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Discriminative Stimulus Effects of Intravenous Nicotine in Squirrel Monkeys

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TAKADA, K., T. J. HAGEN, J. M. COOK, S. R. GOLDBERG AND J. L. KATZ. *Discriminative stimulus effects of intravenous nicotine in squirrel monkeys.* PHARMACOL BIOCHEM BEHAV **30**(1) 243–247, 1988.—Three squirrel monkeys were trained to emit one response after IV administration of nicotine (0.1 or 0.18 mg/kg depending on the subject) and a different response after IV administration of saline. Subjects emitted nicotine-appropriate responses with substitutions of higher doses, but only emitted saline-appropriate responses after substitutions of lower doses. Discrimination performance was then maintained at 0.1 mg/kg of nicotine in all subjects. Neither morphine nor cocaine substituted for the effects of nicotine in any subjects across a range of doses up to those that suppressed responding. Ethyl-β-carboline-3-carboxylate, an inverse agonist at the benzodiazepine receptor, substituted or partially substituted for nicotine in both subjects in which it was studied.

Nicotine Drug discrimination Morphine Cocaine Ethyl-β-carboline-3-carboxylate (β-CCE)

THE discriminative stimulus effects of nicotine have been studied extensively, and almost exclusively, in rodents (for recent reviews, see [16,20]). Other drugs that completely substitute for the discriminative effects of nicotine have been exclusively nicotinic-cholinergic agonists such as its optical isomer *d*-nicotine [12,15] and the nicotine analogs 3-pyridylmethylpyrollidine [4], cytisine and anabasine [14,19]. Cotinine, a major active metabolite of nicotine, did not substitute for the discriminative effects of nicotine [16].

Reinforcing and punishing effects of nicotine have been studied primarily in squirrel monkeys. Nicotine has been shown to function as a reinforcer or as a punisher depending upon the experimental conditions under which it is administered. For example, response-produced nicotine injections maintained responding comparable to that maintained by cocaine in squirrel monkeys under fixed-interval and second-order schedules (for recent review, see [7]). Other studies in squirrel monkeys have demonstrated that response-produced nicotine injections can punish responding [6] or that responding can be maintained by postponement of scheduled nicotine injections [18]. Similar effects have also been observed in humans, i.e., while the subjective effects of nicotine most resembled those of cocaine [9,10], nicotine could function as an aversive stimulus [8]. Since these intricate stimulus effects of nicotine have been demonstrated in squirrel monkeys and since robust reinforcing effects of nicotine have been demonstrated most reliably in this species [7], it seemed desirable to study the discriminative stimulus effects of nicotine in this species. Therefore, in the present study squirrel monkeys were trained to discriminate intravenous nicotine from saline, and several other compounds were tested for their capacity to substitute for the effects of nicotine. The intravenous route was studied since laboratory studies of the reinforcing effects of nicotine have been conducted with this route, both in man and in animals, and since the onset of the effects can be considered to be very close to that of inhalation, the most prevalent method of nicotine intake in man.

METHOD

Subjects

Three adult male squirrel monkeys (*Saimiri sciureus*), which had no experimental history, were used. They weighed 793 to 878 g when they had unrestricted access to food. Their weights were reduced to and subsequently maintained at 80% of the unrestricted-feeding weight by providing sufficient Teklad Monkey Diet (Teklad, Inc., Monmouth, IL) and Purina Monkey Chow (Ralston-Purina Co., St. Louis, MO) after experimental sessions. Water was available at all times in the home cage.

After the initial lever-press training (see below), subjects

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were surgically prepared with intravenous polyvinyl chloride catheters (inside diameter, 0.38 mm; outside diameter, 0.76 mm) under halothane anaesthesia. The catheters were inserted in the external jugular vein to the level of right atrium. When catheters were no longer patent, the alternate external jugular vein was catheterized and if necessary followed by right and left internal jugular and right and left femoral vein catheterization. The distal end of the catheter was guided subcutaneously to exit at the middle of the back of the subject and sealed with a stainless-steel obturator. The subjects wore polyethylene mesh jackets to protect the catheters at all times. Surgical procedures and catheterization techniques have been described by Herd *et al.* [11].

Apparatus

During experimental sessions, monkeys were seated in restraint chairs similar to those described by Barrett [2] which were enclosed in ventilated, sound-attenuating experimental chambers (Model AC-2, Industrial Acoustics Co., Bronx, NY). Two response levers, which could be operated by a downward force of 20 g or more, were mounted on the front wall of the chair, equidistant from a central food receptacle. When either of the levers was operated, an audible click was produced by a relay mounted behind the front wall of the chair. Also mounted behind the transparent front wall were a pellet dispenser (Model D-1, Ralph Gerbrands Co., Arlington, MA) which could deliver 190 mg food pellets (banana flavored, Bioserv Inc., Frenchtown, NJ), and three pairs of red, white and green (left to right) stimulus lights mounted at about eye level. White noise, provided by a speaker attached to the ceiling of the cubicle, was used to mask extraneous sounds during experimental sessions.

For the intravenous injections, the catheter was connected by teflon tubing to a three-way stopcock attached to a syringe containing physiological saline. The syringe could be driven by an injection pump (Sage Instruments, model 355) located on top of the cubicle. The connecting tubing could contain solutions up to 0.8 ml, and drug solutions or physiological saline not exceeding 0.5 ml were injected manually into the tubing through one end of the three-way stopcock before each trial within the experimental session. When a trial was started, the center white lamps were illuminated, and the pump was activated to inject the contents of the connecting tubing. Two ml of saline were flushed at a rate of 0.36 ml/sec; 20 sec after the activation of the pump, the white lights were turned off, and blue and amber lights were both illuminated coincident with the start of the trial. The control of experimental contingencies and data collection were accomplished by a PDP-8E computer; key-press responses were monitored by cumulative recorders (Model C-3, Ralph Gerbrands Co.).

Training Procedure

Subjects were first trained to press the response key by reinforcing with food pellets successive approximations of the response. Subsequently, each response produced food (fixed-ratio or FR 1 schedule). The FR value was gradually increased to five when the subject obtained 50 pellets within the 30-min daily sessions. During this preliminary lever-press training, one of the two levers was removed. The position of the lever was alternated at each FR value when the subject met the above criterion so that it experienced both positions of the lever. After the subjects met the criteria under FR 5 at both lever positions, they were surgically prepared with intravenous catheters. After at least a week for recovery from surgery, discrimination training of intravenous nicotine and saline injections was started.

During discrimination training, daily sessions generally consisted of two trials separated by a timeout. The interinjection interval was set at 30 min, so the duration of timeout varied according to the performance of the subject. Each trial was started with injection of either saline (saline trials) or nicotine (nicotine trials) at an initial training dose of 0.032 mg/kg. Twenty seconds after the injection, the blue and amber stimulus lights were illuminated and consecutive responses meeting the FR requirement on the appropriate lever produced food. Each food presentation was followed by a 5-sec timeout. Responses on the inappropriate lever reset the FR requirement on the appropriate lever. The FR value was increased from one to 20 over successive sessions; increases in the FR requirement were made when 20 pellets had been obtained within 20 min under each condition (nicotine or saline). Thus, each trial ended after 20 min had elapsed or 20 pellets had been obtained. The duration of a trial was decreased to 10 min and the training dose of nicotine was increased to 0.1 mg/kg, when subjects reached FR 20. The appropriate lever for nicotine or saline was arbitrarily selected for each subject; the nicotine lever was on the right of two subjects (S-965, S-974) and was on the left for the remaining subject (S-955).

The sequence of trials in a session was generally either saline-saline, saline-nicotine, or nicotine-nicotine; additional trials were occasionally conducted when the performance of the subject indicated that further training would be beneficial. A saline trial never followed a nicotine trial. Sessions were conducted five days per week. Training continued until each subject met the following discrimination criteria in three consecutive saline and nicotine trials (1) less than 40 responses were emitted before the first pellet delivery, and (2) at least 80% of the total responses were emitted on the appropriate lever.

Drug Testing Procedures

After the subjects met the above criteria, the dose-effects of l-nicotine tartrate were determined. Subsequently, various doses of morphine SO_4, cocaine HCl, and β-CCE HCl (ethyl β-carboline-3-carboxylate) were tested for their capacity to substitute for nicotine as a discriminative stimulus. Test sessions started with a saline trial, with effects of successive doses determined in subsequent trials. Doses were administered in a cumulative manner (cf. [3]) Trials lasted until 10 pellets were presented or 5 min had elapsed. Each trial was separated by a timeout of a length calculated to keep the interval between injections 15 min. Trials were conducted until response rates were markedly decreased or until more than 80% of responses were emitted on the nicotine-appropriate lever. In these test trials, 20 consecutive responses on either lever resulted in a food pellet; switching to the alternate lever reset the FR requirement. Test sessions were separated by at least one, and generally two, training sessions and were conducted only after the criteria above (discrimination criteria) were met during these training sessions. These sessions included at least one nicotine trial. Doses of each drug are expressed in terms of mg of the salt per kg of the body weight of the subject.

The effects of drugs were evaluated in terms of the percent of nicotine appropriate responses emitted (responses on the nicotine-appropriate lever divided by the total number of

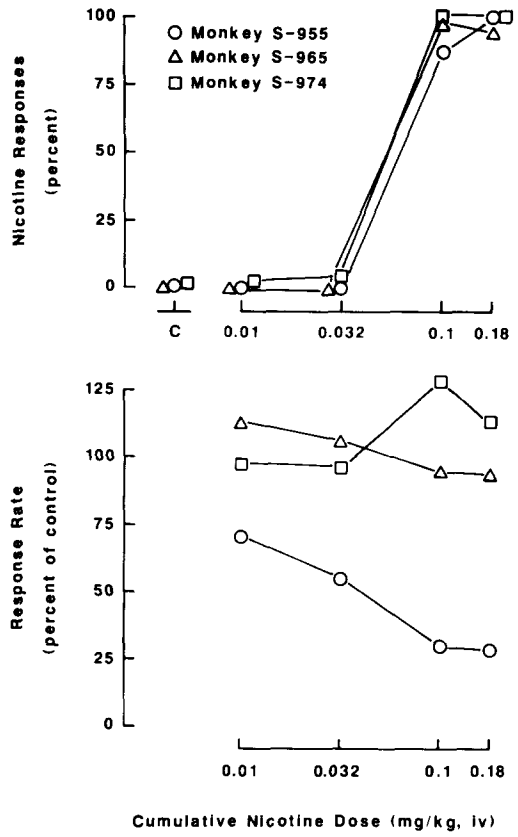

FIG. 1. Dose-effect relationships of intravenous nicotine for individual squirrel monkeys trained to discriminate nicotine at 0.1 (S-965 and S-974) or 0.18 (S-955) mg/kg from saline, IV. Abscissae: dose, log scale; symbols above "C" represent the values from the first saline trial in a test session (see text for detailed explanation). Ordinates: percent of the total number of responses on the nicotine-appropriate lever (upper panel); response rate as a percent of that during the first saline trial in a session (lower panel). Each symbol represents the values from individual subjects as shown in the figure.

responses emitted × 100) and in terms of the rate of responding. Response rates in each trial were expressed as a percentage of the rate during the first saline trial of the test session.

RESULTS

Although subjects readily responded under the FR 5 during the preliminary lever-press training, response rates decreased and subjects failed to meet criteria for increasing the FR requirement during discrimination training. The FR value was decreased for two subjects and then increased again over consecutive sessions. The number of sessions required to reach the FR 20 schedule were 13, 26 and 38 sessions respectively for subjects S-955, S-965, and S-974. To reach the discrimination criteria required 26 and 32 sessions for subjects S-965 and S-974, respectively. The remaining subject (S-955) did not show any sign of discriminative control by nicotine after 15 sessions of training; thus the training dose of nicotine was increased to 0.18 mg/kg. This subject then reached the criteria after 9 sessions. Response rates during the nicotine trials and the saline trials did not differ in subjects with the training dose of 0.1 mg/kg: the average response rates during the last three nicotine trials before they reached the criteria were 2.8 and 2.2 responses per second; rates during the last three saline trials were 2.9 and 2.4 responses per second, for subjects S-965 and S-974 respectively. For subject S-955, the response rates during nicotine trials at the training dose of 0.18 mg/kg were considerably lower than those during saline trials: the average rates were 0.6 and 1.4 responses per second for the last three nicotine and saline trials, respectively.

The dose-effects of nicotine for each subject are shown in Fig. 1. As can be seen from the figure, there was no discernible difference among subjects in terms of the discriminative effects: percent of nicotine-appropriate responses were virtually zero after saline or nicotine 0.01 and 0.032 mg/kg injections, and were in the range of 88–100% after 0.1 mg/kg of nicotine. Response rates were not affected across doses in two subjects, however, the rates of one monkey (S-955), whose training dose was higher than other two subjects, were somewhat lower than other two subjects at the dose of 0.01 mg/kg and decreased in a dose-dependent manner. The training dose for S-955 was decreased and maintained at 0.1 mg/kg after the determination of nicotine dose-effects.

Both morphine and cocaine were studied over a range of doses up to those that decreased response rates. Neither of these drugs produced appreciable responding on the nicotine-appropriate key (Fig. 2). β-CCE was studied in two of the three subjects. In one subject, S-965, the highest percentage of nicotine-appropriate responding was 50% at 0.32 mg/kg; since rates of responding were markedly decreased at this dose, higher doses were not studied. In the second subject, rates of responding were not decreased to the same degree by β-CCE and 0.56 mg/kg produced nicotine-appropriate responding.

DISCUSSION

Discriminative control by intravenous nicotine was established in all three subjects. Although the training dose for one subject differed from the others, the dose-effect curves for the discriminative effects of nicotine were identical among the three subjects. It is possible that if a larger number of sessions was conducted, discriminative control by nicotine in S-955 may have developed without increasing the training dose of nicotine. Indeed, when the training dose of nicotine was later decreased to 0.1 mg/kg in this monkey, discriminative control was maintained.

Results of several previous studies suggested that cocaine, morphine and nicotine may have similar discriminative effects. For example, intravenous administration of nicotine was identified as cocaine in human subjects [10]. Additionally, nicotine, like cocaine and morphine, produced increases in the MBG scale of the Addiction Research Center Inventory of subjective effects of drugs [10]. In rhesus monkeys, morphine produced 80 to 100% drug-appropriate responding in subjects trained with intravenous cocaine as a discriminative stimulus [1]. In rats, cocaine produced approximately 50% drug-appropriate responding in subjects trained with nicotine as a discriminative stimulus [19]. Thus, one might have predicted that cocaine, morphine and nicotine would have similar discriminative effects. However, neither cocaine nor morphine had discriminative effects similar to those of nicotine in the present study. Since the results of studies like the present one can depend on the dose of the drug used to establish the discriminative responding (e.g., [19]), it is possible that cocaine or morphine might produce

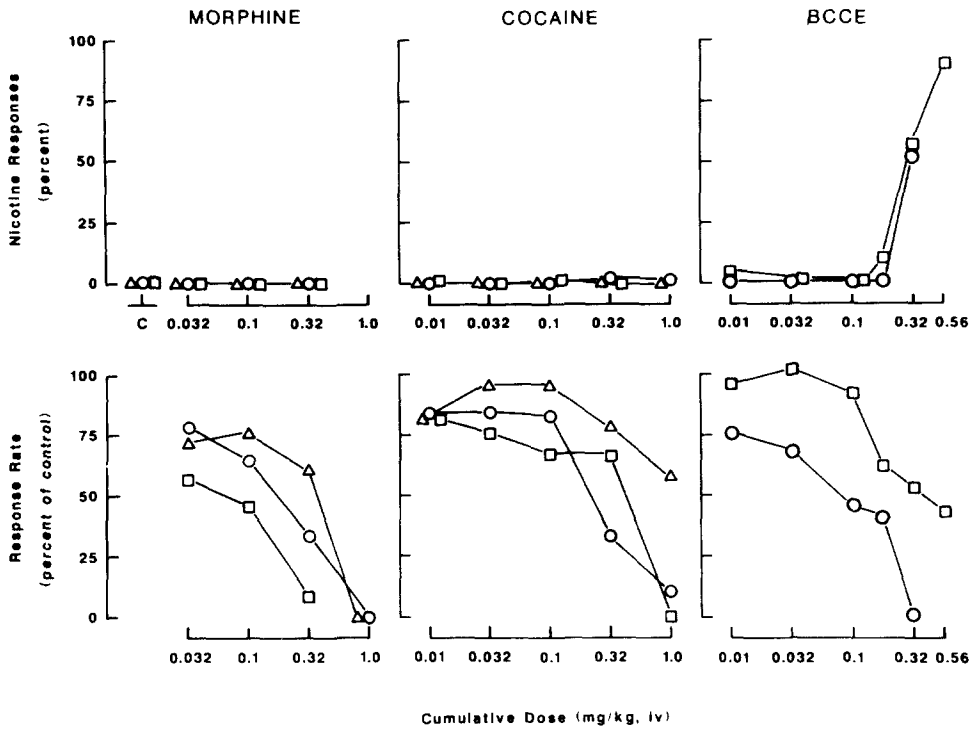

FIG. 2. Effects of various intravenous doses of morphine (left panels), cocaine (middle panels), and ethyl-β-carboline-3-carboxylate (β-CCE; right panels) in squirrel monkeys discriminating nicotine 0.1 mg/kg, IV from saline. Each symbol represents values for an individual monkey. For other details of the figure, see the legend for Fig. 1.

discriminative effects like those of nicotine at a lower nicotine training dose.

In contrast to cocaine and morphine, β-CCE, a benzodiazepine inverse agonist (e.g., [13]), showed some evidence of discriminative effects similar to those of nicotine. Those effects suggest that the discriminative effects are mediated centrally, since β-CCE binds only to benzodiazepine receptors in the brain [4]. Additionally, studies to date also suggest that the stimulus effects of nicotine are mediated centrally. For example, the discriminative effects in rats were antagonized by mecamylamine but not by low to intermediate doses of the peripherally acting antagonist hexamethonium [16,19].

β-CCE has been reported to function as a negative reinforcer in rhesus monkeys [21], and to function as a punisher in squirrel monkeys [22]. Nicotine also has been shown to function as a negative reinforcer [18] as well as a punisher [6]. Cocaine has been reported to maintain responses which postpone its availability [17], but did not function as a punisher in squirrel monkeys [22]. Thus, although nicotine and cocaine share some effects in common with regard to their ability to control behavior, they function differently under some circumstances, and further, it was shown in the present study that they did not share discriminative effects. Therefore it seems that the discriminative stimulus effects of cocaine and nicotine are qualitatively different in squirrel monkeys. The results also suggest that the discriminative effects of β-CCE, under the present conditions, may be related to the punishing or aversive effects of nicotine.

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