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Ethanol Reduces Tolerance, Sensitization, and Up-Regulation of D_2-Receptors After Subchronic Haloperidol

JOCHEN WOLFFGRAMM, HANS ROMMELSPACHER AND ELFRIEDE BUCK

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

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WOLFFGRAMM, J., H. ROMMELSPACHER AND E. BUCK. *Ethanol reduces tolerance, sensitization, and up-regulation of D_2-receptors after subchronic haloperidol.* PHARMACOL BIOCHEM BEHAV **36**(4) 907–914, 1990.—To study the interrelationships between dopamine D_2-receptor density and behavioral responses after chronic treatment with neuroleptics female Wistar rats received haloperidol (HP; 14 mg/l), ethanol (ETOH; 5 vol.%), a combination of both, or tap water as drinking fluids for one or two weeks. Mean intake doses ranged between 1.28 and 1.48 mg/kg/day (HP) and between 3.7 and 4.8 g/kg/day (ETOH). HP administered for one or two weeks raised the number of [^{3}H]spiroperidol binding sites in the striatum by 55%. Concomitant administration of ETOH diminished the increase of B_{max} to 23%. The up-regulation was even reversed when ETOH was added with a delay of one week, although the drug alone had no effect on dopamine-D_2-receptor density. K_D values were not substantially affected. During HP treatment the rats established a tolerance to the motor sedation which was measured by circadian motility recordings. Coadministration of ETOH reduced the development of tolerance, the activity remained at a depressed level. Acute applications of HP (0.3, 0.6, and 0.9 mg/kg, or saline, respectively) also revealed tolerance to the drug for various behavioral responses (exploratory locomotion, rearing, rotarod performance, catalepsy). The tolerance was reduced in all those animals which had received combinations of ETOH and HP. The reduction was most pronounced for the cataleptic response. Pretreatment with ETOH alone had no effect. Sensitization to dopamine agonists was studied by apomorphine-induced stereotypies (licking, sniffing, and forepaw scratching). As expected, chronic HP enhanced the responses. The increased number of stereotypies was reduced in rats pretreated with the combination, although ETOH alone did not affect the response. The reduction was most pronounced for licks. The influence of coadministration of ETOH on both tolerance to dopamine antagonists and sensitization to agonists after chronic HP matches to its effect on D_2-receptors. For catalepsy and stereotyped licking there was a nearly perfect linear correlation between the maximum number of D_2-receptors in the striatum and the behavioral responses. For the other paradigms the degree of reduction of tolerance and sensitization after chronic HP due to concomitant application of ethanol was less than for B_{max}. It is suggested that the development of tolerance and sensitization is coupled to the number of D_2-receptors and that drugs like ETOH interfere with both neuronal and behavioral adaptation during chronic treatment with neuroleptics.

Haloperidol Ethanol Tolerance Sensitization Dopamine Striatum Up-regulation D_2-receptor Rat

CHRONIC treatment with psychoactive drugs leads to adaptive changes in the CNS. Several neuronal functions may be affected like the rate of synthesis and turnover of transmitters, the regulation of transmitter release, the density and sensitivity of presynaptic and postsynaptic receptors, and transmembrane signal transduction (3, 7, 24, 30, 40, 41). On the behavioral level tolerance and cross-tolerance to functionally related drugs develops which may be accompanied by a sensitization to the actions of others (4, 15, 40). Apart from the changed sensitivity to psychoactive drugs both the clinical efficacy and side effects may develop only in the course of chronic treatment (23,30).

Acute application of the neuroleptic compound haloperidol blocks the actions of dopamine agonists at the receptor, stimulates the metabolism of dopamine in the striatum and increases dopamine synthesis (3,31). After some weeks of treatment adaptive changes occur. The initially enhanced rate of dopamine turnover is reduced, the activity of nigro-striatal and mesolimbic dopaminergic neurons decreases, and the number of dopaminergic receptors is augmented (7, 16, 24). During the same time period tolerance develops in particular towards the sedative and cataleptic actions of haloperidol and other dopamine antagonists (3,6), whereas the response to dopamine agonists like apomorphine is sensitized (6, 15, 16).

The temporal coincidence of neuronal adaptation, tolerance and sensitization suggests a causal interrelationship between these three parameters. A simple model to explain both tolerance to antagonists and sensitization to agonists describes pharmacodynamic tolerance as the result of up-regulation of receptors (9, 39, 41). The increased number of receptors attenuates the effects of antagonists and facilitates those of agonists. Although this simplified concept neglects adaptive changes in other receptor systems it seems suitable to predict long-term actions of neuroleptics. As a

consequence, both tolerance and sensitization should be prevented by interferences with the regulation of receptor density. Such an interference is possible by concomitant treatment with ethanol (13,29). Experiments in our laboratory investigating the down-regulation of adrenergic β-receptors and associated behavioral changes following chronic treatment with the tricyclic antidepressant desipramine and their prevention by ethanol revealed a good correlation between receptor density and behavioral parameters related to adrenergic transmission whereas acute effects of desipramine were not affected by ethanol (30).

To test the hypothesis that behavioral adaptation can be caused by an up-regulation of dopaminergic receptors, female Wistar rats were treated for two weeks with haloperidol, ethanol, or combinations, then behavioral responses to acute haloperidol and apomorphine injections were measured and dopaminergic receptor densities (D_2) in striatal membranes were determined.

METHOD

Animals and Housing

The experiments were performed with female Wistar rats (n = 144, breeder: Lippische Versuchstierzuchten, Extertal, F.R.G.), body weight at the beginning of drug treatment ranged from 185 to 205 g. The animals were housed individually in macrolon cages (43 × 26 × 15 cm) with a 12-hr/12-hr LD cycle. Room temperature was 21 ± 2°C, air humidity ranged from 40–60%. Standard diet (Altromin R 1320) was available ad lib. To prevent metabolic interferences after alcohol ingestion 6 g/day of a special diet (Altromin C 200) according to Lieber and di Carli (19) were additionally fed. The purpose of the addition was to offer an excess of requirement of micronutrients (vitamins and minerals) which enables tolerance to higher ETOH concentrations (38). The special diet had no significant effects on receptor regulation since the amount of up-regulation found in this study corresponded to the values which were obtained by similar methods without the diet (13). The drinking fluid consisted of tap water or solutions containing haloperidol (HP) and/or ethanol (ETOH) depending on the experimental conditions. Body weight, food consumption, and fluid intake were measured three times a week.

Subchronic Drug Treatment

The rats were randomly attributed to six experimental groups (n = 24, each). For a period of one week before the treatment all the animals received tap water. During the following two weeks the six groups were supplied with different drinking fluids (Table 1): tap water, ETOH solution (5 vol.%), HP solution (14 mg/l) or combination (14 mg HP diluted in 1 l 5 vol.% ETOH). To determine whether addition of ETOH reverses previous effects of HP treatment the combination was offered to one group after a week of HP supply and to another one after a week of ETOH ingestion without HP. During the treatment period six animals of each group were selected for recordings of circadian activity by means of a motility meter (Rhema, type 2012, Hofheim, F.R.G.). The use of a selective low pass filter allowed to discriminate between components of high and low temporal frequency (corresponding to small rapid body movements and locomotor activity, respectively).

Responses to Acute HP

Six hours prior to the behavioral tests the drinking fluids were removed from the cages to prevent interferences of drug ingestion to the acute application. Thirty to ninety min after the beginning of the dark period a single dose of HP (0.3, 0.6, and 0.9 mg/kg) or saline (0.9% NaCl) was injected subcutaneously under the skin of the neck. Forty-five min later open field behavior, cataleptic response and rotarod performance were subsequently tested. Exploratory activity in the open field (1 × 1 m) was measured by counting square crossings (16 squares; 25 × 25 cm, each) and rearings. Catalepsy was quantified by the time period (up to 30 sec) during which the rat stayed in an unnatural body position at a wooden cube (height: 8 cm). The rotarod task was to stay on a rotating cylinder (8 rotations per min) for 120 sec. During two training sessions at the beginning of chronic treatment and again 30 min before drug injection the animals had been familiarized to the task. Both catalepsy and rotarod performance were tested three times for each rat. The median values were taken for further evaluations.

TABLE 1

SCHEDULE OF SUBCHRONIC TREATMENT BY ADDITION OF DRUGS TO THE DRINKING FLUID

Group	W.W	E.E	E.HE	H.HE	W.H	H.H
1st week	water	ETOH	ETOH	HP	water	HP
2nd week	water	ETOH	HP/ETOH	HP/ETOH	HP	HP

HP: haloperidol 14 mg/l, ETOH: 5 vol.% ethanol, HP/ETOH: haloperidol 14 mg/l diluted in 5 vol.% ethanol.

Apomorphine Stereotypies

Sixteen hours after the application of HP or saline all rats received a subcutaneous injection of apomorphine (1 mg/kg). Between the two tests the groups were supplied only with tap water. Twenty min after injection three kinds of stereotyped behavior (licks, forepaw scratching, and sniffing) were counted during three subsequent periods of 2 min each.

Dopamine Receptor Binding

Four hours after the injection of apomorphine the rats were decapitated by a guillotine. The brains were rapidly removed and cooled over ice. Both striata were excised. To obtain a minimum of 140 mg tissue three striata of rats of the same group of treatment were pooled by chance. After weighing, the tissue was homogenized in cold Tris-HCl buffer (pH 7.7) by use of a glass/teflon homogenizer and centrifuged at 50,000 × g. The supernatant was discarded and the pellet resuspended in fresh Tris-HCl buffer in the same volume as before. Homogenization and centrifugation were repeated once. The resulting pellet was frozen at −20°C and stored for further use (not longer than two weeks). After thawing it was homogenized in 20 ml ice-cold Tris-HCl buffer per 100 mg of fresh tissue.

A mixture containing 800 μl of the membrane suspension, 100 μl of [^{3}H]spiroperidol solution and 100 μl haloperidol (1 μM, additive: 0.15% ascorbic acid) as displacer and ascorbic acid solution, respectively, was incubated at 37°C for 15 min in a metabolic shaker. [^{3}H]Spiroperidol solutions consisted of eight different concentrations (0.053 to 1.9 nM) and ascorbic acid (0.15%). The reaction was terminated by filtration over Whatman GF/B filter and two subsequent washes with 5 ml ice cold Tris-HCl buffer. Radioactivity was measured by a liquid scintillation counter (Packard, type 2660, USA) with 37% efficiency. Specific binding of [^{3}H]spiroperidol was defined as that which could be inhibited by 1 μM haloperidol. It was 70–75% of total binding. No difference was found between the six groups with respect to unspecific binding.

TABLE 2

MEAN VALUES ± SD OF BODY WEIGHT (g), FOOD INGESTION (g), TOTAL FLUID INTAKE (ml), AND DAILY DRUG DOSES (mg/kg b.wt. FOR HP AND g/kg FOR ETOH) DURING THE FIRST AND THE SECOND WEEK OF TREATMENT (BODY WEIGHTS: FIRST AND LAST DAY OF TREATMENT)

Group	W.W	E.E	E.HE	H.HE	W.H	H.H
body weight 1st day	199.4 ± 16.4	199.1 ± 19.5	200.5 ± 20.1	198.0 ± 19.5	197.1 ± 17.7	197.5 ± 15.3
body weight last day	218.0 ± 16.2	217.7 ± 20.0	218.3 ± 7.5	221.1 ± 17.9	219.5 ± 15.7	217.8 ± 13.6
food 1st week	17.6 ± 1.5	14.4 ± 1.3	14.7 ± 1.8	15.1 ± 1.7	18.6 ± 1.6	14.3 ± 2.9
food 2nd week	18.0 ± 1.6	14.6 ± 1.3	13.9 ± 2.4	15.8 ± 2.4	15.1 ± 3.0	17.1 ± 3.2
fluid 1st week	28.9 ± 3.4	24.0 ± 3.5	24.8 ± 3.5	21.0 ± 4.1	30.8 ± 4.8	21.4 ± 3.2
fluid 2nd week	29.8 ± 3.5	25.8 ± 3.4	19.5 ± 2.3	20.6 ± 2.8	22.0 ± 4.3	22.3 ± 3.6
HP 1st week	—	—	—	1.44 ± 0.25	—	1.48 ± 0.22
HP 2nd week	—	—	1.28 ± 0.17	1.34 ± 0.18	1.44 ± 0.27	1.47 ± 0.22
ETOH 1st week	—	4.65 ± 0.59	4.78 ± 0.65	—	—	—
ETOH 2nd week	—	4.79 ± 0.65	3.62 ± 0.47	3.78 ± 0.50	—	—

Protein concentrations were determined by the Biorad micromethod. At a wavelength of 595 nm, extinction was measured using a Zeiss photometer (PMQII). Bovine serum albumin was the protein standard (Behringwerke, Marburg, F.R.G.). The average amount of protein per assay tube was 240 μg (range: 228–254 μg). Six percent of original tissue corresponded to tissue protein.

Data Evaluation, Statistics

The data from housing, behavioral experiments, and receptor binding studies were stored and processed by aids of computerized programs at a Hewlett-Packard 300 table computer. Most of the statistic evaluations concerned differences between groups of treatment. Provided that the prerequisite of homogeneity of variances was met (Bartlett's test) ANOVA statistics were used. Due to the experimental settings (upper time limits) the condition was not met for rotarod performance and catalepsy. For these experiments nonparametric procedures (Kruskal-Wallis H-test) were applied. Interrelationships between different parameters (e.g., receptor density and behavioral responses) were studied by linear and nonlinear regression analysis. For relations of the latter kind an exponential function was adapted by iterative approximation according to the criterion of least mean squares. The data of body weight, food, fluid, and drug intake and of home cage motility consisted in time series. Temporal alterations and trends were tested by ANOVA for repeated measures and Friedman's test. If not stated otherwise the level of significance was $p<0.05$ (two-tailed). Data from binding experiments were processed according to the method first described by Scatchard (32). K_D and B_{max} values were determined from the resulting linear regression line.

Materials

Haloperidol used for chronic ingestion and receptor binding assays was a gift of Janssen GmbH, F.R.G. Acute HP applications were performed with an injection solution of Gry Pharma, F.R.G. Apomorphine was obtained from Woelm Pharma, F.R.G., S(−) sulpiride from Research Biochemicals Inc., Natick, USA, [^{3}H]spiroperidol was purchased from New England Nuclear, Boston, USA with a specific activity of 17 Ci/mmol.

RESULTS

Housing Data

During the two weeks of treatment all groups raised their body weight continuously on an average by 10 g per week (Table 2). Differences between the treatment groups were not significant. Food consumption was stable in controls. Animals treated with ETOH or combination reduced their food intake to 80% of the original consumption due to the caloric properties of the alcohol. The reduction was not significantly modified by HP.

Treatment with HP led to a transient decrease in food consumption (−10%) for 3–5 days. The daily doses of HP and ETOH taken by the rats depended on the fluid intake. Controls drank on an average 29.3±3.4 ml/day. Addition of both ETOH and HP reduced the intake, but combined treatment affected the amount not more than HP alone. Mean doses of HP taken per day ranged from 1.28 to 1.48 g/kg/day, mean ETOH intake was 4.7±0.6 g/kg for single drug and 3.7±0.5 g/kg for combination with HP.

Home Cage Activity

As expected for a night-active species control rats (W.W) revealed high activity scores during the dark period and showed only poor activity with the light on. The circadian pattern was stable for the observation period of two and a half weeks (Fig. 1). It was, in general, similar for locomotion and fine activity. Addition of ETOH or HP to the drinking fluid changed the activity score significantly during the second half of the dark period (Fig. 1), whereas changes during the light period were less pronounced. In the ETOH group (E.E) the activity was attenuated and the transition from dark to light was smoother than in (W.W). When HP was offered for one or two weeks (W.H and H.H) a strong motor depression was observed during the first few days, but, due to the development of tolerance, the activity recovered some days later. Animals treated with the combination (E.HE and H.HE) revealed no tolerance, the activity scores during the dark remained at a significantly lowered level (Fig. 1).

Acute Applications of HP

The effects of 0.3, 0.6, and 0.9 mg/kg HP, or saline on exploratory locomotion and rearing in the open field, rotarod performance and catalepsy were tested 45–90 min after injection. Statistical tests revealed no significant differences between these groups after saline. However, HP-induced responses differed significantly ($p<0.001$). In W.W, HP dose-dependently affected motor coordination (rotarod performance: Fig. 2), stays at the

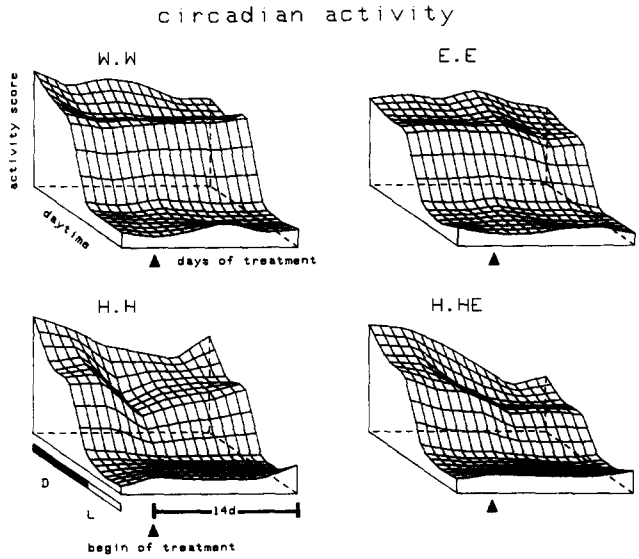

FIG. 1. Three-dimensional plots representing the mean circadian courses of home cage activity of rats receiving water (W.W.), ETOH (E.E), HP (H.H) or successively HP and combination (H.HE) (see Table 1). The activity score (vertical axis) comprises locomotion as well as small body movements. The oblique axis represents 20 hours of a day starting with the dark period, the horizontal axis corresponds to the time period of treatment (2 weeks).

cube (cataleptic rigidity: Fig. 3), and open field behavior including exploratory locomotion and rearing. Subchronic ETOH treatment (E.E) did not significantly alter the responses. HP for one or two weeks (W.H and H.H) induced tolerance to the acute action of HP in all the four paradigms. In the groups that had received a combination of ETOH and HP, tolerance towards HP was developed to a significantly smaller degree (rotarod: $p<0.05$, catalepsy: $p<0.01$, locomotion: $p<0.05$, rearing: $p<0.05$). The reduction was most expressed for the cataleptic response, it did not vary between the combination groups E.HE and H.HE. Thus, even retarded treatment with ETOH was able to reverse the induction of tolerance to chronic HP.

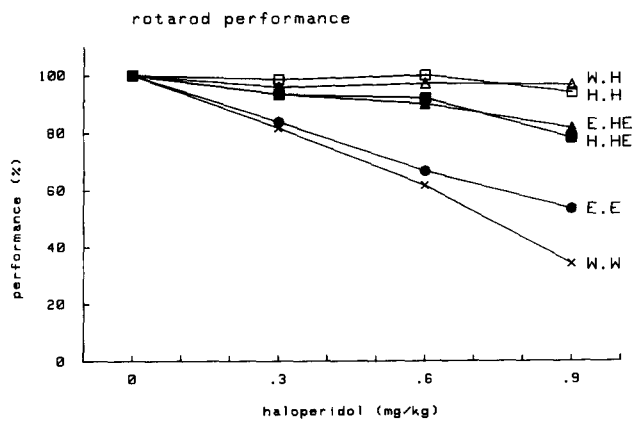

FIG. 2. Dose-response curves for rotarod performance of differently pretreated rats (Table 1) after subcutaneous injection of haloperidol or saline. A performance of 100% means that the rats stayed on a rotating cylinder (8 rotations/min) for at least 120 sec.

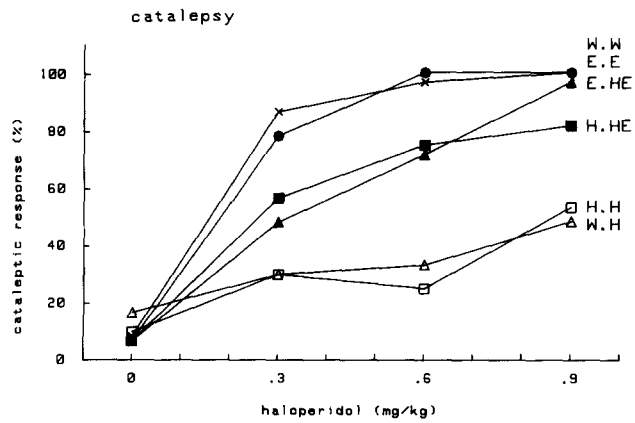

FIG. 3. Dose-response curves for the cataleptic response of differently pretreated rats (Table 1) after subcutaneous injection of haloperidol or saline. A response of 100% means that the rats did not change their unnatural position at a cube for at least 30 sec.

Apomorphine-Induced Stereotyped Behavior

Subcutaneous injection of 1 mg/kg apomorphine caused stereotyped licking, sniffing, and forepaw movements in all groups of treatment. HP-pretreated rats (W.H and H.H) revealed a strong

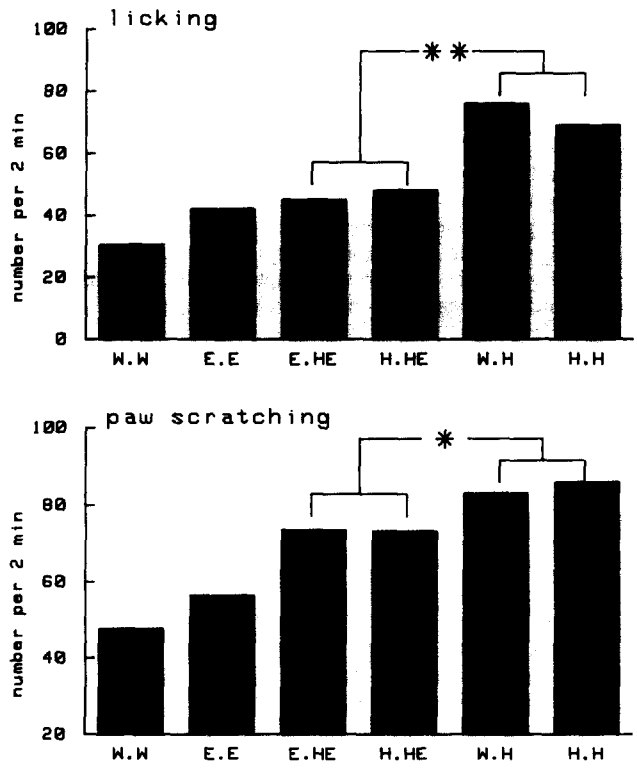

FIG. 4. Stereotyped licking (above) and forepaw scratching (below) after 1 mg/kg SC apomorphine. The types of pretreatment (W.W, E.E, E.HE, W.H, and H.H are listed in Table 1). Significant differences between administration of HP alone and combined treatments are marked by asterisks. *$p<0.01$.

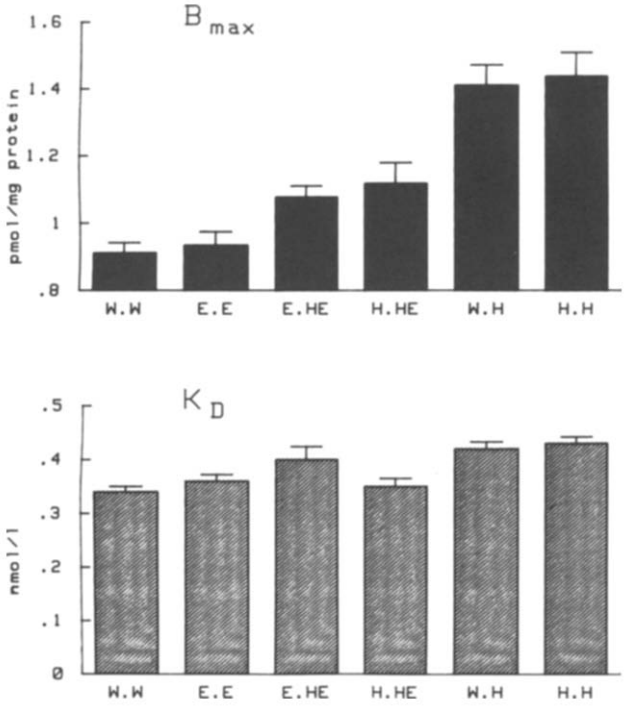

FIG. 5. Average values of B_{max} and K_D ($\pm$SEM) of [^{3}H]spiroperidol binding sites in the striatum of differently pretreated rats (Table 1). The differences of the means between H.H and H-HE and between E.HE and W.H resp. are significant ($p<0.01$).

sensitization of the licking response (average 240% in relation to W.W, $p<0.001$) and also a significant increase of stereotyped paw scratching (185%, $p<0.01$). Single ETOH (E.E) did not alter the responses in comparison to W.W, whereas combined treatments (E.HE and H.HE) significantly reduced sensitization ($p<0.01$, Fig. 4). The alteration was most distinctly expressed for licks. The number of stereotyped sniffings counted 24 min after injection did not statistically depend on the mode of subchronic treatment.

[^{3}H]Spiroperidol Binding

In (W.W) rats the maximum number of dopaminergic D_2 receptors amounted to $B_{max} = 910 \pm 30$ fmol/mg protein, the K_D value was 0.34 ± 0.01 μmol/l (means $\pm$ SEM). The relatively high value of K_D may be explained by the high amount of tissue in the incubation tube which was 4 to 5 times higher than that used by others (34,35). To explain the high density of receptors, the displacement of [^{3}H]spiroperidol by haloperidol and S(−)sulpiride was studied (Fig. 7). The displacement curves clearly show that haloperidol displaces more binding sites at a concentration of 10^{-6} M than S(−)sulpiride. At the higher nanomolar and lower micromolar concentrations the curve with haloperidol shows a plateau. Thus, under the assay conditions used the data referring to the density of binding sites comprise dopamine-2-receptors and in addition about 20% of unidentified binding sites. Pretreatment with ETOH or combinations did not significantly affect the K_D values, whereas in W.H and H.H the affinity to the receptor was slightly reduced ($K_D = 0.42 \pm 0.01$ μmol/l, $p<0.01$, Fig. 5). Treatment with HP for one or two weeks (W.H and H.H) raised B_{max} by more than 50% (Fig. 5). Such up-regulation was significantly ($p<0.01$) reduced or even reversed by concomitant ingestion of HP and ETOH (E.HE or H.HE) although ETOH alone (E.E) had no effect. This result corresponded to the behavioral responses of animals with combined treatment. In the case of catalepsy after acute HP and of apomorphine-induced stereotyped licking the relationship between the number of receptors and the behavioral drug response was linear ($r = -1.00$, and $r = +.97$, $p<0.001$, Fig. 6). For rotarod performance, horizontal as well as vertical exploration and stereotyped forepaw scratching the suppression of tolerance and sensitization, respectively, by chronic ETOH was smaller than the effect on receptor density. In those paradigms an exponential curve shape was more appropriate to approximate the relationship between molecular and behavioral response (Fig. 6).

DISCUSSION

Adaptation after chronic treatment with neuroleptic drugs concerns both transmitters and receptors. In the striatum the acute treatment with neuroleptics induces an increase of dopamine metabolism. After several days of treatment this effect turns to a reduced state (7,24). The time course of the metabolic effect correlates well with the development of behavioral tolerance to antagonists but fails to predict sensitization to agonists. In contrast, the up-regulation of dopaminergic receptors accompanied by a shift to the (high)-affinity state (17) might explain both tolerance and sensitization by the increased receptor concentration (4, 9, 39, 41).

In addition, other transmitter systems are also affected by long-term application of neuroleptic drugs. PCP receptors are up-regulated (8), the density of α_1-receptors in the submandibular glands is increased (27), the secretory response coupled to cholinergic drugs is enhanced (26), and the levels of GABA, glutamate, aspartate, glutamine, substance P, enkephalins, and other peptides in various regions of the brain are altered (5,21). Such actions of chronic neuroleptic treatment may contribute to tolerance and sensitization.

The present study focusses on changes in the dopaminergic system and associated behavioral responses. The hypothesis was tested that experimental interference to the up-regulation of D_2-receptors has analogous consequences for behavioral responses to acute application of agonists or antagonists. Such an interference can be observed by lithium ions (14,28) or by ETOH (6, 13, 29, 30). A chronic ingestion of 4–5 g/kg ETOH per day is sufficient for the effect. Although such a dose is not likely to cause considerable blood concentrations it has been proved to interfere with dopaminergic transmission after chronic administration (11, 13, 18, 22). The mechanism by which the regulation is affected is still unclear but it has been shown in this study that coadministration of ETOH can reverse an up-regulation that has already been established. ETOH when applied without HP revealed only a marginal increase of D_2-receptors which was not significant in our experiments. Other authors controversially reported an increase or decrease of the receptor density with doses that were slightly higher than those used by us (18,22), but these effects did not appear before at least three weeks of treatment. These results confirm that ETOH's interferences to adaptive receptor regulation cannot be regarded as a simple superposition to chronic effects of HP but that they consist in a specific interaction with the process of regulation. Thus, also down-regulation (adrenergic β-receptors) has been shown to be reduced by ETOH (30).

Apart from its effect on receptor regulation chronic ingestion of ETOH has additional consequences for dopaminergic transmission. In particular, the metabolism of dopamine is enhanced by acute and chronic ETOH (11). After chronic administration of ETOH the response is reduced (25). The effect is synergistic to that of chronic HP. Thus, it cannot account for the suspension of the effects of chronic HP by ETOH.

As predicted by the hypothesis, concomitant treatment with

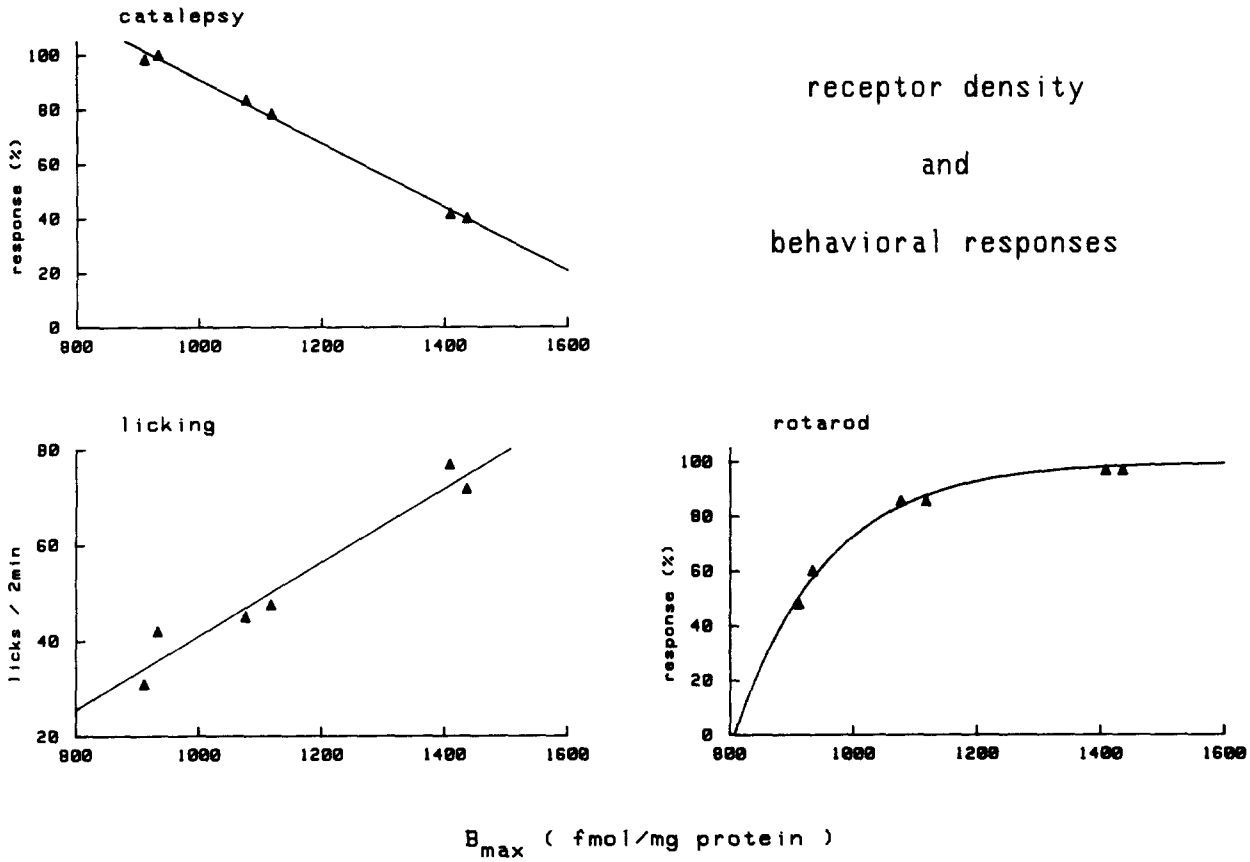

FIG. 6. Correlation diagrams between the maximum number of [^{3}H]spiroperidol binding sites (B_{max}) and behavioral responses. The triangles represent the mean values of the six groups of treatment. For catalepsy and stereotyped licking the regression lines were linear, for rotarod performance an exponential curve was fitting the data. Behavioral responses were determined as the mean reactions after 0.6 and 0.9 mg/kg haloperidol and after 1 mg/kg apomorphine, respectively.

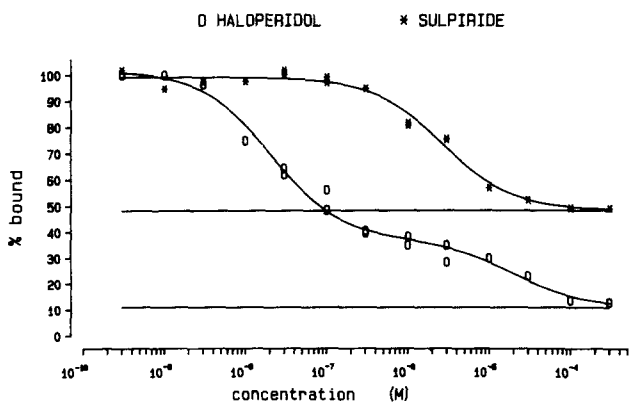

FIG. 7. Inhibition of [^{3}H]spiroperidol binding to rat striatum by haloperidol and S(−)sulpiride. [^{3}H]Spiroperidol was incubated at 0°C in 1 ml 5 mM tris buffer pH 7.7 for 60 min. In contrast to the standard incubation conditions, the incubation mixture contained 100 μM NaCl because the interaction of S(−)sulpiride with dopamine receptors is sodium-dependent (39).

ETOH and HP led to a diminished tolerance to HP effects as well as to a reduced sensitization to apomorphine stereotypies. The only behavioral response which was affected neither by HP nor by combination was stereotyped sniffing. This negative result is probably due to anatomical and physiological constraints. Since the animal cannot concomitantly sniff and lick, it is probable that licking "dominates" the other kind of response at certain latencies after application. Indeed, it has been shown that different types of stereotypies predominate during subsequent observation periods after apomorphine injection (37).

Apart from the acute applications of agonist and antagonist the measures of home cage motility were also related to chronic drug treatment. The circadian time courses of motor activity during the period of drug exposition were in good accordance to the basal hypothesis. After treatment with HP the activity score was dramatically decreased during the second half of the night when the animals had ingested the fluid containing the drug. However, due to the development of tolerance, activity recovered a few days later. When ETOH was coadministered (irrespective whether or not HP treatment had already begun) the recovery was prevented and the activity in the home cage during the dark period remained at a low level.

Although the general prediction of the hypothesis matched with the results of nearly all experiments there existed a marked quantitative differentiation among the measured behavioral responses. Catalepsy and stereotyped licking revealed an extremely

high negative or positive correlation to receptor density. For motor coordination, exploration of a simple novel environment, and stereotyped forepaw scratching, however, the development of tolerance and sensitization was less reduced by ETOH than for receptor density. Consequently the regression line was exponential rather than linear. In these cases expected and observed behavioral responses after combination treatment corresponded only on a qualitative but not on the quantitative level.

Catalepsy and stereotypy are mutually opposite responses which possibly use at least partially the same neuronal pathways (2,12). They may be induced and antagonised by the same drugs (1,12). There is evidence that they are generated mainly in the striatum (2,11). Stimulus properties of apomorphine seem to be mediated by both D_1- and D_2-receptors (33), and the involvement of D_2-receptors has also been shown for the cataleptic reaction. According to the present experiments tolerance and sensitization to catalepsy and stereotyped licking were completely coupled to the number of D_2-receptors in the striatum. If other mechanisms were participating their influence could not be detected.

Locomotion is also influenced by dopaminergic transmission but mainly in mesolimbic areas of the brain [in particular the nucleus accumbens; (10,19)]. Thus, the diminished efficacy of chronic ETOH to reverse tolerance to the motor depression and impairment of coordinative walking as well as sensitization to stereotyped movements of extremities might be due to regional differences between dopaminergic neurons in the brain. On the other hand, tolerance and sensitization to these behavioral responses might to a considerable degree be guided by additional components including compensative neuronal regulation circuits and other transmitter systems. Obviously ETOH affects only parts of the adaptation process but leaves other components unchanged. As a consequence, tolerance is partially reduced but not reversed for those responses which are associated to locomotor activity.

The results obtained by subchronic HP treatment with or without coadministration of ETOH confirm the hypothesis that the density of dopaminergic D_2-receptors in striatal membranes contributes to the development of tolerance and sensitization. The affinity of the receptors to an antagonist (spiroperidol) was not substantially changed but it has been shown that the up-regulation also comprises a shift of the affinity to agonists to the high state (17). To what extent ETOH interferes with such affinity enhancement remains to be clarified.

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