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Generalization of Norcocaine to the Discriminative Stimulus Properties of Cocaine

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MCKENNA, M. L., B. T. HO AND L. F. ENGLERT. *Generalization of norcocaine to the discriminative stimulus properties of cocaine.* PHARMAC. BIOCHEM. BEHAV. 10(2) 273–276, 1979.—In rats trained to discriminate 10 mg/kg cocaine from 1 ml/kg saline, norcocaine, the N-demethylated metabolite, at doses of 2.5 mg/kg, 5 mg/kg and 10 mg/kg, produced a dose response curve similar to that of cocaine and generalized to cocaine at the two higher doses. As with cocaine, the discriminative stimulus produced by the norcocaine was partially attenuated by the dopaminergic antagonist pimozide and the amine depletor reserpine. Benzoylecgonine, benzoylnorecgonine and ecgonine methyl ester in doses of 10 mg/kg and 20 mg/kg did not generalize to cocaine.

Cocaine Norcocaine Benzoylecgonine Benzoylnorecgonine Ecgonine methyl ester Pimozide Reserpine Discriminative stimulus

FOLLOWING systemic administration of cocaine, various human and rodent tissues have been analyzed for the presence of cocaine and its metabolites. Hydrolysis of cocaine by blood and liver esterases produces the cocaine metabolites benzoylecgonine and ecgonine (Fig. 1). Benzoylecgonine is found in human blood [16], urine [6, 16, 22], and digestive tract [16] and in rat urine [17], blood [4,17] and liver [4,17]. Further hydrolysis of benzoylecgonine by esterases produces ecgonine which can be found in blood, brain, liver and in urine [4] as the major excretory product of cocaine. Another esterase product of cocaine recently found in human serum is ecgonine methyl ester [20].

Although the major metabolites formed from cocaine are benzoylecgonine and ecgonine, some N-demethylation of cocaine does occur. The product of N-demethylation, norcocaine, is subsequently metabolized by enzyme hydrolysis to benzoylnorecgonine. Following cocaine administration, norcocaine can be found in rat blood [4,13], brain [4,13] and liver [4], and there is evidence that norcocaine is a cocaine metabolite in humans [10]. Benzoylnorecgonine has been detected in rat liver [4,13] and possibly rat blood and brain [4,13].

Some pharmacological properties of the cocaine metabolites have been studied. Norcocaine, possibly the most active cocaine metabolite, inhibits the uptake of norepinephrine in rat synaptosomal preparations [7], produces rapid heart beat, convulsions and death in rats [14,15], and appears to be 2–3 times more potent a stimulant than cocaine [15]. When administered peripherally, benzoylecgonine, benzoylnorecgonine and ecgonine do not produce these cocaine-like reactions [7, 13, 14].

The purpose of this study was to examine the possibility that cocaine metabolites generalize to the discriminative stimulus properties of cocaine. Animals trained to discriminate cocaine from saline were tested with norcocaine, benzoylnorecgonine, benzoylecgonine and ecgonine methyl ester to determine if these compounds produce a stimulus similar to that of cocaine. The discriminative stimulus properties of cocaine are attenuated by the dopamine antagonist pimozide and the amine depletor reserpine [18]. These metabolites which produced a stimulus like that of cocaine were to be challenged by pretreatment with pimozide and reserpine to determine if there is similarity in the neurochemical nature of their discriminative stimulus properties and that of cocaine.

METHOD

Animals and Apparatus

Twenty-five male Sprague-Dawley rats initially weighing 250–275 g were used as subjects. The animals were food deprived to 85% of their normal free-feeding body weight. Animals were then trained to discriminate 10 mg/kg cocaine from 1 ml/kg saline. Discrimination training was carried out in five two-lever sound attenuated operant chambers (Scientific Prototype Model PLS-1000). Solid state programming equipment (Grason-Stadler 1200 series) was used to control the delivery of reinforcement and to record data generated during test and training sessions.

Preliminary Training

Animals were placed in operant chambers thirty minutes a day for three days on a continuous reinforcement schedule (CRF), followed by two days on a differential reinforcement of low response rate (DRL) 5-second schedule, two days on a DRL-10 sec schedule, and finally, four days on a DRL-15 sec schedule. Noyes standard flavor 45 mg food pellets served as the reinforcement. Responses were reinforced continuously on the CRF schedule and on alternate levers

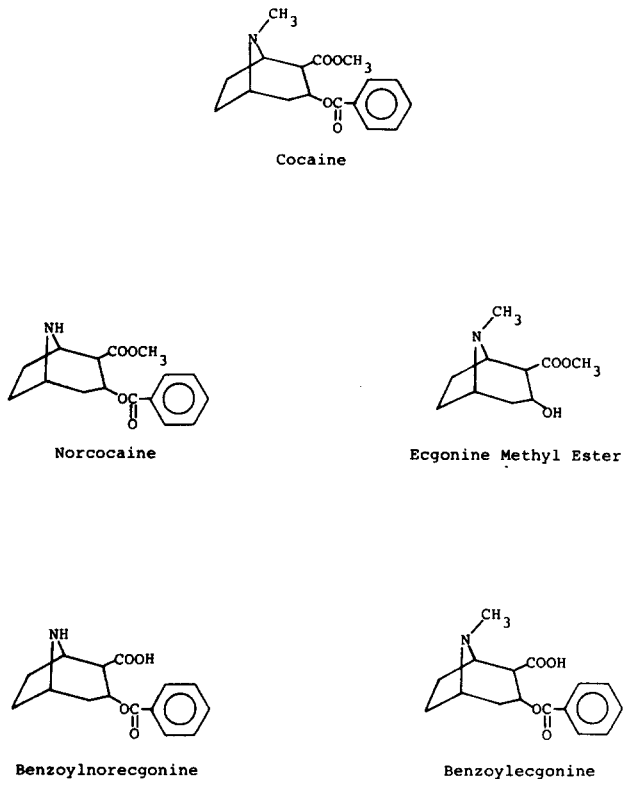

FIG. 1. Cocaine and metabolites.

following the appropriate time interval on DRL schedules. Both levers were operative. Responses made on the inappropriate lever reset the DRL. Training sessions were not preceded by drug or saline injections.

Acquisition of Discrimination

After completion of preliminary training, 10 mg/kg cocaine or 1 ml/kg saline was administered intraperitoneally 15 min before each daily training session. The left lever was designated the cocaine correct lever and the right lever the saline correct lever. Animals were reinforced during training sessions only when they responded on the designated correct lever according to administration of cocaine or saline. Ten-minute extinction tests, during which no reinforcement was available, were given every fifth day following 2 days' training on the cocaine lever and 2 days' training on the saline lever. The order of cocaine and saline injections was randomized weekly. An equal number of tests were performed following cocaine and saline administration. Animals were considered sufficiently trained when more than 80% of the responses were made on the correct lever in four consecutive extinction tests: 2 tests following administration of cocaine and 2 tests following administration of saline. Twelve test sessions were required to achieve the criteria for discrimination.

Generalization Tests

Following acquisition of discrimination, the animals were trained four days a week with the sequence of injections being saline-cocaine-cocaine-saline. On the fifth day the total session consisted of a 10 min test during which no reinforcement was available. Cocaine or norcocaine in doses of 2.5, 5 and 10 mg/kg or benzoylecgonine, benzoylnorecgonine, or ecgonine methyl ester in doses of 10 and 20 mg/kg were administered in random order to establish dose response curves. Dosages of cocaine, norcocaine and ecgonine methyl ester are expressed as hydrochloride salts. Benzoylecgonine and benzoylnorecgonine dosages are expressed as the free base. All drugs except cocaine were prepared in saline solutions on the day they were used. Cocaine was prepared in a saline solution and stored in a refrigerator. The stability of the solution was periodically checked by thin layer chromatography, according to the method of Taylor, Estevez, Englert and Ho [20]. The drugs were administered 15 min prior to the test in a volume of 1 ml/kg.

Those metabolites which generalize to cocaine by producing more than 80% responding on the cocaine lever were subsequently tested following pretreatment with 1 mg/kg pimozide or 2.5 mg/kg reserpine (Serpasil®). Reserpine was administered in a commercially prepared solution. Pimozide, moistened with acetic acid, was solubilized in 30% propylene glycol. All drugs were administered intraperitoneally. Reserpine was administered 24 hr and 15 min and pimozide, 4 hr and 15 min prior to testing.

Drugs

Cocaine hydrochloride and reserpine were purchased from commercial sources. Pimozide was the generous gift of Janssen Pharmaceutical Research Laboratoria. Norcocaine·HCl [2,18], O-benzoylnorecgonine·HCl [5,18], benzoylecgonine [5,18] and ecgonine methyl ester HCl [5,18] were synthesized according to previously published procedures. Identity of the synthesized compounds was confirmed by comparison of their physical and/or spectral properties with those reported in the literature. Purity of the crystalline solids was determined by gas liquid chromatography and thin layer chromatography. The compounds were found to be pure within the limits of these techniques both immediately after synthesis and before administration.

RESULTS

The cocaine dose response curve (Fig. 2) shows the animals acquired the discrimination of 10 mg/kg cocaine from 1 ml/kg saline. Animals receiving the training dose of 10 mg/kg cocaine as well as 5 mg/kg cocaine produced more than 80% responding on the cocaine lever. Decreasing the cocaine dose to 2.5 mg/kg also decreased the percentage of responses on the cocaine lever. All animals responded more than 80% on the saline lever following saline administration. Statistical analysis utilizing a single factor analysis of variance followed by a Newman Keuls test shows no significant difference between 5 mg/kg and 10 mg/kg cocaine, yet they are significantly different with $F(12,78)=26.77$, $p<0.001$, from saline and 2.5 mg/kg cocaine. However, 2.5 mg/kg cocaine and saline are significantly different from each other.

Norcocaine, the N-demethylated metabolite of cocaine, generalized to cocaine (Fig. 2). The two compounds produce the same dose response curves. Norcocaine (5 mg/kg and 10 mg/kg) is not significantly different from cocaine of the same doses, but is significantly different from 2.5 mg/kg cocaine and from saline (Fig. 2). Norcocaine (2.5 mg/kg) is not significantly different from 2.5 mg/kg cocaine, but is significantly different from saline, 5 mg/kg and 10 mg/kg cocaine (Fig. 2).

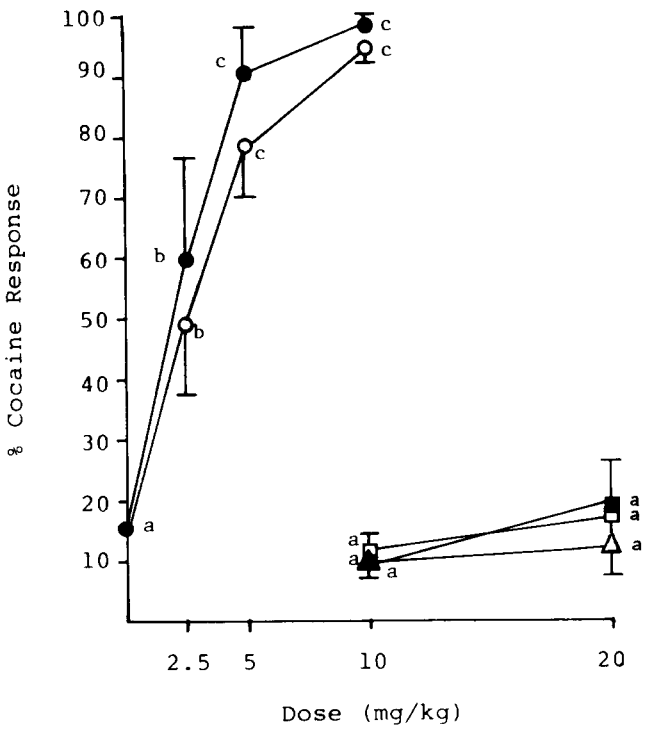

FIG. 2. Generalization tests with cocaine and metabolites. A 10 min test was performed 15 min after administration of each compound. Each point represents the mean response ± SEM of seven rats. The point on the vertical axis indicates the mean saline response. Statistical comparison was by analysis of variance followed by a Newman Keuls test. The level of significance is $p<0.01$. ●—●, cocaine; ○—○, norcocaine; ■—■, benzoylecgonine; □—□, benzoylnorecgonine; △—△, ecgonine methyl ester. (a) Significantly different from drug training condition; (b) Significantly different from drug and saline; (c) Significantly different from saline training condition.

Benzoylecgonine, benzoylnorecgonine and ecgonine methyl ester, formed by blood and liver esterases, do not generalize to cocaine (Fig. 2). In fact, even at a dose of 20 mg/kg, which is four times the threshold dose for cocaine generalization, benzoylecgonine, benzoylnorecgonine and ecgonine methyl ester generalize to saline, not to cocaine (Fig. 2).

Pretreatment with either the dopamine antagonist pimozide or with the amine depletor reserpine attenuates the discriminative stimulus properties of cocaine [8]. The two neurochemical agents likewise attenuate the norcocaine generalization (Table 1). Pimozide significantly reduces the response rate (Table 1). Reserpine significantly reduces the response rate when administered alone, but no alteration of the response rate was observed in animals receiving reserpine prior to testing with cocaine or norcocaine (Table 1).

DISCUSSION

Pharmacologically, norcocaine is as active as cocaine in inhibiting norepinephrine uptake in brain synaptosomes [7], has greater local anesthetic potency [11] and is a more potent analeptic than cocaine [14,15]. The present study shows that systemically administered norcocaine is equipotent to cocaine in producing a discriminative stimulus. The norcocaine and cocaine dose response gradients are similar (Fig. 2). Dopamine depletion and receptor antagonism attenuate the discriminative stimulus of both cocaine and norcocaine (Table 1), indicating the involvement of dopamine in the discriminability of the two compounds. Our data further agree with that of previous drug discrimination studies in which the psychomotor stimulants, amphetamine, methylphenidate and cocaine, were characterized by cross generalization to each other [3,9] and their discriminative stimulus properties were shown to be weakened by dopamine antagonists [19].

TABLE 1

EFFECT OF PIMOZIDE AND RESERPINE PRETREATMENT ON THE DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE AND NORCOCAINE IN RATS TRAINED TO DISCRIMINATE 10 MG/KG COCAINE VS 1 ML/KG SALINE

Pretreatment	Test Condition	N*	Cocaine response (Mean ± SEM)	p†	Total Responses (Mean ± SEM)	p
None	Saline	10	19 ± 2		44 ± 4	
Pimozide (1)	Saline	7	20 ± 9	ns	28 ± 6	0.05
None	Cocaine (10)	10	99 ± 0.6		51 ± 4	
Pimozide (1)	Cocaine (10)	17	59 ± 10	0.001	28 ± 4	0.001
None	Norcocaine (10)	10	95 ± 2		32 ± 6	
Pimozide (1)	Norcocaine (10)	7	64 ± 11	0.02	13 ± 1	0.05
None	Saline	10	10 ± 2		43 ± 4	
Reserpine (2.5)	Saline	5	24 ± 4	ns	15 ± 6	0.005
None	Cocaine (10)	10	99 ± 1		45 ± 9	
Reserpine (2.5)	Cocaine (10)	9	63 ± 13	0.02	49 ± 14	ns
None	Norcocaine (14)	10	96 ± 2		28 ± 7	
Reserpine (2.5)	Norcocaine (14)	6	65 ± 11	0.02	37 ± 10	ns

Doses of the compound in mg/kg are shown in parentheses.
*Number of animals used. These animals were also tested to obtain the data presented in Fig. 2.
†Level of significance based on Student's t-test comparison with the appropriate control values.

It is difficult to choose dosages that deplete or antagonize dopamine but do not affect the alertness and response of the animals. Pimozide (1 mg/kg) has been shown to antagonize the dopamine receptor [1]. Although this dose attenuates the discriminability of cocaine by not more than 40% and that of norcocaine not more than 30%; larger doses were not tried because 1 mg/kg of pimozide caused significant reduction of the response rates in our animals (Table 1). Pretreatment with reserpine (2.5 mg/kg), which depletes brain dopamine 30% and brain norepinephrine 80% [21], reduced discriminability of norcocaine and cocaine approximately 30% (Table 1). Larger doses of reserpine were not tested since our unpublished results show that with larger doses the animals' response rates are greatly reduced.

A possible explanation for the lack of generalization of benzoylecgonine and benzoylnorecgonine to cocaine is as follows: hydrolysis of the methyl ester of the cocaine and norcocaine molecules generates an acidic group (COO^-) which makes the resultant benzoylecgonine and benzoylnorecgonine more polar and thus would hinder their passage across the blood brain barrier or would prohibit their reaching the appropriate site of action. The same rationalization could be applied to ecgonine methyl ester. It is also feasible to argue that certain portions of the cocaine molecule may be essential for its discriminative stimulus properties. The loss of the benzoyl (or at least the phenyl) group as with ecgonine methyl ester, or the conversion of $COOCH_3$ to COO^- as with benzoylecgonine may render the metabolites structurally unfit for binding to the target site.

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