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BRIEF COMMUNICATION

Behavioral Effects of the Reversible Dopamine Antagonist Flupenthixol Are Not Potentiated by *N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline in the Preweanling Rat

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McDOUGALL, S. A. AND C. A. BOLANOS. *Behavioral effects of the reversible dopamine antagonist flupenthixol are not potentiated by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline in the preweanling rat.* PHARMACOL BIOCHEM BEHAV **50**(1) 127–131, 1995. – In the preweanling rat, the irreversible dopamine (DA) receptor antagonist *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) does not diminish behaviors induced by the nonselective DA agonist R(−)-propylnorapomorphine (NPA). To determine whether EEDQ was simply inactivating an insufficient percentage of DA receptors, the NPA-induced behaviors of 17-day-old rats were assessed after treatment with flupenthixol (a reversible DA receptor antagonist) and/or EEDQ. When given alone, flupenthixol (0.04, 0.1, and 0.4 mg/kg, intraperitoneally [IP]) produced a dose-dependent decrease in the behavioral effects induced by 1.0 mg/kg NPA. Unexpectedly, EEDQ (7.5 mg/kg, IP) did not potentiate flupenthixol's actions. This suggests that EEDQ's inability to block the NPA-induced behaviors of preweanling rats was not the result of an insufficient percentage of DA receptors being inactivated.

Dopamine EEDQ Flupenthixol NPA Preweanling rat

N-ETHOXYCARBONYL-2-ETHOXY-1,2-DIHYDROQUINOLINE (EEDQ) is an alkylating agent that irreversibly binds to dopamine (DA) receptors and inactivates them (11). Studies using EEDQ indicate that the DA receptor systems of preweanling and adult rats differ in a fundamental way. In adult rats, EEDQ treatment blocks the behavioral effects normally induced by nonselective DA agonists or selective DA D_2 agonists (2,3,10,11). This behavioral deficit is apparently caused by an EEDQ-induced reduction in DA receptors, and the resulting inability of the agonist to bind to a sufficient number of receptors. EEDQ does not eliminate the DA D_1-mediated behaviors of adult rats, presumably because a large reserve of D_1 receptors is available (2,19). Surprisingly, EEDQ has distinctly different effects in the preweanling rat. For example, EEDQ is unable to block either the D_1 or D_2 mediated behaviors of 11- and 17-day-old rats (13–15,17). Thus, in the rat pup, treatment with either moderate (7.5 mg/kg) or high (15.0 mg/kg) doses of EEDQ does not diminish behaviors induced by the D_1 agonist SKF 38393, the D_2 agonist quinpirole, or the D_1/D_2 agonist R-propylnorapomorphine (NPA). Curiously, age-dependent differences are not typically observed after treatment with reversible (i.e., competitive) DA antagonists, because haloperidol, SCH 23390, and sulpiride induce similar behavior patterns in preweanling and adult rats

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(4,8,12). Therefore, when considered together, experiments using EEDQ show that the DA receptors of preweanling and adult rats differ in a qualitative fashion.

At present, it is uncertain why reversible DA antagonists induce adultlike responding in preweanling rats, whereas an irreversible DA antagonist is behaviorally ineffective in the younger animals. One possibility is that EEDQ may bind to an insufficient percentage of DA receptors in the preweanling rat. Receptor binding studies have shown that EEDQ (7.5 mg/kg) inactivates a moderate percentage of D_1 and D_2 receptors in the 17-day-old (approximately 61–69%); however, this percentage is somewhat less than in the adult (approximately 80–86%) (6,7). Therefore, to determine whether EEDQ inactivates a sufficient percentage of receptors in the preweanling rat, the NPA-induced behaviors of 17-day-olds were assessed after treatment with flupenthixol (a nonselective D_1/D_2 reversible DA receptor antagonist) and/or EEDQ. It was predicted that a low dose of flupenthixol would have a disproportionately disruptive effect on the NPA-induced behaviors of EEDQ-treated rat pups (i.e., because EEDQ has already inactivated a substantial, albeit insufficient, number of receptors). In contrast, a low dose of flupenthixol should only moderately diminish the NPA-induced behaviors of non-EEDQ-treated rat pups (i.e., because these pups have a full complement of DA receptors). Thus, the present hypothesis predicts that EEDQ should potentiate flupenthixol's behavioral effects.

In the first experiment the behavioral effects of flupenthixol (0, 0.04, 0.1, and 0.4 mg/kg) and NPA (0 and 1.0 mg/kg) were assessed in the absence of EEDQ. This initial experiment was necessary to determine a dose range of flupenthixol that would partially, but not completely, block the NPA-induced behaviors of 17-day-old rats.

METHOD

Animals

Subjects were 160 male and female rat pups of Sprague–Dawley descent (Harlan Sprague–Dawley, Indianapolis, IN). Litters were culled to a maximum of 10 pups or a minimum of eight pups at 3 days of age. Pups were kept with the dam throughout behavioral testing. Assignment of subjects to groups was random, with no more than one rat from each litter being placed into a particular group. The colony room was maintained at 23–25°C and kept under a 14 h light–10 h dark cycle. Behavioral testing was conducted during the light phase of the cycle. There were eight subjects in each group, with the mean weight of the rat pups at 34.9 g on the day of testing.

Drugs

All drugs were injected intraperitoneally (IP) and were given at a volume of 5.0 ml/kg. Both NPA and *cis*-(Z)-flupenthixol dihydrochloride were dissolved in distilled water, whereas EEDQ was dissolved in 95% ethanol : distilled water (1 : 4). NPA and flupenthixol were acquired from Research Biochemicals (Natick, MA). EEDQ was acquired from Sigma (St. Louis, MO).

Apparatus

Behavioral testing was done in two activity chambers made of plywood (30 × 30 × 42 cm), with a wood floor and an open top. The floor and walls were painted white.

Procedure

Experiment 1. A total of 64 17-day-old rats were randomly divided into four groups and injected IP with the nonselective D_1/D_2 receptor antagonist flupenthixol (0.04, 0.1, or 0.4 mg/kg) or distilled water. The same pups were injected IP 35 min later with the nonselective D_1/D_2 receptor agonist NPA (1.0 mg/kg) or distilled water. After an additional 5 min, stereotyped (head-down) sniffing and bouts of grooming were assessed during a 20-min testing session. Grooming bouts were measured for the entire 20 min, whereas the occurrence of stereotyped sniffing was determined every 20 s using a time-sampling procedure. Both sniffing and grooming were assessed by a single observer blind to treatment conditions.

Experiment 2. A total of 96 16-day-old rats were injected IP with EEDQ (7.5 mg/kg) or its vehicle. One day later, rat pups were injected with either flupenthixol (0.1 or 0.2 mg/kg) or distilled water and then, 35 min later, with either NPA (1.0 mg/kg) or distilled water. Stereotyped sniffing and bouts of grooming were assessed as in Experiment 1.

Statistical Analyses

Analyses of variance (ANOVA) were used for statistical analysis of stereotyped sniffing and grooming data. When appropriate, Tukey *t*-tests were used for making planned and posthoc comparisons ($p < 0.05$).

RESULTS

Experiment 1

Stereotyped sniffing. Mean stereotyped sniffing counts and grooming bouts of the 17-day-old rats are shown in Fig. 1. Overall, rat pups receiving NPA (1.0 mg/kg) had significantly more stereotyped sniffing counts than pups given distilled water, agonist main effect, $F(1, 56) = 79.81$, $p < 0.001$. Pretreating the pups with flupenthixol reduced NPA-induced sniffing, but only at the greatest dose (0.4 mg/kg) of the antagonist, antagonist main effect, $F(3, 56) = 10.95$, $p < 0.001$; antagonist × agonist interaction, $F(3, 56) = 19.35$, $p < 0.001$. Thus, at the two lower doses (0.04 and 0.1 mg/kg), flupenthixol did not affect the NPA-induced stereotyped sniffing of the rat pups, whereas 0.4 mg/kg flupenthixol completely blocked NPA's effects.

Bouts of grooming. Overall, NPA depressed the grooming bouts of the 17-day-old rats, agonist main effect, $F(1, 56) = 9.72$, $p < 0.01$. NPA's behavior-depressing effects were eliminated by 0.4 mg/kg flupenthixol, as pups receiving flupenthixol (0.4 mg/kg) and NPA had significantly more grooming bouts than pups receiving NPA alone, antagonist × agonist interaction, $F(3, 56) = 2.91$, $p < 0.05$. Moreover, 17-day-olds given both flupenthixol (0.4 mg/kg) and NPA responded similar to pups receiving either no drug treatments or pups receiving flupenthixol (0.4 mg/kg) alone. Curiously, 17-day-olds receiving distilled water and 0.4 mg/kg flupenthixol had significantly fewer grooming bouts than pups receiving no drug treatments. The lower doses (0.04 and 0.1 mg/kg) of flupenthixol did not block NPA's effects.

Experiment 2

Stereotyped sniffing. Figure 2 shows the mean stereotyped sniffing counts and grooming bouts of the 17-day-old rats. Overall, rat pups treated with NPA had significantly more stereotyped sniffing counts than pups given distilled water,

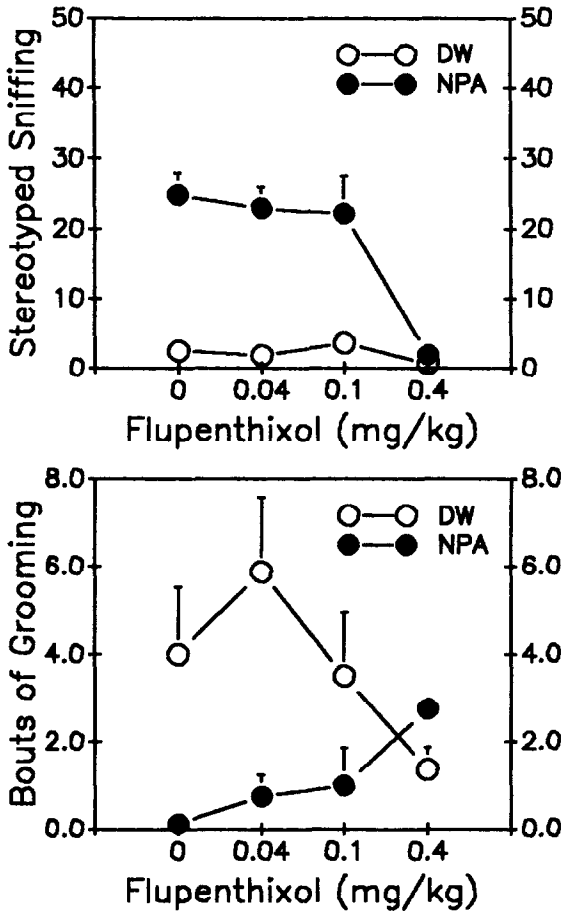

FIG. 1. Mean stereotyped sniffing counts and bouts of grooming (+ SEM) of 17-day-old rats injected IP with either distilled water or flupenthixol (0.04, 0.1, or 0.4 mg/kg; FLU) 35 min before treatment with R(−)-propylnorapomorphine (NPA) (1.0 mg/kg) or distilled water (DW). Behavioral testing lasted 20 min and occurred 5 min after NPA or distilled water treatment.

agonist main effect, $F(1, 84) = 89.79$, $p < 0.001$. Flupenthixol also affected stereotyped sniffing, antagonist main effect, $F(2, 84) = 31.03$, $p < 0.001$, as the reversible antagonist induced a dose-dependent decrease in the sniffing of the NPA-treated rat pups, antagonist × agonist interaction, $F(2, 84) = 28.02$, $p < 0.001$. More specifically, 0.2 mg/kg flupenthixol completely eliminated NPA-induced sniffing, whereas the lower dose of flupenthixol (0.1 mg/kg) only partially blocked this agonist-induced response.

It was originally predicted that flupenthixol would maximally disrupt the NPA-induced behaviors of rat pups in the EEDQ condition, relative to pups in the vehicle condition. Unexpectedly, the stereotyped sniffing of pups in the vehicle and EEDQ conditions did not differ, as the main effect and interaction involving condition as a variable were not statistically significant. Furthermore, planned comparisons indicated that EEDQ-treated pups injected with NPA and 0.1 mg/kg flupenthixol (see the upper-right panel of Fig. 2) did not differ from similarly treated pups in the vehicle condition (see the upper-left panel of Fig. 2), Tukey t-tests, $p > 0.05$. Likewise, the stereotyped sniffing of pups receiving both NPA and 0.2 mg/kg flupenthixol did not differ according to vehicle or EEDQ condition.

Unexpectedly, the mean stereotyped sniffing counts of rat pups exclusively given NPA (i.e., with no flupenthixol) increased substantially across experiments. The reason for this increase is uncertain, but it may have been due to the vehicle (i.e., ethanol-distilled water) injections that pups received in Experiment 2. The heightened sniffing exhibited by pups in the vehicle-NPA group had important ramifications, as the only time 0.1 mg/kg flupenthixol depressed NPA-induced sniffing was in comparison with the vehicle-NPA control group. The mean number of sniffing counts shown by the various flupenthixol-NPA groups was similar across the two experiments (compare Figs. 1 and 2).

Bouts of grooming. Overall, NPA-treated pups had significantly fewer grooming bouts than pups given distilled water, agonist main effect, $F(1, 84) = 15.48$, $p < 0.001$. Flupenthixol was able to block this effect, because pups given both 0.2 mg/kg flupenthixol and NPA did not differ from pups given flupenthixol alone, antagonist × agonist interaction, $F(2, 84) = 11.23$, $p < 0.001$. In contrast, the lower dose of flupenthixol was not sufficient to fully antagonize NPA's effects, as pups given 0.1 mg/kg flupenthixol and NPA had

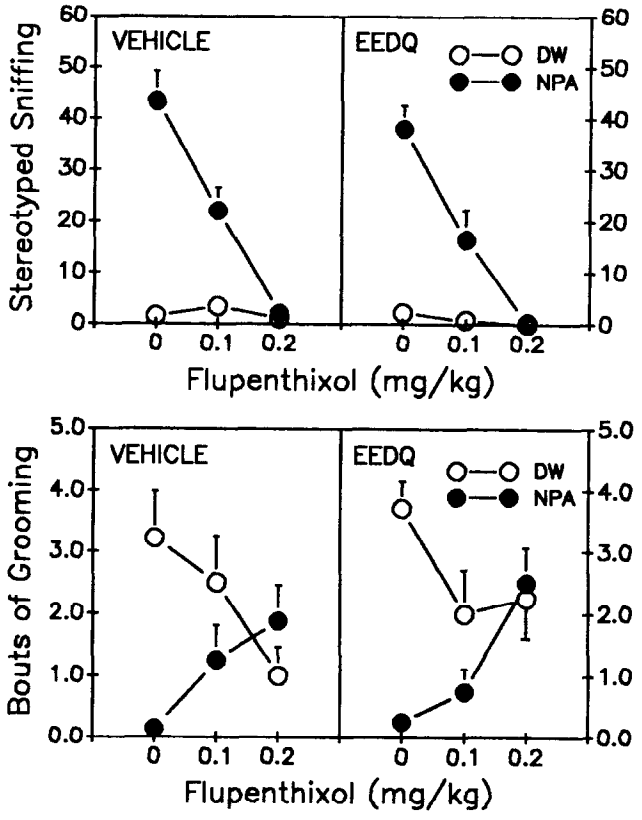

FIG. 2. Mean stereotyped sniffing counts and bouts of grooming (+ SEM) of 17-day-old rats injected IP with flupenthixol (0, 0.1, or 0.2 mg/kg) 35 min before treatment with R(−)-propylnorapomorphine (NPA) (1.0 mg/kg) or distilled water. The NPA injections occurred 24 h after pretreatment with 7.5 mg/kg EEDQ (*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) or its vehicle. Behavioral testing lasted 20 min and occurred 5 min after NPA or distilled water treatment.

significantly fewer grooming bouts than pups injected with 0.1 mg/kg flupenthixol alone.

Once again, neither the main effect nor interactions assessing the condition variable were significant. Moreover, planned comparisons indicated that EEDQ did not potentiate flupenthixol's actions. For example, pups given both 0.2 mg/kg flupenthixol and NPA groomed similarly, regardless of whether EEDQ or vehicle was administered, Tukey *t*-tests, $p > 0.05$. EEDQ did not potentiate the effects of 0.1 mg/kg flupenthixol either, as the comparable groups in the vehicle and EEDQ conditions responded similarly, Tukey t-tests, $p > 0.05$.

DISCUSSION

Previous studies have shown that reversible DA antagonists block the agonist-induced behaviors of preweanling rats, whereas an irreversible DA antagonist is ineffective at blocking these same agonist-induced behaviors (12–15,17). One possible explanation for this unusual effect is that EEDQ inactivates an insufficient percentage of DA receptors in the rat pup. In the present study, we further assessed this phenomenon by determining whether EEDQ would potentiate flupenthixol's behavioral actions. Overall, NPA increased the stereotyped sniffing and decreased the grooming of 17-day-old rats (see Figs. 1 and 2). As predicted, NPA's behavioral effects were diminished by flupenthixol (see Figs. 1 and 2), whereas pretreatment with EEDQ did not affect the NPA-induced stereotyped sniffing or grooming of 17-day-old rats (see Fig. 2). Thus, once again, the behaviors of preweanling rats were found to be unaffected by EEDQ. Unexpectedly, EEDQ did not potentiate flupenthixol's actions, as NPA-treated pups given flupenthixol alone responded the same as NPA-treated pups given both flupenthixol and EEDQ (see Fig. 2). This suggests that EEDQ's inability to affect behavior is not due to an insufficient number of receptors being inactivated, because even a subeffective dose of EEDQ should have potentiated flupenthixol's behavioral effects. This particular result was unexpected and leaves no ready explanation for the age-dependent behavioral differences observed after EEDQ treatment.

More generally, these data are difficult to reconcile with current knowledge of how reversible and irreversible antagonists function, because an irreversible DA antagonist (i.e., EEDQ) should potentiate the behavioral effects of a reversible DA antagonist (i.e., flupenthixol). It is not clear why this did not occur; however, one possibility is that EEDQ and flupenthixol were affecting different receptor subpopulations. Although unlikely, this explanation is tenable because those brain areas (e.g., the striatum and nucleus accumbens) known to mediate unlearned behaviors are neither structurally or functionally homogeneous (1,3,5,9). More specifically, both the density of DA receptors and the behaviors mediated by those receptors varies according to striatal and accumbens subregions. For example, injections of NPA into the anterior ventral striatum of adult rats induce both oral stereotypies and rapid-onset, high-intensity sniffing, whereas injections of NPA into the anterior dorsolateral or posterior ventral striatum induce only slow-onset, low-intensity sniffing (3). Thus, if EEDQ and flupenthixol primarily affected different subregions, the heterogeneous organization of the striatum and accumbens might be responsible for the present results.

An alternate explanation, that rat pups have large reserves of D_1 and D_2 receptors, might also account for these data. A receptor reserve hypothesis has been proposed previously, as EEDQ's inability to block the D_1 mediated behaviors of adult rats has been explained by the presence of a D_1 receptor reserve (2,19). According to this hypothesis, adult rats have an overabundance of D_1 receptors, only a fraction of which are necessary for the mediation of behavior (19). Similarly, it is conceivable that preweanling rats have reserves of both D_1 and D_2 receptors sufficient to compensate for those receptors inactivated by EEDQ. This explanation does not seem to account for the present results, because the receptors remaining after EEDQ treatment should have been particularly susceptible to reversible antagonist blockade (i.e., flupenthixol). Instead, the results showed that flupenthixol did not differentially affect the behaviors of EEDQ- or vehicle-pretreated rat pups. Thus, the basic idea of a receptor reserve hypothesis, that behavior will occur until a critical mass of receptors is no longer available, is not consistent with data from the preweanling rat (see also 15).

Finally, EEDQ's inability to affect the NPA-induced behaviors of preweanling rats does not result from a subeffective dose being used. In two separate receptor binding studies we have shown that 7.5 mg/kg EEDQ inactivates a substantial percentage (i.e., > 60%) of DA D_1 and D_2 receptors in the 17-day-old rat (6,7). In both of these studies, rats were sacrificed 24 h after EEDQ treatment, the same time point used in the present behavioral experiments. Moreover, increasing the dose of EEDQ to 15 or 25 mg/kg (doses > 25 mg/kg were lethal) did not significantly increase the amount of D_1 and D_2 receptor depletion, nor did 15 mg/kg EEDQ affect the NPA-induced behaviors of 17-day-old rats (6,13). EEDQ's actions at DA D_3, D_4, or D_5 receptors have not yet been assessed, so it is possible that these receptors are responsible for mediating NPA-induced behaviors in the preweanling rat.

Various studies have shown that EEDQ preferentially binds to D_1 and D_2 receptors, but will also bind to α-adrenergic, serotonin, and GABA receptors (16,18). To control for the lack of EEDQ specificity, some researchers pretreat half their rats with SCH 23390 and sulpiride (or other reversible DA antagonists) to selectively protect D_1 and D_2 receptors from EEDQ-induced inactivation. These rats (with selectively protected DA receptors) are then compared with rats given EEDQ alone (i.e., rats with depleted DA, α-adrenergic, serotonin, and GABA receptors). By this subtractive procedure EEDQ's nondopaminergic actions can be determined (5,11,13). In contrast, a protection condition was not employed in the present study, because preweanling rats exhibit similar behavioral patterns when no EEDQ is given and when EEDQ follows protection pretreatments (13,14). Moreover, the fact that EEDQ did not affect the behavior of the rat pups suggests that EEDQ's nondopaminergic actions were behaviorally unimportant.

In summary, the present study showed that EEDQ does not combine with flupenthixol to maximally disrupt the NPA-induced behaviors of preweanling rats. This result is difficult to explain, given current knowledge of how irreversible and reversible receptor antagonists function; however, understanding the bases for these age-dependent differences may provide important information about ontogenetic changes in DA receptor structure and function.

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