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Ethanol Intake-Reducing Effects of Ipsapirone in Rats Are Not Due to Simple Stimulus Substitution

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DE BEUN, R., A. LOHMANN, R. SCHNEIDER AND J. DE VRY. *Ethanol intake-reducing effects of ipsapirone in rats are not due to simple stimulus substitution.* PHARMACOL BIOCHEM BEHAV 53(4) 891–898, 1996.—The present series of experiments was conducted to investigate whether the previously reported ethanol intake reducing effects of the 5-HT_{1A} receptor agonist ipsapirone could be based on possible stimulus similarities between both compounds. Rats were trained to discriminate ethanol (12.5% w/v, 1000 mg/kg, IP) from saline in a two-lever food-reinforced drug discrimination (DD) procedure. Discrimination criterion was reached after a mean number of training sessions of 42. In subsequent generalization sessions, a dose-response curve was established for ethanol (125–1000 mg/kg, IP, ED_{50} value: 355 mg/kg). In additional cross-generalization tests with ipsapirone (1–30 mg/kg, IP), stimulus substitution for the ethanol cue was not noted (maximal degree of generalization: 33%, at 10 and 30 mg/kg). To confirm the DD findings that ipsapirone does not substitute for ethanol, an alternative cross-familiarization conditioned taste aversion paradigm (CF-CTA) was utilized. In rats, 1000 mg/kg IP ethanol was used as the reference drug producing a conditioned taste aversion (CTA). It was found that preexposure to ethanol (500–1500 mg/kg, IP) dose-dependently attenuates the CTA produced by this same drug. Full familiarization was noted with 1000 and 1500 mg/kg. In contrast with this, ipsapirone (1–30 mg/kg, IP) failed to abolish ethanol-induced CTA, suggesting again that the ipsapirone stimulus complex is dissimilar to that produced by ethanol. Because the present findings indicate that, in rats, ipsapirone does not substitute for ethanol, it is suggested that the reported ethanol intake-reducing effects of ipsapirone in animal models of alcoholism are not due to simple stimulus substitution.

Alcohol intake Serotonin Ipsapirone Ethanol Drug discrimination Generalization Conditioned taste aversion Rats

EVIDENCE is rapidly growing that central serotonergic (5-hydroxytryptamine, 5-HT) neurotransmission may play an important role in the regulation of ethanol intake (15,25,30, 32,34,39,40,46,51). In line with this, a considerable number of drugs that alter 5-HT activity, such as 5-HT uptake inhibitors and 5-HT releasers, as well as compounds selective for particular 5-HT receptor subtypes, have been found to suppress ethanol intake and to reduce ethanol preference in animal models of alcoholism (7,10,15,25,27,29,30,39,40,46,51). These positive results, deriving from animal research, prompted the idea that drugs acting on 5-HT neurotransmission may be potentially important as pharmacotherapeutics for the treatment of alcohol dependence and craving [see (10,30)]. Although it remains to be determined which 5-HT receptor subtypes provide appropriate targets for serotonergic pharmacotherapy of alcoholism, 5-HT_{1A} receptors appear to be among the most interesting candidates [for review, see (13)]. Thus, it has been demonstrated that a variety of selective 5-HT_{1A} receptor agonists, such as the pyrimidinylpiperazine derivative ipsapirone (7,10,15,39,46,49) and the aminotetralin derivative 8-OH-DPAT (10,15,27,46), are able to suppress ethanol intake and preference in a number of rodent models of alcoholism.

Although 5-HT_{1A} receptor agonists appear to be highly effective in reducing ethanol consumption, the behavioral mechanism underlying such effects are not yet clear (14). One possibility could be that these compounds possess stimulus properties that are similar to those of ethanol, providing the basis for the ethanol intake-reducing effects simply by stimulus substitution. Such an assumption is feasible because it has

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been found that both ethanol and 5-HT_{1A} receptor agonists can function as discriminative [see (2,9,36,45,50)] and affective (i.e., rewarding or aversive) stimulus [see (12,17,31,33,42, 43)]. Obviously, for the potential use in pharmacotherapy, it is important to know whether a given drug that reduces ethanol intake substitutes for the stimulus effects of ethanol. If this should be the case, it might make this particular drug less attractive for its use in the treatment of alcoholism.

The aim of the present studies was to reveal whether or not the 5-HT_{1A} receptor agonist ipsapirone produces discriminative stimulus effects that are sufficiently similar to the stimulus complex produced by ethanol to allow for stimulus substitution. The dose range of ipsapirone was such chosen (1-30 mg/kg) that it fully covered all dose ranges described in the literature as being effective in suppressing ethanol intake in different rodent models of alcoholism (i.e., roughly from 5 to 20 mg/kg) (7,10,15,39,46,49). To detect possible stimulus similarities between ipsapirone and ethanol, a standard two-lever food-reinforced drug discrimination (DD) procedure was applied. In addition to this commonly used method to detect stimulus correspondence between drugs [see (8,36)], the less established cross-familiarization conditioned taste aversion (CF-CTA) procedure was utilized [see (5,20,26,35,48)]. Previous studies have shown that the CF-CTA procedure can be a useful alternative method to detect stimulus similarities between drugs, with results comparable to cross-generalization findings in two-lever DD studies (3,4,11,12), although others have failed to find drug class specificity by applying similar procedures (6,19,47,48). As an extension of the DD generalization tests, CF-CTA studies were conducted with the same dose of ethanol used in the DD studies as the reference drug (inducing a CTA), in combination with various doses of either ethanol itself or ipsapirone as preexposure drug. Applying this alternative method allowed us to confirm the substitution results obtained in DD.

METHODS

Subjects

For the drug discrimination (DD) 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 L : 12 D regime (lights on at 0700 h). Room temperature was maintained at 22-23°C. Throughout the studies, tap water was supplied ad lib 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 GmbH, Soest, Germany).

For the conditioned taste aversion (CTA) and cross-familiarization CTA (CF-CTA) 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 and 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 L : 12 D regime (lights on at 0700 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 GmbH, Soest, Germany) and tap water were supplied ad lib during the adaptation period. At the start of the experiments, the mean body weight of the animals was around 250 g. All procedures were approved by our institutional committee for the use of animals in research, following the guidelines as given by the german government.

Apparatus

Drug discrimination. DD 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 Inc., 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.

Conditioned taste aversion and cross-familiarization conditioned taste aversion. CTA and CF-CTA 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.

Drugs

Ethanol (ethanol absolute, 99.8% v/v) was purchased from Riedel-de Haën AG (Seelze, Germany). Ipsapirone (BAY q 7821) was synthesized by Bayer AG (Leverkusen, Germany). Ethanol was dissolved in physiological saline (0.9% NaCl). Ipsapirone was dissolved in distilled water. All doses of ethanol were injected IP 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 ipsapirone (including vehicle) were injected IP in a volume of 1 ml/kg. The saccharin (2,3-dihydro-3-oxobenzisosulfonazole sodium salt) used for the CTA and CF-CTA experiments was purchased from the Sigma Chemical Company (St. Louis, MO) and dissolved in tap water in a concentration of 0.1% w/v.

Procedure

Drug discrimination. After initial shaping to lever press for food reinforcement, the rats ($N = 8$) were trained to discriminate 1000 mg/kg IP ethanol from saline under a fixed-ratio 10 schedule of reinforcement. Daily sessions were conducted that 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 quasirandom 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 nonreinforced lever) on 10 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 dose-response curve for ethanol, the animals received on test sessions the following doses of ethanol: 0, 125, 250, 500, and 1000 mg/kg IP. For the cross-generalization tests with ipsapirone, the animals were treated with 0, 1, 3, 10, 30 mg/kg IP. For each ethanol and ipsapirone test condition, six animals were randomly selected from the available pool of eight animals and submitted to the respective substitution tests. Each dose of both drugs was tested once. The order of treatment for both the ethanol and ipsapirone 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 10 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.

Conditioned taste aversion. Twenty-four hours 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 CTA 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. This phase of the procedure gave the animals the opportunity to learn to drink a reasonable amount of fluid in a short period of time. For the fifth session (conditioning session), both bottles were filled with a novel, saccharin solution and immediately after completion of this session the animals were injected with either the appropriate vehicle or the test drug. The following drug conditions were tested: for the ethanol CTA, the controls were injected with IP saline and the experimental groups received either 500, 750, 1000, or 1500 mg/kg IP ethanol; for the ipsapirone CTA, the controls received IP distilled water and the experimental groups were treated with 1, 3, 10, 20, or 30 mg/kg IP ipsapirone. Only one dose of a particular drug (or the corresponding vehicle) was tested per animal, making up a total of 15 groups of animals (N = 8 per group). Between the conditioning session and the final test session for CTA, 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. During this last session, one bottle contained the saccharin solution used for conditioning and the other bottle was filled with tap water. 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. By measuring the amount of fluid consumed from both bottles separately, drug-induced CTA could be determined by comparison of the relative saccharin intake in the drug-treated groups and their vehicle-treated controls.

Cross-familiarization conditioned taste aversion. This procedure was identical to the CTA procedure described above, with one essential difference in that the animals were not only injected with a drug immediately after the conditioning session with saccharin (fifth session), but also (with a delay of approximately 1 h) after the four preceding sessions with tap water. In the currently utilized CF-CTA design, the drug injected after the conditioning session is aimed (and known) to induce a CTA, and is designated the reference drug. The drug that is administered after each water session is intended to influence the magnitude of the reference drug-induced CTA and is called the preexposure drug. For the presently described CF-CTA studies, a 1000-mg/kg IP dose of ethanol was used as the reference drug and various doses of either ethanol (500, 750, 1000, and 1500 mg/kg IP) or ipsapirone (1, 3, 10, and 30 mg/kg) served as preexposure drugs. Each subject received only one preexposure dose of a given drug. In this CF-CTA design, animals that were treated four times with a specific preexposure dose of a particular drug and, subsequently, were conditioned with the ethanol reference drug constituted the experimental groups. For each preexposure drug tested, a control group was included that received the appropriate vehicle as both preexposure drug and reference drug. In addition, a reference group was incorporated that 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 CF-CTA experiments were conducted with 17 different groups of animals (N = 8 per group). On the 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 CTA learning was determined by measuring the amount of fluid consumed from both bottles. The capacity of the reference drug to produce a sufficient CTA was verified by comparing the relative saccharin intake in the reference group with the intake in the control group. A substantial CTA effect in the reference group allowed for using this group as baseline for the evaluation of preexposure drug effects on the reference drug-induced CTA, as measured in the experimental groups.

Data Analysis

Drug discrimination. Substitution results for ethanol and ipsapirone 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% drug lever selection (ED_{50}) and its 95% confidence limits. Due to a lack of full generalization, ipsapirone substitution data were not analyzed further.

Conditioned taste aversion. Data obtained from the ethanol and ipsapirone experiments were submitted separately to one-way analyses of variance (ANOVA), with the between-subjects factor Dose (5 and 6 levels, respectively, including the corresponding control groups as 0 mg/kg dose). Additional group-wise comparisons took place with two-tailed independent-samples t-tests. On all occasions, the dependent variable was the ratio of saccharin consumption divided by total fluid consumption. Fluid intake scores were calculated in grams and results were considered significant when $p < 0.05$.

Cross-familiarization conditioned taste aversion. Initially, to check for the required CTA produced by the reference drug

in each experiment, data of control and reference groups were compared with two-tailed independent-samples t-tests. Subsequently, data obtained from the ethanol and ipsapirone preexposure experiments were submitted to separate one-way ANOVAs, with the between-subjects factor Dose (with 5 levels for both drugs). 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 CTA, the dependent variable was the ratio of saccharin consumption divided by total fluid consumption. Fluid intake scores were calculated in grams and results were considered significant when $p < 0.05$.

RESULTS

Drug Discrimination

All animals trained to discriminate 1000 mg/kg ethanol from saline reached discrimination criterion. The mean number of training sessions required to reach criterion was 42 ± 2.27, with a range of 35–53 sessions.

In subsequent generalization sessions, a dose-response curve with ethanol was established (Fig. 1), showing full generalization to the 1000 mg/kg test dose (100% ethanol appropriate responding), with an estimated ED_{50} value of 355 mg/kg. The lower and upper 95% confidence limits were 208 and 604 mg/kg, respectively. A decrease in response rate was not noticed for any of the ethanol doses tested (i.e., response rate less than 50% of vehicle response rate).

Figure 1 also depicts the results obtained in cross-generalization tests with ipsapirone. None of the ipsapirone doses tested (1–30 mg/kg) substituted for the ethanol cue (maximal degree of generalization: 33%, at 10 and 30 mg/kg). A decrease in response rate was not noticed for any of the ipsapirone doses tested (less than 50% of vehicle response rate).

Conditioned Taste Aversion

Due to problems with some drinking spouts during data acquisition, no reliable CTA data could be obtained for four animals and these subjects were excluded from the statistical analyses. Therefore, in the 1500 mg/kg ethanol and in the 3, 10, and 20 mg/kg ipsapirone groups, the number of cases was reduced to seven.

Figure 2 shows the relative amount of saccharin consumed during the final test session, after conditioning with various doses of ethanol or the vehicle. The association of saccharin intake with ethanol injections resulted in a dose-dependent CTA. A significant main effect of DOSE was noted: $F(4, 34) = 4.38$, $p < 0.01$. Additional group-wise comparisons between the control group and the experimental groups revealed that the 1000 and 1500 mg/kg doses of ethanol induced a significant CTA [$t(14) = 4.19$ and $t(13) = 5.58$, respectively, both $p < 0.001$]. The 500 and 750 mg/kg doses were ineffective in producing a CTA.

Also depicted in Fig. 2, the CTA results obtained with five doses of ipsapirone plus the corresponding control group. Ipsapirone was found to induce a dose-dependent CTA. The factor DOSE showed a significant main effect, $F(5, 39) = 9.61$, $p < 0.001$. Subsequent group-wise comparisons revealed that the 20 and 30 mg/kg doses were clearly effective in producing a CTA compared to the control group [$t(13) = 3.42$, $p < 0.01$ for the 20 mg/kg dose, and $t(14) = 4.69$, $p < 0.001$ for the 30 mg/kg dose]. The three lowest doses tested (1, 3 and 10 mg/kg) showed no effect.

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 CF-CTA experiments, the 1000 mg/kg dose of ethanol produced a significant CTA in the reference group (animals only preexposed to the vehicle and injected with ethanol as reference drug) compared to the control group (animals injected with vehicle as both preexposure and reference drug), as can be seen in Fig. 3. For the ethanol and ipsapirone CF-CTA, the results of the group-wise comparisons were $t(14) = 12.59$ and $t(14) = 4.32$ (both $p < 0.001$).

The magnitude of the CTA induced by ethanol was dose

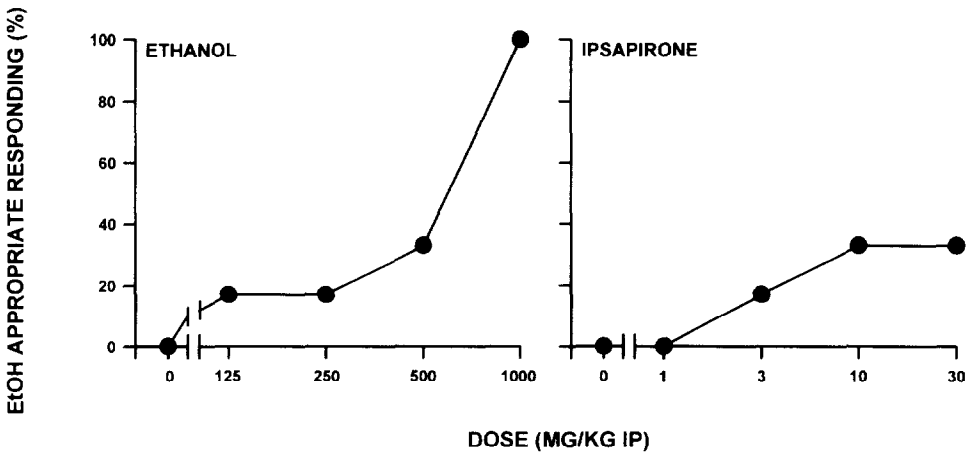

FIG. 1. Substitution results with ethanol (left panel) and ipsapirone (right panel) in rats trained to discriminate 1000 mg/kg IP ethanol from saline. Ethanol-appropriate responding is expressed as percentage of animals selecting the ethanol lever. Depicted are the data obtained with 4 doses of ethanol and 4 doses of ipsapirone administered IP. $N = 6$ per dose.

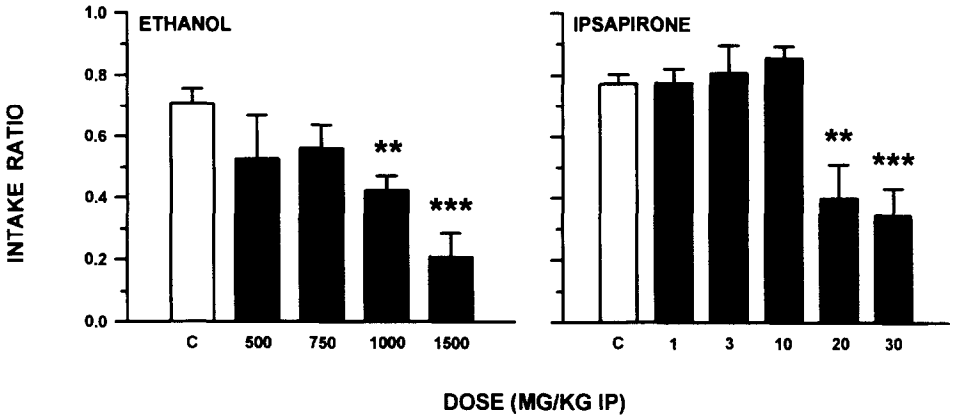

FIG. 2. Saccharin preference expressed as the ratio of saccharin intake divided by total fluid intake during the final test session for CTA, after conditioning with ethanol (left panel) and ipsapirone (right panel). Depicted are the mean + SEM scores for four doses of ethanol and for five doses of ipsapirone, administered IP (filled bars), together with the respective controls (C, open bars). $N = 8$ per group (except for the 1500 mg/kg ethanol dose and the 3, 10, and 20 mg/kg ipsapirone doses; $N = 7$). Significant differences from controls are indicated by asterisks (**$p < 0.01$, ***$p < 0.001$).

dependently attenuated by preexposure to this same drug, indicated by a main DOSE effect: $F(4, 34) = 24.30$, $p < 0.001$ (Fig. 3). Preexposure to 750, 1000, and 1500 mg/kg ethanol significantly suppressed the development of a CTA based on ethanol conditioning. In comparison to the reference group (vehicle preexposure), a marked reduction of the CTA effect was seen [$t(14)$ 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 ethanol-induced CTA. The 750 mg/kg ethanol preexposure dose only reduced the magnitude of the CTA, without preventing its development. This dose not only differed from the reference group, but also differed from the control group, indicating that a significant CTA 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 CTA learning completely. No significant differences could be noted between the 1000 and 1500 mg/kg groups and the control group.

The preexposure effects of ipsapirone on ethanol-induced CTA are also depicted in Fig. 3. No preexposure effects of ipsapirone on CTA learning could be detected, as confirmed by the absence of a main effect of Dose: $F(4, 35) = 1.03$, NS. Not a single preexposure dose of ipsapirone resulted in a significant difference with the reference group with regard to the magnitude of the CTA produced by ethanol.

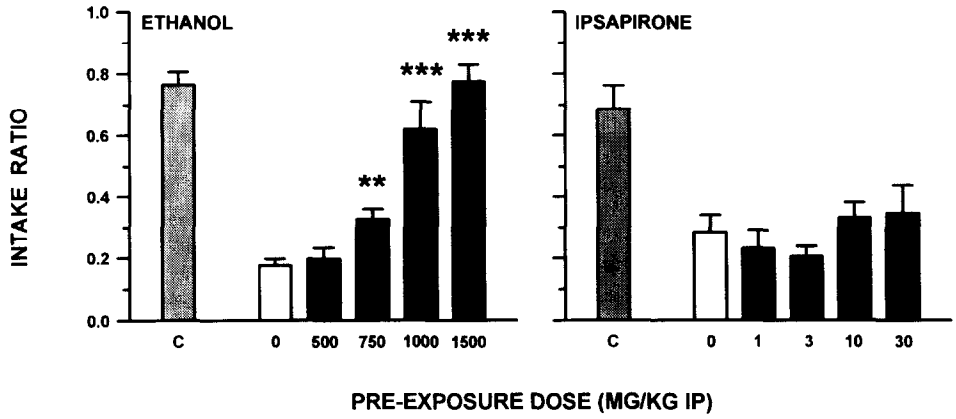

FIG. 3. Saccharin preference expressed as the ratio of saccharin intake divided by total fluid intake during the final test session for CF-CTA, after preexposure to ethanol (left panel) or ipsapirone (right panel) and CTA conditioning with 1000 mg/kg IP ethanol. Depicted are the mean + SEM scores for four preexposure doses of ethanol and for four preexposure doses of ipsapirone, administered IP (filled bars), together with the respective reference groups (vehicle preexposure, the open bars) and the respective control groups (vehicle preexposure and vehicle CTA conditioning, C bars). $N = 8$ per group (except for the 500 mg/kg ethanol preexposure dose; $N = 7$). Significant differences from reference group are indicated by asterisks (**$p < 0.01$, ***$p < 0.001$).

DISCUSSION

The findings of the present study demonstrate that the 5-HT_{1A} receptor agonist ipsapirone does not substitute for the ethanol stimulus complex. In a two-lever drug discrimination (DD) procedure, where rats were trained to discriminate a moderate dose of 1000 mg/kg ethanol from saline, at best only partial generalization from the ethanol cue to the ipsapirone stimulus was found, never exceeding a level of 33% of ethanol-appropriate responding. Absence of generalization from ethanol to ipsapirone could be confirmed in a cross-familiarization conditioned taste aversion (CF-CTA) procedure, where it was found that none of the ipsapirone doses familiarized for the stimulus effect of ethanol.

It has been suggested previously that the discriminative stimulus produced by ethanol comprises a mixed or compound stimulus [see (16,22,23,37)]. Substitution for ethanol has been reported for a variety of drugs acting on different neurotransmitter systems. Thus, NMDA antagonists [e.g., (23,37,41), but see also (1)], as well as agents affecting GABAergic [e.g., (16,28,41)] and serotonergic [e.g., (22,44)] neurotransmission, were found to substitute for the ethanol cue. With respect to the latter neurotransmitter system, however, relatively few substitution studies have focused on drugs interacting selectively with 5-HT receptor subtypes. Until now, positive results have only been reported for the nonselective 5-HT_1 receptor agonist TFMPP. With moderate training doses of ethanol (1000–1500 mg/kg), this compound completely substitutes for the ethanol cue (22,44). The subtype of receptor that may mediate the discriminative stimulus effects of TFMPP that are similar to those of ethanol is, however, not completely clear, although it has been suggested that the 5-HT_{1B} receptor is involved (22,38). In addition, it has been hypothesized that the effects of ethanol that mimic those of the serotonergic agent TFMPP in DD learning are relatively prominent at or near training doses of 1000–1500 mg/kg. At higher training doses, other components of the assumed mixed ethanol cue may overshadow the 5-HT_1 receptor-related effects of ethanol (22,23). The present negative cross-generalization results with ipsapirone after training with a similar moderate dose (1000 mg/kg) of ethanol suggests that 5-HT_{1A} receptors are not involved in the discriminative stimulus effects of moderate doses of ethanol. This conclusion is in accordance with the results of earlier studies with the 5-HT_{1A} receptor agonists 8-OH-DPAT and buspirone, where it was found that these compounds do not to substitute for a low (600 mg/kg, IP) dose of ethanol (44). Taken together, it seems that the 5-HT_{1A} receptor does not appear to be a primary site for central mediation of the ethanol cue.

The positive CTA results obtained with ipsapirone (with an effective minimal dose of 20 mg/kg) extend the findings reported previously with other 5-HT_{1A} receptor agonists in CTA learning. Thus, it has recently been reported that buspirone and gepirone are capable to induce a CTA in rats (31). In addition, these authors were able to show that the quality of the affective stimulus effect is apparently rewarding for both drugs, by applying a conditioned place preference (CPP) paradigm (31). In another paper, CTA effects of 8-OH-DPAT and buspirone were described in mice (12). Similar to buspirone and gepirone, 8-OH-DPAT also appears, at least with relatively low doses, to be rewarding in the CPP paradigm (17,33,43). To our knowledge, no CPP data are at present available for ipsapirone, but based on the already available 5-HT_{1A} data it can be predicted that this 5-HT_{1A} agonist may also show rewarding effects in a CPP design.

After having established that ethanol and ipsapirone have the capacity to act as an affective stimulus in the CTA procedure, CF-CTA studies were conducted with either ethanol itself or ipsapirone as the preexposure drug. As reference drug, producing a CTA towards saccharin, the 1000 mg/kg dose of ethanol provided a suitable baseline to investigate the effects of multiple preexposure to various doses of ethanol itself on the magnitude of the ethanol-induced CTA. Making the animals familiar with the ethanol stimulus, prior to conditioning, resulted in an unambiguous and dose-dependent attenuation of the ethanol-induced CTA. In contrast to ethanol, ipsapirone preexposure failed completely to prevent the development of a CTA induced by ethanol. Within the dose range tested (1–30 mg), none of the ipsapirone doses resulted in a significant difference in saccharin intake as compared to the reference group (vehicle preexposure). Thus, although ipsapirone in itself possesses affective stimulus properties, as indicated by clear CTA learning, the stimulus aspects responsible for this effect seem to be qualitatively different from the stimulus complex produced by ethanol.

Although it has become clear that drug preexposure may influence the formation of a CTA, a variety of explanations for preexposure effects have been proposed (5,6,19). Among these is the suggestion that drug-induced CTAs are, on many instances, mainly based on novelty. The animals avoid a taste associated with the first-time experience of certain (psychotrophic) drug effects, leaving open the possibility that drugs 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 also have the capacity to induce a CTA [e.g., (18,21,24,26)]. Thus, the essential factor is that a drug possesses stimulus properties per se, which need not necessarily be aversive in nature to result in CTA learning. Acceptance of the presumption that novelty of the drug stimulus is a crucial aspect of CTA learning is a prerequisite of the CF-CTA paradigm. By preexposing 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 CTA 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 CTA will be.

Taken together, the present study indicates that ethanol and ipsapirone have stimulus properties of a qualitatively different nature. Demonstration of a lack of substitution—together with the absence of familiarization—of ipsapirone for ethanol is important in light of the consideration that 5-HT_{1A} receptor agonists may provide a suitable pharmacotherapy for alcoholism (13). It has been repeatedly demonstrated that ipsapirone, as well as other 5-HT_{1A} receptor agonists, suppresses ethanol intake and preference in animal models of alcoholism [e.g., (7,10,15,39,46,49)]. The present study suggests that these effects of ipsapirone are not merely the result of stimulus substitution. Further studies are required to elucidate the mechanism underlying this intriguing "antialcohol" effect.

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