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RAPID COMMUNICATION

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Plasma nicotine and cotinine levels following intravenous nicotine self-administration in rats

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Abstract *Rationale*: The route of nicotine administration between animal models and humans is very different and further investigation by determining levels of nicotine entering into the circulatory system is warranted. *Objective*: The present study addresses the validity of the rat self-administration procedure by comparing plasma levels of nicotine in the rat with levels reported in smokers following cigarette consumption. *Methods*: Plasma levels of nicotine and its metabolite cotinine were measured in 17 rats following intravenous self-administration of a range of nicotine doses (0.015, 0.03 and 0.06 mg/kg per infusion). *Results*: The two larger unit doses supported reliable self-administration behaviour with no overall difference in the patterns of nicotine intake. However, the total nicotine intake over the 2-h session was related to unit dose and this correlated highly with nicotine and cotinine levels measured in blood collected from the tail vein. On average, cotinine levels (50–200 ng/ml) were approximately 2-fold higher than nicotine levels (40–120 ng/ml) in plasma. Following an extinction test for one session in which saline was substituted for nicotine, no change in behaviour was observed in the two groups, while plasma levels of nicotine and cotinine dropped to nominal levels. *Conclusions*: The concentrations of nicotine attained following nicotine self-administration appear to be similar to levels reported in smokers after cigarette consumption, providing further validation of this procedure as an animal model of nicotine dependence.

Key words Nicotine · Cotinine · Self-administration · Rat

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Introduction

The intravenous nicotine self-administration paradigm is routinely proposed as the animal model *par excellence* for studying tobacco dependence. Based on the assumption that nicotine is the primary constituent that maintains tobacco smoking, comparisons have been made with smoking behaviour in humans (Rose and Corrigall 1997). For instance, nicotine can support self-administration across various species ranging from rodents to primates, and the doses efficacious in maintaining nicotine self-administration behaviour are very similar (Shoaib 1997). However, the route of administration between the animal model and humans is very different and further investigation by determining levels of nicotine entering into the circulatory system is warranted.

Another feature worthy of consideration is the regulation of nicotine intake. Results from rat self-administration studies reveal that the pattern of nicotine intake differs from that typically observed for other drugs, such as cocaine or heroin. For example, when the unit dose of cocaine is changed, the compensation in behaviour is directly related to the dose reflecting maintenance of a constant level of cocaine (Koob and Goeders 1989). In contrast, changes in the unit dose of nicotine produce an atypical "shallow" dose-response curve on acquisition and maintenance (Corrigall and Coen 1989; Donny et al. 1998), suggesting that animals may be weakly compensating for the change in dose. Smokers also show regulation of nicotine intake, but only partial compensation is observed when cigarettes of varying nicotine content are presented (Ashton et al. 1979; Russell et al. 1982; West et al. 1984).

The present study addresses the validity of the rat self-administration procedure by comparing plasma levels of nicotine in the rat with levels reported in smokers following cigarette consumption (Benowitz et al. 1983). Using a self-administration procedure in rats described previously by Shoaib et al. (1997), blood samples were collected immediately after periods of nicotine self-administration and were assayed for levels of nicotine and its metabolite cotinine.

Materials and methods

Subjects

Male hooded Lister rats (Olac, Bicester) with initial weights between 250 and 300 g were utilised. Once prepared with an intravenous catheter, rats received their daily diet (20–24 g) approximately 1–2 h following the end of the self-administration session. They were individually housed with free access to water, in rooms maintained at 20–22°C with a regular light-dark cycle (light from 0700 to 1900 hours). The studies complied with all local and national ethical requirements and were carried out in accordance with the Animals (Experimental Procedures) Act, 1986 under licence from the UK Home Office.

Surgery

Under anaesthesia (a mixture of medetomidine 0.3 mg/kg and ketamine 70 mg/kg, IP), all rats were implanted with a chronic Silastic catheter into the external jugular vein as described previously by Shoaib et al. (1997). The catheter was connected to an L-shaped connector (Plastics-One, Roanoke, Va., USA) that was mounted with dental acrylic to stainless steel screws imbedded in the skull. Daily flushing with 0.9% physiological saline containing heparin (1.25 units/ml) and gentamicin (0.16 mg/kg) maintained the patency of the intravenous catheter. At least 1 week was allowed for recovery before the start of nicotine self-administration sessions.

Apparatus

Twelve standard operant chambers (Med-Associates, Vt., USA) were used that consisted of a Plexiglas enclosure with one house light, two levers, one tether and fluid swivel. One lever was defined as active and presses on it resulted in fluid infusions; presses on the other lever were recorded but had no programmed consequence. Catheters were connected to an infusion pump (Rasal, Med-Associates, Ind., USA). The operant chambers were controlled by a microcomputer using the Med-Associates (Lafayette, Ind., USA) MED-PC software package.

In 2-h limited access sessions, rats were given the opportunity to lever-press for intravenous infusions of nicotine (0.015, 0.03 or 0.06 mg/kg per infusion) as described previously (Shoaib et al. 1997). Increments to the fixed-ratio (FR) schedule were made with improved accuracy in active lever pressing over self-administration sessions. Once rats exhibited stable levels of nicotine self-administration on an FR5 schedule (less than 30% variability from the mean number of infusions self-administered over three sessions), blood was sampled from the tail vein. Approximately 200 µl of blood was collected from the tip of the tail and the blood was centrifuged for determination of plasma levels of nicotine and cotinine by gas chromatography using nitrogen phosphorous detection with detection limits of 100 pg/ml for nicotine and cotinine using 100 µl plasma (Feyerabend and Russell 1990). Irrespective of the training dose, rats typically required approximately 35 training sessions before satisfying the performance criterion. Several days later, blood was collected again from the tail vein, but this time following the substitution of saline for nicotine (extinction test). The extinction test was only conducted in rats that showed stable levels of responding over the preceding sessions (less than 30% variability over three sessions).

Drugs

Nicotine hydrogen-(+)-tartrate (BDH, Poole, Dorset, UK) was dissolved in isotonic saline. The pH was adjusted to 7 with dilute NaOH. The volume of each infusion was approximately 90 µl delivered over 1 s. Doses of drugs are expressed as those of the base.

Statistics

Correlations between pairs of variables were calculated by means of the Pearson product-moment correlation coefficient (*r*).

Results

The smallest unit dose of nicotine tested (0.015 mg/kg per infusion) failed to support nicotine self-administration in the majority of rats tested. Only two of the 12 rats showed reliable and consistent intake of nicotine. The two higher doses of nicotine were more efficacious in maintaining nicotine self-administration in a greater proportion of animals. Table 1 shows the means±SEM of nicotine and saline injections self-administered by three groups of rats along with the corresponding plasma levels of nicotine and cotinine. Out of the 12 rats trained in each group, nine rats on the 0.03 mg/kg per infusion and six rats on the 0.06 mg/kg per infusion met the criterion for stable responding. A mean of 63±9 responses was made on the active lever compared to 20±5 responses on the inactive for rats on the 0.03 mg/kg unit dose. Slightly fewer responses were recorded for the larger unit dose of 0.06 mg/kg nicotine in which a mean of 58±8 active lever-press responses and 6±3 inactive responses were made. Both of these groups self-administered approximately ten injections during the 2-h session. For the 0.03 mg/kg per infusion unit dose, an average of 60% of the total nicotine injections was earned in the first hour of the session. The average plasma nicotine and cotinine levels displayed a close relationship to the unit doses of nicotine self-administered by rats. Figure 1 contains two scatter diagrams to illustrate the robust correlation between the amounts of nicotine self-administered and the

Table 1 Plasma nicotine and cotinine levels following intravenous nicotine self-administration. Data are means±SEM, except for infusion dose of 0.015 mg/kg per infusion, where individual data are shown

Unit dose (mg/kg per infusion)	*n*	Self-administration	Plasma levels (ng/ml)		Self-administration	Plasma levels (ng/ml)	
		Nicotine injections	Nicotine	Cotinine	Saline injections	Nicotine	Cotinine
0.015	2	41	66.7	99.2	5.0	8.1	23.6
		3	22.5	29.3	1.0	1.9	missing data
0.03	9	10.3±1.1	65.4±6.4	88.6±0.2	7.0±5.4	7.8±1.6	19.8±5.3
0.06	6	10.3±1.4	102.3±16.0	156.3±18.1	13.5±4.0	7.3±1.0	28.0±9.1

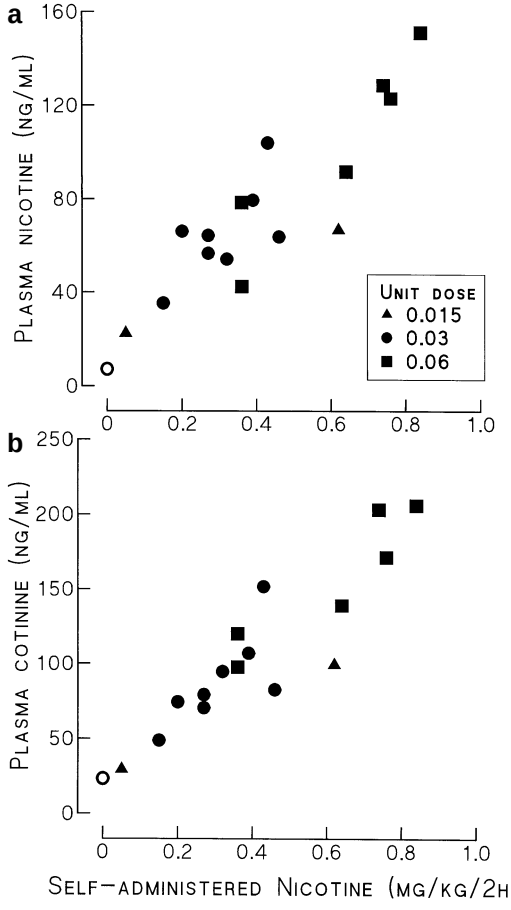

Fig. 1 **a** The scatter diagram illustrates the robust correlation between plasma levels of nicotine and the total amounts of nicotine self-administered by rats. **b** The scatter diagram illustrates the correlation between plasma levels of cotinine and the total amounts of nicotine self-administered by rats. Each point refers to an individual rat self-administering one of three unit doses of nicotine (▲ 0.015, ● 0.03 and ■ 0.06 mg/kg per infusion). *Open circles* (○) indicate mean results from the extinction tests

corresponding plasma levels of nicotine [$r(14)=0.958$, $P<0.001$] and cotinine [$r(14)=0.901$, $P<0.001$]. Exclusion of nicotine from the infusion solution for one session had little effect on behaviour, despite blood levels showing nominal levels of nicotine (Table 1). Plasma levels of cotinine were significantly above detection levels (0.34 ng/ml) in all three groups. No correlations were observed between plasma levels obtained following nicotine sessions and those after a saline extinction test [nicotine: $r(11)=0.316$, NS, cotinine: $r(11)=0.007$, NS].

Discussion

In the present study, two of the three unit doses of nicotine tested maintained intravenous self-administration behaviour in the rat. The range of nicotine doses for investigation was chosen because all three have been shown to maintain responding in rats (Shoaib et al. 1997). However, in the present study the lowest unit dose of nicotine (0.015 mg/kg per infusion) failed to support self-administration of nicotine, a finding consistent with those reported by Donny et al. (1998). The patterns of nicotine intake by rats are not well described, except for the "shallow" dose-response curves reported in the majority of these studies. In the present study, the absence of any compensation with different unit doses of nicotine and nicotine intake across the three groups supports the notion that rats weakly regulate nicotine intake. Further support comes from the robust correlation observed between the total amounts of nicotine self-administered intravenously and the plasma levels of nicotine and cotinine. There was no evidence of any "ceiling" effect on the plasma levels of nicotine. It is plausible that larger unit doses above 0.06 mg/kg per infusion may have been regulated better, but these doses are convulsive and produce marked rate-decreasing effects (Corrigall and Coen 1989).

Results from the extinction test were as expected: it led to very low levels being detected in plasma, indicating that most of the self-administered nicotine was metabolised by the beginning of the next session. In contrast, levels of the metabolite cotinine were significantly above detectable levels although they failed to correlate with nicotine plasma levels from the previous nicotine session. An interesting finding from the extinction test was the lack of any decrease on lever-press responding. However, it should be mentioned that significant changes in behaviour are usually observed following at least the third successive extinction test (Corrigall and Coen 1989; Shoaib et al. 1997).

The original aspect of this investigation is the measurement of plasma levels of nicotine following the voluntary consumption of nicotine. On average, rats self-administered approximately 0.6 mg/kg nicotine resulting in plasma levels accumulating to around 40–120 ng/ml. Typically, plasma levels in humans normally peak at around 15–40 ng/ml following cigarette smoking (Feyeraband et al. 1985; Russell et al. 1986) and the levels decline thereafter to around 10 ng/ml. However, nicotine levels can be much greater depending on how quickly the cigarette is smoked (Benowitz et al. 1983; Herning et al. 1983). Therefore, there may be some concordance in the nicotine levels found in plasma between subjects smoking a cigarette and laboratory animals self-administering nicotine.

Cotinine is the major metabolite of nicotine and plasma levels of cotinine gradually rise during unrestricted cigarette smoking (Benowitz et al. 1983). Typically, cotinine levels accumulate to levels 10 times that of nicotine (260–300 ng/ml) (Benowitz et al. 1983; Hatsukami et al. 1998). However, in the present study, cotinine levels were at best 2-fold higher than nicotine levels. In a recent experiment, chronic infusions of nicotine delivered via osmotic mini-pumps in rats have produced cotinine levels that are at least 5 times that of nicotine blood levels (Winders et al. 1998). The low ratio of cotinine to nicotine in the present experiments may relate to the limited daily access to nicotine as well as possible species

difference. Given that the role of cotinine in tobacco smoking is not clear, and taking into consideration that this metabolite has very little pharmacological activity (Goldberg et al. 1989; Takada et al. 1989), it may not be critical for validation purposes to observe a direct relationship in cotinine levels across the different species.

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