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Evaluation of a Stable CCK Agonist (A68552) in Conditioned Avoidance Responding in Mice, Rats, and Primates: Comparison With Typical and Atypical Antipsychotics

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CCK Cholecystokinin A68552 Avoidance Primates Rats Mice Antipsychotic Clozapine Sulpiride Haloperidol

SEVERAL lines of evidence suggest a functional relationship between dopamine and cholecystokinin (CCK). The two are colocalized in a subset of neurons projecting from the A-10 ventral tegmental area (VTA) to the nucleus accumbens (15). Local microinjection of the sulfated CCK octapeptide, CCK-8-S, into the VTA potentiates the inhibitory actions of dopamine on the firing rate of dopamine-containing cells (4,17,26) and potentiates the behavioral hypoactivity resulting from dopamine injections into that site (7). Administration of CCK-8-S directly into the nucleus accumbens either potentiates or inhibits the behavioral actions of dopamine depending upon where within the accumbens the injections are made (8).

In addition, systemic administration of CCK-8-S has been reported to inhibit dopamine release and also to induce a depolarization block of dopamine neurons as is seen with repeated haloperidol treatment (21). Behaviorally, both ICV and systemic injections of CCK have generally been reported to have dopamine antagonist-like effects in suppressing locomotor activity (3,11,18,27,28) and this effect appears to be mediated by peripheral type (CCK-A) receptors that show selectivity for the sulfated CCK octapeptide (CCK-8-S). There is evidence that systemically administered CCK could produce these CNS effects acting either on vagal afferents or at brain regions with greater exposure to the general circulation. Both

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the appetite suppressant effects and the locomotor effects of systemically administered CCK are attenuated by vagotomy (13,19). It has been suggested that the pathway by which systemically administered CCK produces such behavioral effects involves activation of these vagal receptors and subsequent altercations in neuronal activity going through the nucleus of the solitary tract (9,10).

On the basis of these findings and the fact that existing antipsychotics have dopamine antagonist activity, it has been proposed that CCK or a synthetic CCK agonist could be effective antipsychotics. The conditioned avoidance test has been found to be a reliable predictor of antipsychotic activity of "typical" and, to a lesser degree, "atypical" antipsychotics. CCK has been tested in various conditioned avoidance tests with mixed and conflicting results (5,6,14). One limitation to experiments designed to determine if a CCK agonist could have antipsychotic activity is that the naturally occurring peptide is relatively unstable and may be a poor agonist to use to test the hypothesis.

[(Des-amino)Tyrl, Nle2,5,(*N*-methyl)Phe7]CCK-7 sulfated (A68552) binds with high affinity to both the peripheral type (Type A) CCK receptor (Ki = 3.6 nM) and the cerebral cortical type (Type B) CCK receptor (Ki = 0.26 nM). It is a full agonist in stimulating amylase release and shows 79% the efficacy of CCK-8-S in stimulating phosphoinositol turnover in pancreatic acinar cells (16). In behavioral tests, A68552 administered peripherally suppresses food consumption (22) and locomotor activity within a dose range of 0.01–0.3 mg/kg IP in mice. The motor effect appears to be mediated by Type A receptors as it is blocked by the Type A antagonist, A65186 (Britton et al., in preparation).

These findings suggest the possibility that agonists to the Type A CCK receptor, acting through peripheral mechanisms, might be useful therapeutic agents in conditions such as schizophrenia that are responsive to drugs that alter dopamine neurotransmission. Suppression of conditioned avoidance is highly predictive of antipsychotic activity for agents acting via blockade of dopamine transmission. We studied the effects of A68552 on the acquisition and performance of conditioned avoidance responding in three species.

METHOD

Rat Step-Up Active Avoidance

Subjects. Male CD-1 rats weighing 200–225 g from Charles River Labs (Portage, MI) were used. Animals were individually housed with food (Purina Rodent Chow) and water available ad lib.

Apparatus. Three automated shelf-jump type avoidance boxes (Lafayette Instruments Model 85200, Lafayette, IN) with model 82022SS shock generators were used. The chambers (18 × 16.5 × 20.5 cm) contained a cue light on one end wall, a Plexiglas top and sides, and a stainless steel grid floor. A shelf, 10 cm above the floor, on the opposite end to the cue light had a movable wall that was programmed to push the rat off the shelf at the end of the trial. The chambers were housed in a ventilated, illuminated, sound-attenuated outer chamber. White noise was used in the room to mask any extraneous sounds. An IBM XT computer was used to control the experiment and record responses.

Experimental procedure. Each rat was trained in daily sessions consisting of 10 trials per day. At the start of each trial, the cue light was illuminated and the shelf wall retracted to expose the shelf. The "avoidance" period began with the opening of the shelf and the onset of a cue lamp [conditioned stimulus (CS)] that remained on for 10 s prior to the onset of electric current (0.5 mA) to the floor. The floor remained electrified for up to 20 s during which time an animal that had not already avoided the shock by jumping to the shelf could escape the remaining shock period. An escape or avoidance response terminated the shock and 15 s later the shelf moved forward, forcing the animal back on the floor. The trials were separated by variable intertrial intervals (mean = 30 s; range = 10–60 s).

In general, 10–12 animals per treatment group were tested. In the acquisition studies, clozapine and haloperidol were administered on each training day 60 min prior to the session. A68552 (SC) was given 30 min prior to the sessions. Sulpiride, which has a longer latency to onset of action, was administered 90 min prior to each session. For tests of drug effects on performance of conditioned responding, previously trained rats were treated with saline for 2 days of testing, then divided into equal groups balanced with respect to avoidance performance on the second saline control day. They were then challenged with a test compound.

Mouse Shuttle Avoidance

Subjects. Male CD-1 mice from Charles River Labs weighing 25–30 g at the time of testing were used. Mice were housed six per cage with food and water available ad lib.

Apparatus. Three shuttle avoidance boxes (Campden Industries) were used. They measured 27 × 12 × 12.75 cm and had aluminum ends and top, Plexiglas sides, and a stainless steel rod floor through which foot shock (1.2 mA) could be delivered from a BRS/LVE (Laurel, MD) Model SGS-009 shock generator. Cue lights were placed in the middle of each end of the chamber and a speaker for a tone was centrally placed in the top.

Experimental procedure. Mice were trained to respond to a cue light and tone during the first 5 s of presentation and thus to avoid a foot-shock applied through the grid floor. When the mouse moved to the opposite side of the chamber, the tone, cue light, and shock (if present) were terminated and the timer was reset to begin the next trial. Trials were separated by a variable interval with a mean of 2 s. After the onset of the foot-shock, the mouse could escape by moving to the opposite end of the apparatus. In the absence of an avoidance or escape response, the shock remained on for a maximum of 25 s. Animals were tested for 50 trials a day for up to 9 days. Mice were tested with drugs either during the acquisition of the response or after having achieved an avoidance criterion of at least 80% for testing disruption of performance. Mice were injected IP 15 mins prior to testing with A68552 and 30 min prior with haloperidol and clozapine.

Monkey Conditioned Avoidance

Subjects. Four male cynomolgus monkeys were used. They were housed individually and fed twice daily (Purina Monkey Chow supplemented with fresh fruit). Water was available ad lib.

Apparatus. Two isolation chambers (BRS/LVE) housed the standard monkey chairs, onto each of which was mounted a BRS/LVE primate lever, a speaker connected to a white noise generator, a sonalert tone generator, and a cue lamp. Mild electric shock (2.5 mA) was delivered to two foot plates by a BRS/LVE Model SGS-903 shock generator. An IBM XT computer and interface controlled the operation and timing of the apparatus.

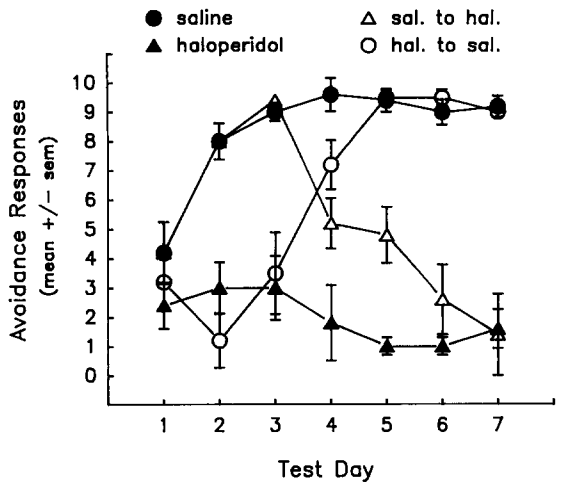

FIG. 1. Effects of haloperidol on acquisition and performance of shelf-jump conditioned avoidance by rats. Four groups of six animals each were treated either with saline or haloperidol (0.15 mg/kg, IP) for the first 3 days of acquisition. Prior to testing on the fourth day, one group from each treatment regimen was switched to the other treatment as indicated in the figure. Haloperidol significantly blocked the initial acquisition of the response ("saline" vs. "haloperidol" on day 3, $p < 0.01$) and suppressed the performance by animals that had learned the avoidance response under saline treatment ("saline switched to haloperidol" vs. "saline" on day 7, $p < 0.01$).

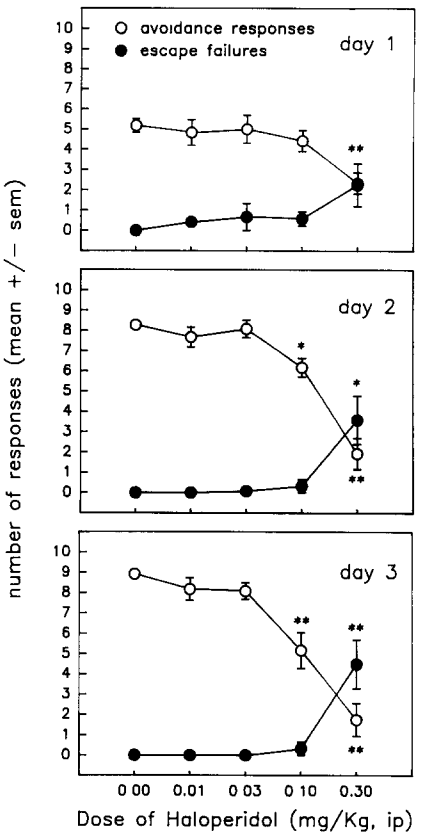

FIG. 2. Effects of varying doses of haloperidol over 3 days of acquisition of a shelf-jump conditioned avoidance response by rats. Open symbols indicate avoidance responses. Filled symbols indicate escape failures. Haloperidol significantly suppressed avoidance responding on day 1, $F(4, 55) = 4.3$, $p < 0.01$, day 2, $F(4, 55) = 24.17$, $p < 0.01$, and day 3, $F(4, 55) = 22.03$, $p < 0.01$. Open symbols indicate escape failures on day 2, $F(4, 55) = 7.87$, $p < 0.05$, and day 3, $F(4, 55) = 12.47$, $p < 0.01$. Significantly different (by Newman-Keuls test) from vehicle control group on that day ($*p < 0.05$, $**p < 0.01$).

Method. The four monkeys used in the study were first acclimated to the primate chairs. Training consisted of 100 trials per session. Each trial began with a CS of 10 s (tone and a cue lamp) followed by the unconditioned stimulus (UCS) (foot-shock), which lasted a maximum duration of 30 s. Trials were separated by a variable intertrial interval (mean = 30 s; minimum 15; maximum 60 s). A bar press response by the animal turned off the conditioned stimuli, as well as the shock if present, and terminated the trial. If the bar press occurred during the CS, it was registered as an avoidance response. A bar press during the UCS was registered as an escape response and no bar press during a trial was deemed an escape failure. Animals were trained with one 100-trial session per day for 5 days per week. When an animal was performing at better than 85% avoidance, saline control injections were given. Drugs were administered IM with at least 2 days between dosing. Haloperidol was administered 30 min prior to the session and A68552 15 min prior.

General Procedures

Statistics. Most data obtained from the mouse and rat experiments were subjected to an analysis of variance (ANOVA) with Newman-Keuls comparisons among groups for avoidance responses and escape failures. In some studies of performance of conditioned avoidance where animals were subjected to several treatments, paired *t*-tests were used comparing performance on a vehicle control day to that on a drug treatment day. Corrections for the level of significance for *t* values for multiple comparisons were made by the method of Bonferroni. In some cases, escape failure data were not normally distributed and were analyzed using Mann-Whitney *U*-tests for comparison of treatment groups with control groups.

Drugs. Haloperidol and sulpiride were obtained from Sigma Chemical Co. (St. Louis, MO) and clozapine was kindly donated by Sandoz Pharmaceuticals (East Hanover, NJ). Haloperidol, sulpiride, and clozapine were prepared for injection by dissolving in a minimal amount of glacial acetic acid, then diluting with 0.9% saline to the desired final volume. A68552 was synthesized at Abbott Laboratories. It was dissolved in distilled water for injections. Animal handling, care, and testing was in accordance with practices set forth in the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

RESULTS

Rat Step-Up Active Avoidance

Rats treated with either haloperidol (0.15 mg/kg, IP) or saline were trained for 3 days in the shelf-jump conditioned avoidance. During this period, rats treated with saline showed acquisition of the avoidance response while haloperidol-treated animals did not improve. Prior to the fourth day of testing, one half of the animals (randomly selected) in each group were switched to the other treatment so that half of the saline-treated animals were maintained on saline and half switched to haloperidol. Likewise, half the haloperidol-treated

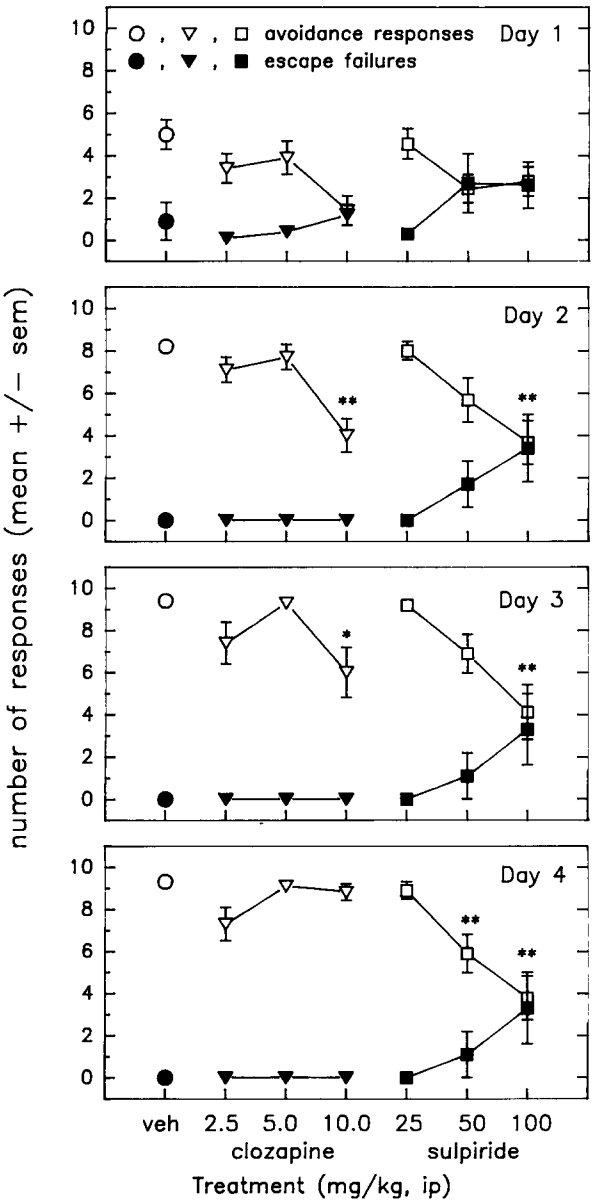

FIG. 3. Effects of varying doses of clozapine or sulpiride over 4 days of acquisition of a shelf-jump conditioned avoidance response by rats. Open symbols indicate avoidance responses. Filled symbols indicate escape failures. Data of all groups analyzed with Newman-Keuls test showed significant effects on avoidance responding on day 2, $F(6, 57) = 6.69$, $p < 0.01$, day 3, $F(6, 57) = 5.50$, $p < 0.01$, and day 4, $F(6, 57) = 9.99$, $p < 0.01$. There was a significant effect on escape failures on day 2, $F(6, 57) = 3.37$, $p < 0.01$. Significantly different from vehicle control group on indicated day by Newman-Keuls comparison (*$p < 0.05$, **$p < 0.01$).

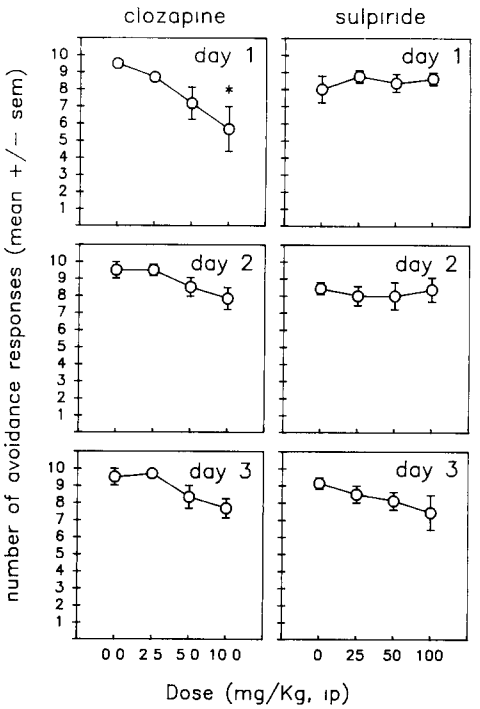

FIG. 4. Effects of varying doses of clozapine and sulpiride on the performance of a shelf-jump conditioned avoidance response by rats over 3 days of drug administration. There was a significant effect of clozapine on avoidance responding on day 1 only, $F(3, 20) = 4.04$, $p < 0.05$. There was no significant effect of sulpiride on avoidance responding and there were no significant differences in escape failures (data not shown) with either drug.

animals were maintained on that treatment and half were changed to saline. Figure 1 shows the effects of this procedure and indicates that haloperidol blocked the acquisition of the response in naive animals and disrupted performance in animals that had received saline prior to the initial training sessions.

A comparison of effects of varying doses of haloperidol, clozapine, and sulpiride on acquisition of avoidance responding are shown in Figs. 2 and 3. Haloperidol treatment (0.01, 0.03, 0.1, or 0.3 mg/kg, IP) over 3 days resulted in a dose-dependent blockade of acquisition (Fig. 2) that was statistically significant at 0.3 mg/kg on day 1 and at both of the higher two doses on days 2 and 3. Haloperidol produced a smaller (relative to effects on avoidance) but nonetheless significantly increase in escape failures at the dose of 0.3 mg/kg on days 2 and 3.

Clozapine at 2.5, 5.0, and 10.0 mg/kg IP produced a different effect than haloperidol on acquisition (Fig. 3). The highest dose tested (10.0 mg/kg) produced a significant suppression of avoidance responding on the first 3 days of treatment, but on day 4 there was no significant difference among the four groups. During the acquisition phase, these doses of clozapine did not increase escape failures. Sulpiride (25.0, 50.0, and 100.0 mg/kg, IP) administered 90 min prior to acquisition on each of 4 consecutive days suppressed avoidance responding at both 50.0 and 100.0 mg/kg on each of the 4 days of testing (Fig. 3) but also tended to increase escape failures. Rats tested after having reached performance criteria of at least 70% avoidance responses failed to show suppression of performance with either clozapine or sulpiride (Fig. 4).

A68552 at 0.11, 0.32, and 1.07 mg/kg IP (equivalent to 0.1, 0.3, and 1.0 μmol/kg) was tested over 3 days of acquisition (see Fig. 5). On each day, the mean number of avoidances by the A68552-treated group was slightly decreased but there

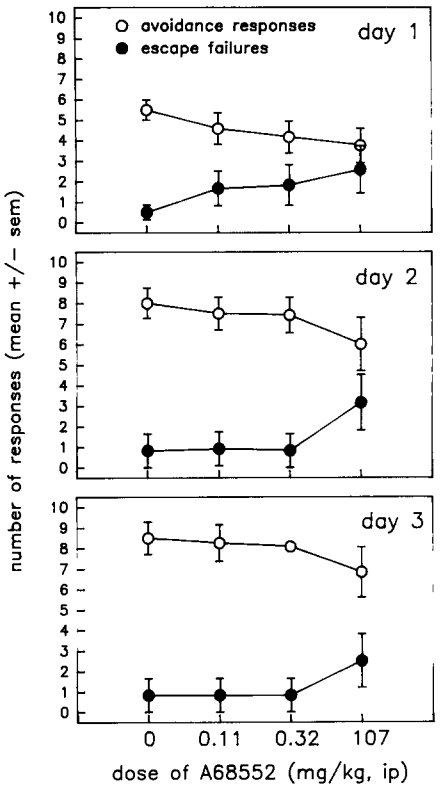

FIG. 5. Effects of varying doses of A68552 over 3 days of acquisition of a shelf-jump conditioned avoidance response by rats. Open symbols indicate avoidance responses. Filled symbols indicate escape failures. There were no significant differences among groups.

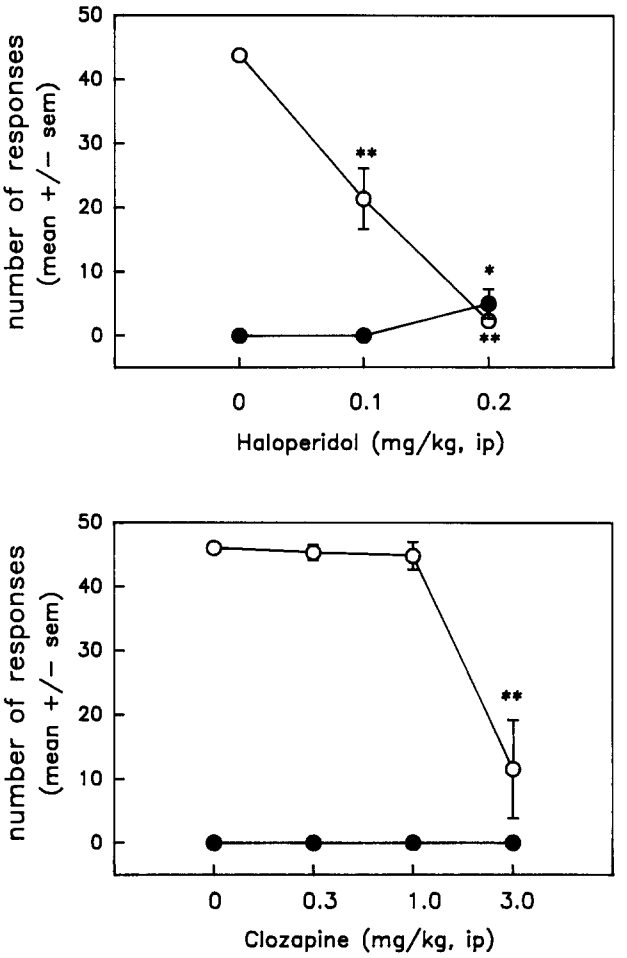

FIG. 6. Effects of haloperidol (upper graph) and clozapine (lower graph) on performance of conditioned avoidance by mice previously trained to a minimum criteria of 80% avoidance responses. Open symbols represent avoidance responses. Filled symbols represent escape failures. Haloperidol significantly suppressed avoidance responding at both doses, $F(2, 15) = 53.1$, $p < 0.01$, and increased escape failures at the higher dose, $F(2, 15) = 4.93$, $p < 0.05$. Clozapine significantly suppressed avoidance responding, $F(3, 20) = 17.47$, $p < 0.01$, with no effect on escape failures. Significantly different from vehicle control (*$p < 0.05$, **$p < 0.01$).

were no significant changes. Escape failures were not significantly altered.

Mouse Shuttle Avoidance

Mice that had been trained to a criterion of 70% avoidance responding were tested for performance following treatment with haloperidol, clozapine, or A68552. Haloperidol at both 0.1 and 0.2 mg/kg IP significantly reduced the mean number of avoidance responses (Fig. 6, upper graph) and slightly increased escape failures at the higher dose. Clozapine was tested at 0.3, 1.0, and 3.0 mg/kg IP. It significantly reduced the number of avoidance responses at 3.0 mg/kg (Fig. 6, lower graph).

A68552 was tested in a study in which each of 24 mice was treated with vehicle 0.11, 0.33, and 1.07 mg/kg IP. The design was counterbalanced with respect to the order in which the doses were given. There was no effect on either avoidance or escape responding (Fig. 7).

Given that studies with rats suggested that acquisition of a conditioned avoidance response was more sensitive to atypical antipsychotics than was performance by well-trained animals, A68552 was tested over the first 5 days of acquisition in mice. At doses of 0.011, 0.107, and 1.07 mg/kg IP, A68552 appeared to moderately suppress acquisition of avoidance responding but also increased escape failures over these same doses. The effects on both avoidance and escape responding were significant for the dose of 0.107 mg/kg on day 5 only (Fig. 8).

Monkey Lever Press Conditioned Avoidance

The effects of haloperidol (0.01 and 0.03 mg/kg, IM) and A68552 (0.02, 0.05, 0.1, and 0.2 mg/kg, IM) are presented in Fig. 9. Avoidance responding was significantly suppressed by haloperidol at 0.03 mg/kg without significant effect on escape responding. A68552 was without effect on avoidance or escape responding at doses up to 0.2 mg/kg. This dose produced an emetic response in two of the four animals receiving it.

DISCUSSION

Traditional neuroleptics of a variety of chemical classes are presumed to exert their therapeutic action by blocking dopamine receptors. Virtually all such drugs have also been shown to be effective in suppressing conditioned avoidance responding (1,20). The atypical agent clozapine, which has

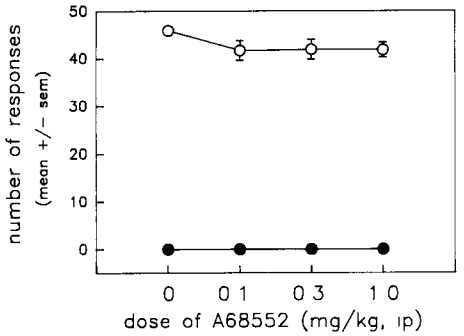

FIG. 7. Effects of A68552 on performance of conditioned avoidance by mice. Each of 24 mice were tested at each of the three doses of A68552 and with vehicle (see the Method section). The vehicle data represent the mean values for each day preceding an A68552 treatment day. There were no significant effects of drug treatment.

dopamine antagonist activity (12) as well as additional pharmacological effects, also blocks conditioned avoidance responding but appears to do so in a manner that is not identical to that of the typical neuroleptics (2,25). However, neither clozapine nor sulpiride exhibited the same type of effect seen with haloperidol—inhibition of both acquisition and performance by well-trained rats. The effects of both clozapine and sulpiride are more apparent in the rat when assessed during the acquisition phase. Consistent with a previous report by Sanger (25), the clozapine effects also appear to show tolerance in that they were no longer apparent by the third day of acquisition. Although sulpiride suppressed avoidance responding without significantly suppressing escape responding, the data suggest that there is not a wide separation between doses that produce these two effects. The effects of sulpiride increased with the duration of treatment. A68552 did not resemble any of the other three drugs on conditioned avoidance by rats and appeared to produce only moderate and general effects on locomotor activity.

Tests with mice showed a significant effect on performance by both haloperidol and clozapine at doses approximately equal to those that have been shown to reduce exploratory locomotion. A68552, which suppresses exploratory locomotion at an ED_{50} of approximately 0.01 mg/kg IP (Britton et al., in preparation), was without effect on avoidance responding at doses up to 1.07 mg/kg IP. Given the evidence that the acquisition phase of testing might be more sensitive to disruption, A68552 was administered to mice over each of the first 5 days of training in the shuttle avoidance test. Under these conditions, very moderate effects were observed on escape and avoidance responding. Avoidance responding was significantly suppressed at a single dose (0.107 mg/kg, IP) on the fifth day and, at this dose, escape failures increased to an equivalent degree (from a mean of 0 for the saline group on day 5 to a mean of 7.3 for the group receiving 0.107 mg/kg A68552).

The extensive literature suggesting a functional interaction between CCK and dopamine has prompted the hypothesis that a CCK agonist might be useful in treating schizophrenia (23,24). To date, both preliminary clinical trials and preclinical animal behavioral studies have been inconclusive. While there have been some reports of alterations in avoidance conditioning (5,6,14), evidence has not been strong that systemically administered CCK-8-S has antipsychotic-like effects in conditioned avoidance. One reason for such paucity of data could be the relative liability of the naturally occurring peptide. A68552 was designed to provide a more stable probe of the CCK system. As such, the present findings fail to provide support for the hypothesis that a Type A CCK receptor ago-

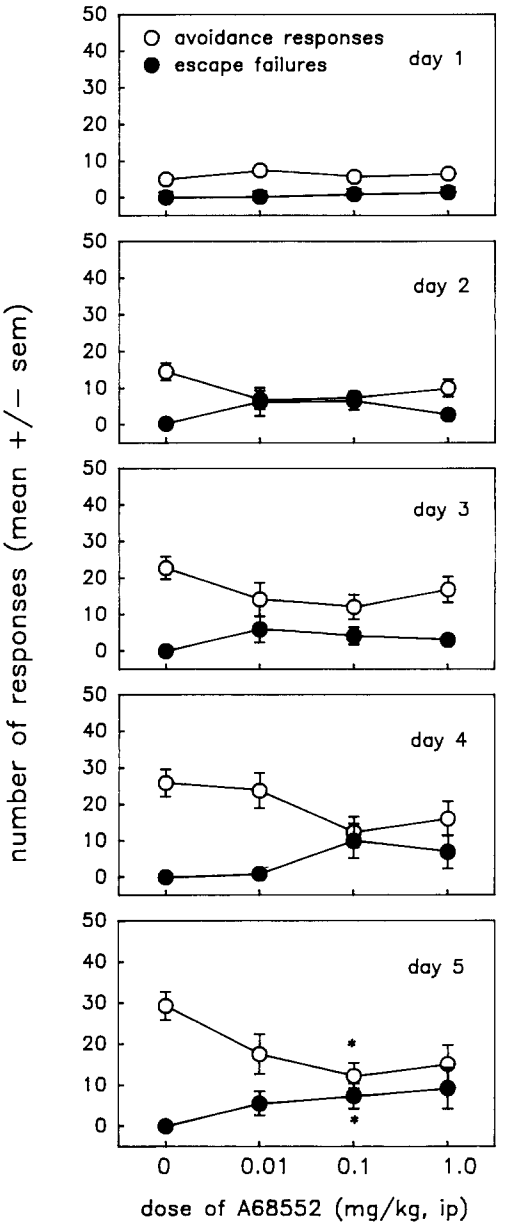

FIG. 8. Effects of varying doses of A68552 over 5 days of acquisition of a conditioned avoidance response by mice. Open symbols indicate avoidance responses. Filled symbols indicate escape failure. ANOVA revealed a significant decrease in avoidance responding on day 5, $F(3, 36) = 3.29, p < 0.05$. Newman-Keuls tests showed a significant effect at the dose of 0.1 mg/kg IP ($p < 0.05$). Escape failures were significantly elevated at the same dose on day 5 ($p < 0.05$ by Mann-Whitney U-test).

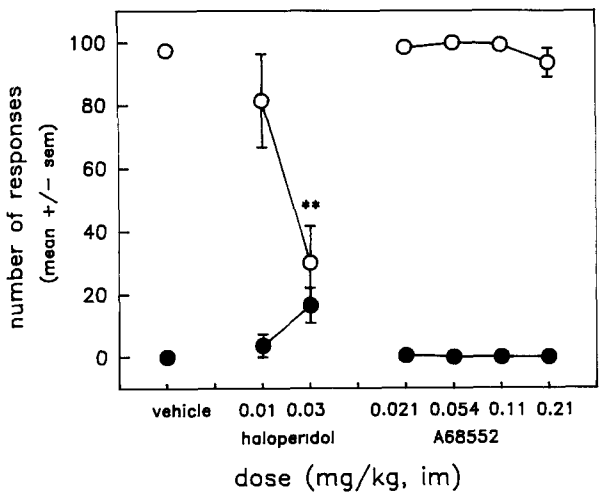

FIG. 9. Effects of haloperidol and A68552 on avoidance responding by cynomolgus monkeys. Data represent mean ± SEM for four animals at all treatments except the two lowest doses of A68552, which represent two animals. Haloperidol significantly decreased avoidance responding (open symbols) at the dose of 0.03 mg/kg. Escape failures (filled symbols) were not significantly changed. There was no significant effect of A68552 on either measure.

nist disrupts conditioned avoidance. In fact, even at doses two orders of magnitude greater than necessary to suppress locomotor activity in mice and rats and doses that produce emesis in monkeys A68552 failed to selectively suppress avoidance responding. The only paradigm in which there was a significant suppression of avoidance responding (a single intermediate dose on day 5 of acquisition in the mouse test) also revealed a suppression of escape responding.

Clearly, the limitations of conditioned avoidance testing as an indicator of antipsychotic activity preclude any definitive rejection of the idea that CCK agonists could have antipsychotic potential by acting peripherally to decrease central dopaminergic tone. The ability of conditioned avoidance tests to predict antipsychotic efficacy is based almost exclusively upon agents that show some degree of dopamine receptor antagonist activity. The predictive utility of conditioned avoidance for compounds acting by other mechanisms remains to be determined. However, the CCK agonist used here, A68552, has high affinity and efficacy at peripheral-type (Type A) CCK receptors and would therefore be an appropriate compound with which to test the hypothesis. The failure of A68552 to exhibit positive effects in conditioned avoidance suggests that either the alterations in dopamine transmission are relatively minor or that other compensatory changes occur that obscure any evidence of a positive effect on avoidance responding.

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