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Comparison of the stimulus properties of ethanol and the Ca^{2+} channel antagonist nimodipine in rats

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Abstract

A variety of L-type Ca^{2+} channel antagonists, including the dihydropyridine derivative nimodipine, have been shown to be effective in reducing ethanol intake and preference in animal models of alcoholism. The behavioral mechanism involved in the anti-alcohol effects of nimodipine are, however, not clear yet. The aim of the present study was to investigate the possibility that the effects of nimodipine on ethanol intake are based on stimulus substitution. Therefore, rats were trained to discriminate ethanol (12.5% w/v, 1000 mg/kg i.p.) from saline in a two-lever food-reinforced drug discrimination procedure (dose range of ethanol tested: 125–1000 mg/kg i.p., ED_{50} value: 488 mg/kg). In cross-generalization tests with nimodipine (0.15–15 mg/kg i.p.), stimulus substitution was not noted. In addition, a cross-familiarization conditioned taste aversion paradigm was utilized. In rats, 1000 mg/kg i.p. ethanol was used as the reference drug producing a conditioned taste aversion. Effects of preexposure to ethanol (500–1500 mg/kg i.p.) and nimodipine (7.5–30 mg/kg i.p.) on the magnitude of the ethanol-induced conditioned taste aversion were investigated as an index for stimulus similarity between preexposure and reference drug. Preexposure to both ethanol and nimodipine prevented the development of a conditioned taste aversion. Contrary to the drug discrimination results, these latter findings suggest that there may be similarities between the stimulus properties of nimodipine and ethanol. Moreover, the apparent discrepancy between the results obtained in drug discrimination and cross-familiarization conditioned taste aversion suggests that different stimulus properties of ethanol control behavior in both procedures. The finding that, under particular conditions, ethanol and nimodipine appear to share common stimulus properties needs to be further evaluated, as this may be related to the reported anti-alcohol effects of nimodipine and other Ca^{2+} channel antagonists.

Keywords: Ca^{2+} channel, L-type; Nimodipine; Ethanol; Substitution; Drug discrimination; Conditioned taste aversion

1. Introduction

A considerable number of studies indicate that voltage-gated L-type Ca^{2+} channels are involved in the regulation of ethanol consumption in experimental animals (e. g., Rezvani and Janowsky, 1990; Rezvani et al., 1991a,b; Fadda et al., 1992; Pucilowski et al., 1994; De Beun et al., 1994b, 1996a). Thus, a variety of Ca^{2+} channel antagonists have convincingly been shown to reduce ethanol intake and preference in rodent and primate models of alcoholism. Drugs in the phenylalkylamine group such as levemopamil and verapamil (Rezvani and Janowsky, 1990; Rezvani et al., 1991a, 1993b; Fadda et al., 1992; De Beun et al., 1996a), as well as the 1,4-dihydropyridine derivatives darodipine, felodipine, GOE 5438 (a 1,4-dihydropyridine substituted 1,6-naphthyridine-3-carboxylic acid ethyl ester), isradipine, nicardipine, nifedipine, nimodipine and nitrendipine (Engel et al., 1988; Fadda et al., 1992; Pucilowski et al., 1992; De Beun et al., 1994a,b, 1996a; Rezvani et al., 1993a; Traber et al., 1993) have all been reported to be effective in decreasing excessive ethanol consumption.

Although the ethanol intake reducing effects of Ca^{2+} channel antagonists are well documented, the underlying mechanism for these effects is still unclear. One possible behavioral mechanism could be that ethanol and Ca^{2+} channel antagonists share common stimulus properties which are sufficiently similar to allow for stimulus substitution. Such an assumption is feasible since it has frequently been reported that ethanol can readily serve as discriminative and affective (i.e., rewarding or aversive) stimulus (see: Barry and Krimmer, 1977; Sherman et al., 1988; Barry, 1991; Samele et al., 1992) and there are first indications that at least some Ca^{2+} channel antagonists

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may also possess stimulus properties (see next paragraph). It is important to know whether L-type Ca^{2+} channel antagonists have the potential to substitute for ethanol, as this may have consequences for the possible use as a pharmacotherapy for alcoholism.

The aim of the currently described studies was to reveal whether nimodipine, as a representative of dihydropyridine derivatives found to be effective in reducing ethanol intake in animals (Rezvani et al., 1993a; Traber et al., 1993; De Beun et al., 1994b, 1996a), has stimulus properties that are sufficiently similar to those of ethanol to allow for stimulus substitution. In this respect, nimodipine seems especially of interest since it has previously been reported that this dihydropyridine shows both affective and discriminative stimulus effects in rats. Conditioned taste aversion, as well as conditioned place preference effects were found with this compound (De Vry et al., 1994; De Beun et al., 1994a, 1996b). In addition, data are available indicating that nimodipine may serve as discriminative stimulus in drug discrimination learning (De Jonge et al., 1993; De Beun et al., 1994a; De Vry et al., 1994). Besides nimodipine another dihydropyridine, isradipine, was recently found to possess discriminative stimulus properties (Schechter, 1995). As for nimodipine, this compound is also effective in reducing ethanol consumption in rats (Fadda et al., 1992; Pucilowski et al., 1994; De Beun et al., 1994b, 1996a). Interestingly, nifedipine and nicardipine, two other dihydropyridine L-type Ca^{2+} channel antagonists effective in suppressing ethanol intake (Engel et al., 1988; Fadda et al., 1992; De Beun et al., 1994b, 1996a; Pucilowski et al., 1994), failed to substitute for the isradipine cue. Only incomplete substitution could be noted with both drugs (Schechter, 1995). However, nifedipine was previously found to substitute completely for the nimodipine cue (De Jonge et al., 1993), thus indicating that this dihydropyridine also has stimulus properties. On the other hand, it has been reported that pre-treatment with isradipine can lead to a complete blockade of the ethanol cue (Colombo et al., 1994), a property of isradipine apparently not shared by nimodipine (De Vry and De Beun, unpublished data). Together, these data indicate that dihydropyridine derivatives have stimulus properties, but they may be qualitatively different, depending on the particular chemical structure. This notion is strengthened by the finding that, in contrast to nimodipine (De Vry et al., 1994; De Beun et al., 1994a, 1996b), isradipine was shown to produce neither a conditioned taste aversion nor a conditioned place preference (Calcagnetti and Schechter, 1994).

In order to reveal possible stimulus similarities between nimodipine and ethanol, a standard two-lever food-reinforced drug discrimination procedure was used. In addition to this generally accepted and well-suited method to detect stimulus similarities between drugs (see: Colpaert, 1986; Samele et al., 1992), the less established cross-familiarization conditioned taste aversion procedure was utilized. Previous studies have shown that this latter paradigm can be a useful alternative method to detect stimulus similarities between drugs with results comparable to cross-generalization findings in two-lever drug discrimination studies (Bluthé et al., 1985; De Beun et al., 1993a,b, 1996c; Berendsen and Broekkamp, 1994), although others have failed to find drug class specificity by applying similar procedures (e. g., Cappell et al., 1975; Goudie et al., 1976; Switzman et al., 1981). As an extension of the drug discrimination generalization tests, the training dose of ethanol used for drug discrimination (1000 mg/kg i.p.) was also chosen to serve as reference drug (inducing a conditioned taste aversion) in the cross-familiarization conditioned taste aversion studies. In addition, the preexposure dose ranges of both ethanol and nimodipine, selected for the cross-familiarization tests of the latter paradigm, were overlapping with the higher doses of the cross-generalization tests in the former paradigm.

2. Materials and methods

2.1. Subjects

For the drug discrimination studies, eight male Wistar rats were purchased from Harlan-Winkelmann (Hsd/Win:WU, Borchen, Germany). Body weight upon arrival at the laboratory was around 200 g, which gradually increased up to about 350 g during the course of the studies. Rats were individually housed in Macrolon type 3 cages under a normal 12 h/12 h light/dark regime (lights on at 07.00 h). Room temperature was maintained at 22–23°C. Throughout the studies, tap water was supplied ad libitum in the home cages, but the animals were (after 1 week of adaptation to the laboratory conditions) food deprived in that food access was limited to daily portions of about 13 g (standard pellets; Snniff Spezialdiäten, Soest, Germany).

For the cross-familiarization conditioned taste aversion studies, male Wistar rats were used (Harlan-Winkelmann; Hsd/Win:WU, Borchen, Germany), eight animals per experimental group. Body weight of the animals was between 220–230 g upon arrival at the laboratory and the animals were throughout the studies maintained in groups of four per cage (Macrolon type 3). A normal 12 h/12 h light/dark regime (lights on at 07.00 h) was operative. Room temperature was held constant at 22–23°C. The animals were allowed to adapt to the laboratory conditions for 1 week prior to the experimental sessions. Food (standard pellets; Snniff Spezialdiäten, Soest, Germany) and tap water were supplied ad libitum during the adaptation period. At the start of the experiments, the mean body weight of the animals was around 250 g.

2.2. Setting and apparatus

Drug discrimination sessions were performed in sound- and light-attenuated standard operant chambers (modular

test cage system, model EW-10 SF, Coulborn Instruments, Lehigh Valley, PA). The chambers were equipped with two levers equidistant from a food tray between the levers. Food reinforcement (45 mg precission pellets; Bio-Serv, Frenchtown, NJ) was delivered by an automated food dispenser located outside of the chamber. Data collection and experimental contingencies were programmed using OPN software (developed by D.G. Spencer, M.W Emmett-Oglesby and D. Arnoult) on a TRS-80 Model III microcomputer interfaced with the operant chambers. Ventilation and masking noise were provided by a fan mounted on the wall of the chamber. A white houselight was switched on during the sessions, which were conducted during the light phase of the day/night cycle.

Cross-familiarization conditioned taste aversion sessions were conducted in a standard Macrolon type 3 cage (37 × 25 × 16 cm) bedded with sawdust. Two bottles (of 300 ml content each) were placed next to each other on top of the cage, near the front wall. The drinking spouts (fitted with double stoppers) protruded about 3 cm into the cage. The distance between the drinking spouts was approximately 15 cm. Fluid consumption was measured by weighting the bottles manually. All sessions were conducted under white light conditions.

2.3. Drugs

Ethanol (ethanol absolute, 99.8% v/v) was purchased from Riedel-de Haën, Seelze, Germany and dissolved in physiological saline (0.9 NaCl). Nimodipine (BAY e 9736; isopropyl-(2-methoxyethyl)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate) was synthesized by Bayer, Wuppertal, Germany and dissolved in a solvent containing 5% v/v Solutol HS 15 (12-hydroxystearic-acid ethoxilate, purchased from BASF, Ludwigshafen, Germany), 5% v/v pure ethanol and 90% v/v saline. All doses of ethanol were injected i.p. in a fixed concentration of 12.5% w/v and, accordingly, the injection volume was varied among doses. The adjusted application volumes for the 125, 250, 500, 750, 1000 and 1500 mg/kg doses of ethanol were 1.04, 2.07, 4.14, 6.20, 8.27 and 12.41 ml/kg, respectively. The saline vehicle of ethanol was in all cases administered in the same volume as the 1000 mg/kg dose of ethanol, i.e., 8.27 ml/kg. All doses of nimodipine (including vehicle) were injected i.p. in a volume of 1 ml/kg. The saccharin (2,3-dihydro-3-oxobenz-isosulfonazole sodium salt) used for the cross-familiarization conditioned taste aversion experiments was purchased from the Sigma Chemical Company (St. Louis, MO) and dissolved in tap water in a concentration of 0.1% w/v.

2.4. Procedure

2.4.1. Drug discrimination

After initial shaping to lever press for food reinforcement, the rats ($n = 8$) were trained to discriminate 1000 mg/kg i.p. ethanol from saline under a fixed-ratio 10 schedule of reinforcement. Daily sessions were conducted which were terminated after either 50 acquired reinforcers or after 10 min. The injection-session interval was 15 min. For half of the animals, responses on the left lever were reinforced after ethanol, for the other half responses on the right lever were reinforced after ethanol. The rats were injected with ethanol or saline in the quasi-random sequence D-D-S-D-S // S-D-S-S-D // D-S-D-S-S // D-D-S-D-S (D = ethanol, S = saline, // = no sessions during the weekends) with repetition. The criterion for discrimination was set at less than 10 incorrect responses (on the non-reinforced lever) on ten consecutive training sessions prior to deliverance of the first reinforcer. Generalization tests were introduced when the number of incorrect responses was less than five on three consecutive training sessions. To establish a generalization gradient for ethanol, the animals received on test sessions the following doses of ethanol: 0, 125, 250, 500 and 1000 mg/kg i.p. For the cross-generalization tests with nimodipine, the animals were treated with 0, 0.15, 0.5, 1.5, 5 and 15 mg/kg i.p. For each ethanol and nimodipine substitution dose, five animals were randomly selected from the available pool of eight animals and submitted to the respective substitution tests (except for the 1.5 and 15 mg/kg test doses of nimodipine: $n = 6$). Each dose of both drugs was tested once. The order of treatment for both the ethanol and nimodipine doses was balanced and all injections were delivered 15 min prior to a session. During test sessions, responding on the selected lever, i.e., the lever on which ten responses accumulated first, was reinforced for the remainder of the session. Substitution tests were separated by at least three training sessions in which vehicle and drug were correctly discriminated.

2.4.2. Cross-familiarization conditioned taste aversion

24 h before the first session, the animals were water-deprived and fluid access was from then on restricted to daily experimental sessions of 15 min, which took place individually in a Macrolon type 3 test cage. After each session, the animals returned to their respective home cages. Food was freely available in the home cages throughout the procedure, but was not available during the sessions. For a given subject, all six sessions required to complete a cross-familiarization conditioned taste aversion took place in the same test cage and the cages were not cleaned between sessions. Animals designated to the same experimental group were run in parallel. During the first four sessions, both bottles contained plain tap water and the animals were injected with the preexposure drug after these sessions (with a delay of approximately 1 h). For the fifth session (conditioning session), both bottles were filled with a, for the animals novel, saccharin solution and immediately after completion of this session the animals were injected the so-called reference drug, which is aimed (and known) to induce a conditioned taste aversion. The

preexposure drug which is administered after each water session is intended to influence the magnitude of the reference drug-induced conditioned taste aversion. For the current cross-familiarization conditioned taste aversion studies, a 1000 mg/kg i.p. dose of ethanol was used as the reference drug and various doses of either ethanol (500, 750, 1000 and 1500 mg/kg i.p.) or nimodipine (7.5, 15 and 30 mg/kg i.p.) served as preexposure drugs. Each subject received only one preexposure dose of the appropriate drug. In this cross-familiarization conditioned taste aversion design, animals which were treated four times with a specific preexposure dose of a particular drug and, subsequently, were conditioned with the ethanol reference drug, built the experimental groups. For each preexposure drug tested, a control group was included which received the appropriate vehicle as both preexposure drug and reference drug. In addition, a reference group was incorporated which was also injected with the vehicle only as preexposure drug, but was (similar to the experimental groups) conditioned with the ethanol reference drug. Thus, the cross-familiarization conditioned taste aversion experiments were conducted with 11 different groups of animals ($n = 8$ per group). Between the conditioning session and the final test session for conditioned taste aversion, the animals were left undisturbed for about 72 h (wash-out period) and during the first 48 h of this period they had free access to tap water in their home cages until they were again deprived of water, 24 h prior to the test session. On this final (sixth) session, where the animals had the choice between a bottle containing the saccharin solution used for conditioning and a bottle filled with tap water, development of conditioned taste aversion learning was determined by measuring the amount of fluid consumed from both bottles. To control for location bias, the saccharin was presented in the left bottle for half of the animals in each group and in the right bottle for the other half. The capacity of the reference drug to produce a sufficient conditioned taste aversion was verified by comparing the relative saccharin intake in the reference group with the intake in the control group. A substantial conditioned taste aversion effect in the reference group allowed for using this group as baseline for the evaluation of preexposure drug effects on the reference drug-induced conditioned taste aversion, as measured in the experimental groups.

2.5. Data analysis

2.5.1. Drug discrimination

Substitution results for ethanol and nimodipine were expressed as the percentage of animals that selected the drug lever (ethanol-appropriate responding). Ethanol substitution data were submitted to least-square linear regression analysis to estimate the dose inducing 50% ethanol-appropriate responding (ED_{50}) and its 95% confidence limits. Due to a lack of full generalization, nimodipine substitution data were not further analyzed.

2.5.2. Cross-familiarization conditioned taste aversion

Initially, to check for the required conditioned taste aversion produced by ethanol in both experiments, data of control and reference groups were compared with two-tailed independent-samples t tests. Subsequently, data obtained from the ethanol and nimodipine preexposure studies were submitted to separate one-way analyses of variance (ANOVA), with the between-subjects factor DOSE (with 5 and 4 levels for ethanol and nimodipine, respectively). Included in the ANOVA were the experimental groups plus the reference group as the 0 mg/kg preexposure dose, but excluded from the ANOVA was the control group. Where appropriate, group-wise comparisons were performed with two-tailed independent-samples t tests. As with conditioned taste aversion, the dependent variable was the ratio of saccharin consumption divided by total fluid consumption. Fluid intake scores were calculated in

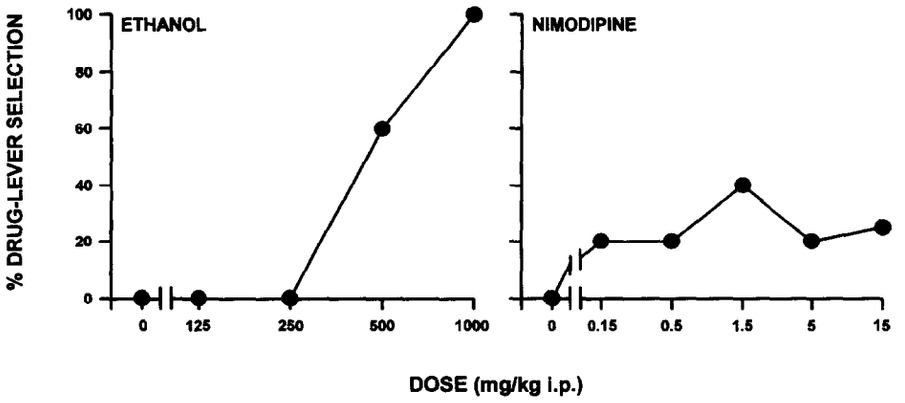

Fig. 1. Substitution results with ethanol (*left panel*) and nimodipine (*right panel*) in rats trained to discriminate 1000 mg/kg i.p. ethanol from saline. Ethanol-appropriate responding is expressed as percentage of animals selecting the drug lever. Depicted are the data obtained with four doses of ethanol and with five doses of nimodipine, administered i.p. Vehicle data are referred to as 0 mg/kg doses. $n = 5$ per dose (except for the 1.5 and 15 mg/kg doses of nimodipine: $n = 6$).

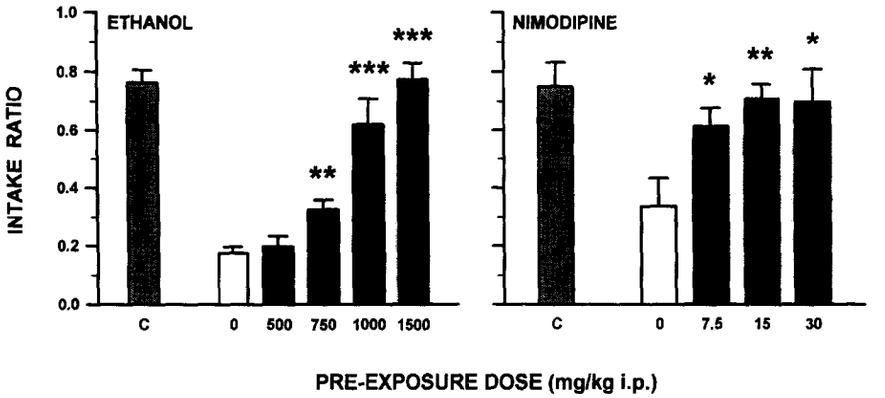

Fig. 2. Saccharin preference expressed as the ratio of saccharin intake divided by total fluid intake after preexposure to ethanol (*left panel*)[1] and nimodipine (*right panel*) and subsequent taste aversion conditioning with 1000 mg/kg i.p. ethanol. Depicted are the mean (+S.E.M.) scores for four preexposure doses of ethanol and three preexposure doses of nimodipine, administered i.p. (filled bars), together with the reference groups (vehicle preexposure, open bars) and the control groups (vehicle preexposure and vehicle taste aversion conditioning, C bars). $n = 8$ per group (except for the 500 mg/kg ethanol preexposure group: $n = 7$). Significant differences from the respective reference group are indicated by asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

grams and results were considered significant when $P < 0.05$.

3. Results

3.1. Drug discrimination

All animals trained to discriminate 1000 mg/kg ethanol from saline reached the discrimination criterion. The mean number of training sessions required to reach the criterion was 40.25 (±3.77), with a range of 27–55 sessions.

In subsequent test sessions, a generalization gradient for ethanol discrimination was established (Fig. 1), revealing partial generalization from the training cue to the 500 mg/kg test dose (60% ethanol-appropriate responding) and full generalization to the 1000 mg/kg test dose (100% ethanol-appropriate responding), with an estimated ED_{50} value of 488 mg/kg. The lower and upper 95% confidence limits were 338 and 704 mg/kg, respectively.

Fig. 1 also depicts the results obtained in cross-generalization tests with nimodipine. Nimodipine induced only partial stimulus substitution at the 1.5 mg/kg dose (40% ethanol-appropriate responding). No substitution for ethanol was found with either lower or higher doses of nimodipine. Response disruption, defined as percentage of animals not selecting a lever, was noted at the 1.5 and 15 mg/kg doses of nimodipine (17 and 33%, respectively). Animals not selecting a lever were excluded from the ethanol-appropriate responding analysis.

3.2. Cross-familiarization conditioned taste aversion

Because of difficulties with the drinking spout during data acquisition, one animal belonging to the 500 mg/kg ethanol preexposure group had to be excluded from the statistical analyses, reducing the number of cases in this group to seven.

In both cross-familiarization conditioned taste aversion experiments [1], the 1000 mg/kg dose of ethanol produced a significant conditioned taste aversion in the reference group (animals only pre-exposed to the vehicle and injected with ethanol as reference drug) as compared to the control group (animals injected with vehicle as both preexposure and reference drug), as can be seen in Fig. 2. For the ethanol and nimodipine cross-familiarization conditioned taste aversion, the results of the group-wise comparisons were, successively: $t_{(14)} = 12.59$, $P < 0.001$ and $t_{(14)} = 3.33$, $P < 0.01$.

The magnitude of the conditioned taste aversion induced by ethanol was dose dependently attenuated by preexposure to this same drug, indicated by a main DOSE effect: $F(4,34) = 24.30$, $P < 0.001$ (Fig. 2). Pre-exposure to 750, 1000 and 1500 mg/kg ethanol significantly suppressed the development of a conditioned taste aversion based on ethanol conditioning. In comparison to the reference group (vehicle preexposure), a marked reduction of the conditioned taste aversion effect was seen with $t_{(14)}$ values of −3.79 ($P < 0.01$), −4.72 and −10.18 (both $P < 0.001$), respectively. Only the lowest preexposure ethanol dose of 500 mg/kg was insufficient to weaken the

[1] The ethanol preexposure cross-familiarization conditioned taste aversion serves as a kind of control experiment, in order to be able to place in context the preexposure effects of different drugs (nimodipine in this case) on the ethanol induced conditioned taste aversion. As such, these data (including the left panel of Fig. 2, depicting the results) have been used in a previous publication (De Beun et al., 1996c). For the sake of clearness, these data are again presented in the current paper.

ethanol-induced conditioned taste aversion. The 750 mg/kg ethanol preexposure dose only reduced the magnitude of the conditioned taste aversion, without preventing its development. This dose not only differed from the reference group, but also differed from the control group, indicating that a significant conditioned taste aversion was still present: $t_{(14)} = 8.29$, $P < 0.001$. However, the two highest preexposure doses of ethanol tested were found to be maximally effective and blocked conditioned taste aversion learning completely. No significant differences could be noted between the 1000 and 1500 mg/kg groups and the control group.

As was the case for ethanol preexposure, the magnitude of the conditioned taste aversion induced by ethanol was clearly attenuated by preexposure to nimodipine (Fig. 2). A main effect of DOSE was found: $F(3,28) = 4.23$, $P < 0.05$. Pre-exposing the animals to 7.5, 15 and 30 mg/kg nimodipine significantly inhibited the development of a conditioned taste aversion based on ethanol conditioning. In comparison to the reference group (vehicle preexposure), a marked reduction of the conditioned taste aversion effect was seen with $t_{(14)}$ values of -2.39 ($P < 0.05$), -3.44 ($P < 0.01$) and -2.43 ($P < 0.05$), respectively. Each dose of nimodipine did not only reduce the magnitude of the conditioned taste aversion produced by ethanol, they prevented completely its development. All preexposure doses of nimodipine tested were found to be maximally effective and blocked conditioned taste aversion learning entirely. No significant differences could be noted between the 7.5, 15 and 30 mg/kg groups and the control group.

4. Discussion

It has been discussed in previous papers that the discriminative stimulus produced by ethanol comprises a mixed or compound stimulus (see: De Vry and Slangen, 1986; Grant and Colombo, 1993a,b; Sanger, 1993). In accordance with this, a variety of drugs acting on different neurotransmitter systems has been reported to substitute for the ethanol stimulus. Thus, *N*-methyl-D-aspartate (NMDA) receptor antagonists, as well as compounds influencing γ-aminobutyric acid (GABA) and serotonin neurotransmitter activity, were found to substitute for the ethanol cue (De Vry and Slangen, 1986; Signs and Schechter, 1988; Grant and Colombo, 1993a,b; Sanger, 1993; Shelton and Balster, 1994; Lytle et al., 1994). However, the extent to which L-type Ca^{2+} channels are involved in the discriminative stimulus properties of ethanol has, thus far, not been investigated. This is surprising, since it has repeatedly been suggested that neuronal L-type Ca^{2+} channels are involved in some of the pharmacological effects of ethanol (for reviews, see: Little, 1991; Littleton et al., 1992). Moreover, it has been reported that dihydropyridine derivatives such as nifedipine (De Jonge et al., 1993), nimodipine (De Jonge et al., 1993; De Beun et al., 1994a; De Vry et al., 1994), and isradipine (Schechter, 1995) possess discriminative stimulus properties. Although it was found that nicardipine and the non-dihydropyridine diltiazem fail to produce complete substitution for the isradipine cue, these Ca^{2+} channel antagonists nevertheless produced 57 and 43 of isradipine-appropriate responding, respectively (Schechter, 1995). These partial generalization results make it conceivable that, in addition to nifedipine, nimodipine and isradipine, other L-type Ca^{2+} channel antagonists may function as discriminative stimulus. The inability of nicardipine and diltiazem to produce complete substitution for the isradipine cue might be due to some subtle differences in the quality of the stimulus properties of the various Ca^{2+} channel antagonists. Such a difference in stimulus quality is also supported by the results of several other studies, mentioned in the introduction (Calcagnetti and Schechter, 1994; Colombo et al., 1994; De Beun et al., 1996b). The finding that nimodipine only induced partial generalization to the ethanol cue suggests that L-type Ca^{2+} channels are not primarily involved in the mechanism underlying the discriminative properties of a moderate dose of ethanol. Because it has been suggested that the quality of the ethanol cue changes with the training dose (Barry and Krimmer, 1977; Barry, 1991; Grant and Colombo, 1993a,b; but see: De Vry and Slangen, 1986), it remains possible that the involvement of Ca^{2+} channels may be different at lower or higher doses of ethanol.

Interestingly, the lack of generalization from ethanol to nimodipine was not corroborated in the additional cross-familiarization conditioned taste aversion studies. Making the animals familiar with the nimodipine stimulus effectively prevented the formation of a conditioned taste aversion after conditioning with ethanol. Within the complete dose range tested (7.5–30 mg/kg), significant conditioned taste aversion effects were eliminated. This preexposure effect of nimodipine suggests that certain aspects of the nimodipine stimulus are similar to the stimulus properties of ethanol involved in conditioned taste aversion learning. This assumption raises the question whether the familiarization effects are symmetrical, i.e., whether ethanol preexposure would equally be effective in attenuating nimodipine conditioned taste aversion learning. As yet, no such data are available for nimodipine or any other Ca^{2+} channel antagonist. However, it should be mentioned here that the ethanol-containing solvent might have been a factor in the cross-familiarization studies with nimodipine. Given the injection volume of 1 ml/kg, the animals received a dose of approximately 40 mg/kg ethanol in combination with different doses of nimodipine. Although a preexposure dose of 500 mg/kg ethanol was found to be too low to attenuate the ethanol-induced conditioned taste aversion, it can nevertheless not be ruled out that nimodipine somehow potentiated the stimulus effects of the ethanol in the solvent. Anyhow, such an interaction effect was not noted in the drug discrimination studies, using the same ethanol-

containing solvent as for the taste aversion studies. Nimodipine (plus 40 mg/kg ethanol) did not substitute for ethanol, although in this procedure ethanol alone substituted at a lower dose (500 mg/kg) as was required for producing ethanol familiarization (750 mg/kg).

Several possible explanations for the divergent results obtained with nimodipine in the two different procedures should be discussed. Firstly, It can be speculated that the cross-familiarization conditioned taste aversion procedure might be capable to reveal stimulus similarities between drugs which remain obscure in two-lever drug discrimination procedures, due to the completely different settings of the paradigms. That is, the learning mechanisms involved in drug discrimination and cross-familiarization conditioned taste aversion may be based on different principles, making some specific components of the stimulus complex produced by a given drug relatively more salient under drug discrimination conditions as compared with cross-familiarization conditioned taste aversion conditions, or vice versa. It is therefore possible that the stimulus properties essential for drug discrimination learning are to a large extent dissimilar for ethanol and nimodipine (as indicated by no, or as in this case, only partial generalization effects). For cross-familiarization conditioned taste aversion learning, other stimulus components might be more important, and these may be shared by ethanol and nimodipine, explaining the full familiarization effect. Secondly, the cross-familiarization conditioned taste aversion procedure could be more sensitive to detect discriminative stimulus properties of (some) drugs than drug discrimination is. Although full generalization was not observed in drug discrimination, the ethanol cue nevertheless generalized partially to nimodipine, and this result was not confounded with possible behavioral toxicity. This stimulus effect of nimodipine may have been sufficient to produce familiarization in cross-familiarization conditioned taste aversion. Thirdly, it has been discussed previously that cross-familiarization conditioned taste aversion paradigms may show a lack of drug class specificity (see: Braveman, 1977; Gamzu, 1977), and that nonspecific drug effects might be responsible for preexposure effects on conditioned taste aversion learning. Among others, explanations based on nonspecific sickness, stress-related physiological changes, tolerance development to certain drug effects essential for conditioned taste aversion conditioning, unnatural need states and competing learning mechanisms, have been discussed elsewhere (e.g., Cannon et al., 1975; Cappell et al., 1975; Goudie et al., 1976, 1982; Braveman, 1977; Gamzu, 1977; Switzman et al., 1981; Grant, 1987; Rabin et al., 1988) and will therefore not be discussed here.

An important interpretation of preexposure effects in conditioned taste aversion which can be regarded as nonspecific, but which could show drug-class specificity as well, may be that drug-induced conditioned taste aversions are mainly based on novelty. Animals avoid a taste associated with first-time experience of certain (psychotrophic) drug effects, leaving open the possibility that also drugs which are shown to function as a rewarding stimulus (as measured with conditioned place preference) or to act as a positive reinforcer (as measured with self-administration), can have the capacity to induce a conditioned taste aversion (Gamzu, 1977; Goudie, 1979; Goudie et al., 1982; Grant, 1987; Hunt and Amit, 1987). Thus, the essential factor is that a drug possesses stimulus properties per se, which need not necessarily be aversive in nature to result in conditioned taste aversion learning. Acceptance of this presumption is a prerequisite of the cross-familiarization conditioned taste aversion paradigm. By pre-exposing animals to the drug effects prior to the actual conditioning with this drug, the novelty of the stimulus complex produced by the drug is eliminated and, as a consequence, the formation of a conditioned taste aversion is weakened or prevented. Extending this rationale to preexposure drugs other than the conditioning drug, the more the stimulus complex produced by the preexposure drug resembles the stimulus of the conditioning drug, the less the magnitude of the induced conditioned taste aversion will be. Thus, previous studies have shown that the cross-familiarization conditioned taste aversion procedure can be a useful method to detect stimulus similarities between drugs, with results comparable to cross-generalization findings in two-lever drug discrimination studies (Bluthé et al., 1985; De Beun et al., 1993a,b, 1996c; Berendsen and Broekkamp, 1994). However, as mentioned before, interpretation of cross-familiarization conditioned taste aversion results in terms of stimulus similarities is not entirely unproblematic since in several other studies failures to find drug class specificity have been reported (e.g., Cappell et al., 1975; Goudie et al., 1976; Switzman et al., 1981). The present results with nimodipine also demonstrate that further research is required to evaluate the validity of the cross-familiarization conditioned taste aversion procedure as an alternative to drug discrimination substitution tests.

As suggested by a variety of animal studies on ethanol intake and preference (Engel et al., 1988; Fadda et al., 1992; Pucilowski et al., 1992, 1994; Rezvani et al., 1993a; De Beun et al., 1994b, 1996a), L-type Ca^{2+} channel antagonists may offer an interesting approach to the pharmacotherapy of alcoholism. It is therefore of importance to characterize in more detail the stimulus properties of these compounds in relation to ethanol. This is of particular interest because both affective (i.e., rewarding or aversive) and discriminative stimulus properties of L-type Ca^{2+} channel antagonists may be involved in the reported ethanol intake reducing effects of this class of drugs. The present investigation with nimodipine suggests that dihydropyridine derivatives and ethanol may have some stimulus properties in common, but further studies are needed to reveal in more detail the possible relationship between stimulus properties of ethanol and Ca^{2+} channel antagonists.

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