Pharmacology Biochemistry & Behavior, Vol. 13, pp. 209–213. Printed in the U.S.A.

Effects of Changing Dosage and Urinary pH in Rats Self-Administering Nicotine on a Food Delivery Schedule

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Received 30 October 1979

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.* PHARMAC. BIOCHEM. BEHAV. 13(2) 209–213, 1980.—The effects of different available dosage and of acidic and alkaline urinary pH have been investigated on the rates of self-administration of nicotine by rats on an FT60 food delivery schedule. Different groups of rats initially received one of 3 doses of nicotine (0.05, 0.1 and 0.25 mg/kg/infusion) contingent upon bar-pressing. The self-administration rates during an initial 6-day period of the 3 groups of rats were significantly different from each other, 26.1 ± 3.2 (SEM), 15.4 ± 1.5 and 9.5 ± 0.9 at doses of 0.05, 0.1 and 0.25 mg/kg/infusion, respectively. However, once the rates of responding were established during the initial period, no significant changes occurred when the doses were changed in all 3 groups after each subsequent 6-day period. These rates of self-administration decreased when saline replaced the available nicotine solution after Day 18. The urinary pH of groups of rats was maintained alkaline (pH 9.0), acidic (pH 5.9) or normal (pH 6.7) by allowing them to drink sodium bicarbonate solution, ammonium chloride solution or water, respectively. The self-administration rates during the initial periods of these 3 groups of rats were also significantly different from each other (4.7 ± 0.66, 17.0 ± 0.76 and 9.4 ± 1.11, respectively). In contrast, however, when the rates of responding were established at normal urinary pH during the initial period when water was available, no significant changes occurred when urinary pH was subsequently changed in either an acidic or alkaline direction. The results suggest that the bar-pressing rates are dependent on the amount of nicotine available or present in plasma during the acquisition phase. Nevertheless, once the rate of bar-pressing is established on a food delivery schedule, it seems that the schedule exerts too powerful an effect on behavior for subsequent changes in nicotine levels to modify responding over the period of these experiments.

Nicotine Self-administration Change in dosage Urinary pH Food delivery schedule

IT has been shown that rats at reduced body weight and on a food delivery schedule self-administer more nicotine than do rats at either reduced or normal body weight without the schedule [6]. The higher rate of responding and the higher intake of nicotine can be considered to be induced by the environmental conditions provided by the schedule. However, other factors including nutritional and pharmacological ones could also affect responding. Singer, Simpson and Lang [11] studied the influence of body weight on nicotine intake by rats self-injecting the drug while on a food delivery schedule. It was concluded that once nicotine intake behavior is established it can be maintained despite changing nutritional factors. The influence of pharmacological factors of dosage and changing urinary pH have been investigated in this study.

The present experiments were designed to investigate the influence of changing dosage in two ways. In Experiment 1A, different groups of rats were allowed to self-inject nicotine at three different dose levels. In Experiment 1B, within each of the three groups of rats, the dose of available nicotine was subsequently changed so that each group self-injected at all three dose levels. The nicotine solution was then replaced with normal saline for self-administration in a final period. Another series of experiments was carried out to study the effect of manipulating urinary pH. The rate of excretion of nicotine is reported to be influenced by the urinary pH [3], less being excreted in alkaline urine and more in an acidic one. In Experiment 2A, different groups of rats self-injecting nicotine were given solutions of sodium bicarbonate or ammonium chloride, or tap water to drink. Their rates of bar-pressing for nicotine were compared. Finally, in Experiment 2B, rats drinking tap water and with normal urinary pH were allowed to bar-press for nicotine injections for 10 days and were then randomly divided into three groups. One group was allowed to drink a solution of sodium bicarbonate, another group drank ammonium chloride solution and the third group continued to drink tap water; each group self-injected nicotine on a food delivery schedule.

METHOD

Animals

Lister hooded rats weighing 250–350 g were used. They were housed in individual cages in a room with a constant 12 hours light-12 hours dark cycle and a temperature of 23 ± 1°C. Rats were reduced to 80% of their free-feeding body weight by keeping them on a diet until the desired

weight was achieved; they were then maintained at this weight throughout the duration of the experiment. The animals were on ad lib water but had no prior exposure to drugs.

Apparatus and Administration of Drugs

Rats were reduced to 80% of their body weight prior to surgery during which a cannula of SP28 polyethylene tubing (Dural Plastics and Engineering) was inserted into their jugular vein, passed underneath the skin and exteriorized approximately midway between the ears. Each rat was fitted with a leather harness which supported the implanted venous cannula connected to a swivel system. The swivel system, designed by Weeks (1977) allowed relatively unrestricted movement. A recovery period of 2–3 days was allowed before the rats underwent experimental sessions in the Skinner boxes (R.S. Hales Equipment) for 1 hour each day. A lever projected into the box from one end which, when pressed, triggered a 5 sec infusion of 83 μl of the drug by a Sage® pump. A National® SC/MP microprocessor controlled all parameters. These included (1) activation of the Sage® infusion pump when the lever was pressed, (2) recording the number of infusions and lever presses and (3) dispensing the food pellet every minute over the 1 hour experimental sessions each day.

Drugs

The anaesthetic used for surgical operations was a mixture of methohexitone (17.6 mg/ml, Brietal, Eli Lilly and Co.) and amylobarbitone (3 mg/ml, Sodium Amytal, Eli Lilly and Co.) given intraperitoneally in a dose of 1.7 ml/kg. Drug solutions were prepared by dissolving nicotine hydrogen (+) tartrate (British Drug Houses Ltd.) in sterile normal saline solution (0.9% NaCl). The sodium bicarbonate (Ajax Chemicals) with sucrose (Ajax Chemicals) solution was made up as 0.15 mol/dm^3 $NaHCO_3$ in 5% sucrose solution and the ammonium chloride (Ajax Chemicals) with sucrose solution was made up as 0.30 mol/dm^3 NH_4Cl in 5% sucrose solution. In both cases, the solvent was tap water.

EXPERIMENT 1: EFFECT OF CHANGING THE DOSE OF NICOTINE SELF-ADMINISTRATION BY RATS ON FT60 FOOD SCHEDULE

In these experiments, the effect of changing the dose of nicotine was studied in two ways. In 1A different groups of rats initially had different doses of nicotine available for self-injection; their rates of self-administration were compared. After initial rates of responding to a particular dose were established by each group of rats, the strengths of the available drug solutions were changed twice so that each group of rats was able to self-inject the different doses of nicotine. In Experiment 1B these rates of responding were again compared over subsequent periods of days.

Procedure

Rats were randomly assigned to three groups: group 1 (8 rats), group 2 (6 rats) and group 3 (6 rats) in Experiment 1. Three different doses (0.05, 0.1 and 0.25 mg/kg) were available for self-injection. At the commencement of the experiment one of the 3 doses of nicotine was available to each group for the initial period of 6 days. After each 6-day period, the concentration of nicotine was randomly changed for each group and was then available for another 6 days, so that after 18 days each group had access to the 3 different strengths of nicotine solution; finally, normal saline replaced the drug for a period of 6 days.

TABLE 1

MEAN INFUSION RATES/HR FOR RATS SELF-INJECTING DIFFERENT DOSES OF NICOTINE OR NORMAL SALINE

Group no.	Means ± SEM over each 6-day period			
	Days 1–6	7–12	13–18	19–24
1 n=8	26.1 ± 3.23 *(0.05)	28.3 ± 3.18 (0.25)	24.3 ± 2.70 (0.10)	5.42 ± 0.55 (Saline)
2 n 6	15.4 ± 1.45 (0.10)	15.9 ± 1.78 (0.05)	11.4 ± 1.10 (0.25)	7.1 ± 1.78 (Saline)
3 n=6	9.5 ± 0.89 (0.25)	13.3 ± 1.22 (0.05)	8.9 ± 0.81 (0.10)	4.4 ± 0.44 (Saline)

*Numbers in brackets indicate doses of nicotine in mg/kg/infusion.

RESULTS

Table 1 shows the means of daily infusion rates over 6-day periods of 3 groups of rats. The mean infusion rates during the acquisition period of the first 6 days were significantly different from each other, $p<0.05$, t test, 10 or 12 df); the lowest dose of 0.05 mg/kg/infusion (Group 1) corresponded with the greatest rate of responding of 26.1/hr (SEM=3.23, n=8), whereas the highest dose of 0.25 mg/kg/infusion (Group 3) corresponded with the lowest rate of responding of 9.46/hr (SEM=0.90, n=6). Within each group, a change in the dosage available in the drug solution did not produce a significant change in the rate of bar-pressing compared to that of the initial 6-day period. However, between Days 19–24, when the saline was substituted for solutions of nicotine, the rate of infusions per hour for all 3 groups fell significantly ($p<0.01$, t test, 10 or 14 df), suggesting that there was a pharmacological effect of nicotine. Results are presented diagrammatically in Fig. 1.

In summary, rats establish different rates of self-administration of nicotine if different doses are available initially. However, once an initial rate is established, changing the strength of the available nicotine does not modify the rate of bar-pressing. When saline replaced nicotine in the final period between Days 19–24, no matter what dose of nicotine the rats were self-injecting, the final rate of infusion of saline was significantly lower than that of nicotine during Days 1–18.

EXPERIMENT 2: THE EFFECT OF CHANGING URINARY pH ON THE SELF-ADMINISTRATION OF NICOTINE BY RATS ON AN FT60 SCHEDULE

The effect of an alkaline or an acidic urinary pH on self-administering behavior was studied in two ways. In Experiment 2A, the urinary pH of groups of rats was altered prior to the acquisition of responding and their rates of intake were compared. In Experiment 2B, after responding to nicotine was established for 10 days in another group of rats, their normal urinary pH was altered and their rates of responding studied for a further 10-day period.

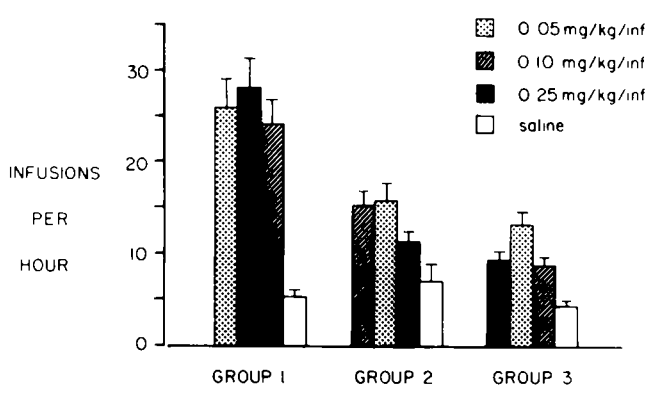

FIG. 1. The figure shows results obtained in Experiment 1. Histograms represent mean infusions per hour (m ± SEM) of different doses of nicotine (0.5, 0.1 and 0.25 mg/kg) and normal saline injected by three groups of rats over successive 6-day periods during the experiment.

Procedure

Nicotine at a dose of 0.1 mg/kg/infusion was available, contingent upon bar-pressing throughout the experiment to rats randomly assigned to three groups, A, B and C. Previous to and during test sessions, rats in Group A (n=6) had sodium bicarbonate and sucrose in their drinking water while those in Group B (n=7) drank a solution of ammonium chloride and sucrose. The rats in Group C were used in test sessions for 20 days, during the first 10 days of which tap water was available for drinking. The rats were then randomly divided into subgroups C_1 (7 rats), C_2 (6 rats) and C_3 (6 rats). Rats in Group C_1 continued to drink tap water, but for those in the other two groups the drinking solutions were changed. The water was replaced by a solution of sodium bicarbonate and sucrose in Group C_2, and by a solution of ammonium chloride and sucrose in Group C_3. The pH of the urine secreted while the rats were in the Skinner boxes was measured throughout the experiment.

RESULTS

Alteration of Urinary pH Prior to the Acquisition of Responding

The rats that initiated responding whilst drinking sodium bicarbonate solution (Group A) self-injected nicotine at a mean daily rate of 4.68 per hour (SEM=0.47, n=6) and had a urinary pH of 9.01 (SEM=0.08, n=6), whilst those drinking ammonium chloride solution (Group B) self-injected nicotine at a mean rate of 17.00/hr (SEM=1.42, n=7) and had a mean urinary pH of 5.90 (SEM=0.03, n=7). Rats drinking tap water (Group C) self-injected at a mean rate of 9.40/hr (SEM=1.11, n=19) over the 10-day period and had a mean urinary pH of 6.74 (SEM=0.04, n=19) (Fig. 2). Statistical analysis of results showed a significant difference between all three groups. Compared with control rats in Group C, Group A rats had a significantly lower rate of infusion ($p<0.05$, t test, 23 df) and Group B had a significantly higher rate of infusion ($p<0.01$, t test, 24 df).

Alteration of Urinary pH After Self-Administration of Nicotine for 10 Days

As reported above, rats in Group C drinking tap water had a mean infusion rate of 9.40/hr (SEM=1.11, n=19) over the first 10 days and their mean urinary pH was 6.74 (SEM=0.04, n=19). Rats in sub-group C_1 which continued to drink tap water over the next 10 days had a mean infusion rate of 14.87/hr (SEM=3.33, n=7) with a urinary pH of 6.71 (SEM=0.07, n=7). Rats in sub-group C_2 continued to self-inject nicotine at a mean rate of 10.32/hr (SEM=1.89, n=6) during the next 10 days when drinking sodium bicarbonate solution; their mean urinary pH was 9.05 (SEM=0.08, n=6) (Fig. 3). Rats in sub-group C_3 self-injected nicotine at a mean rate of 7.82/hr (SEM=1.06, n=6) during the second 10-day period when drinking ammonium chloride solution; the mean of their urinary pH was 5.46 (SEM=0.13, n=6) (Fig. 3). Although the values of the urinary pH of rats in sub-groups C_2 and C_3 were different from each other and that of C_1, there was no statistically significant difference in the rates of self-administration of nicotine.

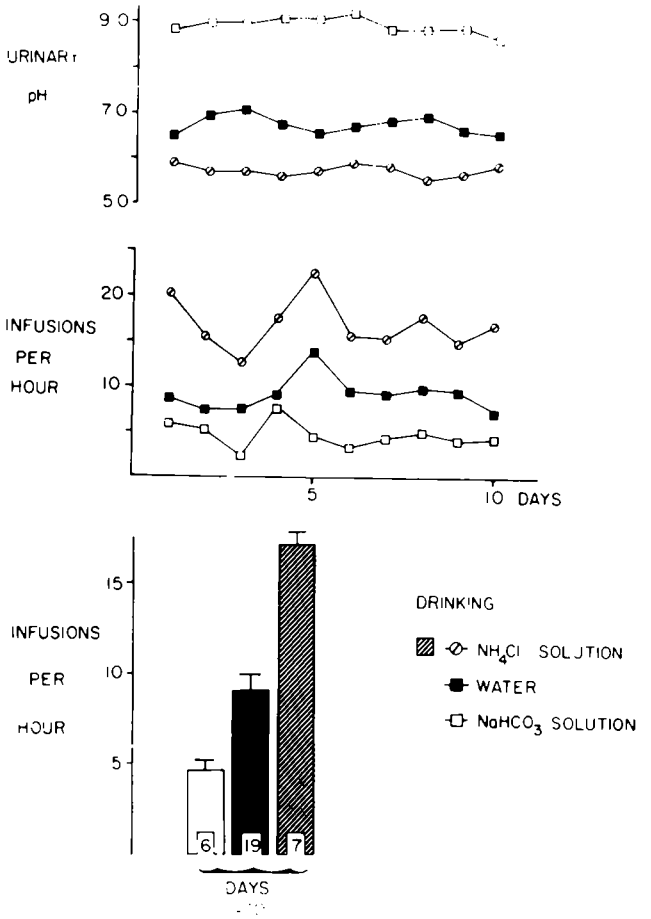

FIG. 2. The figure shows results obtained in Experiment 2A. The upper panel shows means of urinary pH and the middle panel shows mean infusions per hour of nicotine (0.1 mg/kg) by three groups of rats. The bottom panel shows histograms indicating means of daily infusion rates of 0.1 mg/kg of nicotine (m ± SEM) self-injected over the 10-day period by each group of rats. Symbol (○) and open histogram (□) represent Group A rats drinking a solution of sodium bicarbonate. Symbol (⊘) and hatched histogram (▨) represent Group B rats drinking a solution of ammonium chloride. Symbol (●) and closed histograms (■) represent Group C rats drinking tap water.

In summary, rats were found to self-inject nicotine on a

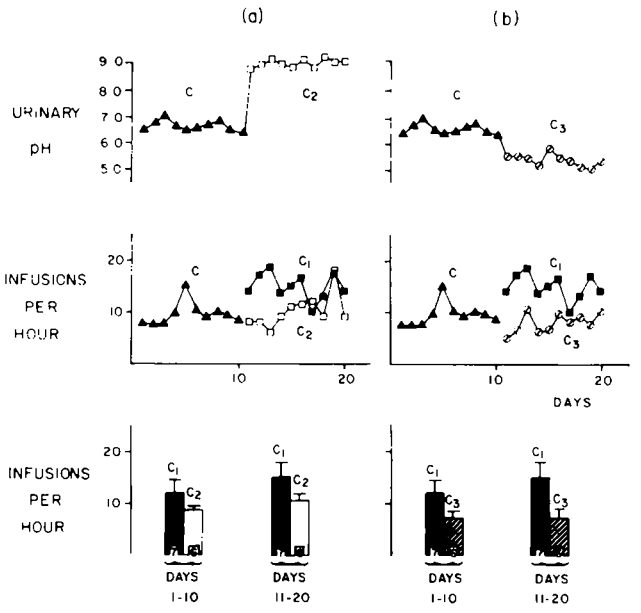

FIG. 3. The figure shows results obtained in Experiment 2B. The upper panel shows means of urinary pH and the middle panel shows mean infusions per hour of nicotine (0.1 mg/kg) by rats in Group C (subdivided into C_1, C_2 and C_3). The bottom panel shows histograms indicating means of daily infusion rates of nicotine (m ± SEM) self-injected over 10-day periods. Symbol (▲) represents Group C rats drinking tap water during the first 10-day period. Symbol (■) and closed histograms (■) represent sub-group C_1 rats which continued to drink tap water during the second 10-day period. Symbol (⁀) and open histogram (□) represent sub-group C_2 rats drinking a solution of sodium bicarbonate during the second 10-day period. Symbol (⊘) and hatched histogram (▨) represent sub-group C_3 rats drinking a solution of ammonium chloride during the second 10-day period.

food delivery schedule at a lower rate when their urine was alkaline and at a higher rate when their urine was acidic than did rats at normal urinary pH. However, once an initial rate of self-administration was established at a normal urinary pH in rats drinking tap water, there was no apparent difference in self-injection rates when their urinary pH was subsequently manipulated.

DISCUSSION

The reasons behind the habit of tobacco smoking are generally believed to involve both psychological and pharmacological factors, and the habit is thought of in terms of drug dependency. Self-administration of a wide variety of substances by animals has been extensively used as a laboratory model to study some of the factors responsible for the phenomenon of drug dependency. However, until the study by Lang *et al.* [6], it had not been convincingly shown that significant amounts of nicotine will be self-administered by rats. In that study, rats at reduced body weight and on a food delivery schedule were found to self-inject significant quantities of the drug. Furthermore, it has been shown in this study that the quantities self-administered can be modified by pharmacological factors.

It was concluded by Schachter [8] that many if not most smokers adjust their smoking rate to keep nicotine at a roughly constant level. It was also claimed that smokers regulate their nicotine intake in such a way that if cigarettes with varied nicotine content were available, so the number smoked varied [1, 2, 4]. There is nevertheless some debate on the role of nicotine levels in modulating the rate of smoking. In one study it was found that doses of nicotine given intravenously failed to affect the ongoing smoking of human subjects even though inhaled amounts of tobacco smoke did reduce smoking [5]. In contrast, other findings demonstrated small, but significant reductions in the numbers of cigarettes smoked following intravenous infusions of nicotine [7].

Our findings show that while under the influence of a food delivery schedule, rats will self-inject nicotine at a rate dependent on the dose available, and that the rates are inversely related to these doses during the initial phase of acquisition. However, once the rate of self-administration of a given dose of nicotine is established in rats subject to the schedule, subsequent alterations of the available dosage did not modify the rate of self-injection. This suggests that while the pharmacological actions of nicotine are important in the behavior, the schedule itself exerts a very strong influence. Nevertheless, the lever-pressing behavior was not maintained when normal saline was substituted for the nicotine injections.

Alteration of urinary pH is known to modify the excretion rate of nicotine [3] and hence the plasma level attained by a given dose of the drug. Changing the pH of urine has been found to affect tobacco smoking behavior. Thus, the rate of smoking in human subjects is decreased after administration of sodium bicarbonate, and increased after administration of ascorbic acid [9,10].

Similarly, in our experiments, rats under the influence of the food delivery schedule self-injected nicotine at a decreased rate when urinary pH was alkaline while they were drinking sodium bicarbonate solution, and at an increased rate when urinary pH was acidic due to their drinking ammonium chloride, compared to normal. This effect was only apparent during the initial acquisition phase, however, and altering the urinary pH by changing the drinking solution did not subsequently change the rate of self-injection. Again this indicates that the schedule does exert a powerful influence but that the intake of nicotine can be modified pharmacologically. No information is available in these experiments on plasma levels of nicotine attained by the rats, but on the basis of the findings of Beckett's group, it seems reasonable to assume that with the given doses of nicotine, higher plasma levels would have been reached when the urine was alkaline and lower levels when the urine was acidic.

The experimental findings suggest that the changes in self-administration behavior by rats taking nicotine bear some similarities to the ways in which man modified his tobacco smoking. Further support is provided therefore that this experimental approach is a useful laboratory model to study phenomena associated with the habit in man.

ACKNOWLEDGEMENT

This work was supported by the Australian Tobacco Research Foundation.

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