Pharmacology Biochemistry & Behavior, Vol. 19, pp. 553–557, 1983. © Ankho International Inc. Printed in the U.S.A.

BRIEF COMMUNICATION

Effects of Urinary pH on the Behavioral Responses of Squirrel Monkeys to Nicotine[1]

NEIL E. GRUNBERG, DAVID E. MORSE AND JAMES E. BARRETT

Uniformed Services University of the Health Sciences, Bethesda, MD 20814

GRUNBERG, N. E., D. E. MORSE AND J. E. BARRETT. *Effects of urinary pH on the behavioral responses of squirrel monkeys to nicotine.* PHARMACOL BIOCHEM BEHAV **19**(3) 553–557, 1983.—The present study evaluated the behavioral effects of nicotine under conditions that manipulated urinary pH. The effects of nicotine were examined on the responding of squirrel monkeys under a multiple fixed-interval, fixed-ratio schedule of stimulus-shock termination when nicotine was administered alone or together with the gastric administration of an acidifier (ammonium chloride) or an alkalinizer (sodium bicarbonate). Responding under the FI schedule was increased markedly across a range of doses of nicotine (0.02–0.20 mg/kg). Responding under the FR was increased to a lesser extent by the lower doses of nicotine (0.02–0.05 mg/kg) and was decreased by doses of nicotine that increased responding under the FI (0.10–0.20 mg/kg). Generally, administration of the acidifier attenuated the effects of nicotine while administration of the alkalinizer potentiated those effects. These findings support the argument that changes in cigarette smoking under conditions that alter urinary pH involve nicotine per se. In addition, a new interpretation of the relationship between urinary pH and cigarette smoking is offered.

Nicotine Urinary pH Ammonium chloride Sodium bicarbonate Fixed-interval schedule
Fixed-ratio schedule Stimulus-shock termination Squirrel monkeys

RECENTLY, a psychopharmacological explanation was offered by Schachter to account for the increases in cigarette smoking behavior that commonly occur in particular situations [10]. For example, under stress there is an increase in urinary acidity which increases the rate of excretion of unmetabolized nicotine from the body [1,2]. As a result of decreased availability of nicotine in the body, it is argued that the smoker is put into a state of partial nicotine withdrawal. In turn, this leads to increased cigarette smoking to replenish nicotine levels which then relieves the unpleasant withdrawal state.

Support for this model was provided by a series of studies that manipulated urinary pH and observed changes in smoking behavior of habitual human smokers [9,10]. However, the role of nicotine per se in altering smoking behavior when urinary pH is changed was reasoned by logical deduction rather than by empirical observation. Despite this important omission, this work has been taken as evidence for the role of nicotine in cigarette smoking and has formed the basis for new approaches to smoking cessation.

More recent studies with both humans and animals have been performed to address the urinary pH/smoking and nicotine issues. It has been demonstrated that changes in urinary pH alter performance of smokers on a detection task (presumably because extent of nicotine deprivation is affected) [12]. Further studies have found that when urinary pH is changed, the amount of nicotine excreted in the urine also changes [3, 7, 8]. However, it has not been clearly demonstrated that changes in urinary pH alter either nicotine self-administration or the behavioral effects of nicotine.

The only data relevant to this point are from studies that examined the effects of urinary pH on nicotine self-administration of rats [6]. Latiff, Smith, and Lang found that rats self-administered nicotine at a lower rate when the urine was made alkaline, whereas rats whose urine was made acidic self-administered nicotine at a higher rate. These data support Schachter's assumption that nicotine mediates the urinary pH/smoking phenomenon. However, once an initial rate of nicotine self-administration was established, changes in urinary pH did not affect self-administration of nicotine by the rats. Latiff *et al.* [6] argue that their findings generally support Schachter's position, but also indicate the importance of schedules in controlling nicotine self-administration. However, there is still a lack of convincing data that either

[1]This research was supported in part by PHS Grant DA-02873.

nicotine self-administration or the behavioral effects of nicotine are related to changes in urinary pH.

The present study evaluated the behavioral effects of nicotine on the responding of squirrel monkeys when nicotine was administered alone or together with a gastric acidifier (ammonium chloride) or alkalinizer (sodium bicarbonate). Previous research has shown that schedule-controlled performances of this type are quite sensitive to the effects of nicotine [11]. Data collected under procedures that also manipulate urinary pH, therefore, would provide necessary information for evaluating the potential modification of the behavioral effects of nicotine by changes in urinary pH.

METHOD

Subjects

Two mature male squirrel monkeys (*Saimiri sciureus*) were used. The subjects were housed individually and, except during experimental sessions, were provided with unrestricted access to food and water. Each subject weighed roughly one kg. The animals previously had been trained on the stimulus-shock termination procedure used in the present study. Neither subject had received any other drugs during the 6 months preceding the present study.

Apparatus

During experimental sessions each subject was seated in a Plexiglas chair [5] which loosely restrained the monkeys at the waist. A BRS/LVE No. 121-05 response lever and three pairs of colored lamps were mounted on the front wall. The tail was immobilized by a small Plexiglas stock which contained a pair of brass electrodes that rested on a shaved portion of the distal end of the tail. Prior to each session, the tail was coated with EKG sol electrode paste to ensure low-resistance contact with the electrodes. Electric shock consisted of a 200 msec 5 mA pulse from a 650 V AC 60 Hz transformer delivered through a variable resistor in series with the tail. The chair was placed inside a sound-attenuating enclosure supplied with white noise and equipped with a ventilating fan.

Behavioral Procedure

The effects of nicotine were evaluated on the responding of squirrel monkeys under a multiple 3-min fixed-interval (FI), 30-response fixed-ratio (FR) schedule of stimulus-shock termination. Under the FI schedule, the first response after 3 min terminated a stimulus (red light) in the presence of which shocks (5 mA, 200 msec) began to occur every 5 sec after the FI had elapsed. Under the FR schedule, the completion of 30 responses within a 15 sec interval terminated a stimulus (white light); failure to complete 30 responses within 15 sec resulted in the presentation of shock every 15 sec until the 30 responses were completed. These two schedules were separated by a 30 sec timeout period during which responding had no consequences and the experimental chamber was darkened. The presentation of two shocks under either schedule automatically advanced the procedure to the 30 sec timeout. Each experimental session (roughly 1.25 hr long) included three separate cycles. Each cycle began with a 5 min period which was followed by four repetitions each of the FI and FR schedules. No stimulus lights were illuminated during the initial 5 min period of the cycle. The FR and FI schedules alternated regularly within each cycle. After the fourth FR within a cycle, the 5 min cycle began again and the schedule sequence was repeated until the three cycles were complete. Experimental sessions were conducted five days per week (Monday–Friday) between 9:00 a.m. and 1:00 p.m.

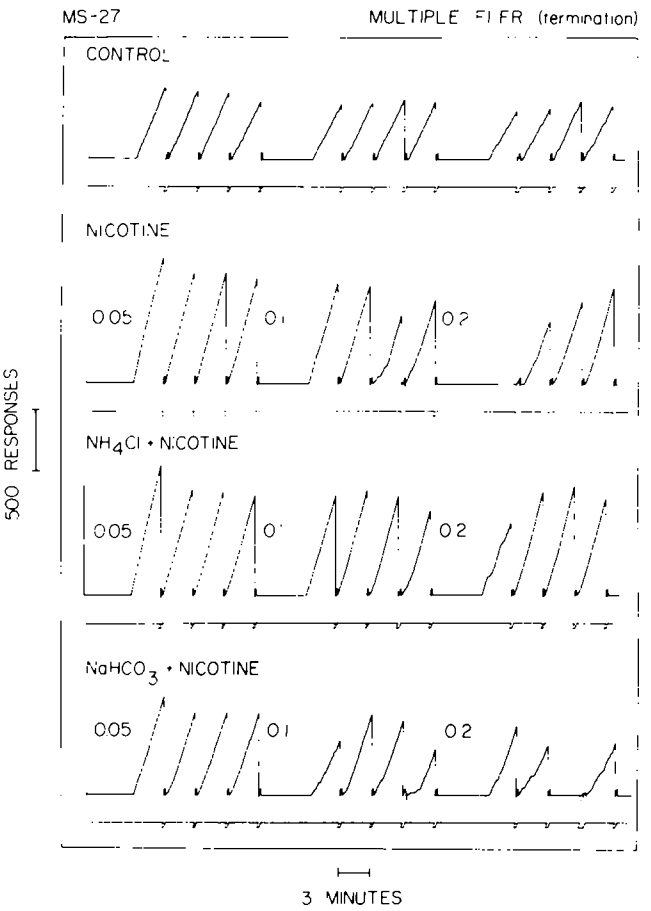

FIG. 1. Cumulative response records showing control performance (top record) and effects of nicotine alone (second record) and effects of nicotine in combination with ammonium chloride and sodium bicarbonate (third and fourth records, respectively). Responding was maintained under a multiple 3-min fixed-interval (FI), 30-response fixed-ratio (FR) schedule of stimulus-shock complex termination. The line beneath the response record was displaced during the FR schedule component. The pen reset at the end of each component when a response produced a 30-sec timeout period during which no shocks were delivered. Shock deliveries are indicated by a diagonal deflection on the response record, e.g., after 0.2 mg/kg nicotine alone. The session was divided into 3 cycles of four components each, separated by a 5-min period. Nicotine was administered during this time at the doses indicated. Note that low doses of nicotine alone increased responding and that the highest doses decreased responding. Ammonium chloride, administered prior to the session, antagonized the rate-decreasing effects of nicotine, whereas sodium bicarbonate potentiated these effects.

Drug Procedure

Nicotine and control injections of saline were administered at the beginning of the 5-min period preceding each cycle. Drug dosing procedures were conducted on Tuesday

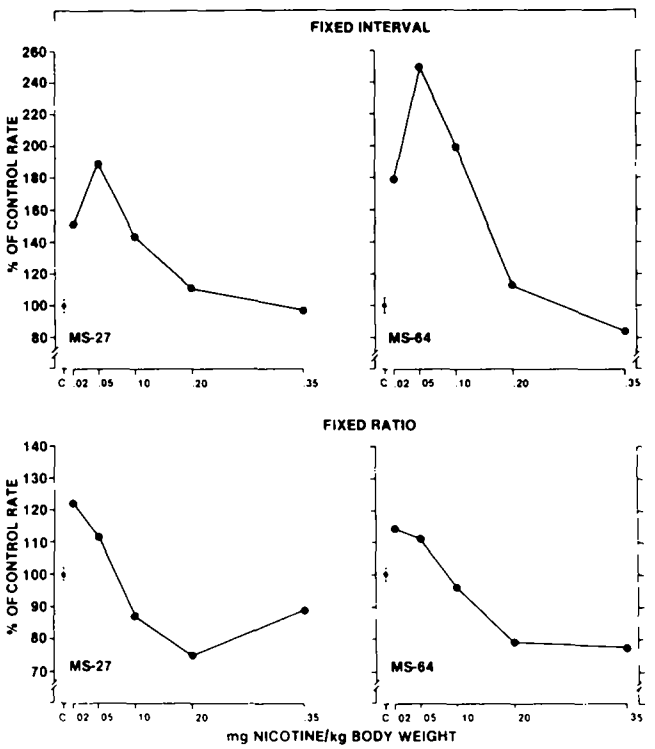

FIG. 2. Dose-response curves of the effects of nicotine on responding of two squirrel monkeys under the fixed-interval and fixed-ratio stimulus-shock termination schedule. Points above C are the control values with ±1 S.E. represented by the vertical bars.

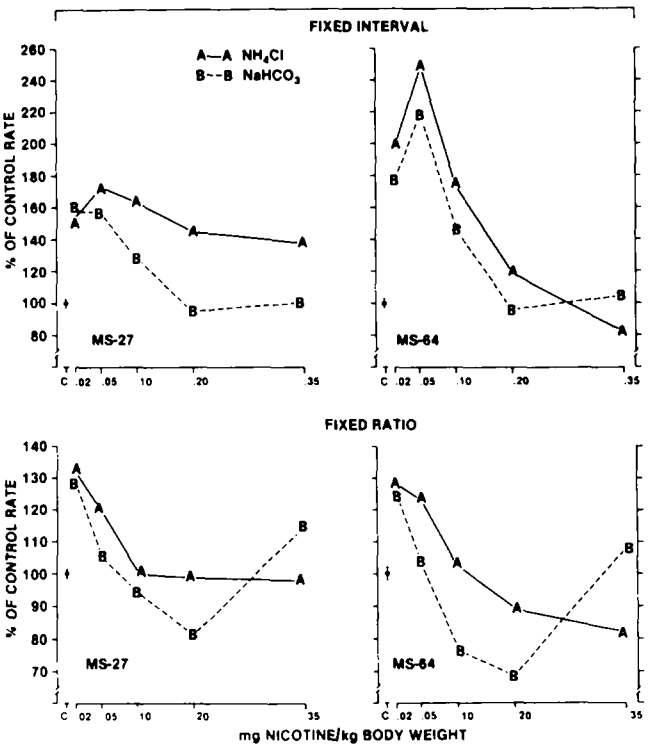

FIG. 3. Effects of intragastric administration of an acidifier (NH_4Cl) and an alkalinizer ($NaHCO_3$) on the effects of nicotine under the fixed-interval and fixed-ratio schedules of stimulus-shock termination. Points above C are the control values with ±1 S.E. represented by the vertical bars.

and Friday of each week, given that performances on Thursday were stable when compared to performances prior to the administration of nicotine. Performances during the Thursday's sessions were used as control values.

Nicotine dihydrochloride (J. T. Baker Company) was dissolved in 0.9% sodium chloride to make the following dosages (computed as nicotine base): 0.02, 0.05, 0.10, 0.20, and 0.35 mg/kg of body weight. Solutions were injected in a volume of 1 ml/kg of body weight. Nicotine solutions were injected subcutaneously in the region of the gastrocnemius muscle according to a cumulative dosing schedule [4,13]. At the beginning of an experimental session, the lowest dose of nicotine to be administered that day was injected. Increasing dosages of nicotine were injected during the 5 minute period preceding each subsequent cycle. No more than three injections were given during each experimental session. On other days, each individual nicotine dose was administered as a bolus injection to insure that the cumulative dosing paradigm was appropriate for the assessment of these doses of nicotine. Saline (0.9%) injections were used as a control.

The effects of nicotine also were examined under conditions that included intragastric infusion of the acidifying agent ammonium chloride (125 mg NH_4Cl/kg of body weight) or the alkalinizing agent sodium bicarbonate (160 mg $NaHCO_3$/kg of body weight). Pre-test data determined that these concentrations of the chemicals substantially altered urinary pH without causing any apparent illness. According to these data, the mean unmanipulated urinary pH of the test animals was 6.60. Intragastric infusion of 125 mg/kg ammonium chloride resulted in a mean urinary pH of 5.58. Intragastric infusion of 160 mg/kg sodium bicarbonate resulted in a mean urinary pH of 8.32. The infusion of nothing or of tap water served as control conditions. All infusions were in a volume of 10 ml/kg of body weight and were made approximately 30 min before the experimental session.

RESULTS

For both subjects control rates of responding under the FR schedule were higher than under the FI schedule (mean 1.50 versus 3.50 for FI and FR, respectively). These performances were stable over the entire time the study was conducted.

Figure 1 presents cumulative records of the typical performance of one animal. The top record represents performance under control conditions (i.e., no nicotine or gastric infusion). The FI schedule maintained lower overall rates of responding that were initiated following a brief pause early in the interval. Under the FR schedule, little or no pausing occurred and responding was maintained at a high rate until the 30 responses were completed and the timeout occurred.

Dose-effect functions under the different conditions are shown in Fig. 2 which presents the mean performance data (each point represents at least eight data points) for each subject under the FI and FR schedules when urinary pH was not manipulated. Under the FI schedule, most doses of nicotine (0.02–0.20 mg/kg) increased responding with the maximum increases occurring at 0.05 mg/kg of body weight; only the highest dose of nicotine (0.35 mg/kg) did not in-

crease responding. Responding under the FR schedule was increased slightly at the 0.02 and 0.05 mg/kg doses of nicotine but decreased under this schedule for doses in the range 0.10–0.35 mg/kg. Doses of nicotine that increased responding under the FI schedule (e.g., 0.1 mg/kg) decreased responding under the FR schedule.

The second record of Fig. 1 shows changes in responding during the cumulative dosing (0.05–0.20 mg/kg) of nicotine. The administration of 0.05 mg nicotine/kg resulted in marked increases in responding; larger doses increased responding less or resulted in a suppression of responding. Further, increasing doses of nicotine resulted in changes in the control patterns of responding (i.e., increased number and duration of pauses during responding and increases in number of shocks received). The effects of nicotine administered cumulatively were comparable to those found when the cumulative individual nicotine doses were given as single bolus injections (not shown).

Figure 3 presents mean responding (each point represents at least eight data points) for each subject under each schedule after administering the urinary acidifier (NH_4Cl) or alkalinizer ($NaHCO_3$). Administration of these agents markedly altered the effects of nicotine under both schedules, shifting the nicotine dose-effect curve in consistent directions. When nicotine was administered with the acidifier, the descending portion of the limb of the dose-effect curve was attenuated. Higher doses of nicotine co-administered with ammonium chloride produced larger increases (FI) or smaller decreases (FR) than when nicotine was administered alone. In contrast, when nicotine and the alkalinizing agent sodium bicarbonate were given together, the intermediate regions (0.05–0.20 mg/kg) of the nicotine dose-effect curves were shifted downwards, indicating that the rate-increasing effects of nicotine were attenuated and the rate-decreasing effects potentiated. At the highest dose of nicotine, 0.35 mg/kg, the otherwise parallel shifts in the acidification and alkalinization curves were typically reversed, with the co-administration of sodium bicarbonate attenuating the rate-decreasing effects of nicotine.

Records 3 and 4 of Fig. 1 present performance after administration of nicotine and either ammonium chloride or sodium bicarbonate. Compared to the effects of nicotine alone, ammonium chloride (third record) attenuated the rate-decreasing effects of nicotine, whereas sodium bicarbonate potentiated the rate-decreasing effects of nicotine. Thus, changes in the physiological state of the organism at the time of nicotine administration resulted in a strong and consistent change in the behavioral effects produced by nicotine.

DISCUSSION

Nicotine administration markedly increased responding under the FI schedule across a range of doses; responding under the FR was also increased, although to a lesser extent. Doses of nicotine that increased FI responding decreased responding under the FR schedule, indicating that FR performance was more sensitive to the rate decreasing effects of this drug. These results are generally similar to those reported previously [11]. Performance under both schedules and under a wide range of nicotine dosages was changed in a consistent manner by gastric administration of agents that alter urinary pH. Perhaps most pronounced were the findings that the gastric administration of an acidifying agent attenuated the rate-decreasing effects of nicotine, while gastric administration of an alkalinizing agent potentiated those effects. These findings support the argument that changes in cigarette smoking under conditions that alter urinary pH involve nicotine per se. Therefore, the present results may be used to support a psychopharmacological explanation of why smoking increases in particular situations [10]. However, the present results also suggest a new, alternative interpretation of Schachter's data and the urinary pH/smoking phenomenon.

The present study found that the administration of acidifying and alkalinizing agents was accompanied by changes in the effects of nicotine on behavior. Therefore, situations that alter urinary pH may affect smoking behavior because the acute effects of given doses of nicotine (as self-administered by the smoker) change. That is, smoking behavior may increase in situations that acidify the urine not because the excretion of unmetabolized nicotine increases (as Schachter argues), but rather because the acute behavioral effects of nicotine decrease from each puff on a cigarette for reasons separate from rate of excretion. Schachter's explanation assumes that the smoker must replenish "lost" nicotine. The present results suggest the alternative explanation that under the situations or conditions that alter urinary pH, the effects of given doses of nicotine change. These changes may result from changes in receptor affinity for nicotine, body fluid nicotine distribution, responsivity to nicotine stimulation, or a host of other possibilities. Further investigations of these mechanisms may contribute substantially to an understanding of cigarette smoking and to the biological and behavioral basis of drug addiction.

ACKNOWLEDGEMENTS

We thank Andrew Baum and David S. Krantz for comments on the manuscript.

REFERENCES

1. Beckett, A. H., M. Rowland and E. G. Triggs. Significance of smoking investigations of urinary excretion rates of amines in man. *Nature* **207:** 200–201, 1965.
2. Beckett, A. H. and E. G. Triggs. Enzyme induction in man caused by smoking. *Nature* **216:** 587, 1967.
3. Feyerabend, C. and M. A. H. Russell. Effect of urinary pH and nicotine excretion rate on plasma nicotine during cigarette smoking and chewing nicotine gum. *Br J Clin Pharmacol* **5:** 293–297, 1978.
4. Hanson, H. M., J. J. Witoslawski, E. H. Campbell and A. G. Itkin. Estimation of relative antiavoidance activity of depressant drugs in squirrel monkeys. *Archs Int Pharmacodyn Ther* **161:** 7–16, 1966.
5. Hake, D. F. and N. Azrin. An apparatus for delivering pain shock to monkeys. *J Exp Anal Behav* **6:** 297–298, 1963.
6. Latiff, A. A., L. A. Smith and W. J. Lang. Effects of changing dosage and urinary pH in rats self-administering nicotine on a food delivery schedule. *Pharmacol Biochem Behav* **13:** 209–213, 1980.
7. Matsukura, S., N. Sakamoto, K. Takahashi, H. Matsuyama and H. Muranaka. Effect of pH and urine flow on urinary nicotine excretion after smoking cigarettes. *Clin Pharmacol Ther* **25:** 549–554, 1979.
8. Russell, M. A. H. and C. Feyerabend. Cigarette smoking: A dependence on high-nicotine boli. *Drug Metab Rev* **8:** 29–57, 1978.

9. Schachter, S. Pharmacological and psychological determinants of smoking. *Ann Intern Med* **88:** 104–114, 1978.
10. Schachter, S., B. Silverstein, L. T. Kozlowski, D. Perlick, C. P. Herman and B. Liebling. Studies of the interaction of psychological and pharmacological determinants of smoking. *J Exp Psychol (Gen)* **106:** 3–40, 1977.
11. Spealman, R. D., S. R. Goldberg and M. L. Gardner. Behavioral effects of nicotine: Schedule-controlled responding by squirrel monkeys. *J Pharmacol Exp Ther* **216:** 484–491, 1981.
12. Taylor, D. H. and P. N. Blezard. The effects of smoking and urinary pH on a detection task. *Q J Exp Psychol* **31:** 635–640, 1979.
13. Wenger, G. R. Cumulative dose-response curves in behavioral pharmacology. *Pharmacol Biochem Behav* **13:** 647–651, 1980.