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Effects of Desipramine on Rat Behavior are Prevented by Concomitant Treatment With Ethanol

HANS ROMMELSPACHER, JOCHEN WOLFFGRAMM AND SHIRLY WIDJAJA

Department of Neuropsychopharmacology, Free University, Ulmenallee 30, D-1000 Berlin 19, F.R. Germany

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ROMMELSPACHER, H., J. WOLFFGRAMM AND S. WIDJAJA. *Effects of desipramine on rat behavior are prevented by concomitant treatment with ethanol.* PHARMACOL BIOCHEM BEHAV 32(2) 533–542, 1989.—Ethanol prevents the decrease of the number of β-adrenoceptors in the cerebral cortex induced by chronic treatment of rats with desipramine. The activation of the adenylate cyclase, the second messenger, by β-adrenergic agonists is reduced somewhat less than after treatment with desipramine alone. The present paper examined the hypothesis that ethanol inhibits the neuronal adaptation to desipramine chronic treatment at the functional level as well. Desipramine reduced exploratory behavior (crossings, rearings) as did ethanol. Combined treatment attenuated the effect of desipramine. Cognitive performance was investigated using an active avoidance paradigm. Desipramine-treated rats did not learn the task in contrast to control animals. Again, combination treatment with ethanol improved the ability of the rats to perform the task. The activity of cerebral β-adrenergic mechanisms was assessed by injection of salbutamol, a β-adrenoceptor agonist in rats pretreated with 5-hydroxytryptophan (5-HTP). The augmentation of the 5-HTP-induced wet dog shake behavior by salbutamol was observed in all animals independent of the chronic treatment. However, rats treated with desipramine were less active than those treated with tap water or ethanol. The effect of desipramine in the presence of a high concentration of salbutamol was attenuated by ethanol. The observed increase of the number of wet dog shakes correlates with the function of these receptors. In two paradigms, spontaneous motility and apomorphine-induced hypothermia, ethanol did not affect the action of desipramine. It is noteworthy that desipramine acted in both situations within a short time period (minutes to hours). The findings strongly suggest that ethanol can prevent adaptive changes in the brain induced by chronic treatment with the antidepressant desipramine. This is of special interest since the adaptation of β-adrenoceptors is thought to be critical for the antidepressant efficacy of various therapeutic interventions applied in psychiatric practice.

Desipramine Ethanol Apomorphine Open field Active shock avoidance Wet dog shaking Hypothermia β-Adrenoceptors Adenylate cyclase

CHRONIC treatment of rats with tricyclic antidepressants like desipramine causes a decrease in the number of β-adrenoceptors of the cerebral cortex and hippocampus (1). In addition, activation of adenylate cyclase by β-adrenoceptor agonists is reduced (48). In other animal models which simulate treatments which have antidepressant efficacy in humans, a reduction in cerebral β-adrenoceptors is also found. Examples include chronic treatment with monoamine oxidase inhibitors like pargyline, clorgyline and tranylcypromine (33, 43, 52), and repeated electroconvulsive treatment (3,18). The specificity of β-adrenoceptor action for the antidepressant effect is underscored by the observation that substances which inhibit high affinity uptake of noradrenaline but lack antidepressant efficacy do not induce a reduction in β-adrenoceptors. This holds true for cocaine and nisoxetine (43,44). On the other hand, substances with antidepressant efficacy like iprindole which do not affect high affinity noradrenaline uptake cause a decrease of β-adrenoceptors (33,43). Interestingly, down regulation of β-adrenoceptors has also been found after chronic treatment with zimelidine, a specific inhibitor of 5-hydroxytryptamine high affinity uptake (29). Mianserin, an atypical antidepressant, causes a down-regulation of adenylate cyclase function without changes in the number of β-adrenoceptors (29). Thus, both components of the signal transducing process should be measured when investigating the actions of antidepressants.

It is of interest that the desipramine-induced down-regulation of β-adrenoceptors in cerebral cortex membranes is prevented by concomitant treatment with ethanol (39). The present study investigates whether ethanol affects β-adrenoceptors not only with respect to number but also function. Behavioral and neurochemical experiments were performed following chronic treatment of rats with ethanol, desipramine and a combination of both drugs.

METHOD

Behavioral Studies

Animals and treatment. Female Wistar rats (Hagemann,

Boesingfeld, F.R.G.) weighing 180–200 g at the beginning of treatment were maintained individually in macrolon cages (43×26×15 cm) in an air-conditioned (21±2°C; air humidity 50±5%) animal room with a 12/12 hr light/dark cycle. One week after the rats had been housed individually, the experiment began with a 7-day adaptation phase during which the animals received standard dry rat chow (Altromin R 10) as well as a special diet (6 g/day) similar to that described by Lieber and DeCarli [(17); Altromin G 0200] and tap water ad lib. Thereafter, the rats received either tap water (group A, controls), ethanol (group B, 6%, v/v), desipramine HCl (group C, 16.5 mg/100 ml), or desipramine together with ethanol (group D, same amounts as in groups B and C) for 3 weeks. Every other day, the rats were weighed, the consumption of rat chow and liquid recorded and the ingestion of drugs calculated. During the 4-week period, motor activity was recorded continuously using a motility meter (Rhema, Type 2012, Hofheim F.R.G.). The two-channel recorder was equipped with a computer for storing the data. The recorder allowed the measurement of crude motility (locomotion) and fine motility (short, fast movements). After the 4-week period, behavioral and biochemical experiments were performed whereby the rats received the drug treatment further. All behavioral experiments were performed by the same observer.

Open field. The open field was a 100×100 cm platform separated by light lines into 16 equal compartments. The number of crossings and rearings was counted for 3 min. The experiment was performed at the beginning of the dark period separately for each rat (n=12 per group).

Active shock avoidance. The learning of the avoidance reaction was performed using a covered shuttle box (Rhema, type 337500, F.R.G.), which was divided into 2 equal compartments (24×21×21 cm each). Both compartments were accessible to the experimental animal by a hole, 9 cm in diameter. The floor consisted of a grid, connected to a shock generator.

The experiments comprised either 50 cycles (acquisition) or 25 cycles (retrieval). Each cycle began with a 22-sec intertrial phase followed by a conditioning light signal and 3.5 sec later by a foot shock (2 mA) with a maximum duration of 4.5 sec. The animal could avoid the shock by moving into the other compartment. The time which elapsed between the onset of the light signal and the movement into the adjacent compartment was registered by an automatic reflex conditioner (type 7501, Rhema; n=10 rats per group).

Wet dog shaking. The 5-hydroxytryptophan-induced (5-HTP) wet dog shakes can be intensified by application of β-adrenoceptor agonists like salbutamol and clenbuterol (8) which allows assessment of β-adrenoceptors activation in the CNS (17).

Each group was divided randomly into 4 subgroups (n=8 per subgroup) which received benserazide (56 mg/kg) and increasing amounts of salbutamol (0, 0.9, 1.8, 2.7 mg/kg) in 0.9% saline, respectively, by subcutaneous injection. All animals received 5-HTP (90 mg/kg IP) 30 min later. Thirty min after 5-HTP injection, 4 rats from each subgroup were placed together in the open field. The number of wet dog shakes was counted for 3 min. Counting was resumed for 3 min, 60 and 90 min after 5-HTP injection.

Apomorphine-induced hypothermia. Desipramine prevents the hypothermia induced by certain doses of apomorphine (32). To examine whether chronic treatment influences the effect of desipramine as reported in acute experiments, the 4 groups were divided in 4 subgroups (n=8 rats per subgroup) which were treated in the following manner: Subgroups A_1, B_1, C_1, D_1 received 0.9% saline SC, 0.9% saline IP. Subgroups A_2, B_2, C_2, D_2 received saline SC and apomorphine (2 mg/kg IP). Subgroups A_3, B_3, C_3, D_3 received saline SC/apomorphine (10 mg/kg IP). Subgroups A_4, B_4, C_4, D_4 received desipramine (20 mg/kg SC) and apomorphine (10 mg/kg IP). All SC and IP injections were made 30 min apart. All drugs were dissolved in 0.9% saline.

To measure body temperature, a thermosonde (Atmos, Kopenhagen, Denmark), was inserted about 5 cm deep into the rectum. The measurement was performed immediately before the IP injection as well as 20, 40 and 60 min thereafter.

Biochemistry

β-Adrenoceptors in the cerebral cortex. The behavioral experiments required 3 days. The next day, the rats were decapitated, the brains were rapidly removed and the cortex excised over ice. The white matter was carefully removed and the tissue placed on dry ice. The tissue was stored at −80°C until the binding experiments were performed (not longer than 4 weeks). The cortex was homogenized in 100 vol. of 0.32 mmol/l sucrose containing 10 mmol/l Tris HCl buffer (pH 7.4 at 24°C) using a glass/teflon homogenizer. The homogenate was centrifuged at 20,000×g for 10 min, the supernatant discarded and the pellet resuspended in 10 mmol/l Tris HCl buffer containing 0.154 mol/l NaCl (pH 7.4). The incubation mixture was composed of a 150 μl membrane suspension (15–30 μg protein), 50 μl [^{125}I]iodocyanopindolol (20,000–50,000 cpm), 50 μl buffer (10 mmol/l Tris HCl + 0.154 mol/l NaCl + 1.1 mmol/l ascorbic acid, pH 7.4) and propranolol (5μmol/l final concentration), respectively, as displacer. The vials were incubated at 37°C for 55 min in a metabolic shaker.

The reaction was terminated by filtration over Whatman GF/B filter followed by 4 washes with 5 ml Tris/NaCl buffer. The radioactivity was counted by a multidetector-gamma-counter (Packard, Crystal 5400, USA) at 75% efficiency.

Adenylate-cyclase activity. Cortex tissue dissected as described above, was homogenized in 10 vol. (w/v) of triethanolamine buffer (50 mmol/l triethanolamine, 100 mmol/l NaCl, 5 mmol/l EDTA, 1 mmol/l dithiothreitol, pH 7.7) with a glass/teflon homogenizer. The homogenate was centrifuged at 10,000×g for 10 min. The resulting supernatant was centrifuged at 20,000×g for 20 min. The pellet was resuspended in 10 vol. of a triethanolamine buffer, pH 7.7 (50 mmol/l triethanolamine, 5 mmol/l EDTA, 1 mmol/l dithiothreitol) and centrifuged at 45,000×g for 10 min. Finally, the pellet was resuspended in 600 μl of 10 mmol/l triethanolamine + 1 mmol/l dithiothreitol, pH 7.7, and stored at −80°C.

The activity of the adenylate cyclase was determined according to the method described (37) using [^{32}P]-ATP as a tracer. The frozen tissue was thawed and centrifuged at 45,000×g, 10 min at 2°C. The pellet was resuspended in 10 mmol/l triethanolamine buffer, pH 7.7. The protein concentration of the incubation mixture was 10 to 20 μg. Other components were magnesium chloride, IBMX, cyclic 5-AMP, [^{32}P]-ATP, creatin phosphate, creatin kinase, bovine serum albumin, and water which was replaced in some assays by stimulating drugs [(−)isoproterenol bitartrate (10 μmol/l), guanylimidodiphosphate (100 μmol/l), DL-propranolol (100 μmol/l), sodiumfluoride (10 mmol/l), forskolin (30 μmol/l)]. Following a preincubation period of 5 min at 30°C in a metabolic shaker the reaction was initiated by

adding the tissue. The incubation period was 10 min. It was terminated by precipitation of ATP by zinc carbonate. The supernatant was transferred to short columns containing aluminum oxide. The eluent was counted in a liquid scintillation counter (Packard, model 3380).

Measurement of the protein concentration. Protein concentration was determined using the Biorad micromethod. Extinction was measured at 595 nm with a Zeiss photometer (PMQ II). The protein standard was bovine serum albumin (Behringwerke, Marburg).

Statistical Analysis

Differences between groups were assessed with parametric (ANOVA) or nonparametric (Kruskall-Wallis) tests (4). The latter test was used whenever the prerequisite for parametric analysis, homogenity of variance (assessed by the Bartlett test) was absent. If necessary, distinct samples were compared to each other by means of U-statistics. Repeated measurements like motor activity were compared by using the Wilcoxon-test or paired *t*-test.

Data comprising time series were submitted to linear interpolation to enable the calculation of courses of the means. Linear trends were assessed by calculating the regression coefficients. Multiple repetitions were compared by means of Friedman's test.

The level of significance was $p=0.05$ (two-tailed) if not stated otherwise in the text.

All calculations were performed with the aid of computer programs (Hewlett-Packard, type 9816, series 200).

Materials

All reagents were purchased from readily available commercial sources. [^{125}J]iodocyanopindolol (spec.act. 2100 Ci/mmol) was from Amersham Buchler, Braunschweig, F.R.G., [^{32}P] from New England Nuclear, Boston, MA.

[α-^{32}P]ATP was synthesized in the department of pharmacology, Free University, by W. Rosenthal. Benserazide was a gift of Dr. Kapp of Hoffmann-La Roche, desipramine HCl of Dr. Maître, Ciba Geigy, apomorphine of Woelm Pharma, DL-propranolol HCl was purchased from Aldrich Chemie.

RESULTS

The animals treated with water or ethanol showed a continuous increase in body weight, whereas rats treated with desipramine or desipramine and ethanol did not change their body weight beginning with the first day of drug treatment. At the end of the treatment period the differences between the groups (n=28 animals per group) were significant ($p<0.01$); controls (236±20.2 g) vs. desipramine (206±18.3 g) as well as vs. the combination (204.5±17.1 g) and $p<0.05$ ethanol (229.5±17.1 g) vs. desipramine as well as vs. the combination (the values are the means ± S.D.). The weight of ethanol-treated rats was not significantly different from controls. Thus, ethanol did not affect the physiological increase in body weight under the conditions used. The weight of desipramine-treated rats was not significantly different from those treated with the combination.

The ingestion of food was reduced by all 3 treatments (ethanol 17.49±6.7 g/day; desipramine 16.39±6.7 g/day; combination 16.49±7.5 g/day) as compared with controls (20.9±7.9 g/day; $p<0.01$).

During the adaptation period the rats drank an average of 30 ml tap water/day. Beginning with the first day of drug treatment, the rats drinking ethanol, desipramine and the combination ingested less fluid. The decrease leveled off at day 3. Thereafter, the ethanol-treated animals drank 25.4±10.1 ml of fluid per day, those treated with desipramine 15.6±5.5 ml and those treated with the combination 17.9±9.9 ml.

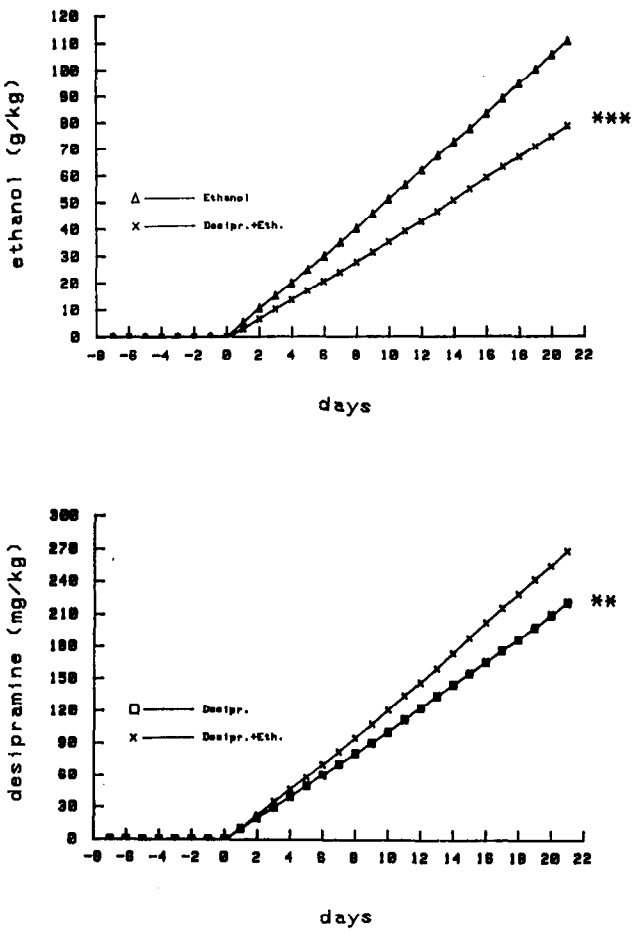

FIG. 1. Upper panel: Means of the cumulative consumption of ethanol of groups of rats treated with ethanol or the combination of ethanol and desipramine (n=28 per group). The ethanol consumption of the group treated with ethanol was significantly higher than that of the group treated with combination ($p<0.001$). Lower panel: Consumption of desipramine of rats treated either with desipramine or in combination with ethanol. The latter group consumed more of the antidepressant ($p<0.01$). The negative numbers on the abscissa indicate the days of the adaptation phase when the rats received tap water.

The cumulative recordings of the ethanol as well as the desipramine intake are depicted in Fig. 1. Rats treated with the combination ingested less ethanol during the treatment period compared to those treated with ethanol alone (29%, $p<0.001$; 3.7 g/kg vs. 4.7 g/kg and day, upper panel, Fig. 1). The total amount of ingested desipramine was 21.6% more in the group of the combined treatment compared with that of desipramine alone (12.8 mg/kg vs. 9.6 mg/kg/day, lower panel, Fig. 1).

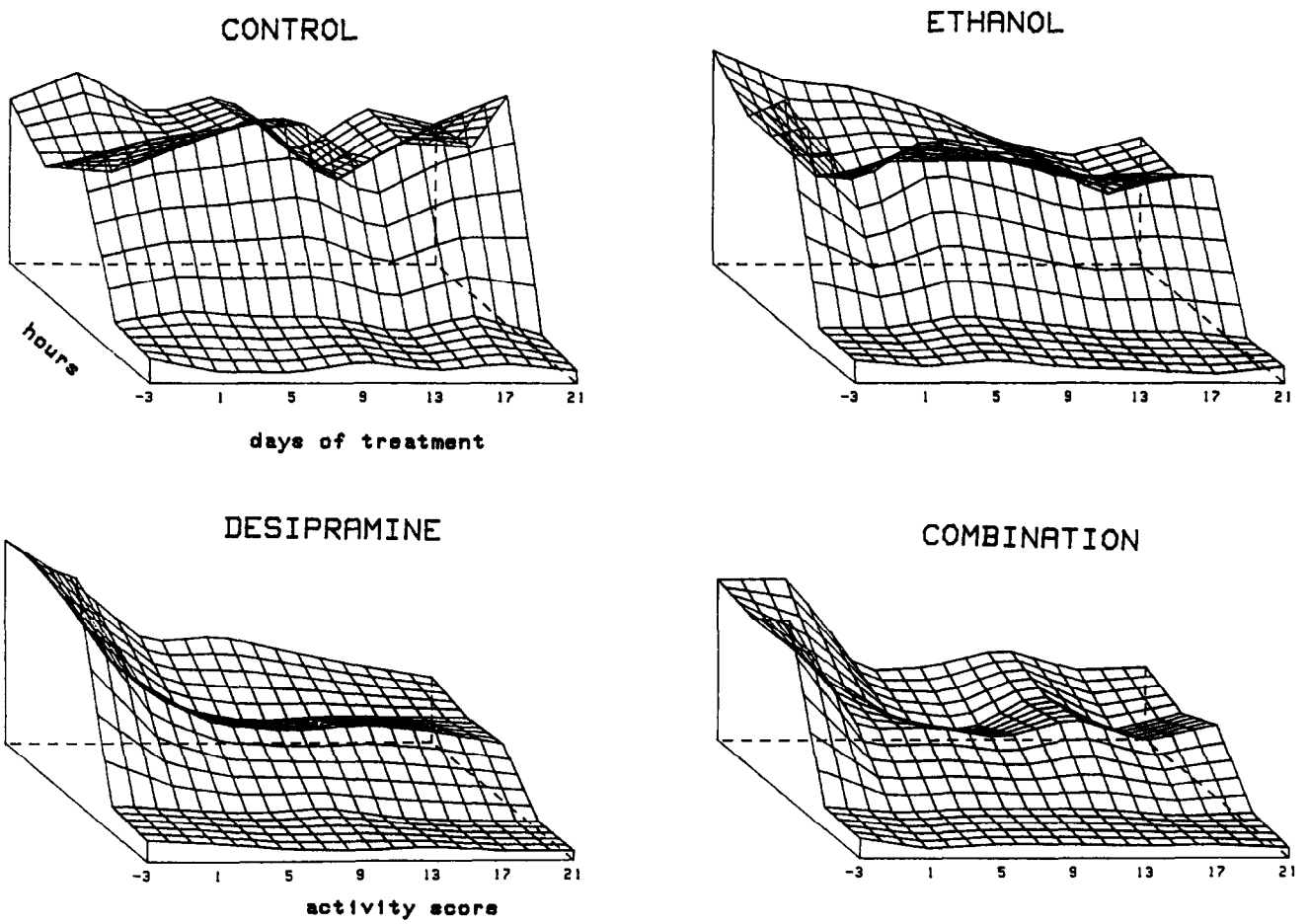

FIG. 2. Three dimensional presentation of the recording of the crude motility (see the Method section for details). The horizontal axis indicates the days of treatment, the transverse axis represents the hours of the day beginning with the onset of the dark phase at 6 p.m. until 2 p.m. The vertical axis demonstrates the normed equivalents of the motility. The net-like presentation was calculated from the means of 4-hour time periods by interpolation. Significant differences are described in the text.

Spontaneous Motor Activity

Control rats did not show changes in motor activity during the treatment period (Fig. 2). Motor activity was reduced in rats treated with desipramine and the combination, both during the light and the dark period ($p<0.001$). Animals treated with ethanol were less active during the light period ($p<0.05$) as well as the second half of the dark period ($p<0.05$) beginning with day 17 of treatment. Both 'crude' and 'fine' motor activity were affected in the same manner.

Since the groups treated with desipramine and the combination were affected to the same extent, ethanol did not significantly alter the motility reducing effect of desipramine.

Comparisons among the groups revelaed that rats treated with desipramine showed lowered motility scores even on the first day of treatment. The alterations were greatest during the second half of the dark period when the animals had already ingested considerable amounts of the drug ($p<0.02$, compared with pretreatment period). Beginning with the 5th day of treatment, the motility in the first half of the dark period was reduced as well ($p<0.01$). In animals treated with the combination the changes were even more pronounced.

Open Field Behavior

In a simple novel environment, control rats were more active than animals from all the other groups (Fig. 3). The changes in exploratory activity included both locomotion (crossing) and static exploration (rearing). Animals with the combined treatment were more active than animals treated with desipramine alone ($p<0.01$). This enhancement could not be attributed to a stimulatory effect of ethanol since the activity of rats treated with ethanol alone was greatly reduced ($p<0.001$).

Active Shock Avoidance

All groups treated with drugs showed a reduced capability to learn the task during the acquisition phase (Fig. 4). The effect of desipramine was attenuated by ethanol ($p<0.05$). A detailed analysis revealed that the difference between these two groups was significant from cycle 21 on ($p<0.05$). Not only reaction latencies were measured, as presented in Fig. 4, but also the number of rats showing no response during a single cycle, as well as those escaping the shock by moving

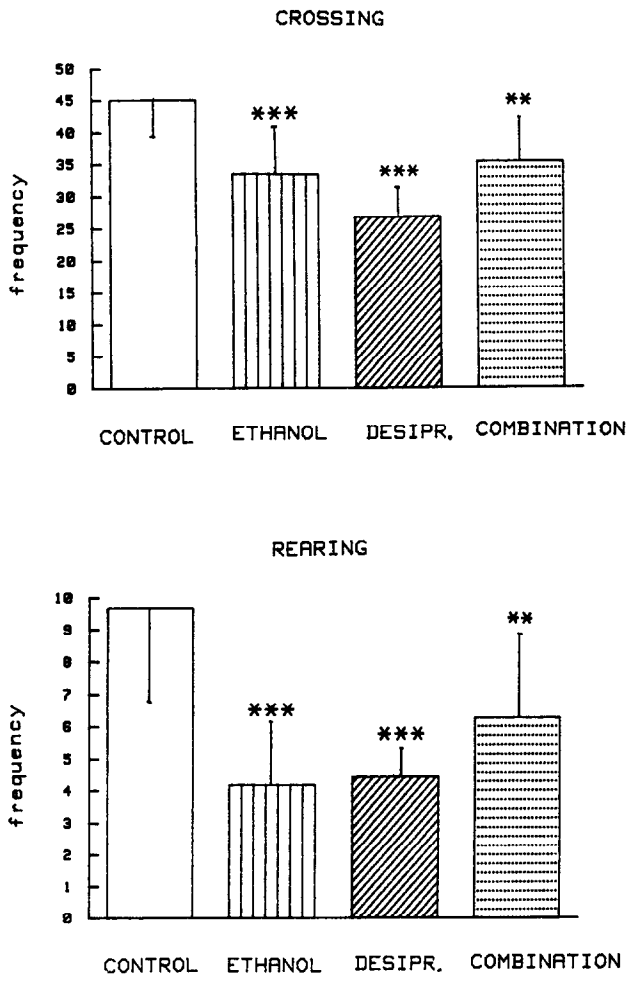

FIG. 3. Upper panel: Means of the umber of crossings during an observation period of 3 min in an open field (1×1 m; n=12; ±SEM). Asterisks indicate differences from controls. Additionally, the means of rats treated with desipramine differ from those with the combination treatment ($p<0.01$). Lower panel: means of the number of rearings. The conditions are the same as in the upper panel. Means between desipramine-treated rats and the rats treated with combination of drugs are different ($p<0.05$).

into the adjacent compartment after the onset of the conditioning stimulus. The number of animals not responding was higher among the desipramine-treated rats than all other groups including the combination ($p<0.05$). The number of animals escaping the footshock was highest among controls. Again, more animals treated with the combination were able to escape during the last 10 cycles than those treated with desipramine alone ($p<0.05$, one-tailed t-test).

In a retrieval experiment, the control animals were immediately able to accomplish the task from the beginning (Fig. 4). Rats treated with desipramine hardly improved during the 25 cycles ($p<0.05$ compared with controls), whereas animals treated with ethanol and with the combination learned the escape behavior quickly. At the end of the session no differences between controls, ethanol-treated, or the combination-treated animals could be detected.

Modulation of 5-HTP-Induced Wet Dog Shaking Behavior by Salbutamol

The 5-hydroxytryptamine precursor 5-HTP induces compulsive shaking described either as a head twitch response or shaking of the trunk, primarily cranially. β-Adrenergic agonists enhance the effect of 5-HTP in mice (17,31).

Pilot experiments in this laboratory revealed a 3-fold increase in the number of 5-HTP-induced wet dog shakes in rats by salbutamol (1.5 mg/kg). The first attacks were observed 30 min following salbutamol injection, the maximum number after 90 min. Under the experimental conditions, none of the pretreatments affected the baseline number of shaking episodes induced by 5-HTP (Fig. 5), suggesting that serotonergic mechanisms were not influenced by the various treatments ($p>0.1$).

Treatment with desipramine reduced the number of 5-HTP/salbutamol attacks ($p<0.001$). The higher doses of salbutamol caused stimulation above baseline in all groups suggesting the existence of functionally intact β-adrenergic receptors in the brain. The dose-response curve of the control group and of the ethanol group did not differ under the examined conditions. Rats treated with the combination did not differ from the animals treated with desipramine alone with respect to the two low doses (0.9 and 1.8 mg/kg). However, the highest dose of salbutamol (2.7 mg/kg) induced a 50% increase in the number of attacks compared with rats treated with desipramine alone ($p<0.05$). Thus, these experiments represent a further example of the suppressive effects of ethanol on chronic desipramine actions.

Apomorphine-Induced Hypothermia

At the end of the treatment period, the body temperature was lower in animals treated with desipramine than in controls ($p<0.001$) or ethanol-treated rats ($p<0.001$). Simultaneous treatment with both drugs did not diminish the effect of desipramine.

Low and high doses of apomorphine caused hypothermia in rats and mice (11,12). Other authors found that desipramine antagonizes the effect of a high dose of apomorphine (32). We obtained similar results. As shown in Fig. 6, apomorphine reduced body temperature in all 4 groups. Desipramine antagonized the effect of apomorphine. Statistical calculations did not reveal any significant differences between the treatment groups. Thus, neither apomorphine-induced hypothermia nor the suppressing action of acutely injected desipramine were affected by chronic ingestion of desipramine, ethanol, or both.

β-Adrenergic Receptors of the Cerebral Cortex

The maximum number of β-adrenergic receptors decreased in cerebral cortical membranes from rats treated chronically with desipramine (184.0±15.9 fmol/mg protein, mean±S.E.M.), compared with controls (269.8±23.4 fmol/mg protein; $p<0.001$; Fig. 7). In contrast to these findings, treatment with neither ethanol (289.2±36.3 fmol/mg protein) nor the combination (227.8±21.6 fmol/mg protein) reduced the maximum number of binding sites. The combination treatment attenuated the effect of desipramine ($p<0.05$, one-tailed t-test). None of the treatments affected the apparent dissociation constant (controls, 3.11±0.5 pmol/l; ethanol-treated, 2.89±0.2 pmol/l; desipramine-treated, 2.54±0.3 pmol/l; combination 2.09±0.3 pmol/l).

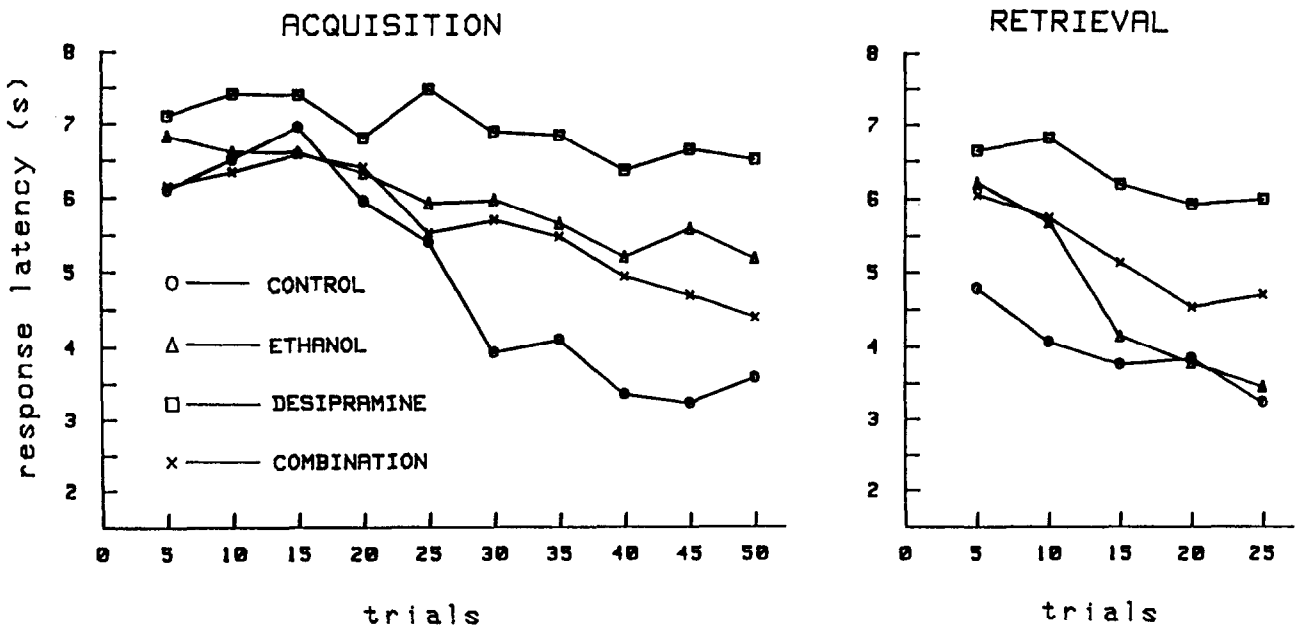

FIG. 4. Active shock avoidance experiment. The means of the response latencies (n=8) during a 50 cycles comprising acquisition phase (left diagram) and a 25 cycles comprising retrieval phase, 1 day later (right diagram). Response latencies indicate the time which elapsed between the onset of the conditioning light stimulus and the appearance of the rat in the adjacent compartment. If the rat did not move into the neighbouring compartment it was rated "no response" and scored as 8 sec. Significant differences are given in the text.

Activity of the Adenylate Cyclase

The basal activity of the enzyme was not changed due to the various treatments. As shown in Table 1, (−) isoproterenol stimulated the activity of adenylate cyclase. The effect of isoproterenol was blocked by (±)propranolol. Gpp(NH)p, sodium fluoride and forskolin activated the enzyme as well. Treatment with desipramine attenuated the stimulation by isoproterenol ($p<0.001$), Gpp(NH)p ($p<0.01$) and NaF ($p<0.05$), whereas the effect of forskolin was not altered.

Under the conditions of the present experiments, ethanol did not change signal-transduction at any level investigated. Combined treatment slightly reduced the stimulation of the enzyme by isoproterenol. The effect was less pronounced than that following the chronic treatment with desipramine alone ($p<0.05$).

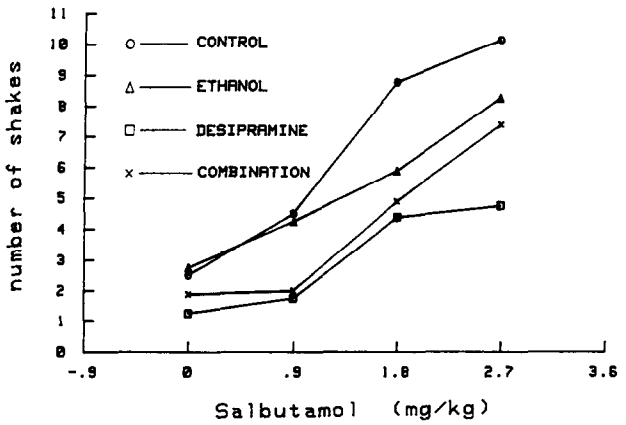

FIG. 5. Dose-response curves of the augmenting effect of salbutamol on the 5-hydroxytryptophan-induced wet dog shake behavior (n=8). Values represent the means of the number of attacks during 3 min of 3 periods 30, 60, and 90 min after the injection of vehicle and salbutamol, respectively.

DISCUSSION

Down-regulation of the β-adrenoceptor-adenylate cyclase-mediated signal transduction in cerebral neurones is proposed to play an important role in the efficacy of antidepressant treatment [for review: (37)]. The target for antidepressant drugs can be the receptor (tricyclic antidepressants), a level beyond the receptor (mianserin) or the phosphodiesterase [rolipram (49)]. Ethanol affects the activation of the adenylate cyclase by drugs both after acute [facilitation (24, 35, 36)] as well as after chronic [reduction (15,40); no change (34,39)] treatment. Based on the effect of each substance alone, one would expect a chronic ethanol treatment to potentiate the antidepressant activity of desipramine. However, in a previous study, down-regulation of β-adrenergic receptors was not observed in the cerebral cortex of animals which received both desipramine and ethanol (39). These findings alone do not unequivocally suggest an "anti-antidepressant" action of ethanol without further investigation under in vivo conditions. In the present study, various experiments were performed in which treatment with desipramine interfered with the respective behaviors measured. Cerebral β-adrenergic neuronal function is presumably involved in some of them. In 3 of the paradigms

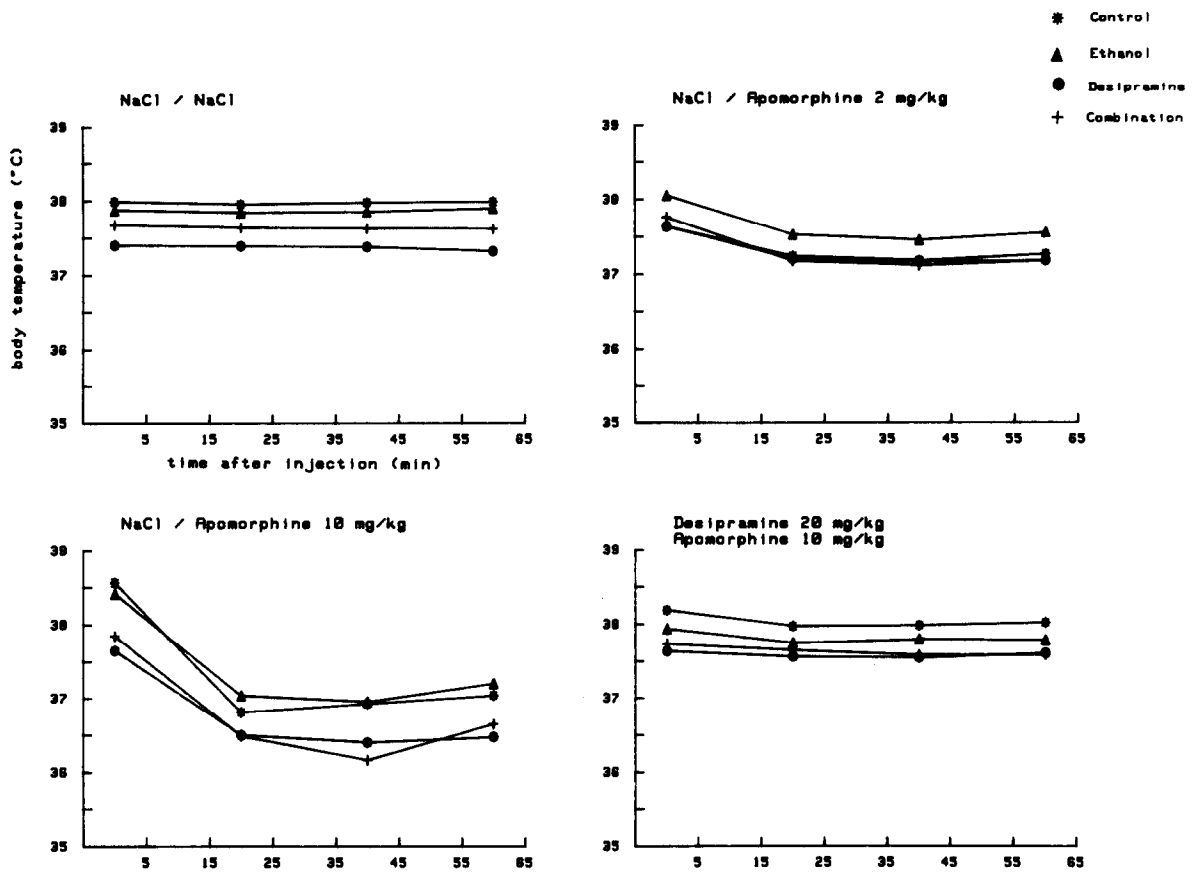

FIG. 6. Time course of the body temperature after the IP injections of the various solutions. The diagrams represent the acute treatments as indicated of the four treatment groups (n=8 per curve). No significant differences between the groups were found.

investigated ethanol diminished the action of desipramine. Ethanol was unable to antagonize the effect of desipramine on spontaneous motility or apomorphine-induced hypothermia.

It is noteworthy with respect ot the amount of drug consumed during the treatment period that the group of rats treated with the drug-combination actually drank significantly more desipramine than the desipramine-treated group and performed better. It was not determined whether the blood and brain levels of the drug were higher in the former group. To the best of our knowledge there is not any report in the literature dealing with the effect of ethanol on the pharmacokinetic of desipramine in rats. However, alcoholics treated with imipramine showed no differences of desipramine levels in blood plasma compared with depressed nonalcoholics (6). Thus, it may be assumed that under the conditions of this study ethanol affects the desipramine concentrations only slightly if at all. The rats in the "combined group" drank less ethanol than the ethanol group yet this dose was sufficient to partially overcome the effects of desipramine.

Low doses of antidepressants have little effect on spontaneous behavior. Medium and high doses exert central depressant actions indicated by a reduction in locomotion, exploratory behavior, and the EEG arousal reaction (9,13).

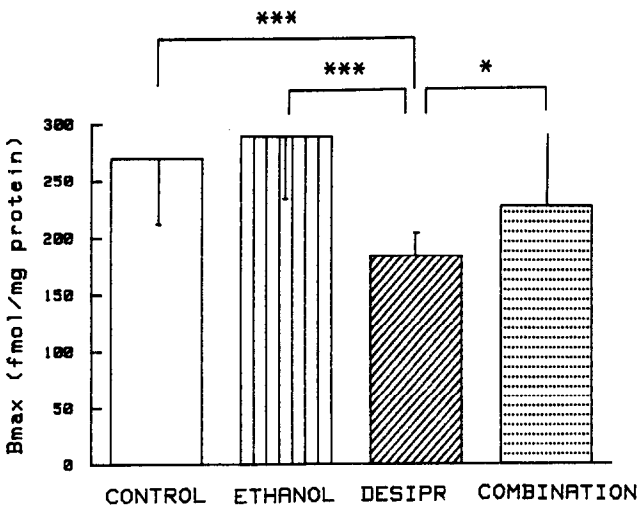

FIG. 7. Maximum number of β-adrenoceptors in the cerebral cortex of rats after chronic treatment. Animals were treated with the respective drugs until decapitation. Values are the means±S.D. of 8–10 independent experiments. [^{125}I]iodocyanopindolol was used as ligand.

TABLE 1

EFFECT OF VARIOUS CHRONIC TREATMENTS ON THE ACTIVATION OF THE ADENYLATE CYCLASE IN RAT CORTEX MEMBRANES

	I (10 μM)	Gpp(NH)p (100 μM)	I (10 μM) + P (100 μM)	NaF (10 mM)	Forskolin (30 μM)
Controls	114.9 ± 8.6	140.4 ± 15.4	96.3 ± 5.4	275.4 ± 33.4	870.6 ± 156.5
Ethanol	115.7 ± 6.6	155 ± 18.4	99.4 ± 3.9	293.1 ± 23.1	823 ± 61.3
Desipramine	103.6 ± 4.6‡	126.3 ± 6.9†	92.7 ± 6.9	245.8 ± 24.9*	826.7 ± 171.9
Desipramine + Ethanol	106.4 ± 8.0*	130.9 ± 12.9	96.3 ± 8.1	245.3 ± 35.0*	750.1 ± 110.8*

*$p<0.05$, †$p<0.01$, ‡$p<0.001$ compared with controls (two-tailed Student's t-test).
I: isoproterenol; Gpp(NH)p: guanylimidodiphosphate; P: propranolol; NaF: sodium fluoride.

These observations were confirmed with respect to motor activity, and two components of exploratory activity, locomotion (crossing) and static exploration (rearing). Desipramine suppressed all three activities. Ethanol acts biphasically on locomotor activity. Low doses increased the behavior (5, 10, 50), whereas high doses reduced it (16, 22, 27, 28, 38, 47). Other authors (45) reported that 0–2 g/kg ethanol increased motor activity. However, nonlocomotor activities like rearing and digging were reduced. Under our experimental conditions ethanol suppressed exploratory behavior. It did not affect the attenuating effect of desipramine on spontaneous motility. However, ethanol antagonized the effect on exploratory behavior (crossing, rearing).

Cerebral noradrenergic neurones are involved in learning or retention of passive or two-way active avoidance tasks [for review (26)]. Furthermore, chronic treatment with desipramine reduces the rate of acquisition of a lever pressing response (23). Thus, to support the hypothesis that ethanol antagonizes the action of desipramine with respect to cognitive performance, it was of interest to examine the effect of the various drug treatments in an active-avoidance test. Control rats learned the task in the course of the session, whereas desipramine-treated animals did not. In support of the hypothesis, animals treated with combined drugs learned the task somewhat slower but eventually nearly as well as control animals. That the suppressing effect of desipramine on motility plays a role in the avoidance deficit cannot be excluded. However, indirect evidence that motility plays no crucial role is provided by the motility experiments. Both desipramine and ethanol diminish motor activity, whereas ethanol prevents learning in the active shock avoidance paradigma less than desipramine. Furthermore, the observation that the number of nonresponders (animals sitting in the chamber without a reaction to the conditioning signal) was higher in desipramine-treated rats than in those treated with the combination support the notion of an essential contribution of factors other than motility. Similar results were found with respect to the retrieval of the learned task.

β-Adrenergic-agonist-augmented wet dog shaking behavior is a paradigm more directly related to central β-adrenergic receptor function. The effect is observed only if animals have been pretreated with 5-HTP (8, 30, 31). β-Adrenoceptor antagonists reduce the number of attacks (17). The reduced number of β-adrenoceptors in the cerebral cortex of rats treated with desipramine correlates well with a reduced activation in the behavioral test. In contrast to the findings with chronic treatment in the present study, acute desipramine treatment facilitates 5-HTP-elicited attacks [2 and 4 mg/kg, mice (25)]. This observation can be explained by an increase in neurotransmitter concentration in the synaptic cleft. Animals treated chronically with the combined treatment reacted less to low doses of salbutamol compared to controls and ethanol-treated rats. The highest dose elicited a significantly higher (50%) number of attacks in these rats than in those treated with desipramine alone.

The 4 groups of animals did not differ with respect to the activation of shaking behavior by 5-HTP supporting the view that desipramine affects noradrenergic and not serotonergic mechanisms (51).

Desipramine antagonizes apomorphine-induced hypothermia when given acutely (14, 41, 46). The effect depends on the dose of apomorphine (32). Although we could reproduce these findings we did not observe differences among the chronically-treated animals. This might be explained by the important role which α_1-adrenoceptors play in the effect of the loading dose of desipramine. The α_1-adrenoceptor agonist St587 diminishes apomorphine-induced hypothermia (7,19).

The activation of adenylate cyclase by (−)isoproterenol was reduced in desipramine-treated rats. The reduction was somewhat less pronounced in animals treated with the combined treatment. Thus, the oral treatment was sufficient to produce the adaptive changes in β-adrenergic receptors and adenylate cyclase seen following daily bolus injections (39).

Some authors reported a decrease of the density of β-adrenergic receptors of approximately 10% by chronic treatment with ethanol (2,34). We did not observe any changes. This contradiction cannot be explained easily. It is conceivable that daily handling of the animals during 31 days in the present study might have induced adaptive changes of these receptors. The number of peripheral β-adrenoceptors is decreased by moderately stressful dietary treatment (20). The treatment-induced changes in signal-transduction at the cellular level are in good agreement with the changes found in several behavioral tests: exploratory activity in the open field, active shock avoidance and intensification of wet dog shake behavior by β-adrenergic agonists.

In none of the paradigms investigated could an antagonistic effect of desipramine or ethanol alone explain the findings following the combined treatment. For example, both desipramine and ethanol worsen avoidance learning while ethanol partially attenuated the desipramine-induced deterioration.

In the paradigms in which ethanol did not have an antagonistic effect (motility, apomorphine-induced hypothermia) the suppressant effects of desipramine occurred within minutes or hours. Thus, these desipramine actions are not due to long-term adaptation processes.

In view of the fact that the down-regulation of neuronal β-adrenergic mechanisms following treatment with antidepressants represents a chronic adaptation process, the observed interference of ethanol with desipramine-induced changes in receptor function and behavior supports the view of a common basis for both phenomena. These findings have both scientific significance, for understanding the mechanisms underlying antidepressant drug actions, and clinical relevance, for psychiatric treatment.

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