Pharmacology Biochemistry & Behavior, Vol. 32, pp. 607–611. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Reinforcing and Discriminative Stimulus Effects of Ca-Acetyl Homotaurine in Animals

K. A. GRANT[1] AND W. L. WOOLVERTON[2]

Drug Abuse Research Center, The University of Chicago, Department of Psychiatry
5841 S. Maryland Avenue, Chicago, IL 60637

Received 12 August 1988

GRANT, K. A. AND W. L. WOOLVERTON. *Reinforcing and discriminative stimulus effects of Ca-acetyl homotaurine in animals*. PHARMACOL BIOCHEM BEHAV **32**(3) 607–611, 1989.—Ca-acetyl homotaurine (Ca-AOTA) has been proposed as an adjunct for ethanol detoxification. The purpose of the present experiment was to determine whether Ca-AOTA would be predicted to have abuse potential. Rhesus monkeys that were experienced in the intravenous self-administration of cocaine (n = 2) or pentobarbital (n = 2) were given the opportunity to self-administer various doses of Ca-AOTA or its vehicle (0.9% saline). Ca-AOTA (1.0–10.0 mg/kg/injection, intravenously) was not self-administered above saline levels. The discriminative stimulus effects of Ca-AOTA were evaluated in a drug discrimination procedure in which animals were trained to make one response after a training drug and a different response after saline. Rhesus monkeys trained to discriminate *d*-amphetamine (n = 3) or pentobarbital (n = 3) from saline were tested with doses of Ca-AOTA ranging from 10 to 100 mg/kg (PO by nasogastric tube) and at 3 different pretreatment times (1, 2, or 4 hr). Ca-AOTA failed to engender drug-appropriate responding at any dose or pretreatment condition in either group of monkeys. In addition, Ca-AOTA was tested in 4 pigeons trained to discriminate pentobarbital from saline. Ca-AOTA administration did not result in pentobarbital-appropriate responding in doses ranging from 30–300 mg/kg (IM) and pretreatment times ranging from 30 to 240 min. The lack of both reinforcing properties and discriminative stimulus properties similar to *d*-amphetamine or pentobarbital suggests that Ca-AOTA has little or no abuse potential.

Homotaurine Self-administration Drug discrimination Monkeys Pigeons

ADMINISTRATION of Ca-acetyl homotaurine (Ca-AOTA), a taurine derivative and a gamma amino butyric acid (GABA) receptor ligand, has been shown to decrease voluntary ethanol consumption in rats (1,3). Decreased ethanol consumption using a preference procedure was found in rats that have been chronically treated with ethanol and those without chronic treatment (9). In addition, daily administration of Ca-AOTA has been reported to increase the percentage of alcoholics abstaining from ethanol 6 months after they had been weaned from alcohol (11). These reports have prompted some investigators to propose the use of Ca-AOTA as an adjunct in treatment of alcoholism (11).

The purpose of the present series of experiments was to determine the effects of Ca-AOTA in two behavioral procedures that have been used to predict the abuse potential of psychoactive compounds. The first experiment investigated the reinforcing properties of Ca-AOTA in a self-administration procedure. Self-administration procedures make drug delivery contingent upon the animal emitting a specific behavior, or a series of behaviors. There is a strong correspondence between drugs that are self-administered by animals and those that are abused by humans (8). The second experiment investigated the discriminative stimulus properties of Ca-AOTA using a drug discrimination procedure. Drug discrimination procedures require the animal to respond on one lever after receiving a training drug and on a different lever after receiving the drug vehicle (2). The discriminative stimulus properties of the drug determines whether the animal responds on the drug-associated lever or the vehicle-associated lever. If the animal responds on the drug-associated lever after receiving a novel compound, the compound is said to have discriminative stimulus properties similar to the training drug. There is a strong relationship between the subjective effects of drugs reported by humans and the discriminative stimulus properties of drugs in animals (8,13). Therefore, a test drug that shares discriminative stimulus properties with a drug of abuse may also share subjective effects. To the extent that subjective effects play a role in abuse potential, drug discrimination may be a predictor of drug abuse.

[1]Present address: Unit for Special Projects, National Institute on Alcohol Abuse and Alcoholism, 12501 Washington Ave., Rockville, MD 20852.
[2]Requests for reprints should be addressed to W. L. Woolverton, Ph.D., Drug Abuse Research Center, Department of Psychiatry, The University of Chicago, 5841 S. Maryland Ave., Chicago, IL 60637.

METHOD

Self-Administration

The subjects were one male and three female rhesus monkeys *(Macaca mulatta)* that weighed between 3.8 and 8.4 kg. The monkeys had previous experience with intravenous (IV) self-administration of stimulants and anxiolytics. Each monkey was fitted with a stainless-steel restraint harness and spring arm that was attached to the rear of the experimental cubicle (70 cm wide × 84 cm deep × 80 cm high) in which the monkey lived for the duration of the experiment. Two response levers (BRS/LVE, PRL-001, Beltsville, MD) were mounted on the inside front of each experimental cubicle 10 cm above the floor and a food dish was mounted between them. Four jewelled stimulus lights, two red and two white, were mounted directly above each lever. In addition, two houselights, one white (34-W) and one red (15-W), were mounted on the ceiling of the cubicle and covered with translucent Plexiglas. Drug injections were delivered by a peristaltic infusion pump (Cole-Parmer Co., Chicago, IL). All programming and recording of experimental events were accomplished by solid state equipment located in an adjacent room. The monkeys were fed 150 to 200 g of monkey chow after each session and given a chewable vitamin tablet 3 days/week.

Each monkey was prepared surgically with a chronic indwelling venous catheter under pentobarbital anesthesia [approximately 30 mg/kg, IV; see (7)]. Experimental sessions, signalled by the illumination of all white lights, were 2 hr in length and were conducted 7 days a week. During baseline sessions the animals were allowed to press the right lever to receive IV injections of cocaine (0.03 mg/kg/injection; monkeys 5024 and 5025) or pentobarbital (0.3 mg/kg/injection; monkeys 4002 and 5019) under a schedule requiring 10 lever presses per injection (fixed-ratio 10; FR 10). During injections the white lights were extinguished and the red lights were illuminated. After the rates of responding became stable under these baseline conditions (less than 10% variation in total number of injections per session for at least three consecutive sessions), 0.9% saline was substituted until responding declined to low, stable levels (3–10 sessions). Subsequently, the animal was returned to baseline conditions for 1 to 2 sessions to ensure that responding approximated previous levels. When responding was again stable under baseline conditions, a dose of Ca-AOTA was made available (substituted) for intravenous self-administration during at least the same number of sessions that had been required for responding for saline to decline, and until there was neither an increasing nor decreasing trend in total injections per session.

Four doses of Ca-AOTA, ranging from 1.0–10.0 mg/kg/injection, were substituted for the baseline drug in each monkey in a mixed order with baseline conditions reinstated between doses of Ca-AOTA. The number of injections over the last two sessions of a Ca-AOTA substitution period was used in data analysis. These values were compared to the same values for the last two sessions of the corresponding saline substitution period. A dose of Ca-AOTA was considered to be a positive reinforcer in a particular monkey if the mean number of injections for the last two sessions of a test period exceeded the mean value for saline injections, and the ranges did not overlap.

Drug Discrimination

Monkeys. The subjects were one female and five male rhesus monkeys *(Macaca mulatta)* that weighed between 6.5 and 12.1 kg. All monkeys had previously participated in studies of IV drug self-administration and drug effects on schedule-controlled responding. Additionally, they had extensive experience with the present drug discrimination procedure before starting this experiment. They were housed individually in stainless-steel cages in which water was available continuously. They were fed 150 to 200 g of monkey chow after each session and were given a chewable vitamin tablet 3 days/week. During experimental sessions the monkeys were seated in a Plas-Lab restraining chair and placed in a wooden cubicle (175 cm high × 85 cm wide × 65 cm deep) containing two response levers mounted 110 cm above the floor. A 34-W white houselight was mounted on the ceiling. The monkey's feet were placed into shoes, the bottoms of which were fitted with brass plates which could deliver electric shocks. All programming and recording of experimental events were accomplished by solid state equipment located in an adjacent room.

The monkeys had been trained previously to discriminate *d*-amphetamine (7037, 7039, 8002) or pentobarbital from saline (1006, 2036, 9067) in a two lever, discrete trial procedure to avoid electric shock (4). One hour after an intragastric infusion of the training drug (0.56–1.0 mg/kg amphetamine or 10.0 mg/kg pentobarbital) or saline, the houselights and lever lights were illuminated (trial) and responding on one lever (the correct lever) avoided electric shock and extinguished the lights. Responding on the incorrect lever started a 2-sec change over delay during which correct lever responding had no consequence. If a correct response was not made within 5 sec of onset of the lights, shock (250 msec duration, 5.0 mA intensity) was delivered every 2 sec until a correct response was made. After a correct response, there was a 55-sec intertrial interval, after which a new trial began. The session lasted for 30 trials or 40 min, whichever came first. The correct lever was determined by the infusion that was administered before the session. For three monkeys the right lever was correct after drug infusions and the left lever was correct after saline infusions. This condition was reversed for the other three monkeys.

Monkeys were considered to be stable in the discrimination when more than 90% of the trials were completed on the correct lever for six consecutive sessions. At this point testing was begun with the training drug and Ca-AOTA. Two 5-day sequences alternated drug, vehicle and test sessions so that the first test session was preceded by two training sessions, one with saline and one with drug pretreatment and the second test session of the sequence was preceded by either vehicle or drug pretreatment. In the event that the criterion for stimulus control was not met during the training sessions, the training sequence was continued. During test sessions, both levers were operational, i.e., shock could be avoided by responding on either lever.

Saline and at least three doses of the training drug, three doses of Ca-AOTA, and three pretreatment times were evaluated under the test conditions for each monkey. The percentage of trials that were completed on the drug lever is presented for each test session. In addition, the average time between the onset of a trial and a lever press (average latency) was calculated for each test session.

Pigeons. The subjects were four White Carneaux pigeons maintained at 80% of their free-feeding weights and housed individually with water and grit freely available. Purina Pigeon Checkers were provided after the session to maintain stable body weights. The experiment was conducted in two ventilated custom-made operant chambers (inside dimensions 32 × 28 × 32 cm). The front and back panels were aluminum and the side walls were transparent plastic. Each chamber was equipped with two translucent response keys (2.5 cm diameter; G6315, Ralph Gerbrands Co., Arlington, MA) which were transilluminated during the experimental session by white 7-W lamps (IEE, Van Nuys, CA) located behind the keys. The keys were 12.5 cm apart and located approximately 24 cm above the floor of the chamber. Purina Pigeon Checkers were made available from a food magazine (G5610A, Ralph Gerbrands Co., Arlington, MA) centered be-

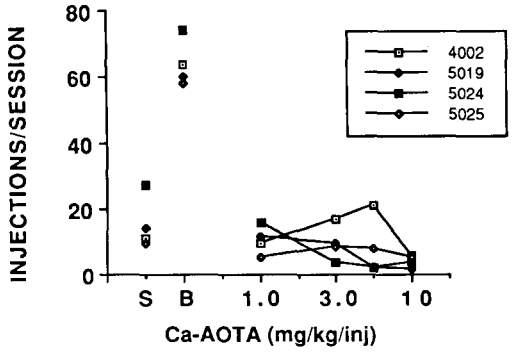

FIG. 1. Evaluation of the reinforcing effect of Ca-AOTA in rhesus monkeys. Each point represents the mean number of infusions of Ca-AOTA over the last 3 days of a period of substitution for each monkey. The points above B represent the grand mean for self-administration of the baseline drug (4002, 5019: 0.3 mg/kg/infusion pentobarbital; 5024, 5025: 0.03 mg/kg/infusion cocaine). The points above S represent the levels of saline intake. Ranges are omitted for clarity but were usually less than 10% of the mean.

TABLE 1

PERCENTAGE OF TRIALS COMPLETED ON THE DRUG-APPROPRIATE LEVER FOLLOWING DIFFERENT DOSES OF THE TRAINING DRUGS*

	Pentobarbital-Trained		
PB (mg/kg)	Monkey 1006	Monkey 2036	Monkey 9067
Saline	0	0	0
1.0	0	0	0
3.0	0	0	0
10.0	**100**	**100**	**100**
	d-Amphetamine-Trained		
AMPH (mg/kg)	Monkey 7037	Monkey 7039	Monkey 8002
Saline	0	0	0
0.03	(—)	0	3
0.1	(—)	97	0
0.3	0	(—)	97
0.56	**100**	100	**100**
1.0	100	**100**	100

*Bold lettering indicates the training dose (see text). (—) = not tested.

tween and below the response keys, 7.6 cm above the floor. This food magazine was illuminated during food delivery. A 6-W white houselight, located behind the back panel, provided indirect illumination during experimental session. Programming and data collection were accomplished using a Rockwell AIM 65 microcomputer and cumulative recorders (Ralph Gerbrands Co., Arlington, MA) which were located in an adjacent room.

The pigeons had been trained previously to discriminate 10.0 mg/kg pentobarbital from saline using a drug discrimination procedure described by Evans and Johanson (5) and had been tested with a variety of drugs. Ten minutes after an intramuscular injection of the training drug (10.0 mg/kg pentobarbital) or saline, the houselights and key lights were illuminated and responding on the injection-appropriate key under a FR 30 schedule resulted in a 3-sec access to food. Responding on the incorrect key reset the FR requirement on the correct key. Each session lasted until 50 reinforcers were delivered or until 30 min had elapsed, whichever occurred first. For pigeons 25 and 32 the left key was correct after PB administration and for pigeons 23 and 26 the right key was correct after PB. The opposite key was designated correct after saline injections. The drug-key-reinforcer relationship was maintained throughout the experiment. Training sessions were conducted 6 or 7 days a week. The injection preceding each session was selected from a pseudorandom sequence, with the restriction that no condition would occur for three or more consecutive sessions.

The pigeons were considered to be stable in the discrimination when the percentage of total responses on the correct key was above 90% and the number of responses emitted on the incorrect key before the first reinforcer was delivered was less than 30 for seven consecutive sessions. At this point, testing was begun with the training drug and Ca-AOTA. Throughout a test session, 30 consecutive responses on either the pentobarbital-appropriate or saline-appropriate key resulted in food delivery. In all other respects test sessions were identical to training session. On the days between test sessions, training sessions continued. Pentobarbital and saline under training conditions were administered in a double alternation sequence with test sessions inserted every third session, i.e., saline, pentobarbital, test, pentobarbital, saline, test, etc. If an animal failed to meet the training criteria during a training session, further testing was postponed until the animal met these criteria on at least two consecutive training sessions.

Saline, at least three doses of the training drug, three doses of Ca-AOTA and 5 different pretreatment times were evaluated under the test conditions for each pigeon. The data are presented as the percentage of total responses emitted on the pentobarbital key for individual pigeons during test session. If no reinforcers were obtained during the test session, the data were not included in the analysis. In addition to recording the distribution of responses, response rate (responses/second) on the two keys was determined for each test session. Doses of Ca-AOTA up to 300 mg/kg were tested.

RESULTS

Self-Administration

The baseline drug, either cocaine (0.03 mg/kg/injection) or pentobarbital (0.3 mg/kg/injection), maintained responding for all monkeys (Fig. 1). The average number of infusions per session over the 4 times the monkeys were given access to their baseline drug was 58 ± 4 (mean $\pm$ sd) and 74 ± 4 for the cocaine baseline monkeys and 60 ± 12 and 64 ± 12 for the pentobarbital baseline monkeys. When saline was substituted, responding declined in all monkeys over a period of 3 to 10 sessions.

Ca-AOTA did not maintain responding above rates maintained by saline in 3 of the 4 monkeys (5019, 5024, 5025) at any of the doses tested. For these monkeys the pattern of responding for Ca-AOTA was similar to the pattern of responding when saline was substituted for the baseline drug, with most of the injections occurring during the first half of the session (data not shown). In the fourth monkey (4002), two doses of Ca-AOTA, 3.0 and 5.6 mg/kg/injection, maintained responding slightly above saline levels and responding in this monkey was evenly spaced over the session. At the highest dose tested, 10.0 mg/kg/injection, all monkeys had lower response rates compared to rates when saline was available. Thus, the higher doses of Ca-AOTA available for self-administration were apparently behaviorally active and resulted in response suppression.

Drug Discrimination

When pentobarbital was tested in the monkeys trained to discriminate pentobarbital from saline, the percentage of responses

TABLE 2

THE EFFECT OF DOSE AND PRETREATMENT TIME ON PERCENTAGE OF TRIALS COMPLETED ON THE DRUG LEVER BY RHESUS MONKEYS ADMINISTERED Ca-AOTA

	Pentobarbital-Trained		
Ca-AOTA (mg/kg)	Monkey 1006	Monkey 2036	Monkey 9067
1-Hr Pretreatment			
10.0	0	0	0
17.0	—	—	0
30.0	0	0	0
56.0	0	0	0
100.0	0*	0	0
2-Hr Pretreatment			
30.0	0	0	3
100.0	0*	0	0
4-Hr Pretreatment			
30.0	0	0	0
100.0	0	0	0
	d-Amphetamine-Trained		
Ca-AOTA (mg/kg)	Monkey 7037	Monkey 7039	Monkey 8002
1-Hr Pretreatment			
10.0	0	0	0
30.0	7	0	0
100.0	0	0	0
2-Hr Pretreatment			
30.0	3	0	0
100.0	0	0	0
4-Hr Pretreatment			
30.0	0	0	0
100	0	7	3

*Indicates the animal became ill or vomited.

TABLE 3

PERCENTAGE OF RESPONSES THAT OCCURRED ON THE PENTOBARBITAL-APPROPRIATE KEY (% DK) AND RATE OF RESPONDING (RESPONSES/SEC) FOLLOWING DIFFERENT DOSES OF PENTOBARBITAL (PB) AND Ca-AOTA WITH A 10 MINUTE PRETREATMENT TIME, AND VARIOUS PRETREATMENT TIMES (PT) FOR THE DOSE OF 300 mg/kg Ca-AOTA

	Pigeon 23		Pigeon 25		Pigeon 26		Pigeon 32	
	% DK	Rate	% DK	Rate	% DK	Rate	% DK	Rate
PB (mg/kg)								
saline	0	1.5	20	1.8	0	1.8	0	2.3
0.1	—	—	0	2.4	—	—	—	—
1.0	0	1.4	44	2.4	0	1.9	0	1.8
3.0	100	2.0	34	2.6	100	2.9	98	2.3
10.0	98	1.8	100	3.5	100	2.8	100	2.7
Ca-AOTA (mg/kg)								
30.0	0	1.5	0	1.9	0	1.9	2	2.0
100.0	0	1.2	8	1.8	0	1.9	1	2.2
300.0	0	1.3	14	2.2	0	1.9	0	1.2
300 (mg/kg) Ca-AOTA								
PT (min)								
30	0	1.0	0	1.8	0	1.8	46	1.7
60	0	1.2	0	2.1	0	1.8	76	2
120	0	1.3	0	1.7	0	1.9	0	1.9
240	0	1.4	0	1.4	0	2.0	0	1.6

that occurred on the drug appropriate lever following pentobarbital administration ranged from 0% at 1.0 mg/kg to 100% at 10.0 mg/kg (Table 1). No drug lever responses were seen when Ca-AOTA (10–100 mg/kg, 1, 2, or 4 hours presession) was administered to both groups of monkeys (Table 2). Similarly, when *d*-amphetamine was tested in the monkeys trained to discriminate *d*-amphetamine from saline, the percentage of responses that occurred on the drug-appropriate lever ranged from 0 at 0.03 mg/kg to 100 at 0.56 mg/kg (Table 1). The doses of Ca-AOTA administered were as high as could be tested without the monkeys showing emesis. Ca-AOTA did not affect average latency to respond in any monkey at any dose.

When pentobarbital was tested in pigeons trained to discriminate pentobarbital from saline, the percentage of responses that occurred on the drug-appropriate lever ranged from 0% following 0.1 mg/kg to 100% following 3.0 or 10 mg/kg (Table 3). The rate of responding was unaffected by pentobarbital. Little or no drug-appropriate responding was seen when Ca-AOTA (30 to 300 mg/kg) was administered 10 min prior to the session. Varying the pretreatment time from 30 to 240 min with a dose of 300 mg/kg Ca-AOTA still resulted in 3 of the 4 pigeons never responding on the drug-appropriate lever. The remaining pigeon (No. 32) emitted 46% and 76% of its responses on the pentobarbital-appropriate lever following 300 mg/kg Ca-AOTA after 30- and 60-min pretreatment time, respectively. Response rate was not systematically altered by Ca-AOTA under any conditions.

DISCUSSION

Ca-AOTA was not self-administered by 3 of 4 rhesus monkeys who reliably self-administered cocaine or pentobarbital. In the fourth monkey two doses of Ca-AOTA maintained responding slightly above saline levels, but well below responding maintained by the baseline drug, pentobarbital. In drug discrimination procedures, Ca-AOTA was found not to have stimulus properties similar to either pentobarbital or *d*-amphetamine in monkeys or pigeons. The overall lack of pentobarbital-like discriminative stimulus properties shown in the present study and the lack of hypnotic effects in the absence of ethanol in previous studies (6,10) suggests that Ca-AOTA is not a sedative. Similarly, Ca-AOTA does not appear to be *d*-amphetamine-like in its CNS actions. The complete lack of behavioral effects of Ca-AOTA in this paradigm raises the possibility that insufficient doses were tested. However, further increases in dose were avoided because of the illness observed in one monkey. In addition, self-administration responding was suppressed in all monkeys at 10 mg/kg suggesting that there were CNS effects at this dose when administered intravenously. Therefore, based on these results presented here, it appears that Ca-AOTA has a relatively low abuse potential, and thus would not be expected to be ingested on the basis of its psychoactive effects.

ACKNOWLEDGEMENTS

This research was supported in part by NIDA Grant DA-00250. The Ca-AOTA was supplied by Lipha Pharmaceutical Co., Lyon, France. The authors gratefully acknowledge the assistance of Dr. Suzette Evans in conducting these experiments.

REFERENCES

1. Boismare, F.; Daoust, M.; Moore, N.; Saligaut, C.; Lhuintre, J. P.; Chretien, P.; Durlach, J. A homotaurine derivative reduces the voluntary intake of ethanol by rats: Are cerebral GABA receptors involved? Pharmacol. Biochem. Behav. 21:787–789; 1984.
2. Colpaert, F. C. Drug discrimination: Behavioral, pharmacological and molecular mechanisms of discriminative drug effects. In: Goldberg, S. R.; Stolerman, I. P., eds. Behavioral analysis of drug dependence. New York: Academic Press; 1986:161–194.
3. Daoust, M.; Lhuintre, J. P.; Saligaut, C.; Moore, N.; Flipo, J. L.; Boismare, F. Noradrenaline and GABA brain receptors are co-involved in the voluntary intake of ethanol by rats. Alcohol Alcohol. 1:319–322; 1987.
4. de la Garza, R.; Johanson, C. E. Discriminative stimulus properties of intragastrically administered d-amphetamine and pentobarbital in rhesus monkeys. J. Pharmacol. Exp. Ther. 243:955–962; 1987.
5. Evans, S. M.; Johanson, C. E. Amphetamine-like effects of anorectics and related compounds in pigeons. J. Pharmacol. Exp. Ther. 241:817–825; 1987.
6. Ferko, A. P. Ethanol-induced sleep time: Interaction with taurine and a taurine antagonist. Pharmacol. Biochem. Behav. 27:235–238; 1987.
7. Johanson, C. E.; Balster, R. L. A summary of the results of a drug self-administration study using substitution procedures in rhesus monkeys. Bull. Narc. 30:43–54; 1978.
8. Johanson, C. E.; Woolverton, W. L.; Schuster, C. R. Evaluating laboratory models of drug dependence. In: Meltzer, H. Y., ed. Psychopharmacology: The third generation of progress. New York: Raven Press; 1987:1617–1626.
9. Le Magnen, J.; Tran, G.; Durlach, J.; Martin, C. Dose-dependent suppression of the high alcohol intake of chronically intoxicated rats by Ca-acetyl homotaurine. Alcohol 4:97–102; 1987.
10. Le Magnen, J.; Tran, G.; Durlach, J. Lack of effects of Ca-acetyl homotaurinate on chronic and acute toxicities of ethanol in rats. Alcohol 4:103–108; 1987.
11. Lhuintre, J. P.; Moore, N. D.; Saligaut, C.; Boismare, F.; Daoust, M.; Chretien, P.; Tran, G.; Hillemand, B. Ability of calcium bis acetyl homotaurine, a GABA agonist, to prevent relapse in weaned alcoholics. Lancet 1:1014–1016; 1985.
12. Woolverton, W. L.; Schuster, C. R. Behavioral and pharmacological aspects of opioid dependence: Mixed agonists and antagonists. Pharmacol. Rev. 35:33–52; 1983.