Neuropharmacology, Vol. 18, pp. 981 to 989
Pergamon Press Ltd 1979. Printed in Great Britain

DRUG-INDUCED CUES IN STUDYING MECHANISMS OF DRUG ACTION*

J. A. ROSECRANS and R. A. GLENNON
Departments of Pharmacology and Pharmaceutical Chemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, VI 23298, U.S.A.

Summary—This paper describes how drug-cues can be used to study psychoactive drug mechanisms. Several overall approaches can be utilized. In one such approach, drug-cues can be either (1) compared with other pharmacological agents, or (2) the pharmacological mechanism of the cue can be evaluated by attempting to antagonize it with various receptor antagonists. A second approach involves studying the mechanism of a drug-cue by central neuropharmacological manipulations. In this approach the site of action of the cue can be characterized by comparing the peripherally generated cue to drug administered into various brain area sites. In addition, the mechanism of action can be studied by attempting to mimic the peripherally-generated cue by stimulating various CNS sites electrically, or one can attempt to alter the cue by the selective neurotoxic lesioning of various brain area sites. This latter approach can provide much information concerning possible neurochemical mechanisms involved in how the drug cue is elicited. Finally, one can compare drug cues with other pharmacological models of drug action such as the rat fundus pA_2 model which has the value of allowing a better understanding of possible structure activity relationships of various psychoactive drugs. The drug-cue provides a very good model of a psychoactive drug effect, which can assist in obtaining important information concerning drug-receptor interactions using an *in vivo* rat preparation.

One of the main objectives of research conducted in these laboratories has been to determine the site and mechanism of action of psychoactive drugs, especially those which tend to be abused by humans. The overall philosophy of these studies has been to evaluate drug mechanisms using *in vivo* pharmacological animal models which would also provide information that could be correlated to human drug effects. In order to carry out this goal, a pharmacological model of psychoactive drug effects was needed, which would be specific and sensitive, and which could be easily manipulated and evaluated by a variety of neuropharmacological techniques.

Initial approaches attempted to correlate neurochemical events with the behavioral disruptive effects of various psychoactive drugs. These studies involved the cholinergic drug physostigmine and clearly showed that this drug disrupted avoidance behavior by increasing acetylcholine levels at central muscarinic sites. However, there were two major problems with this approach. First, disruption of behavior is a relatively non-specific pharmacological measure. For example, it has been shown that (+)-amphetamine, morphine and arecoline all disrupt food-reinforced behavior (variable interval schedules; VI-15 sec) within the same dosage range and it is quite difficult to show any specificity across drug class. Drug specificity can be demonstrated, but the behavioral procedures used to do so are extremely time consuming; this tends to defeat the purpose of trying to correlate behavior with neurochemical events. Secondly, in order to correlate neurochemical events, animals have to be sacrificed, or a different group of similarly handled animals has to be used, the latter producing other problems. Another difficulty encountered when attempting to correlate behavioral effects with neurochemical drug-effects concerns the drug being studied. Again, a similar series of investigations was initiated which attempted to study the effects of nicotine on serotonin turnover. The difficulty encountered with this drug was that its behavioral effects tend to be dependent upon the baseline rates of behavior prior to drug administration. Thus, it was not possible to consistently predict whether nicotine would facilitate or inhibit behavior, and this also, did not always correlate with neurochemical events.

Therefore, there was a need to develop a pharmacological model of a given drug effect which would not present the problems of non-specificity and unpredictability of drug responses. After much searching of the literature it was decided to experiment with the discriminative stimulus properties of drugs as a possible means for circumventing these problems (Schechter and Rosecrans, 1971). Working with this technique it became quite apparent that what was called for was exactly this procedure.

The ability to assume discriminative control of behavior has been observed to be a property of several psychoactive drugs. Within a discriminative stimulus (DS) paradigm, an experimental subject (animal or human) comes under the stimulus control of a drug such that correct responding in a choice situation is contingent upon the "drug state" of the subject at that time. Thus, in a DS procedure a subject is trained to emit one response (such as to press one lever in a two-level operant chamber to obtain a food reward)

* This research was supported by U.S.P.H.S. grants DA-01642-02 and DA-00296-05.

when in one drug state, while the same subject must make the opposite response (press the other lever) when in the non-drug or a different drug state. These behavioral responses are dependent upon the ability of the animal to detect a specific drug state, which is similar to requiring a human subject to report the subjective effects after receiving a psychoactive drug.

Thus, this model is pre-eminently a detection procedure whereby an animal must be able to recognize a given drug state, and likewise, the drug serves as a cue to the animal enabling it to choose the correct lever for a positive reinforcement. By the nature of the task, an animal must develop behavioral tolerance to the disruptive effects of the drug in order to be able to learn the task. In contrast to the necessity to develop behavioral tolerance to disruption, an animal cannot develop tolerance to the cue or else the drug could no longer serve a discriminative stimulus function. Thus, we have an extremely stable behavioral model of a pharmacological effect which is relatively resistant to many of the disruptive and tolerance problems experienced with most psychoactive drugs.

It is necessary to consider the pharmacological properties of the drug cue. First, the drug cue is quite specific. Thus, if a rat is trained to detect morphine vs saline it will perceive other narcotic agonists like morphine, but will make a non-morphine response if given (+)-amphetamine or pentobarbital. Thus, drug cues are pharmacologically class specific and several investigators have used this technique to classify many psychoactive drugs (see Colpaert and Rosecrans, 1978). Once the power of this procedure was recognized a long series of studies was started, which were designed to study the pharmacology of the nicotine cue in order to develop a case for the use of drug discrimination techniques as a means of evaluating mechanisms of psychoactive drug action. The nicotine cue demonstrated the classical pharmacological principles of exhibiting time-duration and dose-response characteristics, which in addition, correlated extremely well with brain nicotine levels (Rosecrans and Chance, 1977, 1978). The nicotine cue was antagonized by the central nicotinic receptor antagonist, mecamylamine, but was not antagonized by the peripherally acting antagonist, hexamethonium. Receptor specificity was also shown by the fact that the muscarinic antagonist, atropine, was unable to block the nicotine cue. More recently, the stereospecificity of the nicotine cue was observed and the (+)-isomer found to possess about one-eighth of the potency of the (−)-isomer (Meltzer, Rosecrans, Aceto and Harris, 1979). The specificity of the cue has also been demonstrated by the fact that except for the chemical isomer, 3-pyridylmethyl-pyrrolidine, few other drugs have stimulus properties similar to nicotine (Rosecrans *et al.*, 1978; Chance, Kallman, Rosecrans and Spencer, 1978).

Therefore, the drug cue appears to provide a rigorous and sensitive model of psychoactive drug effects. In this review some of the approaches employed in using drug cues as a means of evaluating mechanism of drug action will be described. For this purpose it will focus on the study of several narcotic and hallucinogenic drugs.

METHODS

In these studies, male Sprague–Dawley rats were trained to discriminate a specific drug vs saline using a two-lever operant procedure as described by Chance, Murtin, Krynoch and Rosecrans (1977). After initial shaping of behavior to bar press for a food reward, drugs (double alternation) and a schedule of reinforcement were introduced. The following drug regimens were used: 5-methoxy-*N*,*N*,-dimethyltryptamine (5-OMeDMT; 1.5 mg/kg i.p.; $t = 15$ min), morphine (3 mg/kg s.c.; $t = 30$ min), methadone (1.5 mg/kg s.c.; $t = 30$ min), and LSD (96 μg/kg i.p.; $t = 15$ min). Rats were placed in the operant chamber after a specific drug regimen and reinforced on either the left or right lever contingent upon the solution administered. For half the subjects the left lever was reinforced under a specific drug state, and for the other half of the subjects, the right lever was reinforced under the same drug state. The schedule of reinforcement used in most studies was a variable interval of 15 sec (VI-15 sec) and training sessions were 15 min in duration. Performance of the discrimination task was evaluated during 2.5 min non-reinforced extinction tests followed by 12.5 min training sessions. In some studies these tests were conducted daily, while in others, they were conducted weekly. Rats learned a given drug discrimination within 20 daily sessions under each drug and saline condition. At this point, responding during test sessions averaged 80–90% on the drug-correct lever following drug administration, while responding following saline averaged 10–20% on the drug-correct lever. Studies designed to antagonize or mimic the discriminative stimulus (DS) were conducted during 2.5–5.0 min extinction test sessions. These tests were conducted weekly. Drug discrimination training was maintained between test sessions. Chemical injection cannulae or electrodes were implanted when rats had learned the discrimination, and presurgical performance was regained before any specific study was conducted. Brain area sites of cannula and electrode implantation were verified by histological techniques at the termination of each study (Krynock and Rosecrans, 1979).

DRUG-CUE SUBSTITUTION STUDIES

One approach which has been utilized by several investigators to study drug mechanisms involves determining whether one drug cue will substitute for drugs of a specific class. The rationale of such an approach implies that an animal trained to discriminate a given drug will generalize only to chemicals having a similar mechanism of action. That is, animals trained to detect a specific drug will recognize

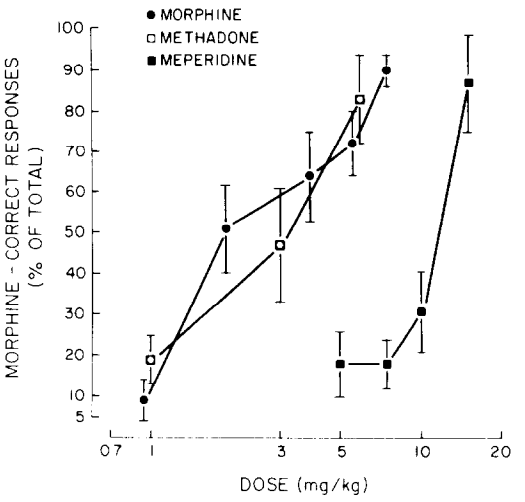

Fig. 1. An example of a cue-substitution experiment. Rats were trained to discriminate between morphine (7.5 mg/kg, s.c.) and saline conditions utilizing a two-lever operant procedure. Data was calculated as percentage correct responses on the morphine lever and was determined during 2.5 min extinction sessions. Rats made 91% of their responses on the morphine-correct lever (MCL) following a 7.5 mg/kg dose of morphine but percentage responses on the MCL declined as the dose approached 1 mg/kg. Thus, the strength of the morphine cue was dose-related. Methadone and meperidine produced similar dose-response profiles indicating that the rats perceived these drugs as being similar to morphine. Meperidine, however, was significantly less potent than morphine (from Hirschorn and Rosecrans, 1974).

drugs acting similarly to the training drug (Fig. 1). In addition, some investigators have implied that substitution potential may also be a predictor of a given drug effect in humans as well. While these concepts are for the most part true, there are some exceptions to the rule. For example, LSD which is a potent hallucinogen will substitute with the drug quipazine (White, Kuhn and Appel, 1977) which appears devoid of any such effects in humans (Villarreal, personal communication). In addition, (±)-cathinone, which appears to be one of the active constituents of

Table 1. Drug-cue cross substitutions between LSD and 5-OMeDMT

Dose LSD (μg/kg)	5-OMeDMT trained rats (N = 24)	Dose 5-OMeDMT (mg/kg)	LSD trained rats (N = 18)
0	11.2 ± 4.0	0	8.1 ± 4.4
24	42.1 ± 4.9	0.5	29.2 ± 6.4
48	69.3 ± 7.5	1.0	68.7 ± 4.6
96	85.6 ± 3.5	1.5	77.6 ± 3.9
5-OMeDMT 1.5 mg/kg	89.1 ± 1.4	LSD 96 μg/kg	91.3 ± 2.3

Rats were trained to discriminate either 5-OMeDMT or LSD using a two-lever operant procedure. Data is presented as percentage correct responses on the drug-correct lever (LSD or 5-OMeDMT lever) during 2.5 min extinction sessions.

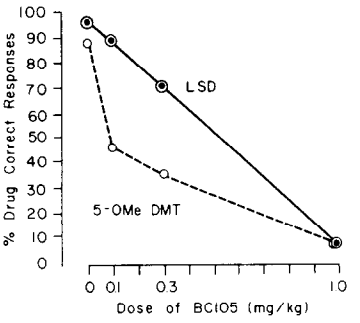

Fig. 2. Antagonism of LSD- and 5-OMeDMT induced cues by various doses of BC105. BC105 was administered 45 min prior to each drug and the animals were placed in the operant chamber 10–15 after for a 2.5 min test session (see Methods).

KHAT which is widely used in Eastern Africa for its stimulant effects has been studied. It was found that (±)-cathinone was twice as potent as (+)-amphetamine but did not appear to be acting by stimulating dopamine neurons as does (+)-amphetamine (Rosecrans, Campbell, Dewey and Harris, 1979; Colpaert, Niemegeers and Janssen, 1978). While drug substitution experiments do provide important information concerning the range of possible drug mechanisms, a complete understanding must await other experiments involving more specific approaches. Thus, this approach is the first step in the process of determining mechanisms of drug action and is extremely useful in classifying chemicals into specific pharmacological classes.

DRUG-CUE ANTAGONISM STUDIES

Once the chemical substitutions that can be achieved for a drug is known, then it is possible to begin the process of characterizing receptor type. The general approach used in these experiments attempts to characterize the receptor type upon which a given agonist may be acting. Two procedures have been used. In the first, the drug cue is challenged by increasing doses of a suspected antagonist until the drug cue has lost its effect. In this approach the training dose is held constant. In the experiment presented here, 5-OMeDMT and LSD, which are hallucinogens in man, substituted for each other as cues (Table 1). In order to determine whether 5-hydroxytryptamine (5-HT) systems were involved with these cues, each drug was challenged with the 5-HT antagonist, BC105 (Winter, 1978). The LSD cue was antagonized by increasing doses of BC105 in a dose-response fashion suggesting that LSD was acting on a 5-HT receptor (Fig. 2). Similar effects were observed with 5-OMeDMT (Glennon, Rosecrans, Young and Gaines, 1979), but the shape of the dose-response antagonism was different, appearing to have more than one component. This finding suggested that 5-OMeDMT might also be acting upon a 5-HT

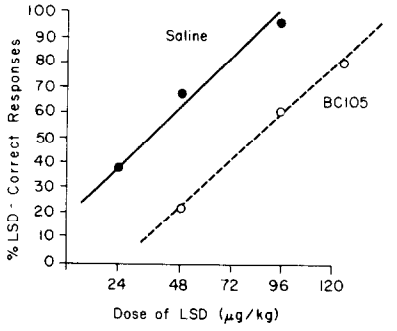

Fig. 3. Antagonism of the LSD-cue by BC105. Saline or BC105 (0.3 mg/kg) were administered 45 min prior to various doses of LSD. Time parameters etc. are described in Fig. 2.

receptor but that the interaction may be quite different to that observed with LSD. This effect while difficult to explain, brings us to a second approach to studying cue-antagonisms. The second approach evaluates agonist dose-response relationships in both the presence and absence of a constant dose of the antagonist. Using this procedure a shift of the dose-response curve to the right can be observed (Fig. 3). A parallel shift indicates a competitive interaction. On the other hand, if the dose-response shifts are not parallel then other non-competitive mechanisms may also be present. This kind of an evaluation is extremely important and antagonism studies should be conducted using both procedures, especially in studies with a drug like 5-OMeDMT (Fig. 2). That is, drug-cue dose response experiments should be conducted in the presence of several doses of the antagonist. This kind of experiment will generate a family of dose-response agonist-antagonist curves which will allow the analysis of the receptor interaction mechanism in depth. This kind of data is now being obtained with 5-OMeDMT in an attempt to explain its mechanism at 5-HT receptors.

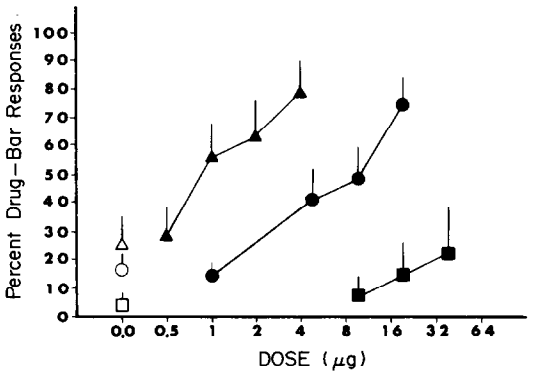

Fig. 4. Generalization of peripherally-generated drug-induced cues to drugs injected directly into the raphe nuclei. Open symbols represent intracerebral (i.c.) injections of 0.5 μl saline. Solid triangles: intracerebral injection of morphine in rats trained to discriminate systemic morphine from saline; solid circles: intracerebral injection of LSD in LSD trained rats; solid boxes: intracerebral methadone in methadone trained rats. Vertical bars represent standard errors of the means (from Rosecrans *et al.*, 1978).

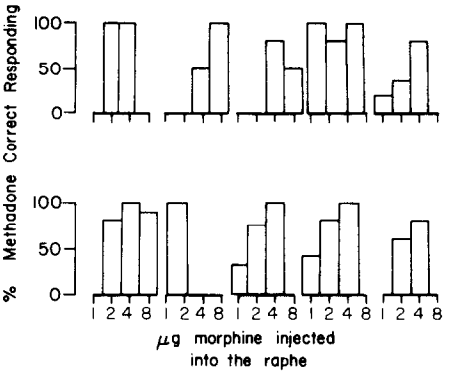

Fig. 5. Generalization of the methadone-induced cue (1.5 mg/kg) to morphine injected into the dorsal raphe sites. This Figure represents the dose at which morphine elicited methadone-correct responding. Methadone was insensitive when injected at these sites. The training dose of methadone elicited 89.1 ± 6.7% correct responding (given s.c.-30 min) while morphine at an average dose of 2.3 ± 0.4 μg injected into the raphe elicited 91 ± 4% methadone correct responding ($N = 10$).

CENTRAL SITES OF DRUG ACTION

Data obtained in this laboratory from cue-substitution and antagonism studies indicated that both LSD and morphine were acting on some central 5-HT receptor. To evaluate this relationship further it was decided to study drug cues directly at the midbrain raphe nuclei which are the major site of origin of 5-HT neurons projecting to the more rostral parts of the brain (Rosecrans *et al.*, 1978). Thus, a series of experiments was initiated in which the raphe nuclei were manipulated neuropharmacologically in animals trained to discriminate LSD and/morphine (Rosecrans *et al.*, 1978; Krynock and Rosecrans, 1979). In addition, animals were also trained to discriminate between electrical stimulation of the raphe and no stimulation.

Administration of drugs into the dorsal raphe nuclei

In this experiment three groups of rats were trained to discriminate either morphine, methadone or LSD via peripheral administration. These animals were then implanted with chronic dorsal raphe injection cannulae and the dose at which each rat perceived the centrally administered drug as similar to the training dose was then determined (Fig. 4). Morphine administered in doses of 3–4 μg, given centrally, mimicked the peripheral cue (3 mg/kg), while the substitution dose for LSD (96 μg/kg) was 20 μg. Interestingly, methadone substitution did not occur when administered centrally. These effects were largely all or none, but a clearer picture evolves if the maximally effective generalization dose for each animal was calculated. Thus, in morphine-trained rats, 2.3 μg administered to

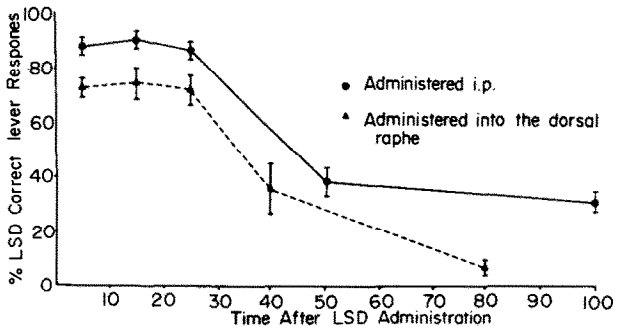

Fig. 6. A comparison of the duration of LSD cue induced peripherally vs time course of cue given directly into the dorsal raphe nucleus. This comparison was made in two groups of LSD trained rats.

the raphe generated 91% morphine-like responding while this factor reached 95% LSD-like responding in rats given 17.5 μg/kg. While methadone did not generalize centrally, morphine administered into the raphe did generate methadone-correct responses in these animals (Fig. 5).

This study indicated that the raphe was extremely sensitive to morphine but not to LSD or methadone. The ratio of central to peripheral effects was 1/650 for morphine and 1/2 for LSD. The LSD effect was quite unexpected as several workers have suggested that LSD's major effects occur at the raphe nuclei (Haigler and Aghajanian (1974). While these data suggest that LSD and methadone are relatively inactive at this site, there are some methodological questions which must be answered. The major question concerns the mobility of drug at the site of injection. Lysergic acid diethylamide is extremely lipid soluble and may leave the site very rapidly. In addition, peripheral vs central effects of LSD have been compared and it was found that the time course of the cue was similar regardless of the time after drug administration (Fig. 6). At this point, no explanation can be offered and attempts are being made to study the distribution of LSD centrally following administration.

Methadone has likewise been studied and as yet it has not been possible to elicit methadone responding by injecting this drug into the raphe. The effects of methadone at this injection site have been studied in relation to the ability of narcotics to block ovulation and it has been found that methadone is capable of producing an effect but at half the dose of morphine (Maughan, Johnson and Rosecrans, 1977). Thus, methadone does appear to affect the raphe but this area may not be an important site at which methadone elicits stimulus control of behavior. This is especially interesting since both morphine and methadone cues cross-substitute for each other in almost exactly the same doses. Thus, the ED_{50} substitution doses of a variety of narcotic agonists are the same when compared to either methadone or morphine cues when both drugs are matched for equal potency (Table 2). Thus, morphine and methadone cues appear similar when compared peripherally, but not when compared centrally. The fact that morphine will elicit methadone-like responding (Fig. 5) gives further support that the morphine-induced cue or its ability to generalize to other narcotic agonists is in part generated via the dorsal raphe nuclei.

Electrical stimulation of the dorsal raphe as a discriminative cue

Another approach to studying mechanisms of drug action is to mimic a drug cue by the electrical stimulation of specific brain areas. In the experiments reported in the previous section, it was possible to

Table 2. Morphine- and methadone-induced cues: generalization to other narcotic agonists

Narcotic studied	ED_{50} doses of various narcotic drugs Rats trained to discriminate morphine (3 mg/kg) $N = 18$	Rats trained to discriminate methadone (1.5 mg/kg) $N = 18$
Morphine	1.3 (0.73–2.2)	1.4 (0.87–2.2)
Methadone	0.6 (0.39–0.91)	0.6 (0.34–0.85)
Codeine	6.6 (4.7–9.1)	7.5 (4.7–11.9)
Propoxyphene	7.0 (3.4–12.1)	8.6 (5.6–13.2)
d-Methadone	12.3 (6.8–22.4)	9.1 (4.8–17.3)
l-Methadone	0.4 (0.27–0.65)	0.5 (0.38–0.76)
l-Acetyl-methadol	0.5 (0.30–0.84)	0.6 (0.38–0.85)

* All drugs were administered (s.c.) 30 min prior to 25 min test session. ED_{50} values were determined using the Litchfield and Wilcoxon method (1949).

Table 3. Effects of electrolytic lesions of the dorsal raphe nucleus on morphine-induced-antinociception and the morphine cue

	Control $N = 6$	Lesioned $N = 6$
Tail-flick* latency % MPE	59 ± 12	7 ± 11
Morphine cue % Correct responses	81 ± 7	88 ± 9
Saline cue % Correct responses	7 ± 2	9 ± 12

* Tail-flick latencies measured 120 sec after 10 mg/kg morphine, s.c.

mimic the morphine or LSD cue by injecting drugs directly into the dorsal raphe. Thus, it would be logical to attempt to accomplish the same generalization by stimulating the raphe electrically.

In this experiment rats were initially trained to discriminate between electrical stimulation of the dorsal raphe and the non-stimulated state (Hirschhorn, Hayes and Rosecrans, 1975). Rats learned the discrimination within 40 sessions and drug substitution studies were then commenced. The first experiments indicated that rats trained to detect raphe stimulation perceived LSD (75–100 μg/kg) as the stimulated state but not morphine. This experiment was important as it was the first demonstration that a drug state could be mimicked by stimulating a brain area.

Replication of this effect was not successful. Morphine and LSD produced a partial substitution but it was not possible to mimic completely the raphe stimulation by LSD administration. This was not surprising since, besides 5-HT containing neurons, the dorsal raphe nucleus contains other amine-containing neurons and the probability of hitting the exact area may be low. However, the initial demonstration that a drug state could be produced by electrical stimulation is important and suggests that this approach should be pursued more vigorously.

Altering drug cues by brain area lesions

If a drug is producing a cue by acting at a specific brain area site then it should be possible to alter the cue (enhance or inhibit) by lesioning that site. Two approaches were used. In the first, electrolytic lesions were produced in the raphe nuclei to see if the morphine cue could be altered. Chance, Krynock and Rosecrans (1978) noted that medial raphe lesions significantly reduced morphine-induced antinociception. One study was conducted in rats trained to detect morphine (3 mg/kg) but no effects on the cue were observed (Krynock and Rosecrans, unpublished observations). More recently a second study was made and this time the dorsal raphe nucleus was lesioned (Table 3). This lesion reduced the analgesic effect of morphine, but again did not alter the strength of the morphine cue. This data is difficult to understand especially since the dorsal raphe appeared to be extremely sensitive to morphine (Fig. 4). However, this may also point to the redundancy in the brain and the fact that the dorsal raphe may be only one site at which morphine may be acting.

The second approach used in this series of experiments involved the production of neurotoxic lesions in neonate rats (Elchisak and Rosecrans, 1979). In this procedure rats are injected intracisternally with 6-OH dopamine (150 μg) at 14 days. When given alone, both brain norepinephrine (NE) and dopamine (DA) are depleted when these animals become adults. By administering either DMI (30 mg/kg) 30 min before 6-OHDA or by giving low doses of 6-OHDA (50 μg) at days 1 and 7 selective depletion of DA or NE can be achieved. The initial research indicated that morphine-induced antinociception could be significantly reduced in DA-depleted rats and thus, this research technique was incorporated into the drug-induced cue studies.

The major experiment involved studying (−)-amphetamine (0.9 mg/kg), morphine (4 mg/kg) and nicotine (400 μg/kg) cues in rats depleted of either brain dopamine (60–70%) or norepinephrine (Rosecrans, Chance and Schechter, 1976; Spencer and Rosecrans, 1977). The findings obtained were as follows:

1. Dopamine-depleted rats learned the morphine and *d*-amphetamine cue faster than control rats. These animals did not appear more sensitive pharmacologically, and it was suggested that these animals learned the cue faster because they were less disrupted behaviorally as a result of a reduction in sensitivity to DA neurons.

2. The nicotine cue was not altered unless central generalization experiments were conducted (Table 5). In these studies, control rats trained to detect 400 μg/kg nicotine (s.c.) responded as if they were given nicotine when 4 μg was injected in the dorsal

Table 4. A summary of drug-cue experiments conducted in dopamine (DA↓) and norepinephrine (NE↓) depleted rats

	Control	DA↓	NE↓
Brain DA/NE* ± SE	3.8 ± 0.3	1.7 ± 0.3	5.4 ± 0.4
Nicotine† ED_{50} dose (μg/kg)	85	97	142
Mecamylamine‡ AD_{50} dose (μg/kg)	690	390	492
d-Amphetamine§ ED_{50} dose (μg/kg)	221	308	426
d/*l*-Amphetamine ED_{50} dose-relationship	1.5	1.5	0.63

* The DA/NE ratio was determined in 18 rats treated similarily with 6-OHDA as described.

† ED_{50} doses were calculated from the regression lines, probit vs log dose.

‡ The antagonist dose which reduce the nicotine cue to 50% was calculated as were ED_{50} calculations.

§ *d*/*l*-Amphetamine ratio was accomplished by comparing ED_{50} values obtained for each isomer under each experimental condition.

hippocampus. This generalization did not occur in the DA-depleted rat. Thus, this experiment indicated that DA was involved in how the nicotine cue was generated, but also indicated that the hippocampus was not the only site at which nicotine was acting, especially since DA-depleted rats learned the nicotine cue.

3. Lastly, the potency of the (+)- and (−)-amphetamine cues were significantly altered by the neonatal depletion of NE neurons (Table 5). Interestingly, the percentage discrimination was reversed. Thus, the (+)-isomer was almost twice as potent in DA-depleted control groups than its enantiomer, whereas the (−)-amphetamine cue was more potent than the (+)-isomer in the NE depleted group. This finding supports at least a partial role for NE in the (+)-amphetamine cue even though it can be readily antagonized by the dopamine antagonist haloperidol (Colpaert *et al.*, 1978).

The use of selective neurotoxins to alter drug cues appeared to be the most profitable in terms of evaluating how drugs may be affecting the brain. The problems encountered with the cannulae (except for morphine), the lipid solubility of drugs, and the non-specificity of electrical stimulation present many problems. Besides the results discussed, an additional finding should be mentioned. In addition to evaluating the nicotine cue in amine-depleted rats, the ability of mecamylamine to antagonize the nicotine-cue in the same animals was also evaluated. As indicated in Table 5, the AD_{50} values for mecamylamine were reduced in both DA-depleted and NE-depleted rats. This indicates that the amine depletion may have reduced the number of nicotinic receptors and thus, less mecamylamine was needed to reduce the nicotine cue. This observation may be extremely helpful in evaluating drug cues in rats depleted of various amines. An analogous experiment is being performed with LSD and 5-OMeDMT in which the dorsal and medial raphe is lesioned with 5,7-dihydroxytryptamine, a selective 5-HT neurotoxin. Thus, with the use of such lesions it should be possible to determine sites of LSD action when studied in conjunction with the 5-HT antagonists, such as BC-105(pisotifen).

COMPARING PERIPHERAL RECEPTOR DATA WITH CENTRAL EFFECTS

Steinsland and Heible (1978) have recently demonstrated a similarity between the interaction of dopamine antagonists (e.g. phenothiazines, butyrophenones, etc....) with a peripheral DA receptor system (rabbit ear artery) and their ability to bind to high-affinity binding sites in brain homogenates. Glennon (1979) has found a similarity between the 5-HT receptor affinities of a small series of tryptamine analogs (using a peripheral rat fundus preparation) and their brain binding data as reported by Whitaker and Seeman. Perhaps there is a certain amount of congruency between particular peripheral receptor systems and central binding (receptor ?) sites.

The blocking experiments described above suggest that the behavioral effects of LSD and 5-OMeDMT are mediated, at least in part, by a serotonergic mechanism. Studies with the rat fundus preparation have led to the conclusion that the most behaviorally active tryptamines and phenalkylamines possess high 5-HT receptor affinity while lesser active or inactive agents possess lower affinities (Glennon and Gessner, 1975; Glennon, Liebowitz and Mack, 1978). Furthermore, the 5-HT receptors of this preparation demonstrate stereoselectivity with respect to affinity. These findings suggested an investigation as to whether or not the 5-OMeDMT stimulus would generalize to the stimulus produced by DOM (STP), a phenalkylamine or amphetamine analog which possesses an affinity very similar to that of 5-OMeDMT. Generalization was found to occur in a dose-related manner (Glennon *et al.*, 1979). Generalization studies are now in progress using other tryptamine and related analogs for which 5-HT receptor affinities have already been determined (Fig. 7).

It might be noted that affinities (pA_2 values) are determined by treating these compounds as antagonists of 5-HT. While these compounds are all partial agonists, i.e. mixed agonist-antagonists, antagonism appears to be competitive. Whether or not the structure-activity (affinity) relationships (SAR) of these compounds as competitive antagonists in the peripheral receptor assay parallels their behavioral (agonistic ?) effects remains to be determined.

SOME OBSERVATIONS AND COMMENTS

What has been attempted in this review is to describe some of the approaches which have been used to study drug mechanisms where drug cues are employed as the pharmacological model of a psychoactive drug effect. The experience gained has led to decisions as to how future studies will be conducted, especially in attempting to evaluate sites of drug action. The use of neurophysiological and central drug injection techniques has been less successful mainly because a better technology has not been developed. The strategies proposed are workable, all that is needed are new and fresh approaches.

The peripheral drug research will focus on drug substitution and antagonism studies employing some of the experimental approaches already described (Figs 1 and 2). In addition, the medicinal chemist will play an important role in designing new compounds which can be applied in behavioral studies. For studying CNS sites of drug action experiments will be designed to alter drug cues employing specific neurotoxin lesions. This technique, in conjunction with properly designed substitution and antagonism experiments, will enable sites of drug action to be localized. Other more sophisticated neurophysiological experiments such as stimulating or recording electrical activity from such sites can then provide more specific

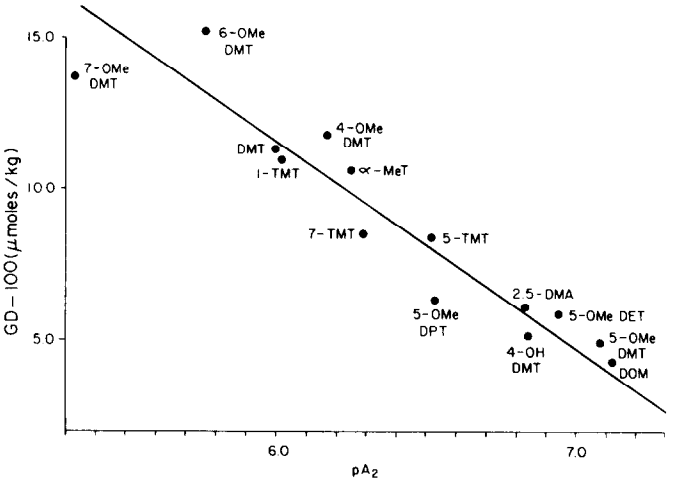

Fig. 7. A correlation between the discriminative stimulus (DS) properties and pA_2 values of a series of tryptamine and phenylisopropylamine analogs. The GD-100 value represents the equivalent dose at which each compound generalized with the training dose of 5-OMeDMT (1.5 mg/kg) when it was used as the DS. Each drug was administered at various doses 15 min prior to being placed in the operant chamber for a 15 min test session (see Methods).

information. Central chemical injection techniques will also be of great assistance at this stage.

Finally, a comment should be made concerning what the drug cue is measuring. As was pointed out earlier, LSD substitutes for the drug quipazine which does not appear to produce hallucinations in human subjects. Thus, a major issue is whether, in fact, drug cues are good measures of a drug's CNS actions which can be translateable to effects in man. The hypothesis generated in these laboratories suggests that in the case of LSD, 5-OMeDMT and quipazine we are not measuring hallucinogenic potency but serotonin agonist potency. Thus, all drugs substituting with LSD would not be expected to be hallucinogens, only that they are 5-HT agonists. Therefore, the drug cue may be basically a direct measure of the interaction between drug and central pharmacological receptor. It may be that drugs have the potential of acting on more than one receptor and this action is most likely dose- and time-dependent. Thus, drug cues may provide a good measure of CNS agonist-receptor interactions much like the rat fundus can provide important information concerning the effects of drugs on the serotonin receptor (Glennon and Gessner, 1979).

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