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Isradipine Combined With Naltrexone Persistently Reduces the Reward-Relevant Effects of Cocaine and Alcohol

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CRAMER, C. M., L. R. GARDELL, K. L. BOEDEKER, J. R. HARRIS, C. L. HUBBELL AND L. D. REID. *Isradipine combined with naltrexone persistently reduces the reward-relevant effects of cocaine and alcohol.* PHARMACOL BIOCHEM BEHAV **60(**2) 345–356, 1998.—Previous studies have revealed that the combination of small doses of isradipine and naltrexone (ISR&NTX) blocks the ability of cocaine to enhance pressing for rewarding, lateral hypothalamic brain stimulation. Further, such combinations also reduce rats' intakes of alcoholic beverages. Here, we asked whether ISR&NTX would lose its ability to reduce the reinforcing effects of cocaine and alcohol when given daily. Specifically, after almost 2 months of daily injections, ISR&NTX blocked the expression of a cocaine-induced conditioned place preference (CPP). By themselves, ISR and NTX were not effective at blocking cocaine's effects. Subsequent to the CPP procedures, the rats continued to receive daily injections for another 3 weeks. During this time, they were given access to water and an alcoholic beverage for 2 h a day. As expected, placebo controls gradually increased their daily intakes until they were taking about 2 g/kg of ethanol daily. ISR, NTX, and ISR&NTX blocked the typical pattern of intakes. At the end of the 3-week period, the rats had received 80 consecutive daily injections. The data suggest that the salient effects of ISR&NTX do not wane. The data support the idea that ISR&NTX would be a useful pharmacotherapy for poly drug abuse. © 1998 Elsevier Science Inc.

Cocaine Ethanol Isradipine Naltrexone Conditioned place preference Pharmacotherapy Drug abuse Rats

ISRADIPINE (ISR) is an L-type calcium channel inhibitor useful in treating hypertension (5). Naltrexone (NTX) is a long-acting selective opioid antagonist useful in treating heroin and alcohol use disorders [for reviews, see (20,22)]. Using rats as subjects, we have recently demonstrated that combinations of small doses of ISR and NTX block the reward-relevant effects of cocaine (27) and reduce the intake of alcoholic beverage under the circumstances in which large intakes usually occur (11). An important issue, in terms of its usefulness in treating substance use disorders, is whether the initial effects of ISR&NTX persist or wane with repeated administrations. Here, we address that issue by using conditioned place preference (CPP) procedures for indexing cocaine's reward-relevant effects.

EXPERIMENT 1

Conditional place preference (CPP) procedures were developed to assess the affective consequences associated with injections of drugs (3,4,28,32). The basic procedure involves an alley having two distinct places. Before the experience of a drug, a rat's preferences for the two places are known (e.g., preference for the places assessed during a baseline measure). With the beginning of conditioning trials, a divider is placed in the alley so that a rat will experience only one place in the alley at a time. Then, the drug in question is injected before a rat is placed in one side of the alley (the place or side of putative drug conditioning). The time in the alley is usually about 30 min, and corresponds to the period of time in which the drug is having its pharmacodynamic effects. On another occa-

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sion (generally 24 h later), an injection of the carrier of the drug (placebo) is given before the rat is placed on the other (alternative) side of the alley. The procedure of giving drug or placebo before being placed into their respective sides is often repeated a number of times.

Subsequent to conditioning, there is a test for the rat's place preference. The usual test occurs without injections of any kind and is a measure of preference for the places in the alley. When the effects of drugs that are typically abused by people are paired with a particular place (and the dose and time after dosing are arranged so that the salient drug effect can be associated with that place), rats change their preference for that place as manifest by spending more time there than they would have otherwise. Because drugs that become the focus of substance use disorders produce characteristic effects with CPP testing, the procedures of CPP testing can be used to assess the effectiveness of potential pharmacotherapies for substance use disorders (1).

Usually, during a test for a CPP, no drug is given before testing. Bozarth (3) found, however, that when the drug was also given before testing, there was an enhanced preference for the place that was previously paired with the drug's effects. The interaction between the CPP and the drug effect itself, producing an enhanced CPP, is thought to reflect the apparent enhanced motivation for more drug that is often manifest when an addict samples a drug (3,19). Here, subsequent to tests for CPP associated with cocaine's effects, we also ran a test in which cocaine was given before testing.

METHOD

Subjects. Sixty male Sprague–Dawley rats were purchased from Taconic Farms (Germantown, NY) when they weighed about 185 g. When the rats arrived, they were housed in individual hanging cages where they always had standard laboratory chow and water. The windowless room housing the rats was maintained at about 22 ± 2°C and had 12 h of incandescent light daily beginning at 0700 h.

Drugs and injections. The doses of cocaine HCl (from Sigma) were 5 and 20 mg/kg. ISR (from Novartis), 3 mg/kg, was given to one group of rats and NTX HCl (from DuPont Merck), 3 mg/kg, was given to another group. One group received 1 mg/kg of ISR plus 3 mg/kg of NTX, i.e., the combination, ISR&NTX. All injections were 1 ml/kg, given intraperitoneally.

The carrier of cocaine was physiological saline (0.9% NaCl). The carrier of the other agents was a solution of 9% Tween 80 (polyoxyethylenesorbitan monooleate) in physiological saline. Placebos associated with cocaine and the other drugs were injections of their respective carriers.

A 3 mg/kg dose of ISR blocks cocaine's enhancement of pressing for rewarding brain stimulation (14), but neither 1 mg/kg of ISR nor 3 mg/kg of NTX, by themselves, effectively reduce cocaine's ability to enhance pressing [(14); unpublished observations]. However, when 1 mg/kg of ISR and 3 mg/kg of NTX are given in combination, cocaine's usual enhancement of pressing for rewarding brain stimulation is blocked (27).

Apparatus. The apparatus is 12 nearly identical alleys and is described in detail elsewhere (26). The two halves have distinct visual and textural cues. One-half has walls painted solid gray, while the other has black and white horizontal stripes. Steel rods that form the floor of the alley are perpendicular to the length of the alley in one side (gray side) and horizontal to the length in the other side. Two dividers are used, on different occasions, to separate the halves of the alley: one has a large hole (12 cm in diameter), allowing the rats free access to both sides; the other has no hole, and is used to confine a rat to one side of the alley.

Each half of the alley has an adjustable light overhead. When the brightness of the sides of the alley are nearly the same, rats show no reliable preference for one side over the other. In these procedures, however, one side was made brighter than the other which, in turn, produced a preference, at baseline, for the darker half of the alley. For six of the alleys, the side with gray walls was the brighter place; and, for the other six, the side with striped walls was brighter. It was the bright side of the alley that the effects of cocaine were paired and, hence, is referred to as the putative side of conditioning.

During tests for place preference, the rats have access to the entire alley, and their position is monitored by a computer-based system. In brief, when a rat moves from one side of the alley to the other an electrical circuit is completed. The computer software developed for this system (26) automatically tabulates the amount of time that the rat spends on each side of the alley.

Procedure. The specific procedures spanned 62 days. Across days 1–3, the rats were habituated to the general procedures of the experiment. This included weighing the rats and extensive handling while transporting them to and from an adjacent room which housed the apparatus. Additionally, each rat was given an injection of cocaine, 5 mg/kg, just before being put back into their home cages on each of these 3 days. This was done to ensure some experience with cocaine's effects for all subjects (i.e., all rats had an opportunity for cocaine sensitization). The rationale for giving all rats some experience with cocaine was to control for the potential for ISR, NTX, or ISR&NTX to interact with or block the processes of cocaine sensitization. Although such effects would surely be interesting, our goal is to find medicines for cocaine abuse that will be effective among people who have experience with cocaine's effects. Also, because group membership had not yet been determined, it was necessary to give all rats cocaine during the initial 3 days of the procedure. It is doubtful that this preexposure would affect the place preference among controls that never experience cocaine in the apparatus.

Day 4 was the first time the rats were placed in the alleys. Using the divider with the hole in it, the rats were allowed access to both sides and the amount of time spent on each side of the apparatus was tabulated (first baseline). Using this baseline measure, the rats were divided into five groups (n = 12) so that the groups' mean preferences for the side of putative conditioning were nearly equal. Then, a treatment was randomly assigned to the groups.

On days 5–34, the rats were weighed periodically and injected daily. Two of the groups received placebos (control groups). One group (NTX group) received a daily dose of 3 mg/kg of NTX. Another group (ISR group) received 3 mg/kg of ISR. The last group (ISR&NTX group) received 1 mg/kg of ISR and 3 mg/kg of NTX. All of these injections were given during the late afternoon. During the 30-day period of injections, one rat of both the ISR and ISR&NTX groups died of unknown causes; and consequently, n = 11 for these two groups. Before the rats' deaths, there was no evidence of ill health and the rats were gaining weight regularly.

On day 34 and before their daily injections, the rats were again placed in the alley with access to both sides to obtain a second baseline measurement. At this stage of the procedures and just before conditioning associated with CPP testing,

there were five groups of rats (*n*s = 11 or 12) that had nearly identical experiences except for the different kinds of injections they received, daily, for 30 days. The daily injections of the first 30 days continued, as before, and were given 30 min before conditioning sessions.

During conditioning, the alleys were separated by the divider preventing movement from one end of the alley to the other. On days 35 and 37, all rats were given saline and then placed on the darker side of the alley for 30 min. On days 36 and 38, all rats, except for those of one group, were given cocaine, 5 mg/kg, and placed on the putative side of conditioning (the brighter side) for 30 min. The injections associated with cocaine were given about 1 min before the conditioning sessions. The one group that did not get cocaine received saline before placement in the putative side of conditioning (placebo controls). The placebo control group was the group that only received placebos during the 30 days of injections.

Day 39 was a test for CPP. On the test day, rats had free access to both halves of the alley, for 30 min, while time spent on side of putative conditioning was recorded. The rats were not given their usual daily injections until at least 1 h after the test was completed. On days 40–41, there were no special treatments, except the continuing daily injections of the previous days.

The cycle of four conditioning sessions, a test, and 2 days of no special procedures was repeated across 3 more weeks, except that on days 57 and 59, the dose of cocaine was increased to 20 mg/kg, for the four groups previously receiving 5 mg/kg of cocaine. The rationale for increasing the dose of cocaine was to ensure that an effective dose of cocaine was given, because cocaine's positive effects are known to wane with repeated dosing. Placebo controls continued to receive injections of saline. Thus, tests 2, 3, and 4 occurred on days 46, 53, and 60, respectively.

As a consequence of these procedures, the following measures were taken: (a) two baselines, taken 30 days apart, indexing preferences before conditioning, and (b) four tests potentially reflecting a place preference. Test 4 occurred after 16 conditioning trials (8 on the putative side of conditioning and 8 on the alternate side) and after 55 days of daily injections of test drugs or their placebos.

On day 60, rats received their usual daily injections after test 4. On day 61, there was no special procedure, but rats did receive their usual daily injection. On day 62, rats again re-

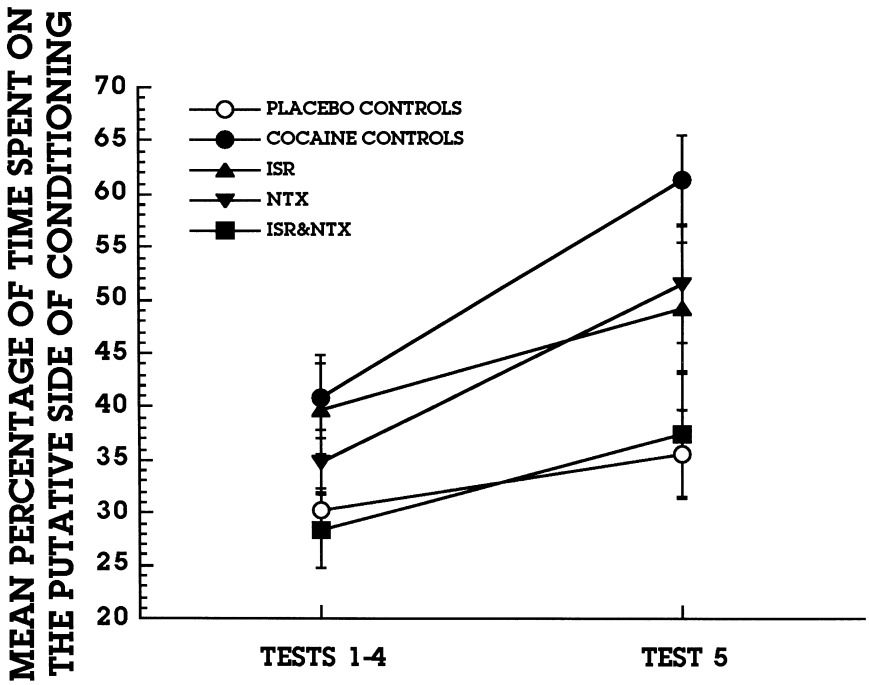

FIG. 1. Depicted are the mean percentages of time spent on the putative side of conditioning of a 30-min test session. Across a 30-day period before any conditioning, and throughout the remainder of these procedures the rats received, daily, either Tween 80 in saline (placebo and cocaine controls), 3 mg/kg of isradipine (ISR), 3 mg/kg of naltrexone (NTX), or a combination of 1 mg/kg of ISR and 3 mg/kg of NTX (ISR&NTX). In brief, the rats received their 58th consecutive day of these particular injections on the day of test 5. The data points to left side of the figure are mean scores across four weekly tests after conditioning sessions with cocaine (cocaine controls and the ISR, NTX, and ISR&NTX groups) or saline (placebo controls). The dose of cocaine before tests 1–3 was 5 mg/kg, whereas, before test 4, the dose was 20 mg/kg. Immediately before test 5, all rats except placebo controls received 5 mg/kg of cocaine. On all test days, the daily injections of Tween 80, ISR, NTX, or ISR&NTX were given after the test session. Notice that ISR&NTX blocked the expression of a cocaine CPP (tests 1–4) and the ability of cocaine to enhance the expression of a cocaine CPP (test 5). These data suggest that the ability of ISR&NTX to block cocaine's reward relevant effects do not wane with repeated administrations. Error bars are standard errors of the means.

ceived their usual injection and then another test was programmed. In addition, all rats, except the placebo controls, received an injection of cocaine, 5 mg/kg, just before being placed in the alley. The placebo controls received an injection of saline.

Data reduction and statistics. The data were expressed as the percentage of time spent on the putative side of conditioning across a 30-min period. Across the procedures, there were seven measures of preference for the putative side of conditioning: two baselines, four tests associated with conditioning but with no injections before testing, and one test after extensive conditioning but with an injection before the testing. There were five groups of subjects.

The data of tests 1–4 conform to a 5 × 4 analysis of variance (ANOVA), having repeated measures, with factors of the five groups and the four tests. The results of that ANOVA indicate that the scores of the groups did not differ reliably across the four tests, $F(3, 159) = 0.6$, $p = 0.63$. Furthermore, the interaction term is not a reliable source of variance, $F(12, 159) = 1.56$, $p = 0.11$. Given these results, it can be concluded that increasing the dose of cocaine had no discernible effect on rats' preferences. Consequently, the scores of tests 1–4 were averaged to get a single index of test scores for each group. Summarizing the test scores in this manner, of course, best reflects the factor associated with groups, which is a reliable source of variance, $F(4, 53) = 2.66$, $p = 0.04$. The scores of the two baselines also do not differ, $F(1, 54) = 0.5$, $p = 0.47$. Consequently, the scores of the two baselines were averaged to get a single index of the groups' preferences before conditioning.

With these reductions in data, the scores conform to a 5 × 3 ANOVA, having repeated measures, with factors of groups (different histories of drugs) and tests (baseline, mean of tests 1–4 and test 5), respectively. That ANOVA yields: (a) for the factor of tests, $F(2, 106) = 17.3$, $p < 0.0001$, (b) for the factor of groups, $F(4, 53) = 3.2$, $p = 0.02$, and (c) for the interaction, $F(8, 106) = 3.0$, $p = 0.005$. Given that the groups' mean baseline scores are not reliably different and the observation that the scores of the placebo controls did not reliably shift across tests, it is apparent that the scores of interest are those of the two kinds of tests (i.e., means of tests 1–4 and test 5). Those data conform to a 5 × 2 ANOVA, having repeated measures, with factors of groups and tests, respectively.

RESULTS

A summary of the results appear in Fig. 1. The ANOVA of the data of Fig. 1 reveals reliable main effects of groups and tests, $F(4, 53) = 4.9$, $p = 0.002$, and $F(1, 53) = 28.7$, $p < 0.0001$, respectively. The interaction is also a reliable source of variance, $F(4, 53) = 1.5$, $p = 0.22$. During tests 1–4 and during test 5, compared to placebo controls, the cocaine controls spent reliably more time on the putative side of conditioning, $t(22) = 2.41$, $p = 0.02$, and $t(22) = 4.36$, $p = 0.0002$, respectively. In addition, the cocaine control's mean test 5 score is reliable greater than their mean test 1–4 score, $t(11) = 3.93$, $p = 0.002$. In brief, with these procedures, cocaine established a CPP that was enhanced by giving cocaine just before test 5. The next question is whether ISR, NTX, or ISR&NTX produced scores more similar to those of placebo controls or cocaine controls.

The scores of the ISR group indicate that ISR did not reliably modify cocaine's ability to establish a CPP. For both kinds of testing, the ISR Group's scores are similar to those of the cocaine controls, both ts$(22) < 1.0$. There are indications that the ISR group's scores were greater than those of placebo controls: (a) for the tests without injections, $t(22) = 2.06$, $p = 0.052$, (b) for the test with cocaine injections, $t(22) = 1.86$, $p = 0.08$. In brief, these data provide minimal support for the conclusion that ISR alone has enduring effects in terms of blocking cocaine's reward-relevant effects. Taken together, these findings and those of a previous report, showing that ISR blocks cocaine's effects with ISR's initial administrations (14), indicate that ISR's effects wane with repeated administrations.

The scores of the NTX group indicate that NTX had some, but not marked, effects. The comparison between cocaine controls and NTX group across both kinds of testing yielded ts$(22) < 1.5$, ps > 0.16. The scores of NTX group are not different from those of placebo controls across tests 1–4, $t(22) = 1.25$, $p = 0.23$, but are different at test 5, $t(22) = 2.2$, $p = 0.03$. In general, these outcomes provide only minimal support for the conclusion that NTX mutes a CPP associated with cocaine (2,13).

Across tests, the scores of ISR&NTX group are not reliably different from those of placebo controls, both ts < 0.50, ps > 0.60. The scores of ISR&NTX are, however, reliably less than those of cocaine controls: (a) for the tests without injections, tests 1–4, $t(22) = 2.38$, $p = 0.03$, (b) for the test with injections of cocaine, test 5, $t(22) = 3.39$, $p = 0.003$.

Figure 2 presents a summary of the rats' body weights across a 63-day period that includes the 62-day period of specific procedures and one day after (i.e., the 59th day of daily injections). Notice that the rate of body weight gain of the subjects getting ISR is slightly less than the other groups. Also, notice, however, that the rats getting ISR&NTX gained weight throughout the period. Further, these subjects appeared healthy. In general, the conclusion, from the perspective of these data, is that the combination is no more toxic than either drug alone.

DISCUSSION

Subsequent to 30 daily injections of ISR&NTX, the combination was effective in blocking cocaine's ability to establish a CPP. The conclusion is that the combination's effects do not wane with repeated administrations. In brief, ISR&NTX is remarkably effective at blocking the reward-relevant properties of cocaine.

EXPERIMENT 2

Through the day of the fifth test, the subjects of Experiment 1 had 58 consecutive days of injections of either placebos, ISR, NTX, or ISR&NTX. Additionally, many of the subjects had received 12 doses of cocaine. It was decided to capitalize on that history and further study the effects of these daily administrations. Consequently, with their daily injections continuing, these subjects were placed on a daily regimen involving intake of alcoholic beverage.

The essence of the daily regimen is the presentation of water and an alcoholic beverage for only 2 h a day. Food was always available. The alcoholic beverage was a sweetened 12% ethanol solution. Ordinarily, when rats are placed on this daily regimen of limited access to water and alcoholic beverage, they take very little alcoholic beverage at first, but gradually escalate their intakes until they are taking, on average, over 2 g of ethanol per kg of body weight (g/kg) daily. Also, when first placed on this daily regimen, rats take a considerable amount of water, but not a sufficient amount to grow across days. However, they quickly learn to take a sufficient amounts. It is as if the rats need time to learn, across the first

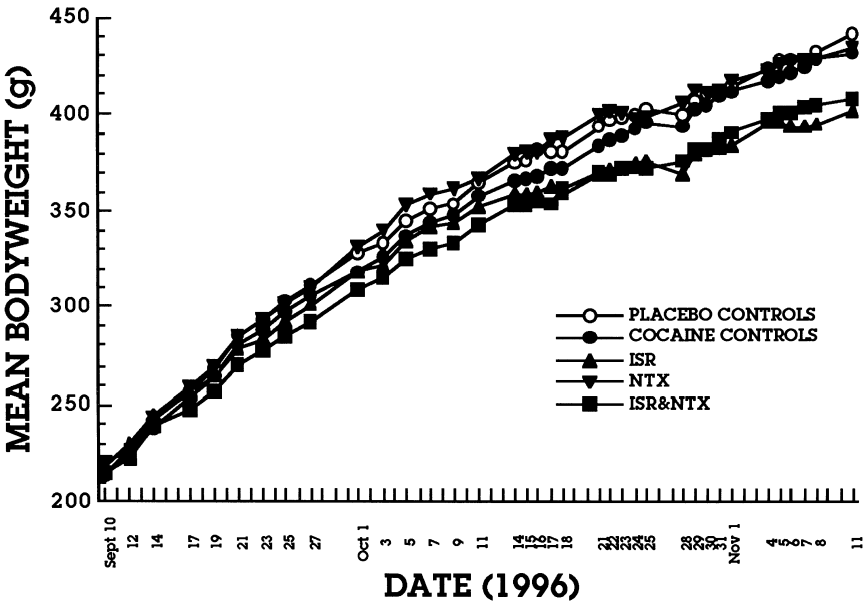

FIG. 2. Depicted are the mean body weights of the rats of the 5 groups of Fig. 1. The daily injections of Tween 80, ISR, NTX, or ISR&NTX began on 14 September and continued throughout the remainder of the procedures. Conditioning began on 14 October. Tests 1–4 occurred on 18 October, 25 October, 1 November, and 8 November, respectively. Test 5 occurred on 10 November 1996. Notice that the rate of body weight gain was slightly slower for the rats of the ISR and ISR&NTX groups.

few days, to take sufficient water to sustain their usual rate of gain in body weight.

There have been extensive studies of the effects of drugs, particularly opioids, on intake of alcoholic beverage with rats on this daily regimen after they have developed high levels of intake of ethanol (18,23,25). Moderate to large doses of naloxone and NTX reduce intakes. Small doses of morphine increase intakes.

There have been fewer studies of the effects of drugs on the acquisition of an appetite for alcoholic beverages. Naloxone seems to prevent the development of an appetite. Small doses of morphine seem to accelerate the development of and enhance the appetite for alcoholic beverages (16). Given these circumstances, it is hypothesized that NTX will also prevent the development of an appetite for alcoholic beverage. On the other hand, the dose of NTX that is being used is small (3 mg/kg), in terms of appetitive behavior, and it will have been given for many days before the rats are presented alcoholic beverage. Some of our recent data (12) indicate that small doses of NTX, when given day after day, do lose their ability to suppress intake of alcoholic beverage.

Both ISR (8,11) and ISR&NTX (11) suppress intakes of alcoholic beverages after an appetite for ethanol has been established. The prediction, therefore, is that they will also suppress the development of the appetite. On the other hand, both agents will have been given many days before the opportunity to take alcohol.

Because many persons having cocaine use disorder also drink excessive amounts of alcoholic beverage, it would be desirable to have a pharmacotherapy that would be effective in suppressing the appetitive features of both cocaine and alcohol. Based on the available evidence, ISR&NTX seems to do that, but we do not know if ISR&NTX will be effective when given day after day.

METHOD

Subjects. The 58 rats that ended Experiment 1 began these procedures without interruption of daily injections. The subjects of the ISR, NTX and ISR&NTX groups continued to receive their respective injections. Half of the placebo controls and half of the cocaine controls were randomly assigned to a new control group (to continue to receive placebos daily). The other halves of those groups were assigned to the ISR&NTX group.

Apparatus and alcoholic beverage. The rats were presented water and alcoholic beverage for 2 h a day in their home cages. The fluids were presented by way of bottles equipped with ball-point sipping tubes. The bottles were weighed before and after their presentation and the differences, corrected for spillage, were taken as the index of amount consumed. This measure correlates well with blood ethanol levels (17). The alcoholic beverage was a flavored, 12% solution of ethanol: 100 g of the beverage contained 12.00 g of absolute ethanol, 0.25 g of saccharin, and 87.75 g of tap water. The rationale for the use of this and similar alcoholic beverages has been discussed (9,23,29).

Procedure. On the 59th day of daily injections, a daily regimen involving 22 h of fluid deprivation followed by a 2-h drinking session was established. During the daily drinking session, the rats were allowed to drink water and alcoholic beverage from 1200 to 1400 h. Food was always available.

The procedures reported here are associated with the initial 21 days of being maintained on the daily regimen. The daily injections associated with Experiment 1 were continued throughout these procedures and were given 0.5 h before the daily drinking session.

ISR&NTX is toxic when paired with initiation of a limited fluid access schedule. Within the first week of this procedure, 9 of the 23 rats getting ISR&NTX displayed problematic,

even lethal, weight loss because they did not take sufficient fluids to sustain their health. ISR&NTX produced toxic effects among some rats with and without a history of ISR&NTX, but the rats without a history of the combination seemed to be affected the most. With the first signs of lethal effects, we ended the participation of the subjects that were naive to ISR&NTX and any other subject whose health appeared in jeopardy. These rats were immediately given unlimited access to water and, within a few days, some of these rats' body weights returned to their previous levels. Also, one rat of the control group escaped from its cage and was not found for a few days. Consequently, these subjects' data were not used. Also, recall that one rat was lost from the ISR and ISR&NTX groups with the injections of Experiment 1. With those reductions in numbers, there were four groups of subjects: placebo controls (n = 11), ISR (n = 11), NTX (n = 12), and ISR&NTX (n = 8). Please note that with the 21st daily opportunity to take alcoholic beverages, all of these subjects had received their respective injections across 80 consecutive days.

Daily, the rats were weighed about an hour before presentation of fluids. Using these scores plus scores of intake of alcoholic beverage, g/kg were calculated. To simplify the presentation of the results, the data were collapsed into 3-day means for each measure. Thus, the data of each measure conforms to a 4 × 7 ANOVA, having repeated measures, with factors of groups and blocks of 3-day means, respectively.

RESULTS AND DISCUSSION

The subjects' body weights, summarized in Fig. 3, increased across days, $F(6, 228) = 3.14$, $p = 0.006$. The statistic associated with the differences among groups is $F(3, 38) = 1.52$, $p = 0.22$, and with the interaction between groups and blocks is $F(18, 228) = 0.98$, $p = 0.48$. After (a) the first few days of limited access to fluids, and (b) after the rats had learned to drink enough water during its limited availability, the data of body weights indicates that the drugs produced no toxic reactions.

Figure 4 summarizes the results of water intake. The ANOVA of the data of water intakes yields: (a) for groups, $F(3, 38) = 5.99$, $p = 0.002$, (b) for blocks, $F(6, 228) = 23.5$, $p < 0.0001$, and (c) the interaction, $F(18, 228) = 5.21$, $p < 0.0001$. Most of the variance of these reliable effects can be accounted for by the rapid increase in water intake during the first days of the procedure. Also, notice that groups getting NTX did not take as much water as the other groups during the initial days on the schedule. Specifically, compared to controls, the NTX and ISR&NTX groups took reliably less water during block 1, $t(21) = 3.13$, $p = 0.005$, and $t(17) = 4.54$, $p = 0.0003$, respectively. However, tests for simple main effects reveal that across blocks 2–7 the ISR, NTX and ISR&NTX groups all took reliably more water than controls (all $ps \leq 0.03$).

Figure 5 presents the data of intake of ethanol in terms of mean g/kg, and the relevant ANOVA yields: (a) for group (kinds of drugs), $F(3, 38) = 9.13$, $p = 0.0001$; (b) for blocks, $F(6, 228) = 10.9$, $p < 0.0001$; and (c) for the interaction, $F(18, 228) = 3.14$, $p < 0.0001$. The groups did not behave similarly with respect to intake of alcoholic beverage. Only the placebo controls developed an appetite for alcohol characteristic of rats on this daily regimen. Indeed, during block 7, independent t-tests reveal that the ISR, NTX, and ISR&NTX groups all took reliably less ethanol than Controls (all $ps < 0.003$). In brief, ISR&NTX kept intakes of alcoholic beverage below

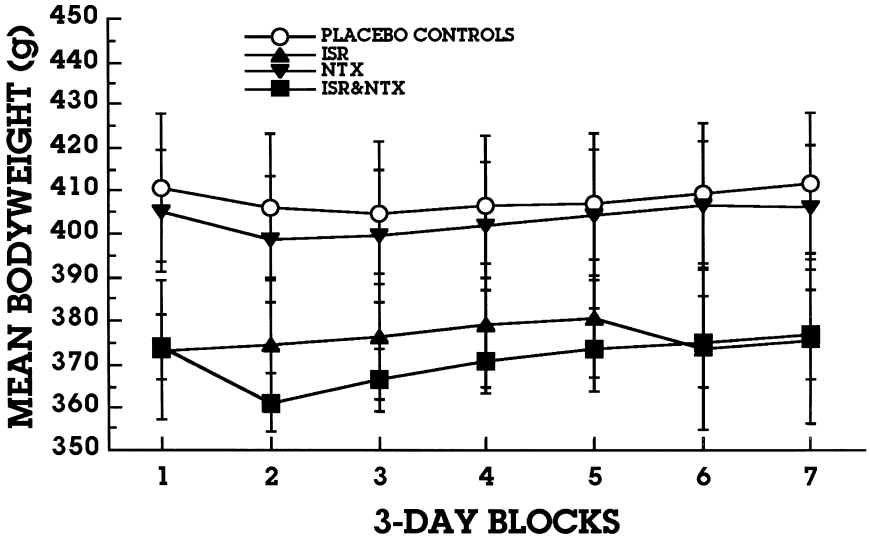

FIG. 3. The mean body weights of the four groups across the 21 days of alcohol availability are presented. These were the rats of the Experiment 1. Group labels refer to daily injections of agents and are described in Fig. 1. In brief, without interruption they continued to receive daily injections of Tween 80 (placebo), ISR, NTX, or ISR&NTX. Note that on day 21 of these procedures (i.e., the last day of block 7) the rats had received 80 consecutive daily injections. In these procedures, the rats were maintained on a schedule involving limited (2 h) daily access to fluid. During the daily drinking session the rats had the opportunity to drink water and an alcoholic beverage. Notice that body weights were generally stable across days of the procedure. Also notice that the scale of the ordinate is truncated making differences appear larger than they would otherwise. Error bars are standard errors of the means.

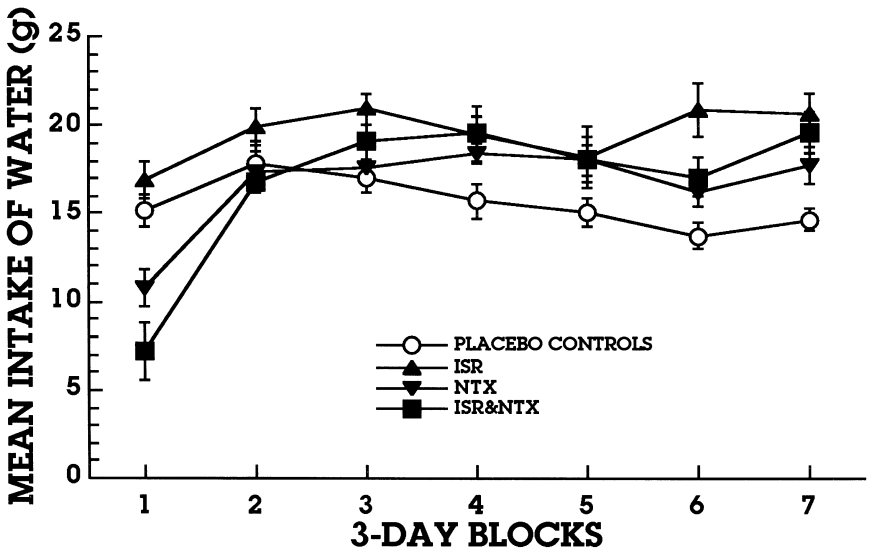

FIG. 4. Depicted are mean intakes of water across the 21-day period described in Fig. 3. Group labels refer to daily injections of agents and are described in Fig. 1. Error bars are standard errors of the means.

values that are apt to produce a meaningful pharmacological effect of ethanol.

Total intakes of fluids are summarized in Fig. 6 The ANOVA of those data yields: (a) for groups, $F(3, 38) = 5.83$, $p = 0.002$, (b) for blocks, $F(6, 228) = 54.1$, $p < 0.0001$, and (c) the interaction, $F(18, 228) = 4.22$, $p < 0.0001$. Subsequent analyses reveal that the reliable interaction term is due to the data of block 1. Recall that during block 1, the groups getting NTX took less water than the other groups, an effect that, of course, was also manifest in total intake of fluids.

An ANOVA of the data of total fluid intake associated with blocks 2–7 fails to yield a reliable interaction term ($p > 0.08$). However, that ANOVA does yield a reliable main effect of groups, $F(3, 38) = 3.26$, $p = 0.03$. Across blocks 2–7 the groups' mean (±SEM) total intakes of fluids were as follows: (a) placebo controls, 20.3 g ± 0.59; (b) ISR, 22.1 g ± 0.93; (c)

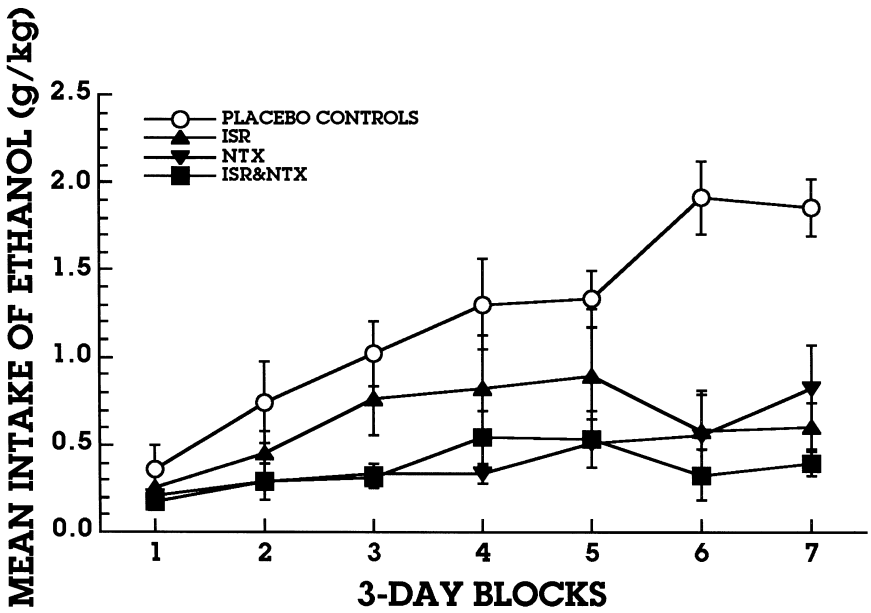

FIG. 5. Depicted are rats' mean g/kg intakes of ethanol across the initial 21 daily opportunities to sample an alcoholic beverage. Group labels refer to daily injections of agents and are described in ggg. 1. Notice that ISR, NTX, and ISR&NTX blocked rats acquisition of a pattern of daily intakes of ethanol. The placebo controls acquisition curve is typical, given the extant procedural circumstances (18,23,25). Error bars are standard errors of the means.

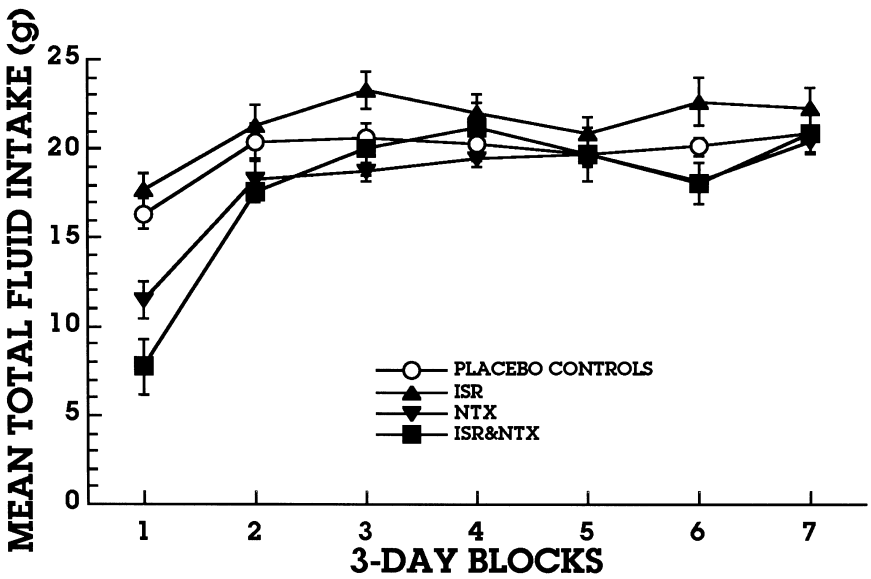

FIG. 6. Depicted are mean total fluid intakes across the 21-day period described in Fig. 3. Group labels refer to daily injections of agents and are described in Fig. 1. Error bars are standard errors of the means.

NTX, 19.1 g ± 0.49; and (d) ISR&NTX, 19.6 g ± 0.92. Only the ISR and NTX groups' mean total intakes of fluids were reliably different across blocks 2–7, $t(21) = 2.87$, $p = 0.009$. However, although the ISR group tended to take 2 to 3 g more, in terms of total intake of fluids, than the other groups across blocks 2–7, the groups' total fluid intakes were, in general, similar after block 1.

EXPERIMENT 3

The subjects getting ISR&NTX experienced considerable toxicity when they were first put on the schedule of limited opportunity to take fluids. Is this toxicity related to the low levels of fluid intake that comes with the introduction of the limited access to fluids? The subjects of Experiment 2 provide an opportunity to obtain information germane to answering the question. The control group had never experienced ISR&NTX throughout Experiments 1 and 2, but had learned to drink sufficient amounts of water during 2 h to maintain their body weights. The question is whether they would show marked reductions in body weights with the introduction of injections of ISR&NTX.

METHOD

The subjects were those of Experiment 2. The daily regimen of presenting alcoholic beverage and water was sustained for another 6 days. Across those 6 days, the subjects previously receiving ISR, NTX, and ISR&NTX stopped receiving injections. The placebo controls of Experiment 2 were given injections of ISR&NTX, 30 min before the opportunity to drink.

As in Experiment 2, the data were reduced to 3-day means. Thus, the reduced data are those of the eighth and ninth 3-day mean scores associated with being maintained on the daily regimen. We include the data of the seventh block of days, from Experiment 2, in the presentation of the results.

RESULTS AND DISCUSSION

Figure 7 presents mean body weights for the four groups from the end of the procedures of Experiment 2 through the procedures of this experiment. The ANOVA of those data failed to reveal reliable main effects of groups or blocks (ps < 0.13). The interaction term, however, is a reliable source of variance,

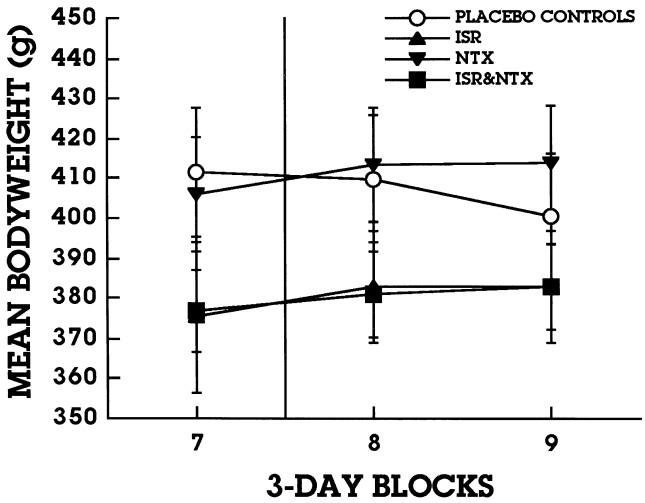

FIG. 7. The mean body weights of the four groups of subjects are presented. The left data points are the last days of procedures of Experiment 2 (block 7) and are presented for easy comparison to the other data. Group labels are described in Fig. 1. The data to the right of the vertical line represent body weights with the introduction of new procedures. The former placebo controls (open circles) received placebos during block 7 and ISR&NTX during blocks 8 and 9. The other groups received no injections during blocks 8 and 9. Error bars are standard errors of the means.

$F(6, 76) = 2.46$, $p = 0.03$. A one-way ANOVA revealed that with the introduction of ISR&NTX to the group that had previously received only placebos, there was only a slight, yet reliable, reduction in body weights, $F(2, 20) = 29.9$, $p = 0.000001$, but no other signs of toxicity. The other groups tended to gain weight across the procedures, $F(2, 56) = 3.74$, $p = 0.03$.

Figure 8 presents the data associated with intakes of water. The ANOVA of those data yields: (a) for groups, $F(3, 38) = 6.23$, $p = 0.002$, (b) for blocks, $F(2, 76) = 16.6$, $p = 0.000001$, and (c) for the interaction, $F(6, 76) = 2.49$, $p = 0.03$.

Upon inspection of Fig. 8, it is apparent that water intake among the group labeled placebo controls was not markedly affected by the introduction of ISR&NTX. However, the slight reduction in their mean intakes of water, 14.6 g during block 7 to 12.3 g across blocks 8 and 9, does meet standards of statistical significance, $t(10) = 2.56$, $p = 0.03$. The fact that there was no marked reduction in water intakes is very different than what happened when rats naive to ISR&NTX were first placed on the schedule of limited access to water associated with the beginning of Experiment 2.

With the termination of injections the ISR group's mean intake of water decreased from a mean of 20.6 g during block 7 to a mean of 15.8 g across blocks 8 and 9, $t(10) = 5.17$, $p = 0.0004$. Among the NTX and ISR&NTX groups there was no marked changes in intakes of water.

Figure 9 presents the mean g/kg of ethanol for the four groups. The ANOVA of the data of Fig. 9 yields: (a) for groups, $F(3, 38) = 1.13$, $p = 0.35$, (b) for blocks, $F(2, 76) = 3.60$, $p = 0.03$, and (c) for the interaction term, $F(6, 76) = 9.90$, $p < 0.0000001$. Notice that with the termination of injections, the three groups previously receiving ISR, NTX, and ISR&NTX began to take alcoholic beverage.

The postinjection data provides further support for the idea that the drugs were holding in check the rat's usual propensity to increase intake of alcoholic beverage. Notice that the introduction of ISR&NTX to the group that had previously been receiving placebos lead to a marked decline in intakes of ethanol. The decrease in intakes of the group introduced to ISR&NTX and the increase in intakes of the other three groups is reflected in the significant interaction term revealed by the ANOVA. A comparison of the g/kg of the group introduced to ISR&NTX from block 7 to block 8, by way of dependent t-test, yields a $t(10) = 3.24$, $p = 0.009$. The reduction in intakes of alcoholic beverage seen with this group confirms other observations (10).

EXPERIMENT 4

A drug could reduce the apparent rewarding effects of another drug by merely making the rats ill or by inducing some other negative affective state. If a drug induces an aversive state, it is not apt to be an effective medicine for treating substance use disorders, because it would be difficult to get patients to take it across a meaningful period of time. Here, we assess a dose of ISR&NTX for its ability to establish a negative affective state by way of CPP testing.

One rationale for using a combination of drugs is to reduce the possibility of slight, yet problematic, side effects that may limit compliance with the prescription of larger doses of drugs. The idea is that small doses of each drug would combine to be effective in blocking cocaine and alcohol's rewarding effects, but not combine to induce aversive side effects. With ISR and NTX that is a possibility, because they have some common effects on the processes induced by addictive drugs, but can have different side effects because they are different kinds of drugs.

There is some evidence that supports the idea that ISR&NTX does not produce an aversive state, at least in the doses used in Experiments 1–3. For example, the doses of ISR

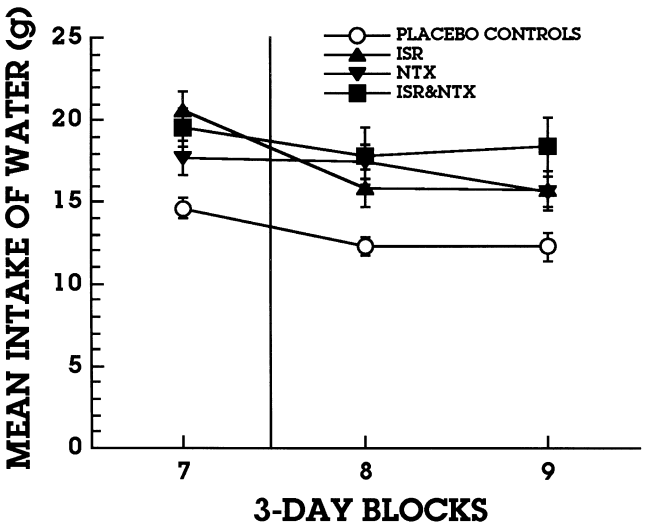

FIG. 8. The mean water intakes of the four groups of subjects are presented. The left data points are the last days of procedures of Experiment 2 (block 7). Group labels are described in Fig. 1. The data to the right of the vertical line represent intakes with the introduction of new procedures. The former placebo controls (open circles) received placebos during block 7 and ISR&NTX during blocks 8 and 9. The other groups received no injections during blocks 8 and 9. Error bars are standard errors of the means.

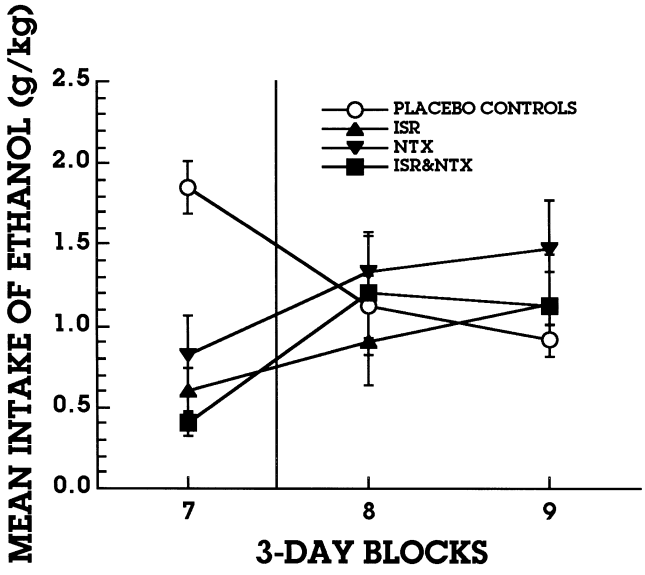

FIG. 9. The mean intakes of alcoholic beverage in terms of g/kg for the four groups of subjects are presented. Group labels are described in Fig. 1. The left data points are the last days of procedures of Experiment 2 (block 7). The data to the right of the vertical line represent intakes with the introduction of new procedures. The former placebo controls (open circles) received placebos during block 7 and ISR&NTX during blocks 8 and 9. The other groups received no injections during blocks 8 and 9. Error bars are standard errors of the means.

and NTX used in Experiments 1–3 do not by themselves reduce high rates of pressing for rewarding brain stimulation [(14); unpublished results]. Further, ISR in doses larger than those used in combination with NTX do not establish a conditioned place aversion (6). Finally, doses of ISR&NTX similar to those used here do not reduce pressing for rewarding intracranial stimulation (27).

CPP testing can be used to assess the negative affective states induced by drugs as well as the positive affective states (7,30). Here, we assess the potential aversive effects of ISR&NTX. The procedure used should be sensitive to any aversiveness of the combination. In these procedures, the side of putative conditioning was the dark side of the alley because rats initially prefer that side given the extant lighting conditions (about 70% of the 30-min baseline period was spent on the dark side). Further, because it is almost impossible to suppress some exploration of the alley, the procedures used in this test are probably not particularly sensitive to potential positive affective states that might be induced by the combination (i.e., it is difficult to go from a 70% preference to a 90% preference, because the rats will explore). Stated in a slightly different way, there is a ceiling effect limiting the possibility that this test will show that ISR&NTX induces a positive affective state. That limitation, however, increases the likelihood that any aversive state induced by ISR&NTX will be indexed. In any case, any potential the combination may have for showing addiction liability would probably have emerged in our tests involving rats pressing for rewarding intracranial stimulation (24).

METHOD

The same methods used in Experiment 1 were used here with only a few, but notable, exceptions. First, the drug effect associated with the putative side of conditioning were those of ISR&NTX rather than those of cocaine. Second, the dose of ISR was 2 mg/kg, rather than 1 mg/kg of Experiments 1–3, because (a) it is a dose that might produce a desirable reduction of blood pressure (Dr. David Gauvin, University of Oklahoma, personal communication), and (b) it is apt to be the dose used in some additional tests. Third, the side of putative conditioning was the dark side of the alley rather than the bright side. Fourth, no drugs were given as putative blocking agents. If ISR&NTX is aversive, then one could expect the "preference" of subjects getting ISR&NTX to be less than the controls.

There were 24 subjects similar to those of Experiment 1. Twelve of these subjects served as controls, receiving only placebo before all conditioning sessions. The other 12 received ISR&NTX before conditioning sessions in the dark side of the alley (i.e., the putative side of conditioning) and placebo before conditioning sessions in the bright side of the alley. There were 3 weeks of conditioning and testing.

Across these procedures there were place preference measures were taken on four occasions. Thus, the initial analysis of the data involved a 2×4 ANOVA, having repeated measures, with factors of groups and tests (i.e., baseline and the three tests subsequent to conditioning), respectively.

RESULTS AND DISCUSSION

The outcomes of this experiment are presented in Fig. 10. An inspection of the means presented in the figure indicates that ISR&NTX might produce a slight aversive state, because there is a trend toward less time spent in the putative side of conditioning across tests. The ANOVA of the data of Fig. 10, however, fails to reveal either a significant difference between groups, $F(1, 22) = 1.75$, $p = 0.20$, or a significant interaction, $F(3, 66) = 1.66$, $p = 0.18$.

There is a possibility that with continued conditioning and testing a statistically significant difference between the control and ISR&NTX groups might emerge. Further testing, however, may not be that instructive. Suppose that with further conditioning and testing a slight difference between the controls and the ISR&NTX groups emerged. It would be concluded that this dose of ISR&NTX is not markedly aversive, but has some potential for problematic side effects. That is the same conclusion that we draw from the data of Figs. 2 and 10. These data, combined with other data reported here, lead to the conclusion that the combination is generally safe and does not produce, at these doses, marked aversiveness. Nevertheless, there are sufficient problematic effects to see if smaller doses might be less problematic, but just as effective.

GENERAL DISCUSSION

Both ISR and NTX are drugs that have been approved by the US Food and Drug Administration for indications in which people are apt to take them for many days. Therapeutic doses of these drugs are generally regarded as safe. Together they might, however, produce some toxicity that neither one has alone. That does appear to be the case when they are given to rats that have for the first time been put on a schedule of limited access to water (Experiment 2). The toxicity associated with initial experience with a limited access schedule of fluids is limited to the initial days when rats must learn to drink adequate amounts of fluid. After rats have learned to take sufficient fluid, the toxicity is no longer apparent (Experiment 3).

ISR does tend to reduce body weight gain, but the effect is very small (Fig. 2). Assessments during that last days of Experiments 2 and 3, in fact, fail to reveal statistically significant difference among groups in body weights. The data of Experiment 4 combined with the data showing that generally ISR&NTX does not modify pressing for rewarding intracranial stimulation [(27); unpublished observations] indicate that the combination does not produce a markedly aversive state. There may, however, be some negativity associated with injections of the combination as manifest in slight reductions in body weights and the trend in outcomes seen in Experiment 4. The degree of negativity associated with ISR&NTX is probably not sufficient to account for the combination's ability to modify cocaine and alcohol's reward-relevant effects.

ISR, by itself, in the smaller doses similar to those used here has effects with its initial injections that reduce rats' pressing for intracranial stimulation (14). These effects, however, are temporary and limited to initial injections. When higher doses of ISR, for example, 10 mg/kg, are injected, hypotension is evident. The degree of apparently aversive effects associated with injections of ISR by itself and ISR&NTX may be much smaller when the drugs are given orally, as they would be if given to people. The knowledge that these doses of ISR&NTX have some aversive effects leads to the question of whether the doses of the two drugs can be lowered and still be effective in terms of reducing cocaine and alcohol's effects.

Using rats, there are a number of tests that have a strong potential for predicting salient events associated with people's cocaine use disorders (4). Each of those tests has limitations, therefore, conclusions are strengthened when results from more than one kind of test converge to support a conclusion

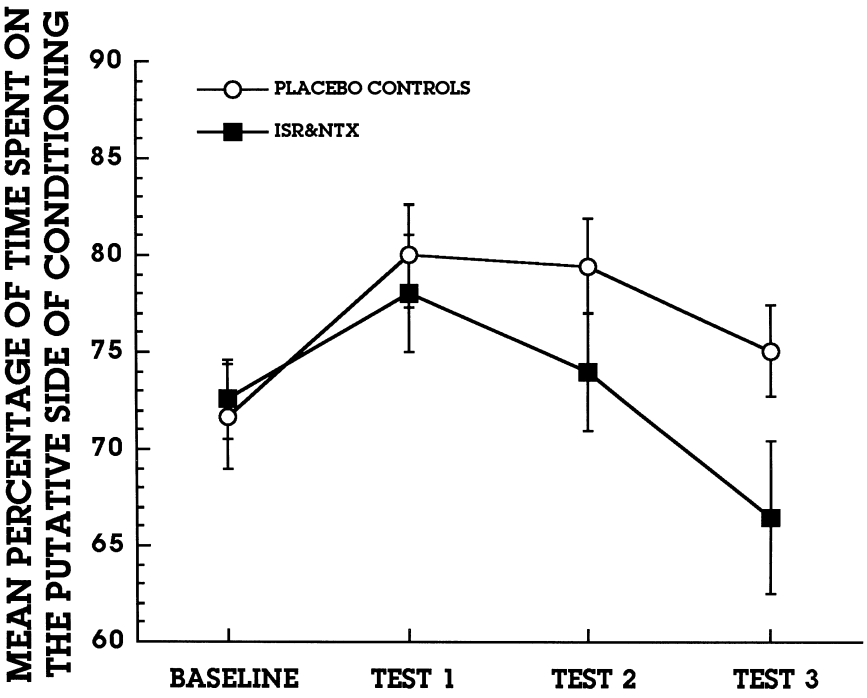

FIG. 10. Depicted are the results of an assessment of the ability of the combination of 2 mg/kg of ISR and 3 mg/kg of NTX (ISR&NTX) to produce a conditioned place aversion. The data are in terms of mean percentages time spent on the putative side of conditioning of a 30-min test session. In this procedure, the amount of time, before any injections, spent on the darker (less bright) side of an alley served as a baseline measure. Subsequently, the rats received either Tween 80 in saline (placebo) or ISR&NTX before being confined to the darker side on days of putative conditioning. On other days (i.e., days of alternate conditioning) all rats received placebo before being confined to the brighter side of the alley. Tests were performed once a week without injections. Although analyses did not reveal a statistically significant effect associated with ISR&NTX, there was an apparent trend for the ISR&NTX group to spend less and less time in the putative side of conditioning across tests. Error bars are standard errors of the means.

(24). Using rats and testing involving cocaine's ability to enhance pressing for rewarding brain stimulation, the conclusion was derived that ISR&NTX blocked cocaine's reward-relevant effects (27). The results of CPP testing confirm the conclusion derived from the initial test. Further, the results from CPP testing indicate that the effects of ISR&NTX retain their ability to block cocaine's effects after more than 30 daily administrations.

Experiment 2 indicates that ISR&NTX blocks the development of an appetite for palatable alcoholic beverage in circumstances where an appetite usually develops. Further, ISR&NTX was capable of doing that after more than 60 daily administrations. We have data (11) showing that ISR&NTX reduces intakes of alcoholic beverage subsequent to the development of high levels of intake similar to that of Experiment 3. So, although the observations with respect to alcohol are not from converging operations as with testing for cocaine's effects, the results of these tests do confirm the notion that ISR&NTX might be an effective agent to use as an adjunct to other treatments for alcohol use disorders.

The small dose of NTX was sufficient to block the development of large intakes of alcoholic beverage. That same dose of NTX, however, loses its ability to suppress intake of the alcoholic beverage once large intakes have been routine for a number of days (12). To overcome the tendency of NTX to lose its effectiveness, larger doses can be given (10) or ISR can be given concurrently [Experiments 2 and 3; (11)]. The concurrent prescription of ISR with NTX for persons with alcohol use disorder will not only be effective in terms of suppressing propensity to take excessive amounts of alcoholic beverage, but may be beneficial in other ways. Many persons routinely using alcohol also have high blood pressure (21), and ISR is a medicine for hypertension.

The conclusions that might be drawn from these initial trials with ISR&NTX are limited, because the available data are, indeed, limited, as they must be with initial trials. We do not have, for example, extensive dose–response relationships. The available data indicate that smaller doses might be as effective as those used here (27). Further, there are limited tests with different doses of cocaine and different circumstances in which alcohol is taken. Nevertheless, a certain consistency emerges from the initial tests. ISR&NTX reduces cocaine and alcohol's effects that seem salient to their ability to sustain their own use.

Many persons being treated for either cocaine or alcohol use disorders use both cocaine and alcohol. Small doses of ISR&NTX may be a reasonable treatment for those individuals. Extrapolating from the available data, the expectation is that persons under the influence of the combination will not drink excessive amounts of alcoholic beverage or take large amounts

of cocaine. Reducing the reinforcing effects of these drugs will provide individuals an additional way to control their use of these drugs. Further, the side effect profile of the combination may even be beneficial in terms of reducing the hypertension often seen among alcoholics (21) and the cerebral vascular deficits often seen with cocaine abuse (15,31,33,34).

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