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Effects of dihydropyridine Ca^{2+} channel blockers on the discriminative stimulus and the motor impairing effects of (±)-Bay K 8644

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Abstract

Functional interactions between the dihydropyridine Ca^{2+} channel activator, (±)-Bay K 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethyphenyl)-pyridine-5-carboxylate), and several dihydropyridine L-type Ca^{2+} channel blockers were investigated on rotarod performance in mice and in rats trained to discriminate between (±)Bay K 8644 and saline. When administered alone, (±)-Bay K 8644 produced dose-dependent impairments of rotarod activity with an ED_{50} of 1.3 mg/kg. Pretreatment with nifedipine (10–30 mg/kg) produced dose-dependent rightward shifts of the (±)-Bay K 8644 dose–response curve. In contrast, pretreatment with several other dihydropyridine L-type Ca^{2+} channel blockers, including nicardipine, nimodipine, isradipine and nitrendipine, did not modify the (±)-Bay K 8644 dose–effect function. Rats learned to discriminate between (±)-Bay K 8644 (0.5 mg/kg) and saline in an average of 65 training sessions. In substitution tests, the Ca^{2+} channel activator engendered dose-related increases in the percentage of rats selecting the drug-associated lever with an ED_{50} of 0.19 mg/kg. Pretreatment with nifedipine (10 mg/kg) produced a rightward shift of the (±)-Bay K 8644 dose–response function. Pretreatment with nicardipine (2.5 mg/kg) only partially antagonised the training dose of (±)-Bay K 8644 whereas nimodipine (0.6–10 mg/kg) did not affect the (±)-Bay K 8644 discriminative stimulus. The results of the present study show that the behavioural effects of the dihydropyridine Ca^{2+} channel activator are differentially modified by dihydropyridine L-type Ca^{2+} channel blockers. These results may suggest that dihydropyridine blockers possess different intrinsic activities or act at different binding sites.

Keywords: Ca^{2+} channel; Dihydropyridine; Motor effect; Drug discrimination

1. Introduction

Of the different classes of voltage-dependent Ca^{2+} channels (L, T, N and P) identified, L-type channels have been the best characterised by molecular, electrophysiological and pharmacological studies (Janis et al., 1987; Spedding and Paoletti, 1992). L-type channels contain several binding sites sensitive to selective ligands: the dihydropyridines, the benzothiazepines and the phenylalkylamines. Most of these drugs act as inhibitors which block Ca^{2+} flow through the channels. They are well known for their cardiovascular effects. In particular, nifedipine (dihydropyridine), diltiazem (benzothiazepine) and verapamil (phenylalkylamine) are used in the treatment of angina, tachyarrythmia and hypertension (for review see Janis et al., 1987). Evidence also exists that they have central effects. Thus, in rodents, Ca^{2+} channel blockers show some protective effects against chemically-induced convulsions, antidepressant-like actions and antiaddictive effects (for review see Pucilowski, 1992).

Ca^{2+} channel blockers interacting with different sites on L-type channels have been shown to exhibit different pharmacological properties. They possess distinct cardiovascular effects which were the basis for their initial classification (Vanhoutte and Paoletti, 1987). They also differ in their psychotropic effects. In most studies, only drugs selectively interacting with dihydropyridine sites display anticonvulsant and antidepressant-like actions (Mogilnicka et al., 1987; Palmer et al., 1993; Cohen et al., 1997; reviewed in Pucilowski, 1992). They can also be distinguished on the basis of their interactions with cocaine and D-amphetamine as it appears that dihydropyridine drugs attenuate hyperactivity induced by these stimulant drugs whereas benzothiazepine and phenylalkylamine drugs have given mixed results (reviewed in Rosenzweig-Lipson and Barrett, 1995). In contrast, few functional studies have compared the pharmacological activities of Ca^{2+} channel

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blockers within the dihydropyridine group (Palmer et al., 1993; De Beun et al., 1996a). A recent study by Schechter (1995) showed that the discriminative stimulus effects of isradipine, a dihydropyridine drug, were only partially mimicked by other dihydropyridines, such as nifedipine and nicardipine. These results suggest that dihydropyridine drugs differ in their pharmacological activities. This notion is further supported by the finding that, in contrast to isradipine, the dihydropyridine drug, nimodipine, produced conditioned taste aversion and place preference (Calcagnetti and Schechter, 1994; De Beun et al., 1996b).

(±)-Bay K 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethyphenyl)-pyridine-5-carboxylate), a dihydropyridine Ca^{2+} channel activator, has been shown to produce pressor effects, increases in dopamine and serotonin turnover, motor deficits and discriminative stimulus effects (Bolger et al., 1985; Gladstein et al., 1987; Bourson et al., 1989). These actions were blocked by nifedipine (Bolger et al., 1985; Woodward and Leslie, 1986; Gladstein et al., 1987; Bourson et al., 1989). However, few data are available on the interaction between other dihydropyridine channel blockers and (±)-Bay K 8644 (Woodward and Leslie, 1986, Palmer et al., 1993, Rosenzweig-Lipson and Barrett, 1995). Therefore, the present study was carried out to evaluate the capacity of several dihydropyridine drugs to antagonise the behavioural effects of (±)-Bay K 8644. Nifedipine, nimodipine, nicardipine, isradipine and nitrendipine were studied in combination with the channel activator in a model sensitive to motor deficits (rotarod performance). Nifedipine, nimodipine and nicardipine were also evaluated for their ability to antagonise the discriminative stimulus effects of (±)-Bay K 8644. Drug discrimination procedures are particularly useful to characterise the pharmacological properties of many psychotropic drugs including opiates, psychomotor stimulants, anxiolytics and sedative drugs (Colpaert, 1986). Through the use of appropriate tests of substitution for, and antagonism of, a training drug, it is possible, using drug discrimination, to make precise comparisons between drugs and to investigate their mechanism of action.

2. Materials and methods

2.1. Rotarod performance

Male CD1 mice (Charles River, France) weighing between 20 and 26 g were used. The animals were housed in an animal facility for at least 4 days prior to behavioural testing (with food and water available ad libitum). The lights in the colony room were turned on between 7.00 a.m. and 7.00 p.m.. Animals were housed and tested in accordance with current French legislation on animal experimentation.

2 h before the experiments, mice were given a pre-test on the rotarod apparatus. The rod (diameter, 3 cm) rotated at a constant speed (10 rpm). Animals which remained on the rotarod for 2 min were selected for drug testing. If a mouse dropped from the rotarod during this time period, it was given another trial. Mice unable to stay on the apparatus during these two trials were discarded. The mice were injected intraperitoneally (20 ml/kg, i.p.) with different doses of one of the test drugs or with its vehicle. 15 min later, they were injected i.p. with one of several doses of (±)-Bay K 8644 or with saline. After 15 min, they were replaced on the rotarod. The time for which each mouse stayed on the rotarod was recorded up to a maximum of 2 min. At least 8 mice were tested per group. (±)-Bay K 8644 dose–effect function on rotarod performance was determined first. Interaction studies were then conducted, starting with doses of (±)-Bay K 8644 which decreased performance (1 and 3 mg/kg) and completed with a higher dose (5 mg/kg) when an antagonism was found or a lower dose (0.3 mg/kg) when no antagonism emerged. The dose of 1 or 3 mg/kg was replicated which explains differences in the number of mice per group.

The ED_{50} value for the potency of (±)-Bay K 8644 to impair rotarod performance (i.e. the dose that reduced rotarod performance to 50% of control values) was calculated using log–probit analysis. The effects of treatments were evaluated using nonparametric statistics because maximum recording time (2 min) was consistently reached in mice treated with low doses of (±)-Bay K 8644. Analyses were carried out on the (±)-Bay K 8644 data alone and for each dose of (±)-Bay K 8644 in combination with different doses of each pretreatment drug using the Kruskal–Wallis test.

2.2. Drug discrimination

Thirty seven male Wistar rats obtained from IFFA CREDO (L'Arbresle, France) were used. They weighed 180–200 g when obtained from the suppliers and were allowed to gain weight during the experiment so that by the end they weighed between 400 and 600 g. Animals were restricted to the food obtained during sessions and a daily ration of 15–20 g of standard laboratory food given at the end of each day and over the weekend. Housing was in individual cages.

The animals were trained to press both levers in standard two-lever operant test chambers (MED-Associates, Georgia, VT) to obtain 45 mg food pellets (Noyes, formula P, Lancaster, NH). Initially, daily sessions were 30 min in duration and every lever press produced a pellet. The schedule requirement was then gradually increased until 10 lever presses were required for each pellet (fixed ratio 10: FR10). At this stage the daily session duration was reduced to 15 min and injections were started. Rats were given injections of either a dose of 0.5 mg/kg of (±)-Bay K 8644 or physiological saline 15 min before sessions in the daily sequence SDDSS//DSSDD (D = drug, S = saline,

//= no sessions during the weekends), with repetition. The animals were assigned randomly to one of six test chambers. In three cages, responding on the right lever after drug injection and the left lever after saline injection was reinforced with food. For the other three chambers, this relationship was reversed. Responses on the inappropriate lever were counted but were not reinforced with food. This training procedure was continued until the following criterion was met for a period of 10 successive days: the total number of responses on both levers before the first reinforcement was less than 15.

Once a rat met the criterion for successful discriminative control, test sessions were carried out on this particular rat. A staggered start was, thus, used. Test sessions were conducted in a daily sequence of STDTS//DTSTD (D = drug, S = saline, T = Test, //= no sessions during the weekends) as long as performance on the training sessions between tests remained at the criterion for stimulus control. When a rat made an inaccurate choice during a control session, test sessions were stopped until the accuracy criterion was met for a period of 5 successive sessions. During test sessions the animal was placed in the test chamber at the appropriate time after injection and was reinforced after the first ratio of 10 responses had been completed on either lever. For the remainder of the 15 min session, responding on the lever on which the first 10 responses had occurred continued to be reinforced according to the FR10 schedule. Substitution tests with several doses of (±)-Bay K 8644 were first carried out in each rat which learned the discrimination. These rats were then randomly assigned to substitution tests with novel compounds and antagonist tests with Ca^{2+} channel blockers. In substitution tests, rats were given an injection with one dose of the test drug and returned to the home cage for the appropriate delay before being placed in the test chamber for the start of the session. The drugs used in substitution tests were (±)-Bay K 8644, cocaine, D-amphetamine, nicotine and physostigmine which were injected 15 min before the start of the sessions and apomorphine, oxotremorine and fluoxetine which were administered 30 min before testing. In antagonism tests, the Ca^{2+} channel blockers (nifedipine, nicardipine and nimodipine) were injected 45 min before testing and (±)-Bay K 8644 was given 15 min pre-session. Rats were returned in the home

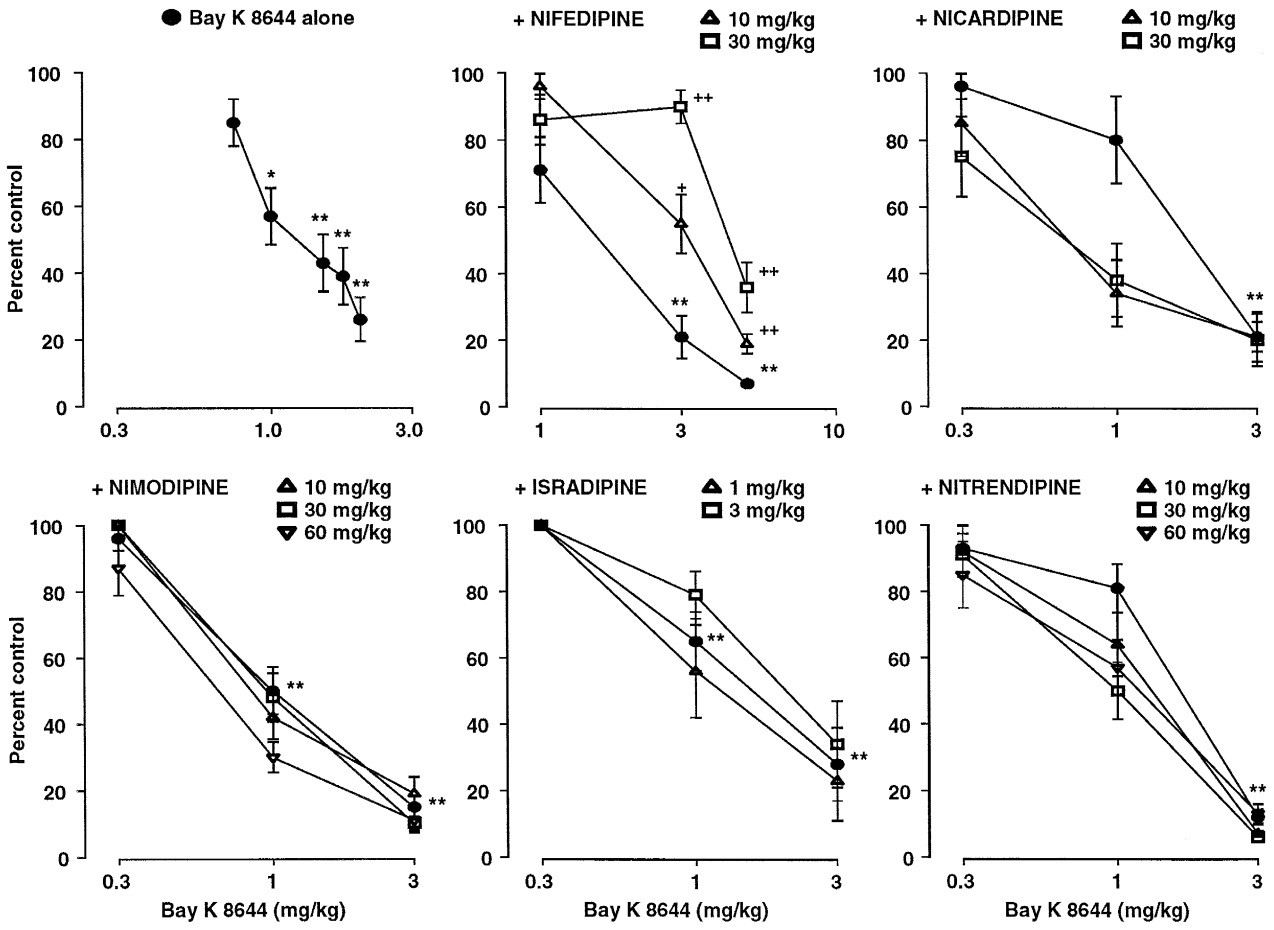

Fig. 1. Effects of (±)-Bay K 8644 alone and after pretreatment with several Ca^{2+} channel blockers on rotarod performance in mice. Data represent the time for which mice stayed on the rotarod expressed as a percentage of maximum performance. Points are means ± SEM based on 8–24 mice. Analyses were carried out on the (±)-Bay K 8644 data alone: * $P < 0.05$; ** $P < 0.01$ compared to control and for each dose of (±)-Bay K 8644 in combination with different doses of each pretreatment drug: + $P < 0.05$; ++ $P < 0.01$ compared to corresponding (±)-Bay K 8644 dose.

cage after each injection until it was placed in the chamber immediately before the start of the session. All injections were given i.p. (1 or 2 ml/kg).

The lever chosen and the total number of lever presses were recorded during each 15 min session. During tests of substitution and antagonism the results were expressed as the percentage of rats choosing the lever associated with the training dose of (±)-Bay K 8644 (percent Bay K 8644 lever) and the rate of responding expressed as a percentage on the mean rate of the preceding saline sessions (percent control rate). If a rat did not respond at least 10 times on one of the levers, the data for that session were not included in the calculation of the percentage of rats selecting the drug associated-lever but were included in the response rate determination. Drug effects on rates of lever pressing were analysed statistically using Friedman analyses of variance followed by Wilcoxon matched-pairs signed-ranks tests. ED_{50} values for the potency of drugs to produce (±)-Bay K 8644-lever responding (i.e. doses at which 50% of rats tested chose the (±)-Bay K 8644 associated-lever) or to decrease rates of responding (i.e. doses that reduced response rates to 50% of control values) were calculated using probit analysis.

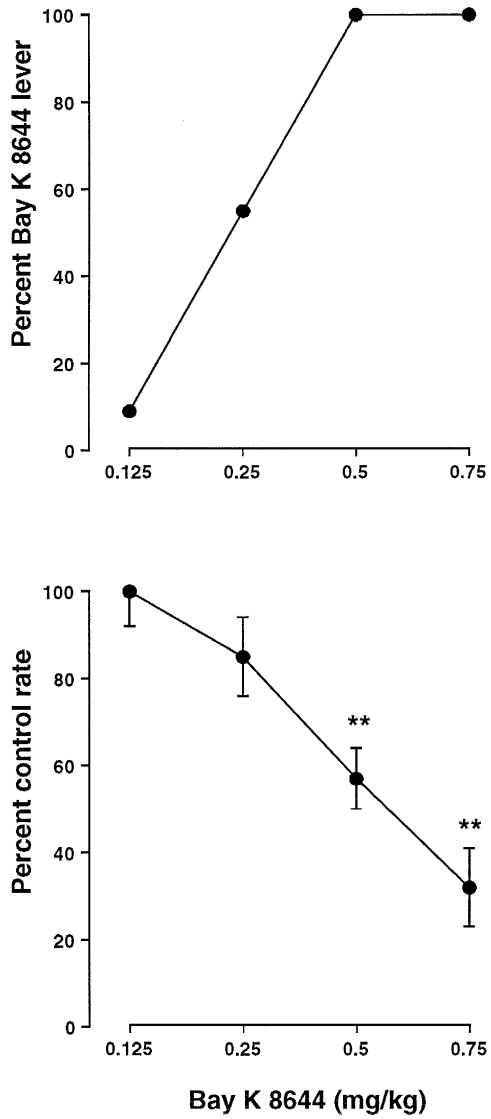

Fig. 2. Effects of (±)-Bay K 8644 in rats trained to discriminate (±)-Bay K 8644 from saline. Data represent the percentage of rats selecting the (±)-Bay K 8644-associated lever and the rates of responding expressed as a percentage of control rates obtained from the sessions preceding the drug sessions. Points are means ± SEM based on 20 rats. ** $P < 0.01$ compared to saline control values.

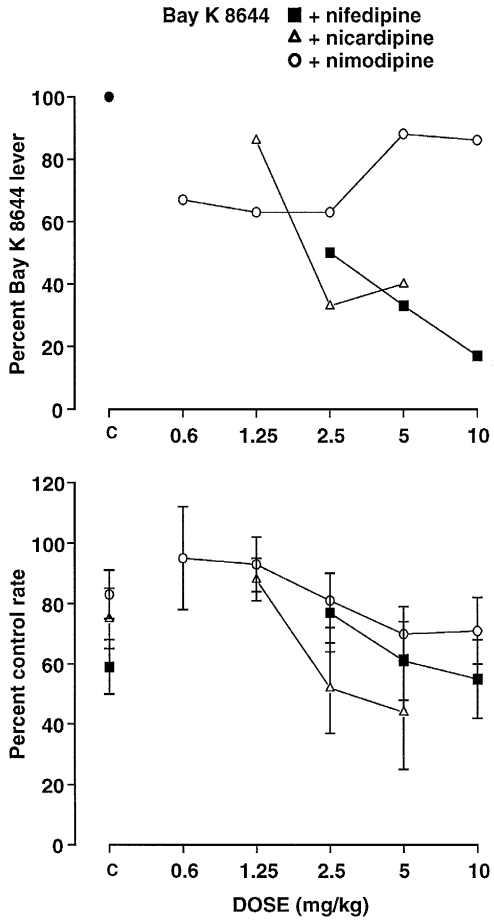

Fig. 3. Effects of the training dose of (±)-Bay K 8644 after pretreatment with several Ca^{2+} channel blockers in rats trained to discriminate (±)-Bay K 8644 from saline. Data represent the percentage of rats selecting the (±)-Bay K 8644-associated lever and the rates of responding expressed as a percentage of control rates obtained from the sessions preceding the drug sessions. Points are means ± SEM based on 5–8 rats.

2.3. Drugs

All doses are expressed as the base. The drugs were made up as solutions in saline or suspensions in saline to which two drops of Tween 80 had been added. Nifedipine, nicardipine hydrochloride, nimodipine, physostigmine, cocaine hydrochloride, apomorphine hydrochloride and nicotine tartrate were purchased from Sigma (St. Louis, MO, USA), nitrendipine, isradipine and (±)-Bay K 8644 from Research Biochemicals International (Natick, MA, USA),

oxotremorine fumarate from Laboratoire Auclair (France), D-amphetamine sulfate from Laboratoires du Bac (France), fluoxetine was donated by Lilly (Indianapolis, IN, USA).

3. Results

3.1. Rotarod performance

All control mice were able to remain on the rotarod for 2 min when they were retested following saline injections 2 h after the pre-test. (±)-Bay K 8644 produced a dose-dependent impairment of rotarod performance (Fig. 1, top left). The ED_{50} value for this motor deficit was 1.3 mg/kg (95% confidence interval: 1.1–1.7 mg/kg).

Several Ca^{2+} channel blockers were evaluated for their capacity to antagonise the effects of (±)-Bay K 8644 on rotarod performance. The doses of the different drugs used in the present study were chosen on the basis of the published literature or preliminary studies. Pretreatment with the Ca^{2+} channel blocker, nifedipine, resulted in a rightward shift in the (±)-Bay K 8644 dose–response effect (Fig. 1, top middle). Nifedipine (10 mg/kg and 30 mg/kg) significantly antagonised the impairment of rotarod performance induced by (±)-Bay K 8644 at 3 mg/kg and 5 mg/kg. In contrast to nifedipine, pretreatment with nicardipine produced a small but not significant potentiation of the effects of (±)Bay K 8644 on rotarod performance (Fig. 1, top right). Pretreatment with nimodipine, isradipine and nitrendipine did not significantly affect the motor impairing effects of (±)-Bay K 8644 (Fig. 1, bottom). When given alone, none of the Ca^{2+} channel blockers decreased rotarod performance at the doses tested (data not shown).

3.2. Drug discrimination

Thirty seven rats were used in the drug discrimination experiment. Six of these rats were discarded because they did not lever-press following the training dose of (±)-Bay K 8644 (they were, however, neither prostrated nor sedated) and eleven rats were dropped from the experiment when it became apparent that they were not acquiring a discrimination. Thus, twenty rats achieved the accuracy criterion after a mean total of 65 (range 37–108) training sessions.

(±)-Bay K 8644 (0.125–0.75 mg/kg) engendered dose-related increases in the percentage of rats selecting the drug-associated lever (Fig. 2, top). The dose of (±)-Bay K 8644 calculated to elicit 50% drug-lever choice (ED_{50}) was 0.19 mg/kg (95% confidence interval: 0.15–0.24 mg/kg). Response rates after (±)-Bay K 8644 doses of 0.5 and 0.75 mg/kg were significantly different from saline sessions, with the training dose of (±)-Bay K 8644 (0.5 mg/kg) producing a 43% decrease in rates of responding (Fig. 2, bottom). The ED_{50} value for decreased rates of lever pressing was 0.55 mg/kg (95% confidence interval: 0.50–0.61 mg/kg).

To examine the pharmacological specificity of (±)-Bay K 8644 discrimination, several reference compounds were evaluated for (±)-Bay K 8644-like discriminative stimulus effects. Table 1 shows results for dopaminergic, choliner-

Table 1
Results of substitution tests in rats trained to discriminate (±)-Bay K 8644 from saline. n/N = Number of rats responding at least 10 times on one of the levers/number of rats tested whose data are included in mean response rate

Drug	Dose (mg/kg)	n/N	% (±)-Bay K 8644 lever	% control rate (±) S.E.M
D-amphetamine	0.25	9/9	22	94 (±15)
	0.5	9/9	44	71 (±19) [a]
	1	7/9	28	39 (±15) [b]
Cocaine	4	6/6	0	79 (±18) [a]
	6	6/6	17	69 (±16) [b]
	8	6/6	0	57 (±15) [b]
Apomorphine	0.16	6/6	33	90 (±9)
	0.32	5/6	40	47 (±18) [b]
Physostigmine	0.1	10/10	30	78 (±12)
	0.2	10/10	70	48 (±13) [b]
	0.3	6/9	67	20 (±10) [b]
Nicotine	0.1	6/6	0	93 (±10)
	0.2	6/6	33	88 (±9)
	0.4	6/6	0	95 (±10)
Oxotremorine	0.01	6/6	0	93 (±13)
	0.03	5/6	0	80 (±22)
	0.1	5/6	20	68 (±17)
Fluoxetine	5	6/6	17	87 (±12)
	10	5/6	20	54 (±13) [b]

[a] $P < 0.05$ compared to saline control values.
[b] $P < 0.01$ compared to saline control values.

gic and serotoninergic agonists. Physostigmine was the only drug tested to produce more than 50% responding on the (±)-Bay K 8644-associated lever.

Three Ca^{2+} channel blockers were evaluated for their capacity to antagonise the discriminative stimulus effects of (±)-Bay K 8644. As shown in Fig. 3, pretreatment with nifedipine dose-dependently and nicardipine partially antagonised the stimulus effects of the training dose of (±)-Bay K 8644. In contrast, nimodipine failed to consistently antagonise the (±)-Bay K 8644 cue. Pretreatment with nifedipine, nicardipine and nimodipine did not significantly affect response rates following administration of (±)-Bay K 8644 (Fig. 3, bottom).

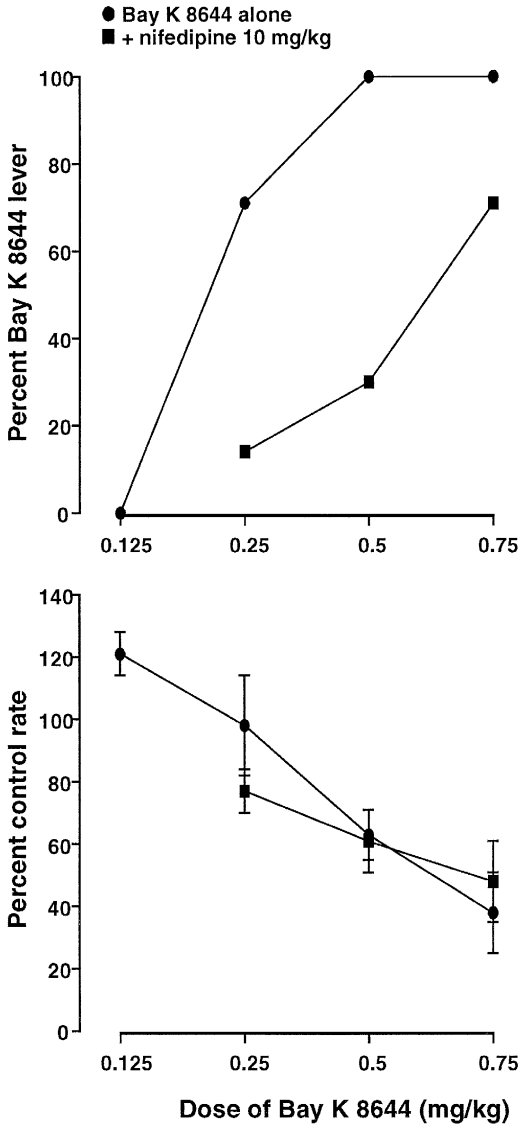

Fig. 4. Effects of (±)-Bay K 8644 after pretreatment with nifedipine (10 mg/kg) in rats trained to discriminate (±)-Bay K 8644 from saline. Data represent the percentage of rats selecting the (±)-Bay K 8644-associated lever and the rates of responding expressed as a percentage of control rates obtained from the sessions preceding the drug sessions. Points are means ± SEM based on 7–10 rats.

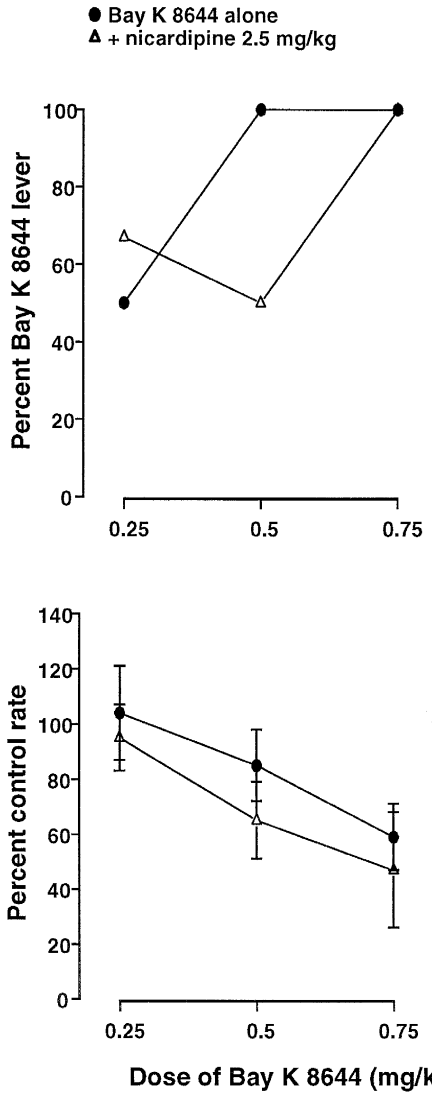

Fig. 5. Effects of (±)-Bay K 8644 after pretreatment with nicardipine (2.5 mg/kg) in rats trained to discriminate (±)-Bay K 8644 from saline. Data represent the percentage of rats selecting the (±)-Bay K 8644-associated lever and the rates of responding expressed as a percentage of control rates obtained from the sessions preceding the drug sessions. Points are means ± SEM based on 4–8 rats.

Administration of a fixed dose of nifedipine (10 mg/kg) in combination with several doses of (±)Bay K 8644 (0.25–0.5 and 0.75 mg/kg) resulted in a rightward shift in the (±)Bay K 8644 discrimination dose–response curve (Fig. 4, top). In contrast, nifedipine did not modify the rate decreasing effect of (±)-Bay K 8644 (Fig. 4, bottom). Fig. 5 shows the effects of nicardipine (2.5 mg/kg) in combination with (±)Bay K 8644. In contrast to nifedipine, nicardipine only partially antagonized the discriminative stimulus effects of the training dose of (±)-Bay K 8644. It did not significantly affect the rate-decreasing effects of (±)-Bay K 8644.

4. Discussion

The results of the present study show that the discriminative stimulus and the impairment of rotarod performance produced by the dihydropyridine L-type Ca^{2+} channel activator, (±)-Bay K 8644, are differentially modified by several dihydropyridine Ca^{2+} channel blockers.

Consistent with previous behavioural observations of motor and postural changes following (±)-Bay K 8644 administration in rodents (Bourson et al., 1989), (±)-Bay K 8644 produced impairment of rotarod performance in mice and decreases of operant responding in rats in the present study. The ED_{50} value for the potency of (±)-Bay K 8644 to impair rotarod performance (1.3 mg/kg) was comparable to that observed in another report (Bolger et al., 1985). In the drug discrimination study, (±)-Bay K 8644 produced dose-dependent decreases of response rates (ED_{50} = 0.55 mg/kg). These results are consistent with previous findings that (±)-Bay K 8644 decreased rates of responding under a schedule of food-reinforcement in rats (De Beun et al., 1996c) and under a schedule of stimulus-shock termination in primates (Rosenzweig-Lipson and Barrett, 1995).

In spite of the rate-decreasing effects of the Ca^{2+} channel activator, it was possible to train rats to discriminate a dose of 0.5 mg/kg of (±)-Bay K 8644 from saline. However, six of the thirty seven rats used in the drug discrimination study were abandoned because they failed to lever-press following the administration of the training dose of (±)-Bay K 8644. For the other rats, it appeared that some tolerance developed to the rate-decreasing effects of (±)-Bay K 8644. This finding is consistent with a previous report that chronic administration of the Ca^{2+} channel activator produced tolerance to its effects on rotarod activity in mice (O'Neill and Bolger, 1988).

(±)-Bay K 8644 engendered dose-dependent increases in the percentage of rats selecting the drug-associated lever with an ED_{50} value (0.19 mg/kg) which was lower than the ED_{50} value for reducing response rate (0.55 mg/kg). (±)-Bay K 8644 required a relatively lengthy training period (mean of 65 sessions) to function as a discriminative stimulus.

The discriminative stimulus effects of Ca^{2+} channel modulators have been investigated in previous studies. Thus, Gladstein et al. (1987) successfully trained rats to discriminate a dose a 2.5 mg/kg of (±)-Bay K 8644 following oral administration, and, more recently, De Beun et al. (1996c) trained rats to discriminate a low dose of the agonist enantiomer (−)-Bay K 8644 (0.3 mg/kg, i.p.). Two dihydropyridine L-type Ca^{2+} channel blockers, isradipine and nimodipine, have also been found to serve effectively as discriminative stimuli (Schechter, 1995; De Beun et al., 1994). Taken together, these findings indicate that dihydropyridine L-type Ca^{2+} channel modulators produce discriminative stimulus effects. However, the pharmacological specificity of the discriminations has not been examined in these studies. Because it has been hypothesized that L-type Ca^{2+} channels are involved in regulating neurotransmitter release (Middlemiss and Spedding, 1985; Middlemiss, 1985; Woodward and Leslie, 1986), several dopaminergic, cholinergic and serotoninergic agonists were evaluated, in the present study, for (±)-Bay K 8644-like discriminative stimulus effects. Results indicate that the (±)-Bay K 8644 interoceptive cue is mediated by specific mechanisms. The cholinesterase inhibitor, physostigmine, was the only drug tested to produce partial substitution for (±)-Bay K 8644.

Nifedipine is the dihydropyridine Ca^{2+} channel blocker which has been shown in previous studies to antagonise the effects of (±)-Bay K 8644. It has been found that nifedipine can block the impairment of rotarod performance (Bolger et al., 1985) and the discriminative stimulus induced by (±)-Bay K 8644 (Gladstein et al., 1987). The present study extends these findings by showing that pretreatment with nifedipine produced dose-related rightward shifts of the (±)-Bay K 8644 dose–response function, indicative of competitive-like antagonism. These results are consistent with previous findings that nifedipine antagonises other effects of (±)-Bay K 8644, including increases in DOPAC and HVA levels (Bourson et al., 1989), convulsions (Petersen, 1986) and reductions of locomotor activity (Bolger et al., 1985). In contrast, the dose of nifedipine which blocks the discriminative stimulus effects of (±)-Bay K 8644 did not antagonise the effects of the activator on the rate of operant responding, suggesting that these effects are not mediated by the same mechanisms.

In contrast to the effects of nifedipine, the other dihydropyridine L-type Ca^{2+} channel blockers (nicardipine, nimodipine, isradipine and nitrendipine) did not antagonise the effects of (±)-Bay K 8644 on rotarod performance. In the drug discrimination model, nicardipine but not nimodipine produced a partial antagonism of the discriminative stimulus effects of (±)-Bay K 8644. The failure of these Ca^{2+} channel blockers to antagonise the discriminative stimulus and the motor impairing effects of (±)-Bay K 8644 is not likely to be due to inadequate doses as these doses have been shown to produce neuropharmacological effects in mice and rats such as protection against chemically-induced convulsions and antiaddictive effects (Palmer et al., 1993; De Beun et al., 1996a).

The present results differ from previous reports indicating that the behavioural effects of (±)-Bay K 8644 are antagonised by several dihydropyridine Ca^{2+} channel blockers. For example, the seizures induced by (±)-Bay K 8644 in rodents are reversed by nitrendipine, nicardipine, and nimodipine (Palmer et al., 1993). The decreased rates of responding under a fixed-ratio schedule of stimulus-shock termination produced by (±)-Bay K 8644 in squirrel monkeys are antagonised by nimodipine (Rosenzweig-Lipson and Barrett, 1995). Similarly, nimodipine can reverse the biochemical effects of the Ca^{2+} channel activator (Woodward and Leslie, 1986). However, the present re-

sults are consistent with a previous preliminary report that the discriminative stimulus effects of the agonist enantiomer (−)-Bay K 8644 were not blocked by pretreatment with nimodipine (De Vry et al., 1996). There are also reports showing that dihydropyridine Ca^{2+} channel blockers can potentiate rather than antagonise the effects of a dihydropyridine Ca^{2+} channel activator. Thus, nitrendipine potentiates (±)-Bay K 8644-induced contraction of porcine coronary artery (Dube et al., 1985) and the dihydropyridine Ca^{2+} channel blocker (−)-(R)-202 791 enhances the prolongation of Ca^{2+} channel openings by the dihydropyridine activator (+)-(S)-202 791 in rat heart cell cultures (Kokubun et al., 1986).

The present findings that dihydropyridine Ca^{2+} channel blockers differentially modified the behavioural effects of (±)-Bay K 8644 may suggest that dihydropyridine Ca^{2+} channel blockers display different intrinsic activities. Previous studies have found that dihydropyridine modulators can behave as activators or as antagonists, depending on membrane depolarization or drug concentration (Bechem et al., 1988; Triggle and Rampe, 1989). The present results may also indicate that several subtypes of dihydropyridine-sensitive L-type Ca^{2+} channels exist in the brain (Forti and Pietrobon, 1993). Several dihydropyridine binding sites may also be expressed on the same channel (Bechem et al., 1988; Triggle and Rampe, 1989). Complex interactions between compounds acting at different sites (dihydropyridines, phenylalkylamines and benzothiazepines) on the L-type Ca^{2+} channel have been shown previously. For example, dihydropyridine blockers enhance diltiazem (benzothiazepine) binding and reduce (−)desmethoxyverapamil (phenylalkylamine) binding (Schoemaker and Langer, 1985; Ferry et al., 1984). Similarly, diltiazem enhances and verapamil (phenylalkylamine) reduces dihydropyridine binding (Ferry and Glossman, 1982; Lee et al., 1984; Porzig and Becker, 1988; Wei et al., 1989). These allosteric couplings have been found to result in functional interactions, i.e. potentiation or inhibition (Kokubun et al., 1986; Usowicz et al., 1995; Watson and Little, 1994).

In conclusion, the present results showing that dihydropyridine Ca^{2+} channel blockers differentially modified the discriminative stimulus and the motor impairing effects of (±)-Bay K 8644 suggest that dihydropyridine Ca^{2+} channel blockers display different intrinsic activities and/or act at different binding sites.

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