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Discriminative Stimulus Properties of Isradipine: Effect of Other Calcium Channel Blockers

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SCHECHTER, M. D. *Discriminative stimulus properties of isradipine: Effect of other calcium channel blockers.* PHARMACOL BIOCHEM BEHAV **50**(4) 539–543, 1995.—This study constitutes the first report of a calcium channel blocker used as a drug capable of controlling differential responding in a drug-discrimination paradigm. Male Sprague–Dawley rats were trained to discriminate between intraperitoneally administered 10.0 mg/kg isradipine and its vehicle in a two-lever, food-motivated, operant task. Once trained, rats displayed a dose-related decrease in discriminative responding when tested with lower isradipine doses. An analysis of the dose-response curve indicated an ED_{50} = 5.71 mg/kg. As all training and dose-response testing occurred at 60 min postadministration, experiments were conducted with varying injection-to-test intervals ranging from 15–240 min. Results indicate that the optimum time for discriminative performance was at the time used in training, and that discrimination returned to nondrug (vehicle) levels 2 h postinjection. Administration of other L-type calcium channel blockers, viz., nifedipine (5–50 mg/kg), diltiazem (10–60 mg/kg), or nicardipine (0.5–3.0 mg/kg), as well as a novel antipsychotic that inhibits dopamine release (10–30 mg/kg of CGS 10746B), did not produce isradipine-like discriminative effects. Thus, there was no generalization from the training dose of 10 mg/kg isradipine to any of these other agents, and the results are discussed in light of the possible specificity of the isradipine discriminative stimulus cue as it is produced in the central nervous system.

Behavior Dose-response Time course Rat

CALCIUM channel blockers, by definition, inhibit calcium channels or calcium entry and have been successfully marketed for their vasodilatory and antiarrhythmic properties in the therapy of hypertension, angina, or atrial fibrillation. As a class of drugs, the numerous clinically available calcium channel blockers differ in their ability to cross the blood–brain barrier and, thereby, gain access to the central nervous system after peripheral administration. Nevertheless, many of these drugs do reach the brain in sufficient quantity to exert pharmacological effects (8). Currently, four distinct categories of calcium channel blockers have been identified, viz., L-, T-, N-, and P-type (22), with the L-type having the greatest effect upon channels located on neuronal cell bodies. Drugs that specifically block the movement of calcium in these L-type channels have been reported to decrease the reinforcing properties of ethanol, cocaine, and morphine (6,7,16–18), as well as inhibiting the ability of these three abusable drugs to induce dopamine release in the mesolimbic dopaminergic system (10). This laboratory has been the site of experiments that indicated that the L-type calcium channel blocker isradipine attenuates the discriminative effects of cocaine (20). In light of the fact that isradipine pretreatment also blocks cocaine-induced (17) or morphine-induced (11) conditioned place preference, it has been shown that isradipine, by itself, produces neither a conditioned place preference nor aversive effect (4). Isradipine has also been found to suppress intravenous cocaine self-administration in drug-naive mice (12), as well as preventing cocaine-induced inhibition of dopaminergic neuronal firing rates (5) and ischemia-induced release of dopamine (15).

A behavioral paradigm that is particularly well suited to assess the subjective effects of drugs that enter the central nervous system is the drug discrimination procedure. Indeed, discriminative control of responding in this behavioral task has been observed to be the property of virtually every psychoactive drug tested to date. After a rat has been trained to discriminate the effects of a drug from a nondrug (vehicle) state, it can be tested with different doses of the same drug or at different injection-to-test intervals to allow for a dose-response and a time-course relationship, respectively, to be determined. In addition, once trained, subjects can be tested

with other drugs to determine whether the novel drug effects are similar or dissimilar to the drug used in their training. As of 1991, this behavioral technique had generated 1675 publications (19), but not one of these employs a calcium channel blocker as the drug to control differential discriminative responding. It is, therefore, the purpose of the present investigation to train rats to discriminate the interoceptive cueing properties of the L-type calcium channel blocker isradipine, to determine its dose-response relationship, time course, as well as possible generalization (transfer) of its stimulus discrimination cues to other calcium channel blockers.

METHOD

Subjects

Experimentally naive male Sprague-Dawley rats were purchased from Zivic-Miller (Allison Park, PA) and weighed 220-230 g upon arrival. After a week of isolation, animals were assigned to individual hanging wire cages and kept in a colony facility maintained on a 12 L : 12 D cycle (0600-1800 h) at a constant temperature and humidity. They were given water ad lib in their home cages, as well as daily rationing of commercial rat chow so as to maintain them at approximately 85% of their free-feeding weights as determined by a growth chart from the supplier. This procedure was maintained throughout experimentation to facilitate and motivate operant performance for food reward.

Apparatus

Ten standard rodent operant chambers (Lafayette Instruments Corp., Lafayette, IN), each containing two levers situated 7 cm apart and 7 cm above a metal grid floor, were used as the experimental space. Equidistant between the levers was placed a food receptacle that received delivery of 45 mg Noyes food pellets. Each operant chamber was enclosed in a sound-attenuated cubicle with an exhaust fan and a 9 W houselight. Solid-state programming equipment (Med Associates, St. Albans, VT) was located in an adjacent room and was used to control and record discrimination training and testing sessions.

Drug Discrimination

The behavioral paradigm employed allowed rats to be trained to discriminate between isradipine and its vehicle; the detailed methodology can be found in a previous publication (20). Briefly, the discrimination procedure consisted of training rats to press one of two identical levers 60 min following intraperitoneal (IP) administration of vehicle (two drops of 2.0% Tween 80 in 0.9% sterile saline) administered in a volume of 1 ml/kg. The initial fixed ratio 1 (FR 1) reinforcing schedule was gradually increased, over 9 days, until an FR 10 reinforcement schedule was attained. This procedure was then repeated with presses upon the other lever being reinforced 60 min following IP administration of a similar volume of vehicle containing 10.0 mg/ml of (+)-isradipine (Sandoz Research Institute, East Hanover, NJ) made fresh daily and protected from light. The initial FR 1 reinforcement requirement was gradually incremented on the second lever, over 7 days, to attain an FR 10. Once lever pressing behavior was established on both levers, a biweekly, repeating, injection schedule was employed: V,D,D,V,V; D,V,V,D,D, where V = vehicle and D = 10.0 mg/kg isradipine. For each animal, the choice on any given day was considered correct if the first lever to accumulate 10 presses was state appropriate, i.e., the isradipine lever after isradipine administration and the vehicle lever after vehicle administration. Training was continued until all rats achieved the training criterion of eight correct first-lever selections in ten consecutive sessions.

Dose-Response Experiments

Once the training criterion was achieved, rats were tested with doses of isradipine different from their 10 mg/kg training dose; this allowed a dose-response relationship to be observed. During this series, and in subsequent time-course and generalization experiments (below), isradipine-vehicle discrimination performance was tested and maintained by administering either the training dose of isradipine or its vehicle every second day. During these maintenance sessions, the lever pressed 10 times first was considered the selected lever and rats were allowed to continue pressing the state-appropriate lever 400 times to receive (on the FR 10 schedule) 40 additional reinforcements. In intervening test sessions, rats were placed into the experimental chamber 60 min following IP administration of lower isradipine doses and allowed to lever press until 10 presses were accumulated on either lever. They were then immediately removed without receiving reinforcement to preclude reinforcement/training at a dose different than the dose (10 mg/kg) of isradipine to which they were trained. Each isradipine dose-response test was administered in random order on two occasions, with each test session preceded by one vehicle and one 10 mg/kg isradipine maintenance session at 60 min postadministration.

Time-Course Studies

Once the dose-response relationship was determined, test sessions, between interspersed isradipine or vehicle test-training sessions at 60 min postinjection, were used to investigate the time-course effects of the training dose of isradipine. Thus, rats were injected with the 10 mg/kg dose of isradipine and returned to their home cage for varying lengths of time prior to placement into the test chamber. At that time, they were allowed to press both levers until 10 lever responses accumulated on any one lever. Times earlier than the 60 min injection-to-test interval used in training/maintenance, i.e., 15 and 30 min, as well as two times longer than the training interval, viz. 120 and 240 min, were used on two occasions, with each time delay following one isradipine and one vehicle test session at 60 min postadministration. As with the dose-response experiments, the rats were immediately removed, without receiving food reinforcement, upon pressing either lever 10 times.

Generalization Experiments

Following the dose-response and time-course experiments, the test days interspersed with maintenance testing/training days were used to determine the ability of other drugs to produce isradipine-like discriminative stimuli. Each dose of novel drug was tested in a random order on two occasions, with each test session preceded by one vehicle and one isradipine maintenance session. As with testing isradipine at a dose different than the training dose or at a time different than the postinjection time used in that training, animals were immediately removed without receiving reinforcement upon pressing either the isradipine- or vehicle-appropriate lever 10 times; this procedure intended to preclude reinforcement/training in a drug state different than that to which the rats were trained.

TABLE 1

DOSE-RESPONSE (A) AND TIME COURSE (B) OF DISCRIMINATIVE PERFORMANCE IN RATS (n = 8) TRAINED WITH 10 mg/kg ISRADIPINE (ISR) AND VEHICLE

	Quantal	Quantitative (SD)	
A. ISR Dose at 60 min (mg/kg)			
10.0	84.4	70.3	(3.4)
7.5	75.0	66.8	(2.9)
5.0	37.5	42.5	(3.0)
0.0 (vehicle)	2.5	20.8	(10.4)
ED50 (95% CL)	5.71 mg/kg (4.45-7.33)		
B. 10 mg/kg ISR Injection-to-test Interval (min)			
240	6.3	16.7	(4.7)
120	31.3	36.8	(13.2)
60	81.3	70.3	(9.5)
30	68.8	61.8	(5.2)
15	62.5	58.4	(2.1)
Vehicle at 60 min	6.3	26.3	(6.7)

Data Analysis

The results of discriminative responding are expressed in terms of both quantal and quantitative measurements. The lever pressed 10 times first is designated the selected lever for that day's session and the percentage of rats selecting the drug-appropriate lever constitutes the quantal measure of discrimination. In addition, the total number of responses made on both levers before 10 responses were accumulated on either lever constitutes the quantitative measurement for that session, i.e., the number of responses made on the isradipine lever divided by the total responses made on both levers, × 100. The quantal data for the dose-response results were analyzed by the methods of Litchfield and Wilcoxon (13), which employed probits vs. log-dose effects and yields ED_{50} values as generated by a computerized program (21). In generalization studies, if a drug was seen to produce 80% or more quantal discrimination, i.e., the isradipine lever was selected by 80% or more of the rats tested, that drug would have been considered generalizable. This seemed appropriate, as 80% (8 correct of 10 consecutive sessions) was the level of criterion performance required of the rats to adjudge them capable of discriminating isradipine from saline. Response rates, i.e., time required to complete the FR 10 ratios, were not specifically measured.

Drugs and Treatment Regimens

Isradipine (PN 200-110), as all other drugs, was suspended on the day of testing in the vehicle, viz., two drops of 2.0% Tween 80 in 0.9% saline, protected from exposure to light and administered in a volume of 1 ml/kg intraperitoneally. Nifedipine (purchased from Research Biochemicals International; RBI, Natick, MA) was a second dihydropyridine L-type calcium channel blocker and was tested for generalization in doses of 5, 10, 15, 20, 30, 40, and 50 mg/kg. The benzothiazepine calcium channel blocker diltiazem HCl and nicardipine HCl (both purchased from RBI) are L-type calcium channel blockers and their preparation and administration was similar to isradipine. Lastly, the atypical antipsychotic CGS 10746B (Ciba-Geigy, Summit, NJ) was administered in doses of 10, 20, and 30 mg/kg. All novel drugs were administered at 30 min before generalization testing.

RESULTS

The sessions-to-criterion (± SEM), i.e., the first of 10 consecutive sessions in which the rats correctly chose the state-appropriate lever on eight sessions, was shown to be a mean of 22.3 (4.1) sessions with a range of 7-39 sessions. Thus, by approximately the 50th session, 25 sessions with 10 mg/kg isradipine and 25 sessions with its vehicle, all eight rats were trained to the criterion of 8 of 10 consecutive correct lever selections. The effects of testing lower doses of isradipine, at the same postinjection interval of 60 min, is presented in Table 1(A) and indicates that decreasing doses of isradipine produced decreased quantal and quantitative discriminative performance. Application of the Litchfield-Wilcoxon statistic (13) indicated an ED_{50} value (with 95% confidence limits) of 5.71 (4.45-7.33) mg/kg for the quantal results. In addition, Table 1(B), represents the effects of the training dose tested at different injection-to-test intervals. It indicates that the post-administration interval used for training, i.e., 60 min, allowed for the highest degree of discriminative performance. Thus, isradipine at the 10 mg/kg dose appears to peak at 60 min postinjection and the testing of the rats at 240 min after administration showed a return to vehicle-appropriate discrimination levels. After the time-course experiments, one rat died of an undetermined cause and the generalization studies reflect an n = 7.

Administration of 5-50 mg/kg nifedipine, 10, 30, and 60 mg/kg diltiazem and 0.5-3.0 mg/kg nicardipine did not produce generalization (Table 2). One higher dose of each of

TABLE 2

GENERALIZATION OF OTHER CALCIUM CHANNEL BLOCKERS AS TESTED IN ISRADIPINE-TRAINED RATS (n = 7)

Drug	Dose (mg/kg)	Quantal	Quantitative (SD)	
Nifedipine	5	35.7	34.0	(3.3)
	10	57.1	47.2	(2.3)
	15	64.3	56.9	(5.0)
	20	64.3	57.9	(24.3)
	30	57.1	55.9	(13.0)
	40	64.3	50.0	(9.7)
	50	57.1	53.3	(1.8)
Diltiazem	10	0.0	19.6	(8.4)
	30	28.6	39.3	(2.5)
	60	42.9	51.9	(10.3)
Nicardipine	0.5	21.4	30.8	(2.2)
	1.0	57.1	50.3	(1.8)
	1.5	35.7	39.6	(9.9)
	2.0	21.4	30.4	(11.6)
	3.0	21.4	30.7	(9.6)
CGS 10746B	10	22.2	30.6	(0.6)
	20	22.2	32.1	(4.6)
	30	27.8	41.3	(6.3)
Maintenance Sessions				
Isradipine	10.0	82.1	70.6	(10.9)
Vehicle	–	8.6	21.8	(8.1)

these three drugs was used and was seen to produce behavioral disruption, i.e., rats did not choose either lever for over 30 min after being placed into the experimental apparatus. Likewise, generalization testing with CGS 10746B, at doses of 10–30 mg/kg, did not produce isradipine-appropriate responding.

DISCUSSION

The present investigation is the first to use a calcium channel blocker as a drug to control differential responding in a drug discrimination task, although the prototype calcium L-type channel activator BAY K 8644 has been successfully employed in a previous study (19). Isradipine, at 10 mg/kg IP, was seen to produce discrimination at an optimum postadministration interval of 60 min with a typical dose–response relationship and time-course decay as different doses and injection-to-test times were employed (Table 1). Testing of other calcium channel blockers in animals trained to discriminate isradipine produced no generalization as defined as 80% isradipine-appropriate lever selections. Nevertheless, a dose of each of nifedipine, diltiazem, and nicardipine produced at, or near, 50% of first lever selections on the isradipine-appropriate lever. The possibility exists that isradipine is more potent than other dihydropyridine-derived calcium channel blockers (11) and the dose of each of the other drugs tested reaches behavioral disruptive dose levels that obscure discriminative generalization.

Calcium channel blockers act to attenuate dopamine release at the synapse as they increase dopamine turnover, as typically seen in classical antipsychotic drugs (9). Thus, the possibility is that at least some calcium channel blockers have at least some antidopaminergic properties. In the present study, the dopamine release inhibiting drug CGS 10746B (1,2) was administered to animals trained to discriminate isradipine (Table 2). Doses of 10, 20, and 30 mg/kg were shown to produce a maximum of 27.8% of total responding on the isradipine-correct lever. Previous work with CGS 10746B has allowed animals to be trained with it (14) and has indicated that the training dose of 10 mg/kg CGS 10746B allows for a maximum transfer of 70% at 10 mg/kg isradipine tested 75 min after administration. Thus, there is a symmetrical non-generalization effect between isradipine and CGS 10746B. Although L-type, voltage-dependent, calcium channel blockers have been shown, using microdialysis methods, to suppress basal dopamine release in rat striatum (10), there seems to be different interoceptive discriminative cues produced between isradipine and CGS 10746B, despite the possibility of a common antidopaminergic mechanism of action. Also, the possibility exists that different, and specific, pharmacological profiles of calcium channel antagonists may affect, either by increasing or decreasing, neurotransmitter release as a consequence of relative distribution of different calcium channels at specific brain sites (23).

In the realm of drug discrimination behavior, the reported activity of isradipine in decreasing cocaine discrimination in rats (20) may be a result of the reported and putative antidopaminergic activity of isradipine or it may be the result of the recently reported ability of these agents to specifically inhibit the binding of cocaine-like drugs to specific sites in the central nervous system (3). In either case, the present study indicates that isradipine can function as a drug to control differential responding in a drug discrimination task with decreased performance seen with decreasing doses and a peak time of efficacy. The inability of other L-type calcium channel blockers or a dopamine release inhibitor to produce isradipine-like discriminative stimuli may indicate that the mechanism of isradipine action in the CNS is selective. Continued work with this and other calcium channel blockers will allow for further discovery on this point.

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