Pharmacology Biochemistry & Behavior, Vol. 23, pp. 529–533, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

One-Way Generalization of Clonidine to the Discriminative Stimulus Produced by Cocaine

DOUGLAS M. WOOD, HARBANS LAL, SENKA YADEN AND M. W. EMMETT-OGLESBY[1]

Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107

Received 16 August 1984

WOOD, D. M., H. LAL, S. YADEN AND M. W. EMMETT-OGLESBY. *One-way generalization of clonidine to the discriminative stimulus produced by cocaine.* PHARMACOL BIOCHEM BEHAV **23**(4) 529–533, 1985.—Rats were trained to discriminate the stimulus properties of either cocaine or clonidine using a food reinforced two-lever choice paradigm. After training, cocaine was generalized to the cocaine lever in a dose-dependent manner, and clonidine was generalized to the clonidine lever in a dose-dependent manner. Yohimbine, an alpha-2 antagonist, blocked the clonidine stimulus but not the cocaine stimulus. Cocaine was not generalized to the clonidine stimulus; however, clonidine was generalized to the cocaine stimulus, and this generalization was blocked by yohimbine. The one-way generalization of clonidine to cocaine suggests that clonidine has at least two discrete stimulus components: a major component that is not cocaine-like, and a minor component that can be detected by cocaine-trained subjects. In addition, the yohimbine blockade data suggest that both components of the clonidine stimulus are mediated via alpha-2 receptors.

Cocaine Discriminative stimulus Clonidine Alpha-2 receptors Yohimbine

BOTH cocaine and clonidine can serve as discriminative stimuli [2, 3, 8, 11, 22]. That is, rats can be trained to emit one response when injected under drug conditions (i.e., cocaine or clonidine) and another response when injected with saline. In generalization tests, dopamine releasing compounds produce discriminative stimuli similar to cocaine [3, 9, 16, 22], whereas alpha-2 agonists produce discriminative stimuli similar to clonidine [2]. Further, the behavioral effects of cocaine have been linked primarily to brain dopamine actions (for review see [23]), whereas, many behavioral effects of clonidine have been tied to its alpha-2 mechanism [5, 6, 7, 14, 18]. These findings are consistent with the observation that drugs can be classified according to their disciminative stimulus properties [1].

The evidence that brain dopamine mediates the discriminative stimulus properties of psychomotor stimulants such as cocaine is substantial [3, 16, 17]. For example, apomorphine and other dopamine receptor agonists are generalized to cocaine and amphetamine [4, 12, 16]; dopamine receptor blockers antagonize the cocaine and amphetamine discriminative stimulus [4,16]; and catecholamine synthesis inhibitors block the discriminative stimulus produced by amphetamine [12]. Similarly, the evidence that alpha-2 receptors mediate the discriminative stimulus properties of clonidine is also well established. For example, alpha-2 agonists are generalized to clonidine [2], and alpha-2 antagonists block the clonidine stimulus [2,8].

The role of dopamine mechanisms in the mediation of the clonidine stimulus has not been well established. Likewise, the role of alpha-2 receptors in the mediation of the cocaine stimulus is unclear. In a test of the role of alpha adrenergic receptors in the mediation of the amphetamine cue, D'Mello [8] reported that clonidine generalized to d-amphetamine, but d-amphetamine did not generalize to the discriminative stimulus properties of clonidine. However, the role of alpha-2 receptor stimulation in the partial generalization of clonidine to d-amphetamine was not tested. In the present experiment, the cross-generalization of cocaine and clonidine was investigated. In addition, the role of alpha-2 receptors in mediating the cocaine and clonidine stimuli was tested by determining whether yohimbine, an alpha-2 antagonist, would block the generalization of cocaine or clonidine.

METHOD

Subjects

Ten male Long-Evans hooded strain rats (Charles River Breeding Laboratories, Willmington, MA) were used for cocaine discrimination training, and twenty male spontaneously hypertensive (SH) rats (Taconic Farms, Germantown, NY) were used for clonidine discrimination training. SH rats were used for the discrimination of clonidine because this discrimination has been linked to the reduction of blood

[1]Requests for reprints should be addressed to M. W. Emmett-Oglesby, Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

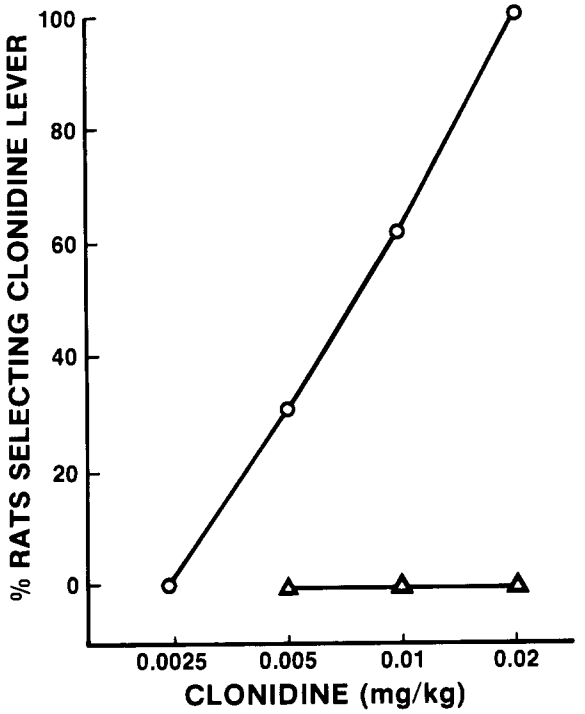

FIG. 1. Generalization of clonidine and clonidine in combination with yohimbine to the clonidine stimulus. Abscissa: dose of clonidine. Ordinate: percentage of rats completing 10 responses on the clonidine lever prior to reinforcement. Rats were trained to detect 0.02 mg/kg of clonidine. Data show clonidine-lever selection obtained for clonidine (○). and clonidine plus yohimbine (△). N = 8 at all points tested.

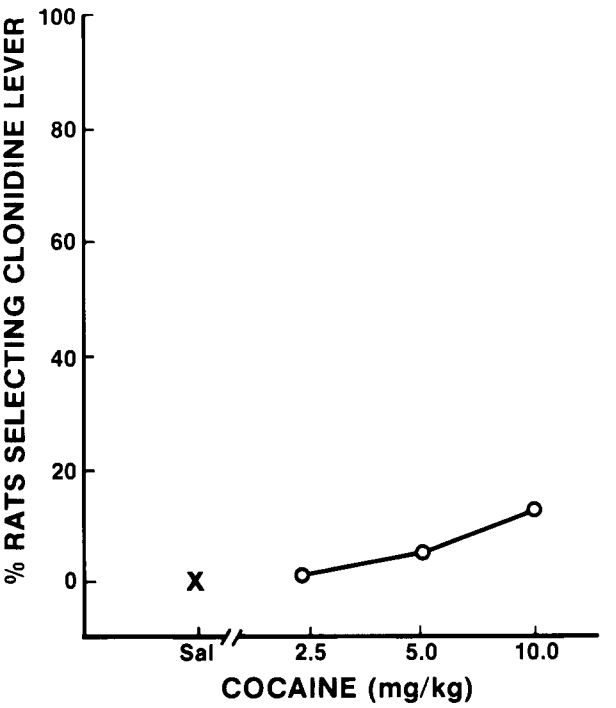

FIG. 2. Generalization of cocaine to the clonidine stimulus. Abscissa: Saline (SAL) or dose of cocaine. Ordinate: percentage of rats completing 10 responses on the clonidine lever prior to reinforcement. N = 8 at all points tested.

pressure [2,14], and SH rats are more sensitive in the detection of this cue. The subjects were housed individually in a large colony room of constant temperature (21 ± 1°C), and body weights were maintained at 320 ± 5 g by limiting food availablity.

Apparatus

Discrimination training was carried out in sixteen standard operant behavioral chambers (Coulbourn Instruments). Each chamber was housed in a light and sound attenuating box that was fan ventilated. A houselight was mounted centrally above a food cup, which was located between two response levers. Food reward (45 mg pellets Noyes Co.) was delivered by a pellet dispenser. Recording of lever responses and scheduling of reinforcement contingencies was performed through TRS-80 Model III microcomputers (Radio Shack) and printers connected to the chambers through LVB interfaces (Med Associates) using a modification of a program developed in this laboratory [10,19].

Discrimination Training

Using food as a reinforcer, subjects were trained to press a lever, and their behavior was shaped progressively until 10 bar-press responses (FR10) were required to obtain each reinforcement. For the cocaine group, the subjects were trained to press one of the levers 15 minutes following cocaine (10.0 mg/kg) intraperitoneal (IP) injection and the other lever following saline (1 ml/kg) injection. Following cocaine injection, only FR10 responses on one of the levers (the drug lever) were reinforced; responses on the saline lever were recorded but not reinforced. Similarly, following injection of saline, only FR10 responses on the saline lever were reinforced, and responses on the cocaine lever were recorded but not reinforced. Cocaine/saline injections were given in an irregular sequence, and no cue other than the effects of the drug was available to guide appropriate lever selection. For the clonidine group a similar procedure was used to train the discrimination of clonidine (0.02 mg/kg) except that clonidine or saline was administered 30 minutes pre-session.

For both training and testing, only responses emitted prior to obtaining the first reinforcement were used to record which lever was selected. To insure accurate discrimination, during training the correct lever (saline following saline injection or drug following drug injection) had to be selected for 10 successive sessions before subjects were considered ready for testing. Subsequently, drug testing occurred only after 4 consecutive sessions in which the correct lever was selected with fewer than 4 incorrect responses.

Generalization tests were conducted for cocaine, clonidine, and yohimbine in rats trained to discriminate cocaine from saline. Generalization tests were also conducted for clonidine and cocaine in SH rats trained to discriminate clonidine. In the cocaine trained rats, yohimbine (2.5 mg/kg) in combination with several doses of cocaine (2.5–10.0 mg/kg) or clonidine (0.005–0.02 mg/kg) were tested. Sim-

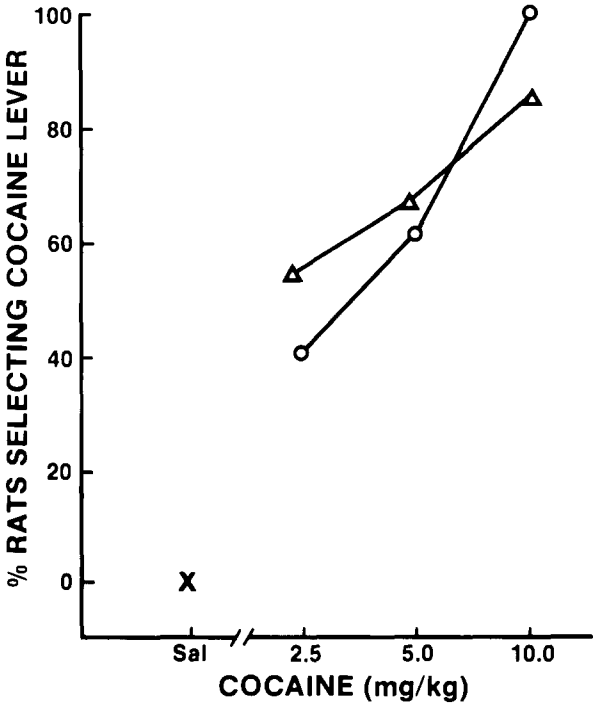

FIG. 3. Generalization of cocaine and cocaine in combination with yohimbine to the cocaine stimulus. Abscissa: Saline (SAL) or dose of cocaine. Ordinate: percentage of rats completing 10 responses on the cocaine lever prior to reinforcement. Rats were trained to detect 10.0 mg/kg of cocaine. Data show cocaine-lever selection obtained for cocaine (○) and cocaine plus yohimbine (△). N = 10 for all cocaine determinations; N = 7 for all points tested.

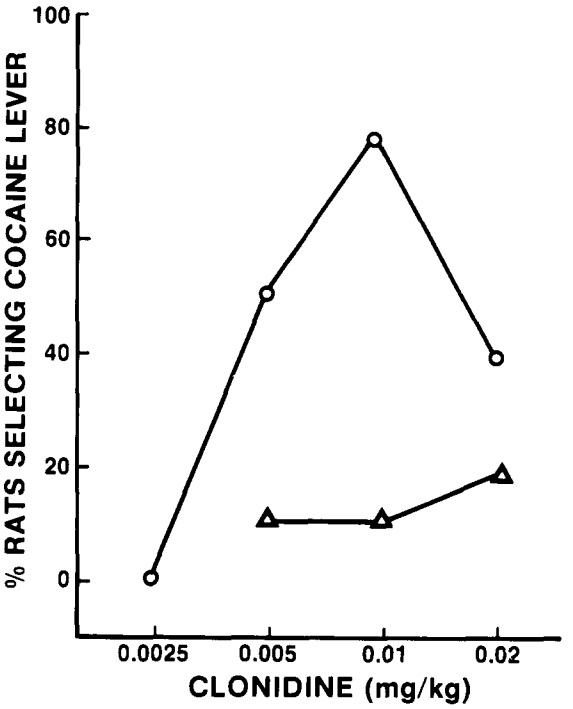

FIG. 4. Generalization of clonidine and clonidine in combination with yohimbine to the cocaine stimulus. Abscissa: dose of clonidine. Ordinate: percentage of rats completing 10 responses on the cocaine lever prior to reinforcement. Data show cocaine-lever selection obtained for clonidine (○) and clonidine plus yohimbine (△). N = 8 for all clonidine tests; N = 10 for all clonidine plus yohimbine tests.

ilarly, in the clonidine trained SH rats, yohimbine (2.5 mg/kg) in combination with clonidine (0.005–0.02 mg/kg) was tested. Yohimbine was administered 5 minutes prior to the cocaine or clonidine injection.

Drugs

Cocaine hydrochloride was obtained from Merck Pharmaceutical. Clonidine hydrochloride was obtained from Boehringer Ingelheim Ltd. Yohimbine hydrochloride was obtained from Sigma Chemical Co. Cocaine and clonidine were dissolved in 0.9% saline; yohimbine was homogenized in a suspension of 86% isotonic saline, 13% propylene glycol and 1% Tween 80. Drugs were injected intraperitoneally in a volume of 1 ml/kg.

RESULTS

Acquisition of the cocaine and clonidine discrimination took approximately 70–80 sessions. Both groups of subjects were under strong stimulus control. In the clonidine trained group, the interoceptive stimulus produced by clonidine was dose dependent (Fig. 1) with an approximate ED50 of 0.0075 mg/kg. The training dose of clonidine produced 100% clonidine-lever selection and saline produced 100% saline-lever selection. The generalization of clonidine was blocked by the alpha-2 antagonist yohimbine (Fig. 1), with 2.5 mg/kg yohimbine completely antagonizing the clonidine stimulus. Doses larger than 2.5 mg/kg yohimbine produced significant behavioral toxicity resulting in no lever selection during the test session. Cocaine was not generalized to the clonidine lever (Fig. 2).

In the cocaine trained group, the interoceptive stimulus produced by cocaine was dose-dependent with an approximate ED50 of 3.5 mg/kg. The training dose of cocaine produced 100% cocaine-lever selection and saline produced 100% saline-lever selection (Fig. 3). Generalization of cocaine was not blocked by yohimbine (Fig. 3). Yohimbine did not generalize to the cocaine discriminative stimulus over a range of doses tested (2.5–10.0 mg/kg). Cocaine-lever selection at any dose was 10%, and disruption of lever responding was observed in 3 of 9 rats tested at 10.0 mg/kg. Clonidine generalized to the cocaine discriminative stimulus with the highest generalization occurring at 0.01 mg/kg clonidine (Fig. 4). The next higher dose of clonidine produced reduced generalization of clonidine to the cocaine discriminative stimulus. Clonidine in a dose of 0.04 mg/kg clonidine produced behavioral toxicity with 7 of 8 rats not responding. Yohimbine (2.5 mg/kg) antagonized the generalization of clonidine to cocaine (Fig. 4).

DISCUSSION

The present experiment confirms that cocaine and clonidine can serve as discriminative stimuli. A high degree of stimulus control was obtained for each drug in approximately 70–80 sessions of training. In generalization tests, clonidine was generalized to cocaine, but cocaine was not generalized to clonidine. These results are in contrast to those obtained

by McKenna and Ho [16], who found no generalization of clonidine to the discriminative stimulus properties of cocaine. However, McKenna and Ho only tested a single dose (0.1 mg/kg) of clonidine, and as shown in the present experiment, doses of clonidine higher than 0.02 mg/kg produced a decreased generalization to the cocaine stimulus (Fig. 4). Because the discriminative stimulus properties produced by amphetamine and cocaine are similar [3, 9, 11, 12, 20, 22] drugs generalizing to one of these compounds should generalize to the other. Thus, the present data support those of D'Mello [8] who obtained a one-way generalization of clonidine to d-amphetamine. Further, the partial generalization of clonidine to the cocaine discriminative stimulus is similar to the partial generalization of clonidine to the amphetamine stimulus obtained by D'Mello.

One-way generalizations between drugs is a relatively less common finding in drug discrimination research. Although no well established principles can be used to explain such a finding, in exteroceptive discrimination paradigms subjects typically attend to only the major component of a compound stimulus [15]. If clonidine produces two discriminative stimuli, then it is possible that a major component may be due to its sedative [2,8] effects, and when trained on clonidine, this becomes the only component of the stimulus that is used as a cue. However, there may be a minor component of clonidine which is cocaine-like in character and can be detected by cocaine-trained rats. Because cocaine does not have sedative or antihypertensive effects, it does not resemble the major component of clonidine; therefore, it is not detected as clonidine-like by clonidine-trained rats. This hypothesis would account for the one-way generalization obtained in the present experiment. Mechanistically, these data could be explained by proposing that clonidine, through initial alpha-2 receptor action, activates a multi-component pathway in the production of the cocaine cue; a one way generalization would then be possible if cocaine produced its stimulus by directly affecting a site in the pathway beyond the alpha-2 receptor.

In rats trained to discriminate clonidine, the alpha-2 antagonist yohimbine blocked the clonidine stimulus. Previously, we have demonstrated that this effect is attributable to yohimbine, and not to the yohimbine-vehicle [14]. These data support the findings of Bennett and Lal [2] and are in agreement with D'Mello [8], who used the alpha-2 antagonist, piperoxane, to block the clonidine stimulus. In rats trained to discriminate cocaine in the present experiment, yohimbine also blocked the generalization of clonidine to the cocaine stimulus. These data indicate that the cocaine-like stimulus properties of clonidine are mediated by alpha-2 adrenergic receptors. In the periphery, alpha-2 receptors have been well established as noradrenergic pre-synaptic autoreceptors [21]. However, it is unlikely that central modulation of norepinephrine metabolism is the primary mechanism that accounts for the generalization of clonidine to cocaine. For example, neither alpha- nor beta-receptor blockers alter the discriminative stimulus properties of amphetamine [12, 13, 16, 17].

Thus, it is likely that brain alpha-2 receptors are not the primary mechanism responsible for the cocaine cue, and clonidine may produce its cocaine-like cue by activating alpha-2 receptors which in turn activate pathways directly involved in the production of the cocaine stimulus.

ACKNOWLEDGEMENTS

This research was supported in part by American Osteopathic Association grant 82-11-045, Organized Faculty Research grant 34117, and NHLBI grant HL07465. We appreciate the expert technical assistance of Blair Kuhnen.

REFERENCES

1. Barry, H., III. Classification of drugs according to their discriminable effects in rats. *Fed Proc* **33:** 1814-1824, 1974.
2. Bennett, D. A. and H. Lal. Discriminative stimuli produced by clonidine: An investigation of the possible relationship to adrenoceptor stimulation and hypotension. *J Pharmacol Exp Ther* **233:** 642-648, 1982.
3. Colpaert, F. C., C. J. E. Niemegeers and P. A. J. Janssen. Discriminative stimulus properties of cocaine: Neuropharmacological characteristics as derived from stimulus generalization experiments. *Pharmacol Biochem Behav* **10:** 535-546, 1978.
4. Colpaert, F. C., C. J. E. Niemegeers and P. A. J. Janssen. Discriminative stimulus properties of cocaine and d-amphetamine, and antagonism by haloperidol: A comparative study. *Neuropharmacology* **17:** 937-942, 1978.
5. Davis, M., J. M. Cedarbaum, G. K. Aghajanian and D. S. Gendelman. Effects of clonidine on habituation and sensitisation of acoustic startle in normal, decerebrate and locus coeruleus lesioned rats. *Psychopharmacology (Berlin)* **51:** 243-253, 1977.
6. Davis, W. M. and S. G. Smith. Catecholaminergic mechanisms of reinforcement: Direct assessment of drug self-administration. *Life Sci* **20:** 483-492, 1977.
7. Delbarre, B. and H. Schmidt. Effects of clonidine and some alpha-adrenoreceptor blocking agents on avoidance conditioned reflexes in rats, their interactions and antagonism by atropine. *Psychopharmacologia* **35:** 195-202, 1974.
8. D'Mello, G. D. Comparison of the discriminative stimulus properties of clonidine and amphetamine in rats. *Neuropharmacology* **21:** 763-769, 1982.
9. D'Mello, G. D. and I. P. Stolerman. Comparison of the discriminative stimulus properties of cocaine and amphetamine in rats. *Br J Pharmacol* **61:** 415-422, 1977.
10. Emmett-Oglesby, M. W., D. G. Spencer, Jr. and D. E. Arnoult. A TRS-80-based system for the control of behavioral experiments. *Pharmacol Biochem Behav* **17:** 583-587, 1980.
11. Emmett-Oglesby, M. W., M. Wurst and H. Lal. Discriminative stimulus properties of a small dose of cocaine. *Neuropharmacology* **22:** 97-101, 1983.
12. Ho, B. T. and J. Huang. Role of dopamine in d-amphetamine-induced discriminative responding. *Pharmacol Biochem Behav* **3:** 1085-1092, 1975.
13. Jarbe, T. U. C. Cocaine as a discriminative cue in rats: Interactions with neuroleptics and other drugs. *Psychopharmacology (Berlin)* **59:** 183-187, 1978.
14. Lal, H. and S. Yaden. Discriminative stimuli produced by clonidine in spontaneously hypertensive rats: generalization to antihypertensive drugs with different mechanisms of action. *J Pharmacol Exp Ther*, in press.
15. Mackintosh, N. J. Overshadowing and stimulus intensity. *Anim Learn Behav* **4:** 186-192, 1976.
16. McKenna, M. L. and B. T. Ho. The role of dopamine in the discriminative stimulus properties of cocaine. *Neuropharmacology* **19:** 297-303, 1980.
17. Schecter, M. D. and P. G. Cook. Dopaminergic mediation of the interoceptive cue produced by d-amphetamine in rats. *Psychopharmacologia* **42:** 185-193, 1975.
18. Shearman, G., M. Hynes and H. Lal. Self administration of clonidine by the rat. *Prog Clin Biol Res* **71:** 259-276, 1981.

19. Spencer, Jr., D. G. and M. W. Emmett-Oglesby. Parallel processing strategies in the application of microcomputers to the behavioral laboratory. *Behav Res Method Instr Comput* **17:** 294-300, 1985.
20. Stolerman, I. P. and G. D. D'Mello. Role of training conditions in discrimination of central nervous system stimulants by rats. *Psychopharmacologia* **73:** 295-303, 1973.
21. U'Prichard D. C. and S. H. Snyder. Distinct alpha-noradrenergic receptors identified by binding and physiological relationships. *Life Sci* **24:** 79-88, 1979.
22. Wood, D. M., H. Lal and M. W. Emmett-Oglesby. Discriminative stimulus properties of cocaine: tolerance and cross-tolerance characteristics. *Neuropharmacology* **23:** 1419-1423, 1984.
23. Woods, J. Behavioral effects of cocaine in animals. In: *Cocaine*, edited by R. C. Petersen and R. C. Stillman. Washington: Department of HEW Publication Number (ADM 77-471), 1977, pp. 63-95.