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Some Methodological Problems in Assessing Dependence-producing Properties of Drugs in Animals

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THE ultimate purpose of an animal test for dependence-producing properties of drugs is to provide information which is helpful in predicting the possibility of abuse and the kinds of social and individual harm to be expected when a drug is abused. For predictive purposes, it is obvious that information on dependence-producing properties alone is not satisfactory, and that information on physicochemical characteristics, pharmacodynamics (particularly pharmacological profiles and psychotropic effects), general and specific toxicities, and pharmacokinetics is necessary. In the case of most therapeutic drugs, this information should usually be available through a number of preclinical and clinical tests. Therefore, only a few tests which are specific to dependence-related properties of a drug may be needed.

Reliability of Animal Tests in Assessment

The dependence-related properties of a drug to be explored in the specific tests are the ability to function as a reinforcer and maintain drug-seeking behavior, the ability to produce physiological dependence and also tolerance, the severity of the withdrawal syndrome and whether it intensifies the drug-seeking behavior of the animal, and, finally, psychotoxic effects at self-administered dose levels (6). The reliability of animal test results depends on the availability of adequate laboratory methods to explore the above effects. Inasmuch as it is difficult to cover all the effects by any single method, comprehensive methods may be necessary.

As in many other animal tests with drugs, species differences are an important problem in tests of dependence-related properties of a drug, and the similarity of animals to man in pharmacodynamic susceptibility and pharmacokinetics greatly influences reliability. For example, it is well known that single doses of morphine in mice or repeated doses of morphine in rats stimulate locomotor activity, but overexcitation never has been a psychotoxic effect of its nonmedical use in man. Other examples are the findings in intravenous and intragastric self-administration studies with alcohol or barbiturates that the rhesus monkey will self-administer the drug to the point of anesthetization, like man, but the rat will not (1, 2, 5). Species differences in pharmacokinetics may also influence the results of tests for other dependence-related properties of a drug, since the quality, intensity, and continuity of the pharmacological effects of drugs play crucial roles in the development of tolerance and physiological dependence on a drug. The higher species of animals such as nonhuman primates are generally believed to be more similar to man in this regard. This is not always the case, however, and the logical choice of species should ideally be determined according to the characteristics of the species in pharmacodynamic susceptibilities and pharmacokinetics. In practice, since the choice of species is considerably limited by the availability of

laboratory methods of testing for the dependence-related properties of a drug, disagreement over the best-suited species and the availability of methods can arise.

The importance of dose-regimen cannot be overstressed as a factor influencing reliability. One of the most noteworthy pharmacological characteristics in the nonmedical use of a drug is the variant use of different drugs regarding route of administration, dose, and dose-intervals; these are determined by the users themselves. Thus, it becomes important to consider carefully the dose-regimen for animal tests according to this variant use. For example, a number of experiments demonstrate that while the short-acting barbiturates do not produce marked physiological dependence by infrequent daily doses for a considerably long period of time, they will produce marked physiological dependence by frequent daily doses (3, 4, 9). It was because of such dose-regimen differences that meperidine was believed to possess only weak or even no dependence liability when it was first introduced. Thus, the use of an inadequate dose-regimen may result in wrong predictions.

In the past, clinical investigators were disappointed by the animal data on the dependence liability of some drugs and may have considered animal tests unreliable. In most cases the disappointment seems to be attributable to conclusions erroneously arrived at on the basis of insufficient or narrow sighted experiments and it is my belief that today the results of animal tests are highly reliable as long as the factors influencing reliability are well managed.

Experimental Criteria for Development of Psychological Dependence in Laboratory Animals

Concerning the term psychological dependence, there have been repeated discussions on such questions as "What is psychic or psychological dependence?", "Is the term necessary?", "How can we demonstrate that state under experimental conditions?", "Would it not be better replaced by the term reinforcement?", and so forth. I will not attempt to answer these questions or criticize these discussions in the present paper, but, instead, will simply consider psychological dependence from an experimental viewpoint. For prediction of psychological dependence potential, it is obvious that the determination of the reinforcing properties of a drug is important, but it is not the only factor to be considered, since the reinforcing effect of a drug, by itself, is neither socially harmful nor self-destructive. For this reason, I have been concerned about the following criteria for the experimental development of psychological dependence:

A. to find out whether animals manifest overt signs of drug effects repeatedly day to day when a certain drug is freely available without time or dose limitations, particularly in animals that have no history of drug self-administration at the beginning of the experiment;

B. to find out whether intensive drug-seeking behavior for a certain drug is demonstrable in animals after they experience self-administration of that drug.

To satisfy these criteria seemed to be essential in laboratory work for the prediction of psychological dependence potential. For this reason, in the early phase of our self-administration studies we used the continuous self-administration procedures without time or dose limitations in the rhesus monkey, especially in drug-naive monkeys, and tried to observe a typical state of psychological dependence that met these criteria. The practical value of the self-administration experiments for predicting the psychological dependence potential was indicated in our early phase of studies when the state of psychological dependence which fulfills criterion A was observed in laboratory animals for the first time in the history of research of this kind. The drugs involved were principle drugs of abuse such as morphine, codeine,

cocaine, *d*-amphetamine, pentobarbital and alcohol (2). There have been discussions as to whether or not these early studies conclusively demonstrated the positive-reinforcing effect of the drugs because of the rather naive experimental procedures employed and the development of physiological dependence on some of these drugs during the course of the studies. Regardless of these discussions, we still believe that this approach is the most valid and essential way of producing a state in laboratory animals that is analogous to human drug abuse.

It was difficult in the case of laboratory animals to demonstrate criterion B. The most promising method has been a progressive ratio technique. With this technique, monkeys were trained to self-administer a test drug under a schedule where 50 lever-pressing responses were required to produce an intravenous injection of the drug (a 50-response fixed-ratio schedule). Subsequently, the response requirement was increased progressively after each injection of drug from the initial ratio of 50 to 100, 150, 200, 300, 400, 600, 800 and so on until monkeys were said to have terminated self-administration of the drug when no intake was observed for 48 hr. Then saline solution was substituted for the drug solution and the response requirement was reduced to one response per injection for 2 to 8 weeks until response rate decreased significantly. This procedure was then repeated with the Latin square design. The preliminary results obtained at The University of Michigan were reported at the 23rd International Congress of Physiological Sciences which was held in Tokyo in 1965 (7). Some of these preliminary results with several drugs are summarized in table 1. Although the experiment was found to be in need of improvement, the final ratio attained with many of the drugs was high enough to say that intensive drug seeking behavior for certain drugs was demonstrated in some monkeys.

Although the analysis of the reinforcement phenomena in the interactions between drugs and subjects is very important and critical for understanding and analyzing the phenomena of psychological dependence, self-administration experiments can also give information on behavioral, toxicological, and drug metabolic changes which develop as a result of free drug intake. This information is as important for the prediction of dependence potential as information on the reinforcing effects of drugs because, from a practical viewpoint, our concern is not limited to the likelihood that a drug is to be abused, but also to the social and individual consequences when the drug is abused. The information necessary for prediction should be collected as much as possible not only by self-administration experiments but also by many other types of experiments. However, only the self-administration experiment can tell us about the self-determined dose regimen which is the most essential determinant for

TABLE 1

*Preliminary results on the progressive ratio test by intravenous self-administration of drugs in rhesus monkeys (7)**

Monkey	Morphine (2.5 mg/kg/inj.)	Alcohol (0.8 mg/kg)	Pentobarbital (15.0 mg/kg)	Cocaine (0.5 mg/kg)	*d*-Amphetamine (0.25 mg/kg)	Caffeine (2.5 mg/kg)
#930 (F) 5.0 kg	4,800	2,400	1,600	6,400	600	800
#982 (M) 3.4 kg	600	1,100	800	3,200	1,600	1,600
#1006 (M) 5.9 kg	150	300	100	1,600	1,100	150
#1022 (M) 4.0 kg	600	600	1,100	1,600	200	150
#1023 (M) 3.2 kg	3,200	1,100	1,100	3,200	3,200	50
#1041 (M) 3.3 kg	300	800	1,100	1,100	1,600	0

* Each number shows the maximal number of lever presses for one dose of drug.

any experiment. From this viewpoint, I should like to stress the primary importance of the continuous self-administration procedures without time or dose limitations in testing the dependence potential of drugs.

Dependence Tests Being Conducted at CIEA Japan

Three main categories of tests are currently being conducted as dependence tests at the Central Institute for Experimental Animals in Japan. The first is dose-range determination and acute tests on the central nervous system and toxic effects of the drug. Basically these experiments are conducted to find out the minimal effective dose, the plateau dose level, the toxic dose level, and a profile of pharmacological effects and duration of effects at each dose level. All routes of administration which are indicated for clinical use and expected for laboratory use are utilized.

The second category of experiments is on physiological dependence potential and in this regard two or three experiments are performed. One is the so-called single dose suppression or cross physical dependence test in which monkeys physically dependent on morphine or barbital are withdrawn; when clear cut withdrawal signs develop a single dose of the test drug is given to the animal and it is observed whether or not withdrawal signs are suppressed. If the drug suppresses the withdrawal signs, the minimal dose required for complete suppression with a certain route of administration is determined for comparison with standard drugs.

Another type of experiment is the precipitation test which is used with analgesics that do not suppress morphine withdrawal signs. This test, which is conducted in morphine dependent and non-withdrawn subjects, is very sensitive for detecting partial morphine-antagonist properties of a drug, and many drugs which do not antagonize morphine in other pharmacological experiments are found to precipitate morphine withdrawal signs. Some results obtained by these tests on several analgesics are shown in table 2.

The third type of experiment is a test for physiological dependence. Naive monkeys receive repeated administrations of the drug one to four times a day for 4 to 12 weeks. Abrupt withdrawal or precipitated withdrawal tests with morphine antagonists are then conducted periodically for observation of the withdrawal syndrome (table 3). Development of tolerance is also examined within the range of gross behavioral observations. Determinations of blood concentration of the drug are occasionally conducted when needed. When frequent administration of a drug is necessary, then the programmed administration technique is used.

The third category of experiments is related to self-administration. The routine procedure is the continuous intravenous or intragastric self-administration of the drug. The intravenous route is the first choice if the drug is water soluble. Sometimes thin solvents such as ethanol, acid buffer or polyethylene glycol are used as vehicles. At the beginning we use a couple of monkeys with a history of self-administering a standard drug and discriminating

TABLE 2

Single dose substitution and precipitation tests in morphine dependent monkeys [3 mg/kg × 4 per day sc (6)]

Drug	Withdrawal Signs		Dose (mg/kg) for Complete Suppression
	Suppression	Precipitation	
Morphine	+	–	3.0 sc
Methadone	+	–	3.0 sc
Oxymethebanol	+	–	3.0 sc
Meperidine	+	–	10.0 sc
Codeine	+	–	16.0 sc
d-Propoxyphene	+	–	16.0 sc
Thebaine	–	+	
Azabicyclane	–(+)*	+	
Propiram	–(+)	+	
Pentazocine	–(+)	+	
Naloxone	–(–)	+	
Saline	–(–)	–	

* ( ) = Suppression in monkeys dependent on low dose of morphine (0.3 mg/kg × 4 per day sc).

TABLE 3

Development of physiological dependence on analgesics (6)

Drug	Route	Dose (mg/kg) × times per day) for 31 days	Grade in Natural Withdrawal*
Morphine	sc	3 × 4	Severe
Oxymethebanol	sc	4 × 4	Severe
Meperidine	sc	5 × 4	Severe
Codeine	sc	16 × 4	Severe
	po	128 × 4	Intermediate
Thebaine	iv	self 1 × 10–35	Severe
Azabicyclane	sc	20 × 4	Severe
d-Propoxyphene	sc	10 × 4	Intermediate
Propiram	sc	16 × 4	Intermediate
Pentazocine	sc	6 × 4	Mild

* Graded by Seevers' criteria (1936).

between the effects of a standard drug and saline; our previous studies have shown that the experienced monkeys are more susceptible to the reinforcing effects of test drugs. First, the vehicle alone (usually saline) is used for the first 7 to 14 days to determine whether the baseline level of responding is low enough to initiate the drug trial. Then, the vehicle is replaced by the testing drug at an injection dose of a quarter to one half of the minimal effective dose; 2 to 4 weeks later, if the monkey is found not to be self-administering the drug at a higher rate than that for vehicle, the dose is increased two to four times and the observations continue for another 2 to 4 weeks. If the animal still does not increase its rate of responding, then a programmed-administration schedule is superimposed on the self-administration schedule for 2 weeks and the response rate during and after this period is observed. After that, the dose is decreased by a quarter to half of the initial dose to determine whether response rate increases. Finally, the monkeys are exposed to the vehicle alone and the process of extinction and possible manifestations of a withdrawal syndrome are observed. During the first exposure to a drug, if significant increases in response rate are observed, observation is continued for more than 8 weeks, and the daily pattern of self-administration and severity of the drug effects are periodically recorded. In this case, changes in the injection dose and programmed administration of drug are not used. If a drug is found to maintain high levels of responding in the first two monkeys studied, then two to four naive monkeys are used for subsequent experiments with the same procedures. If not, two or four experienced monkeys are added and the same experiments are repeated. Some results obtained by this test on several sedative hypnotics are shown in table 4.

A second type of self-administration test consists of a cross self-administration experiment, which is used by many investigators, and is also known as the substitution test. In this test, the drugs are limited to those which can be administered intravenously. We have never tried this method for intragastric self-administration, under the assumption that the slow absorption rate of the drug may obscure the results. The third type of self-administration test is the progressive ratio test described earlier. This test is used when a quantitative assay of the reinforcing effect is needed for a particular drug. When a drug is found to produce substantial physiological dependence, the progressive ratio test is also used to determine the influence of physiological dependence on the reinforcing effects of the drug. To describe briefly the experimental procedure, the monkeys are trained to self-administer *l*-1,2 diphenyl-1-di-

TABLE 4

Psychopharmacological profile of dependence liability of some hypnotic-antianxiety agents (10)

Drugs	Cross Physiological Dependence		Physiological Dependence Producing Capacity	Drug Behavior Reinforcing Capacity	Behavioral Manisfestation During Self-administration
	Capacity	Complete Suppression (mg/kg)			
Barbital	+	75 po	++	Untested	Untested
Pentobarbital	+	>25 iv	++	++ (iv, ig)	Self-anesthesia
Alcohol	+	4,000 po	++	++ (iv, ig)	Self-anesthesia
Chloroform	+		Untested	++ (Inhale)	Self-anesthesia
Meprobamate	+	>200 po	++	Untested	Untested
Diazepam	+	5 po	++	+ (iv)	Ataxia
Chlordiazepoxide	+	20 po	++	+ (iv, ig)	Ataxia
Oxazolam	+	20 po	+	+ (ig)	Sedation
Chlorpromazine	–	–	–	– (iv)	–
Benzoctamine	–	–	–	+ (ig)	–
Perlapine	–	–	–	– (ig)	–

TABLE 5

Progressive ratio test on morphine, cacaine and pentazocine in rhesus monkeys (6, 8)

Drug mg/kg/inj.	Monkey	Pretreated with Test Drug (A)	Pretreated with Saline (B)	A/B Ratio
Morphine, 0.5	#174*	1,600†	1,600	1
0†	#234	12,800	1,600†	8
	#248	12,800†	6,400	2
	#254	6,400	200†	32
Cocaine, 0.11	#281	800	1,600†	1/2
	#334	3,200†	6,400	1/2
Pentazocine, 0.25	#171	3,200	6,400†	1/2
	#364	3,200†	3,200	1
	#412	3,200	6,400†	1/2

* Self-maintained dose level of morphine was very low.

† Tested first.

methyl-aminoethane-HCl (SPA) under a 100-response fixed-ratio schedule with a 15 min time-out period after each injection. The monkeys then receive saline or the test drug by programmed administration for 4 weeks. Self-administration is not conducted during this period. After this period, the monkeys are allowed to self-administer the test drug again for 24 hr under the 100-response fixed-ratio schedule with the 15 min time-outs. Then the response requirement is doubled after every 4 to 16 injections. The number of injections before an increase in response requirement is low for long acting drugs and high for short acting drugs. Some results obtained by these procedures are shown in tables 5 and 6. Note that with morphine (table 5) and alcohol (table 6) high response requirements are often at-

TABLE 6

Progressive ratio test on alcohol in rhesus monkeys

Drug and Dose	Monkey	Maximal No. of Lever Presses for 1 Dose	
		Pretreated with alcohol	Pretreated with saline
Alcohol	#171 M	3,200	3,200
0.8 g/kg/inj.	#425 M	6,400	6,400
	#466 F	6,400	1,600
	#485 F	6,400	3,200

tained after monkeys have received programmed administrations of drug for 4 weeks (pretreatment) which allows the development of physiological dependence. We are presently exploring by computer an automatically-shifting response requirement as a function of the response rate during the preceding experimental period.

Other Tests Conducted at CIEA in Japan

In addition to the experiments described in this paper, several of our other laboratories, such as biochemistry, general pharmacology, behavioral pharmacology, and pathology and toxicoloty, participate in the testing for dependence potential. On many occasions, independent experiments are conducted in order to provide information necessary for prediction of abuse potential. In most cases, these studies are designed to explore the biological effects of the drug in different species, including man.

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