.

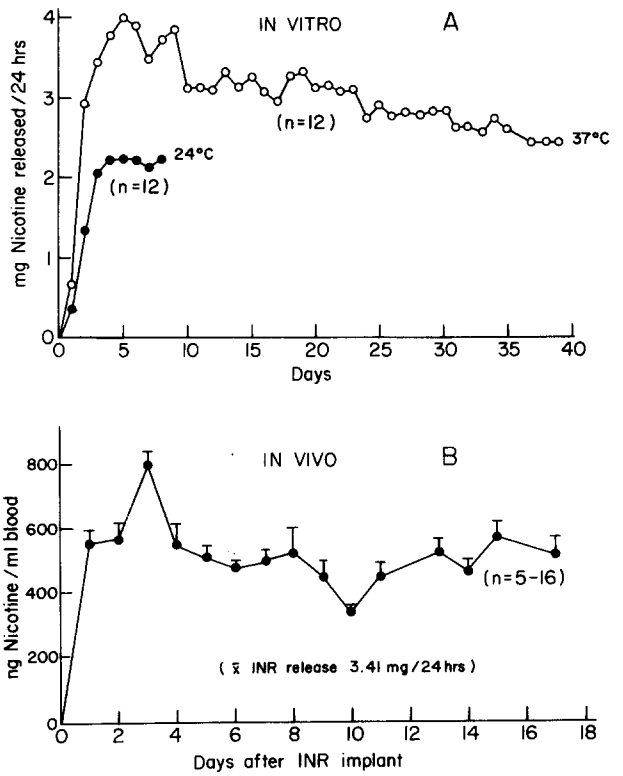

FIG. 2. Characteristics of *in vitro* and *in vivo* release of nicotine from INRs. Panel A illustrates the *in vitro* release of nicotine from INRs incubated in sealed glass tubes rotating in a constant temperature water bath. Analysis of released nicotine was by spectrophotometry. Panel B illustrates blood nicotine levels produced by the devices similar to those in panel A, implanted in female Sprague-Dawley rats. Nicotine in blood was measured gas chromatographically. (Mean INR release rate indicated in Panel B is the *in vitro* release rate before implantation. The variable n in Panel B is due to the fact that blood samples were not taken from every rat at each time period.)

nicotine from a Silastic® tube like the one used for ethanol [5,6] was much too rapid. After preparing several prototypes, we settled on a glass reservoir in the form of a glass cup (Implantable Nicotine Reservoir, INR, Fig. 1). It is 22 mm long × 5 mm i.d., contains 200 μl of nicotine alkaloid, and is sealed with a 10 mm thick plug of Silastic® polymer. The reservoirs were made by carefully cutting glass tubing (7 mm o.d., 5 mm i.d.) at 22 mm after one end was flamed-sealed. The other end was flame-polished. Nicotine alkaloid (200 μl) was placed by syringe into the cup, and the remaining space was filled with Silastic® elastomer mixed with catalyst. The cups were inverted onto a glass plate and cured at room temperature for 2–3 hours.

The reservoir is subcutaneously implantable in the back of a rat, and has been found to release nicotine through the Silastic® plug at a rate proportional to the diameter of the plug. Several prototypes were made, all of which released nicotine at a rate which, when tested *in vivo*, produced marked hypothermia, tremors, rigidity and convulsions in the animals. The reservoir in Fig. 1 has been developed for female Sprague-Dawley rats weighing 200–250 g, and nicotine release into these animals produces a mild hypothermia and no significant effect on normal body weight increments.

Preimplantation In Vitro *Release Studies*

A study on the *in vitro* release of nicotine at different temperatures involved incubation of the reservoirs in 67-ml screw-cap sealed tubes which rotated at 25 RPM in a temperature-controlled water bath. The nicotine released from each INR was measured every 24 hours in samples of water from the large tube surrounding it. Analysis was by ultraviolet spectrophotometry at λ_{max} 261 nm. At each sampling time, the water in the large tubes was replaced with fresh deionized water.

Surgical Procedure

Rats were given light ether anesthesia, and a cut was made in the skin 2 cm behind the ears. A glass probe was inserted to make a track down the side of the animal's body, and the reservoir was placed into the track beneath the skin, with the Silastic plug facing ventrally toward the belly. The incision was closed with wound clips.

Measurement of Blood Nicotine Levels

The *in vivo* accumulation of nicotine was measured by collecting daily tail-tip blood samples and measuring nicotine by a gas chromatographic method. Blood samples were 100 μl, collected in heparinized Microcaps®, and emptied into screwcap centrifuge tubes containing 0.5 ml internal standard, N-ethylnornicotinebis-oxalate, in a concentration of 200 ng/ml. The tubes were vortex-mixed for 20 seconds and then frozen until assay. Blank curves of nicotine in both water and blood were used as external standards. Nicotine was extracted from blood using ether extraction, and 5 μl of the final supernatant was injected into a Hewlett-Packard Model 5710A gas chromatograph with nitrogen-phosphorus detector. The column was 2-ft glass, filled with 2% Carbowax 20 M and 2% KOH on Gaschrom P, 100/120 mesh. Helium carrier gas flow was 30 ml/min, and the oven was 160°C. Retention times were 80 sec for nicotine and 110 sec for internal standard. Blank samples of blood gave small interfering peaks at 80 sec which were subtracted for correction of the nicotine standard curve.

RESULTS

In Vitro *Release Studies*

Figure 2A shows that the release of nicotine from the

INRs is temperature-dependent. Figure 2A also illustrates the sustained release of nicotine at body temperature (37°C) for 40 days. The variability of release during the first 8 days is consistent, yet unexplained. Therefore, we chose to implant INRs in rats 8 days after their production, when relatively stable release had been established. The rate of release at this point averaged 3.4 mg of nicotine per 24 hours.

After implantation of these devices which were already releasing nicotine, the release rate was calculated to be 14 mg of nicotine per kg of body weight per 24 hours, based upon data obtained in the *in vitro* release study. This compares favorably with an *in vivo* release rate of 12.9–20.6 mg/kg/hr, calculated from data on the metabolism of nicotine reported by Miller *et al.* [12], who studied the pharmacokinetics of intravenous Me-^{14}C-nicotine. *In vivo* release rates of nicotine were calculated using total body clearance of nicotine in male Fischer-344 rats. (We realize that there is probably a difference in metabolism of nicotine in male Fischer rats and the female Sprague-Dawley rats used in the present study.)

Sustained Blood Nicotine Levels

Figure 2B shows an initial peak blood nicotine value 3 days after implantation, which occurred in every animal and may be due to a liver insult followed by reduced metabolism. The blood levels soon stabilized at 400–500 ng/ml, however, and the observed minor daily variability thereafter through 18 days may be due to changes in body temperature or metabolism of the animal. When INRs were removed from the animals after 30 days, no tissue pathology was noted in the area of the implantation. Thus we believe that INRs themselves are nontoxic in rats.

DISCUSSION

Comparing the present device with the other sustained-release form available for nicotine, the Alzet minipump, the INR is much less expensive, it releases nicotine continuously for 4–5 times longer than the minipump, and it has been proven to be suitable for producing stable blood nicotine levels. The release rate of the Model 2001–2002 minipump, 10 μg nicotine/hour, is suitable for intraventricular injection of nicotine into the brain [1], but is much too slow for sufficient parenteral release of the drug in toxicological studies. A new minipump (Model 2ML1) offers a release rate of 10 times the Model 2001–2002, and should be more effective for this purpose, however.

Although the INRs reported in this study were made exclusively for use in a certain size, sex (female), and strain of rat, we have made devices which release from 1.5 to 5.0 mg of nicotine in 24 hours, simply by changing the internal diameter of the glass cups. The thickness of the Silastic® plug, in our preliminary studies, did not appear to affect the rate of release of nicotine, only the latency to constant release.

It is clear that the blood nicotine levels produced by the INR in rats are much higher than those achieved after cigarette smoking in humans (400–500 ng/ml vs 9–38 ng/ml) [15]. While species differences may exist, the INR release rate is probably more suitable for toxicological studies than for pharmacological studies. However, the release rate can easily be reduced for studies requiring lower doses. Also, the release rate can be adjusted, with some experimentation, for larger or smaller rats, or for strains of rats which metabolize nicotine more slowly or faster than Sprague-Dawley. The INR thus should be valuable for many studies of the pharmacologic, carcinogenic, teratogenic, and other toxicologic properties of the drug.

REFERENCES

1. Abood, L. G., K. Lowy and H. Booth. Acute and chronic effects of nicotine in rats and evidence for a noncholinergic site of action. *NIDA Res. Monog. Ser.* **23:** 136–149, 1979.
2. Beleslin, D. B. and Z. S. Malobabic. Catalepsy produced by intraventricular injection of nicotine. *Experientia* **28:** 427, 1972.
3. Bretthauer, E. W., S. C. Black, R. L. Satterwhite, E. Compton and A. A. Moghissi. An inhalation device for exposing rats to cigarette smoke. *Archs Envir. Hlth* **25:** 456–458, 1972.
4. Ellison, G., M. S. Eison, H. S. Huberman and F. Daniel. Long-term changes in dopaminergic innervation of caudate nucleus after continuous amphetamine administration. *Science* **201:** 276–278, 1978.
5. Erickson, C. K., K. I. Koch and J. W. McGinity. Subcutaneous silastic implants: Maintenance of high blood ethanol levels in rats drinking a liquid diet. *Pharmac. Biochem. Behav.* **13:** 781–786, 1980.
6. Erickson, C. K., K. I. Koch, C. S. Mehta and J. W. McGinity. Sustained release of alcohol: Subcutaneous silastic implants in mice. *Science* **199:** 1457–1459, 1978.
7. Hall, G. H. Effects of nicotine, carbon monoxide and tobacco smoke on regional blood flow in the cerebral cortex. *Eur. J. Pharmac.* **19:** 385–388, 1972.
8. Haworth, J. C., J. J. Ellestad-Sayed, J. King and L. A. Dilling. Fetal growth retardation in cigarette-smoking mothers is not due to decreased maternal food intake. *Am. J. Obstet. Gynec.* **137:** 719–723, 1980.
9. Hubbard, J. E. and R. S. Gohd. Tolerance development to the arousal effects of nicotine. *Pharmac. Biochem. Behav.* **3:** 471–476, 1975.
10. Lucchesi, B. R., C. R. Schuster and G. S. Emley. The role of nicotine as a determinant of cigarette smoking frequency in man with observations of certain cardiovascular effects associated with the tobacco alkaloid. *Clin. Pharmac. Ther.* **8:** 789–796, 1967.
11. McGinity, J. W. and C. S. Mehta. Preparation and evaluation of a sustained morphine delivery system in rats. *Pharmac. Biochem. Behav.* **9:** 705–708, 1978.
12. Miller, R. P., K. S. Rotenberg and J. Adir. Effect of dose on the pharmacokinetics of intravenous nicotine in the rat. *Drug Metab. Dispos.* **5:** 436–443, 1977.
13. Morrison, C. F. and J. A. Stephenson. The occurrence of tolerance to a central depressant effect of nicotine. *Br. J. Pharmac.* **46:** 151–156, 1972.
14. Peters, D. A. V., H. Taub and S. Tang. Postnatal effects of maternal nicotine exposure. *Neurobehav. Toxicol.* **1:** 221–225, 1979.
15. Russell, M. A. H., C. Wilson, U. A. Patel, C. Feyerabend and P. V. Cole. Plasma nicotine levels after smoking cigarettes with high, medium, and low nicotine yields. *Br. med. J.* **2:** 414–416, 1975.
16. Schecter, M. D. and J. A. Rosecrans. Behavioral tolerance to an effect of nicotine in the rat. *Archs int. Pharmacodyn.* **195:** 52–56, 1972.
17. Singer, G., F. Simpson and W. J. Lang. Schedule induced self injections of nicotine with recovered body weight. *Pharmac. Biochem. Behav.* **9:** 387–389, 1978.