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Behavioral Effects of Irreversible Dopamine Receptor Inactivation in the Preweanling Rat: Assessment of the Receptor Reserve Hypothesis

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Abstract

EEDQ is an irreversible receptor antagonist that eliminates the dopamine (DA) mediated behaviors of adults rats. In contrast, EEDQ does not seem to affect the DA mediated behaviors of preweanling rat pups. One explanation for this age-dependent difference is that rat pups may have a DA receptor reserve, not available to adults, which is sufficient to mediate behavior. Therefore, the purpose of the present study was to determine whether a D_1 and D_2 receptor reserve exists in preweanling rats. A total of 96, 17-day-old rat pups were injected with EEDQ (7.5 mg/kg) or its vehicle immediately after being trained to approach an anesthetized dam on a straight alley for nipple attachment reward. After 18 hours rat pups were then injected with saline, the D_1 agonist SCH 23390 (0.5 mg/kg), or the D_2 agonist sulpiride (50 mg/kg). A final testing session occurred 30 min later. This session consisted of an additional 28 trials on the straight alley, in which responding resulted in either reinforcement or extinction. Results of this experiment indicated that EEDQ and SCH 23390 combined to maximally disrupt the extinction responding of the rat pups. Unexpectedly, EEDQ did not potentiate sulpiride's effects. In general, these results indicate that preweanling rat pups do not have a D_1 or D_2 receptor reserve, but age-dependent differences in DA receptor functioning were apparent.

Biochemical studies have shown that dopamine (DA) receptors can be divided into a number of distinct subtypes: D_1, D_{2S}, D_{2I}, D_3, D_4, and D_5 (Chio, Hess, Graham, & Huff, 1990; Sokoloff, Giros, Martes, Bouthenet, & Schwartz, 1990; Sunahara et al., 1991; Van Tol et al., 1991). The psychopharmacological characteristics of these receptor subtypes are only partially understood, as the behavioral actions of just the D_1 and D_2 receptors have been studied intensively. For example, selective D_2 agonists (e.g., quinpirole and bromocriptine) increase the locomotor activity, rearing, and sniffing of preweanling and adult rats (Arnt, 1987; McDougall, Arnold, & Nonneman, 1990; McDougall, Crawford, & Nonneman, 1993). Conversely, selective D_1 agonists (e.g., SKF 38393) have only a few behavioral effects, the most prominent among them being a dose-dependent increase in grooming (McDougall et al., 1990, 1993; Molloy & Waddington, 1985; Murray & Waddington, 1989). Blocking these D_1 and D_2 receptors has predictable actions, as reversible DA antagonists (e.g., SCH 23390 and sulpiride) eliminate the agonist-induced behaviors of both rat pups and adults (Arnt, 1987; McDougall et al., 1990). When considered together, these studies indicate that D_1 and D_2 receptors mediate different behaviors and that treatment with reversible D_1 and D_2 agonists and antagonists induce similar behavioral effects in preweanling and adult rats.

In contrast, studies using the irreversible receptor antagonist N- ethoxycarbonyl- 2- ethoxy- 1, 2- dihydroquinoline (EEDQ) indicate that the D_1 and D_2 receptor systems of preweanling and adult rats differ in a fundamental way. EEDQ is an alkylating agent that permanently binds to DA receptors and inactivates them (Hamblin & Creese, 1983). In adult rats, EEDQ treatment blocks the behavioral effects normally induced by selective D_2 agonists or nonselective DA agonists (Arnt, Hyttel, & Meier, 1988; Cameron & Crocker, 1989; Giorgi & Biggio, 1990; Hamblin & Creese, 1983; McDougall, Crawford, & Nonneman, 1992a; Meller, Bordi, & Bohmaker, 1989). This behavioral deficit

is apparently caused by an EEDQ-induced reduction in D_2 receptors, and the resulting inability of the agonist to bind to a sufficient number of receptors. After approximately four days (depending on the behavior), the agonist is once again able to induce behavioral effects, presumably because D_2 receptor repopulation is sufficient to mediate behavior. A qualitatively different effect is observed in adult rats after treatment with a D_1 agonist, as EEDQ does not block behaviors induced by SKF 38393 (Arnt et al., 1988; Rosengarten, Schweitzer, & Friedhoff, 1989; Yokoo, Goldstein, & Meller, 1988). This D_1/D_2 dichotomy is not due to differential selectivity of EEDQ, because this irreversible antagonist inactivates approximately the same percentage of D_1 and D_2 receptors in adult rats (Arnt et al., 1988; Crawford, McDougall, Rowlett, & Bardo, 1992; Crawford, Rowlett, McDougall, & Bardo, 1994; Hamblin & Creese, 1983; Saller, Kreamer, Adamovage, & Salama, 1989).

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 (McDougall et al., 1992a, 1993; Mestlin & McDougall, 1993). More specifically, in the preweanling rat, treatment with either moderate (7.5 mg/kg) or high (15.0 mg/kg) doses of EEDQ does not diminish behaviors induced by SKF 38393 (a D_1 agonist), quinpirole (a D_2 agonist), or NPA (a nonselective DA agonist). EEDQ's inability to effect behavior is not due to a lack of drug efficacy, because EEDQ inactivates a substantial percentage (approximately 63-69%) of D_1 and D_2 receptors in the 17-day-old rat (Crawford et al., 1992, 1994). Therefore, when considered together, these results are consistent with the idea that the preweanling rat has large reserves of D_1 and D_2 receptors--reserves which are sufficient to compensate for the EEDQ-induced receptor loss.

In order to determine whether preweanling rats actually have functional reserves of D_1 and D_2 receptors, we trained 17-day-old rats on an appetitive approach task and then injected them with EEDQ or its vehicle. (The appetitive approach task was used because this behavior is very sensitive to DA receptor blockade [McDougall, Crawford, & Nonneman, 1992b; McDougall, Nonneman, & Crawford, 1991]). One day later, pups were given either the D_1 antagonist SCH 23390, the D_2 antagonist sulpiride, or saline, 30 minutes prior to reinforcement or extinction testing. If the receptor reserve hypothesis is correct, SCH 23390 and sulpiride should only moderately diminish the extinction and reinforced responding of the non-EEDQ-treated rat pups. In contrast, the same DA antagonists should more severely disrupt the extinction and reinforced responding of the EEDQ-pretreated rat pups, because the reserve of DA receptors was already inactivated by EEDQ.

Method

Subjects and rearing procedures

Subjects were 96 male and female rats of Sprague-Dawley descent (Harlan Sprague Dawley, Inc., Indianapolis, IN) tested when 16 and 17 days of age. Litters were culled to a maximum of 10 pups or a minimum of 8 pups at three days of age. Rat pups were kept with the dam until initial isolation 16 hours before testing. Assignment of subjects to groups was random according to gender and within each litter. The colony room was maintained at 23-25^0 C and kept under a 14:10-hour light-dark cycle. Behavioral testing was conducted during the light phase of the cycle.

Apparatus

The testing apparatus was a straight alley (40 X 8 X 15 cm) with start and goal boxes (15 X 15 X 15 cm) located at either end. The alley and goal box were painted black and the start box was painted gray. Clear Plexiglas basket cages (45 X 21 X 24 cm) that contained hardwood chipped bedding were used as isolation cages and intertrial interval (ITI) chambers. The isolation cages, ITI chambers, and straight alley were located in a separate experimental room. Both the isolation

cages and the ITI chambers were placed on heating pads so that the rat pups could be maintained at 33^0 C, which is approximate thermoneutrality for pups between 10 and 20 days of age (Conklin & Heggeness, 1971).

Procedure

Approximately 16 hours before testing, rat pups were removed from their mother and placed in an isolation cage without food or water being available. After this 16 hour isolation period, the rat pup was placed in the goal box of the straight alley and allowed 15 seconds of nipple attachment to an anesthetized lactating dam. Anesthetization and blockade of milk production were produced by injections of L. A. Thesia (chloral hydrate [60 mg/ml] and sodium pentobarbital [30 mg/ml]) starting 20 minutes before testing. After the initial 15 seconds of nipple attachment, the rat pup was placed in the start box for the beginning of acquisition training. If the rat pup did not traverse the start box and alley after 60 seconds, then it was gently forced down the alley to the goal box. In either case, a 15 second nipple attachment reward was provided and followed by a 15 second placement in the ITI chamber. Acquisition of the approach response consisted of two, eight trial acquisition sessions that were separated by a 5 minutes placement in the ITI chamber.

The rat pups were returned to their home cages for 4 hours and then injected intraperitoneally (ip) with either EEDQ (7.5 mg/kg) or its vehicle. (EEDQ was dissolved in 95% ethanol:distilled water [1:4] and was given at a volume of 5 ml/kg.) After an additional 18 hour of isolation, the 17-day-old rats were injected ip (5 ml/kg) with SCH 23390 (0.5 mg/kg), sulpiride (50 mg/kg), or saline 30 minutes before a final testing session. (Sulpiride and SCH 23390 were dissolved in saline, with the former drug requiring a small volume of glacial acetic acid.) The final testing session consisted of four acquisition trials followed by either 28 reinforcement trials (responding resulted in 15 second confinement with the dam) or 28 extinction trials (responding resulted in 15 second confinement in the empty goal box). During the testing session, the rat pup was not forced down the alley for nonresponding; rather, after 60 seconds it was given a 15 second placement in the ITI chamber.

Analyses of variance (ANOVAs) with repeated measures were used for statistical analysis of mean latencies to traverse the maze. The ANOVAs were performed across blocks of four trials. Significant two-and three-way interactions were further analyzed using lower order ANOVAs and Tukey tests ($p < .05$).

Results

Extinction responding

Mean latencies to traverse the maze during the single extinction session are presented in Figure 1. Across the initial block of four trials, rat pups receiving both EEDQ and sulpiride had significantly longer response latencies than all other group, Pre X Post interaction, F (2, 42) = 3.96, $p < .05$, and Tukey tests ($p < .05$). In addition, the EEDQ/SCH 23390 groups showed enhanced latencies relative to the VEHICLE/SALINE and VEHICLE/SCH 23390 controls, Pre X Post interaction.

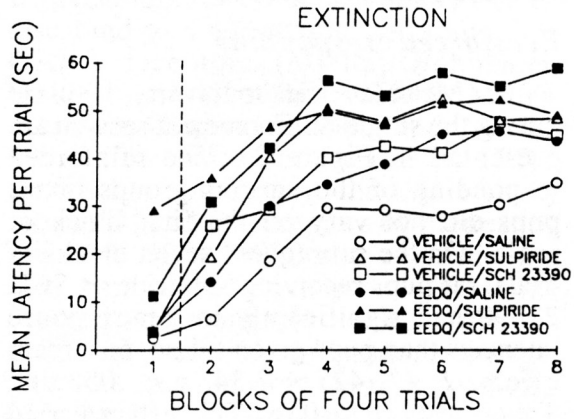

Figure 1. Mean response latencies per trial across blocks of four trials for 17-day-old rat pups injected with EEDQ (7.5 mg/kg) or its vehicle 18 hr prior to the extinction session. The EEDQ and vehicle groups were further subdivided with rat pups being injected with either saline, sulpiride (50 mg/kg), or SCH 23390 (0.5 mg/kg) 30 min prior to extinction testing.

Overall, when collapsed across the remaining seven blocks of trials, rat pups receiving EEDQ had longer response latencies than pups receiving vehicle, pre main effect, F (1, 42) = 6.90, $p < .05$; and rat pups injected with sulpiride or SCH 23390 had longer latencies than those pups given saline, post main effect, F (2, 42) = 8.52, $p < .05$, and Tukey tests ($p < .05$). More specifically, on Blocks 3-8, rat pups receiving both EEDQ and SCH 23390 had longer response latencies than pups in the VEHICLE/SCH 23390 group, Pre X Post X Block interaction, F (12, 252) = 3.11, $p < .05$, and Tukey tests ($p<.05$). Rat pups in the EEDQ/SULPIRIDE group also had longer response latencies than pups in the VEHICLE/SULPIRIDE group, but only on the second block of the extinction session, Pre X Post X Block interaction. Interestingly, rats receiving EEDQ alone (i.e. the EEDQ/SALINE group) had longer latencies than rats in the VEHICLE/SALINE group. The differences between these two groups were significant on Blocks 3, 6, 7, and 8, Pre X Post X Block interaction. Importantly, rat pups in the EEDQ/SALINE group had significantly shorter response latencies than pups from the EEDQ/SULPIRIDE and EEDQ/SCH 23390 groups.

Reinforced responding

Mean latencies to traverse the maze during the single reinforcement session are presented in Figure 2. The reinforced responding of the various groups of rat pups did not vary on the first block of trials. On the subsequent seven blocks of trials, rat pups receiving sulpiride or SCH 23390 had significantly longer response latencies than pups given saline, post main effect, F (2, 42) = 7.34, $p < .05$, and Tukey tests ($p < .05$). This effect varied across blocks, as the differences between the saline-treated rat pups and the SCH 23390-and sulpiride-treated pups were only apparent on Blocks 4-8, Post X Block interaction, F (12, 252) = 2.18, $p < .05$, and Tukey tests ($p < .05$). None of the interactions involving EEDQ and vehicle as a variable were significant.

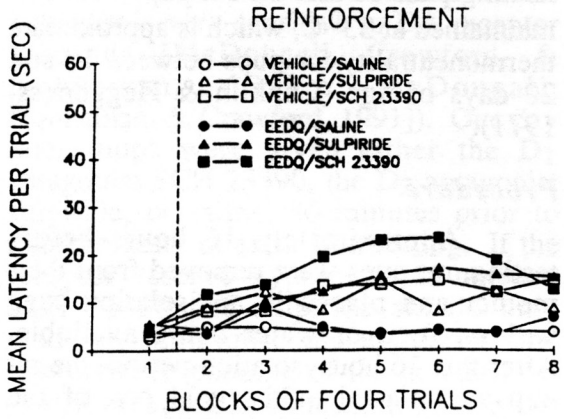

Figure 2. Mean response latencies per trial across blocks of four trials for 17-day-old rat pups injected with EEDQ (7.5 mg/kg) or its vehicle 18 hr prior to the reinforcement session. The EEDQ and vehicle groups were further subdivided with rat pups being injected with either saline, sulpiride (50 mg/kg), or SCH 23390 (0.5 mg/kg) 30 min prior to reinforcement testing.

General Discussion

Previous studies have shown that EEDQ does not block the D_1 and D_2 mediated behaviors of preweanling rats (McDougall et al., 1992a, 1993; Mestlin & McDougall, 1993). Consistent with this, in the present study, EEDQ did not affect the reinforced responding of 17-day-old rats (see Figure 2). In contrast, EEDQ did increase the response latencies of rat pups tested during extinction (see Figure 1). Importantly, rat pups treated with both SCH 23390 and EEDQ had longer extinction latencies than pups given either EEDQ or SCH 23390 alone. Sulpiride also affected extinction responding, however EEDQ did not potentiate sulpiride's effects.

In general, the present results suggest that the D_1 receptors mediating extinction responding do not have an appreciable receptor reserve. More specifically, an EEDQ-induced reduction of DA receptors was sufficient to increase the extinction latencies of the rat pups. This indicates that a reserve of D_1 receptors was not available to replace those lost to EEDQ. However, it is important to realize that SCH 23390 did potentiate EEDQ's actions,

which was expected since both drugs effect the D_1 receptor. In contrast, there appears to be a D_1 receptor reserve for reinforced responding, since EEDQ alone did not affect this behavior. It is unclear why the extinction and reinforced responding of preweanling rats was differentially affected by EEDQ; however, it is possible that extinction and reinforced responding are mediated by different populations of DA receptors. Alternatively, it is also possible that extinction responding is simply a more sensitive measure of performance and was better able to detect drug-induced effects.

Unexpectedly, there was no evidence for a D_2 receptor reserve when either extinction or reinforced responding was assessed. For example, sulpiride disrupted the extinction and reinforced responding of the rat pups, but at no time did sulpiride and EEDQ combine to maximally disrupt responding. This is entirely consistent with other studies showing that EEDQ is unable to block the D_2 mediated locomotor activity, stereotyped sniffing, and rearing of preweanling rats (McDougall et al., 1992a, 1993; Mestlin & McDougall, 1993). Of course, similar studies using adult rats have shown that EEDQ will eliminate D_2 mediated behaviors (Arnt et al., 1988; Cameron & Crocker, 1989; Giorgi & Biggio, 1990; Hamblin & Creese, 1983; McDougall et al., 1992a; Meller et al., 1989). The reason for this age-dependent difference is uncertain, but it is apparently not due to a lack of drug efficacy or quick repopulation rates. More specifically, the D_2 receptors of preweanling rats are reduced by at least 60% when assayed 24 hours after EEDQ treatment. Importantly, this level of depletion is sufficient to significantly disrupt the behaviors of adult rats (Crawford et al., 1992, 1994; McDougall et al., 1992a). More generally, it remains uncertain why an irreversible DA receptor antagonist (EEDQ) did not potentiate the effects of a reversible D_2 receptor antagonist (sulpiride). Previously we have shown that two reversible antagonists will combine to maximally disrupt the behaviors of preweanling rats (McDougall et al., 1991, 1992b). Likewise, Wanibuchi and Usuda (1990) found that YM-09151 (a reversible D_2 antagonist) would potentiate SCH 23390-induced catalepsy in the adult rat. Thus, it is unclear why the present results were obtained. One possibility is that EEDQ and sulpiride were affecting different receptor subpopulations, perhaps within a particular brain region or even within a given population of neurons. The same explanation may also account for why EEDQ did not affect D_1 mediated locomotor activity or grooming (McDougall et al., 1993), whereas EEDQ did affect D_1 mediated extinction responding (see Figure 1). More specifically, those brain areas (e.g. the striatum) mediating locomotor activity are probably different from those brain areas (e.g. the nucleus accumbens and other limbic structures) mediating learned behaviors (Bordi, Carr, & Meller, 1989; Cameron & Crocker, 1989). Not surprisingly, the availability of reserve receptors varies according to the receptor population being assessed (Meller, Enz, & Goldstein, 1988; Yokoo et al., 1988), so EEDQ's differential effects may be due to the characteristics of those receptor populations mediating a particular behavior.

A number of studies have shown that EEDQ preferentially binds to D_1 and D_2 receptors, but will, to a lesser extent, also bind to a-adrenergic, serotonin, and GABA receptors (Meller, Bohmaker, Goldstein, & Friedhoff, 1985; Miller, Lumpkin, Galpern, Greenblatt, & Shader, 1991). To control for the lack of EEDQ specificity, some researchers selectively protect D_1 and D_2 receptors by pretreating rats with SCH 23390 and sulpiride and then comparing those groups to saline pretreated controls (i.e., groups specifically depleted of nondopaminergic receptors are compared to groups depleted of both nondopaminergic and dopaminergic receptors) (Cameron & Crocker, 1989; Hamblin & Creese, 1983; McDougall et al., 1992a). In the present study, DA receptors were not selectively protected because we have previously shown that rat pups given protection pretreatment and EEDQ respond similarly to pups given vehicle alone (McDougall et

al., 1992a, 1993).

In summary, EEDQ alone effected the extinction responding of preweanling rats, indicating the lack of a D_1 receptor reserve for this behavior. In contrast, EEDQ did not affect the D_2 mediated extinction or reinforced responding of the 17-day-olds. However, the latter result does not necessarily indicate the presence of a D_2 receptor reserve, because sulpiride was unable to combine with EEDQ to maximally disrupt behavior. Rather, these results may also reflect an age-dependent difference in the characteristics of DA receptor functioning.

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