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DOPAMINE RECEPTOR INACTIVATION IN THE CAUDATE-PUTAMEN DIFFERENTIALLY AFFECTS THE BEHAVIOR OF PREWEANLING AND ADULT RATS

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Abstract—The irreversible receptor antagonist *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) has been used to study the ontogeny of dopamine (DA) receptor functioning in young and adult rats. Most notably, systemic administration of EEDQ blocks the DA agonist-induced behaviors of adult rats, while leaving the behavior of preweanling rats unaffected. The purpose of the present study was to: (a) determine whether the age-dependent actions of EEDQ involve receptors located in the dorsal caudate-putamen (CPu) and (b) confirm that EEDQ's behavioral effects result from the inactivation of DA receptors rather than some other receptor type. In Experiment 1, EEDQ or DMSO was bilaterally infused into the CPu on PD 17 or PD 84. After 24 h, rats were given bilateral microinjections of the full DA agonist R(−)-propylnorapomorphine (NPA) or vehicle into the dorsal CPu and behavior was assessed for 40 min. In Experiment 2, preweanling rats were treated as just described, except that DA receptors were protected from EEDQ-induced alkylation by administering systemic injections of D1 (SCH23390) and D2 (sulpiride) receptor antagonists. As predicted, microinjecting EEDQ into the dorsal CPu attenuated the NPA-induced locomotor activity and stereotypy of adult rats. In contrast, rats given bilateral EEDQ infusions on PD 17 exhibited a potentiated locomotor response when treated with NPA. Experiment 2 showed that DA receptor inactivation was responsible for NPA's actions. A likely explanation for these results is that EEDQ inactivates a sizable percentage of DA receptors on PD 17, but leaves the remaining receptors in a supersensitive state. This receptor supersensitivity, which probably involves alterations in G protein coupling, could account for NPA-induced locomotor potentiation. It is likely that adult rats to not show a similar EEDQ-induced change in receptor dynamics or DA receptor inactivation was more complete in older animals and effectively eliminated the expression of DA agonist-induced behaviors.

Key words: caudate-putamen, R(−)-propylnorapomorphine (NPA), *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), behavior, ontogeny.

INTRODUCTION

There is a growing understanding that dopamine (DA) agonists and antagonists affect the developing and mature brain differently (Andersen, 2005). Support for this idea is abundant and includes data showing that dopaminergic drugs frequently induce quantitatively different behavioral effects across ontogeny (for a review, see Spear, 1979). In some cases, the potency of DA-acting drugs changes linearly with age, but often the relationship between age and drug responsiveness is nonmonotonic (Spear and Brick, 1979; Campbell and Baldessarini, 1981). For example, Lepekhina and Tsitsurina (2007) reported that apomorphine-induced stereotyped sniffing peaked at postnatal day (PD) 14 and subsequently declined, whereas stereotyped licking increased progressively until 12 months of age. DA agonists occasionally induce qualitatively different behavioral effects depending on age, however these ontogenetic differences usually involve the emergence of age-specific responses (Moody and Spear, 1992).

Most studies examining ontogenetic differences in drug responsiveness rely on systemic administration procedures, however microinjection studies have provided important information about age-dependent changes in the localization of DA-mediated brain function. In most cases, infusing nonselective and selective DA agonists into the caudate-putamen (CPu) affects preweanling and adult rats in a generally similar manner. For example, microinjecting a selective D1 agonist into the CPu increases the locomotor activity of preweanling and adult rats (Kreipke and Walker, 2004; Krolewski et al., 2005; Charntikov et al., 2011). Both age groups also show an intensification of stereotypy when a D1 or D2 agonist is infused into the CPu (Bordi and Meller, 1989; Canales and Iversen, 1998; Waszczak et al., 2002; Kreipke and Walker, 2004;

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Abbreviations: ANOVA, analysis of variance; CPu, caudate-putamen; DA, dopamine; DMSO, dimethyl sulfoxide; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; IP, intraperitoneal; NPA, R-propylnorapomorphine; PD, postnatal day.

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Krolewski et al., 2005; Charntikov et al., 2011). Some interesting age-dependent differences are apparent, however, because selectively stimulating D2 receptors in the dorsal CPu trigger a pronounced locomotor response in preweanling rats (Charntikov et al., 2011), while causing either a reduction (Bordi and Meller, 1989; Canales and Iversen, 1998) or a subtle, biphasic increase in the locomotor activity of adult rats (Van Hartesveldt et al., 1992). Perhaps most importantly, co-activation of D1 and D2 receptors in the CPu of adult rats causes both an intensification of stereotypy and a pronounced reduction in locomotor activity (Bordi and Meller, 1989; Bordi et al., 1989; Waszczak et al., 2002); whereas, preweanling rats continue to exhibit a robust locomotor response and show only a modest intensification of stereotyped behaviors (Charntikov et al., 2008, 2011).

N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an alkylating agent that inactivates DA receptors, has been used to study both the time-course of receptor recovery and the behavioral relevance of D1 and D2 receptor stimulation (Hamblin and Creese, 1983; Arnt et al., 1988; Cameron and Crocker, 1988; Meller et al., 1989). When assessed 24 h after systemic EEDQ (7.5 mg/kg, IP) administration, adult and preweanling rats show an approximately 61–86% reduction in D1 and D2 receptor densities in the CPu, with the percent decline being somewhat greater in adult rats (Crawford et al., 1992; Gnanalingham et al., 1994). Despite a substantial decline in DA receptors, systemically administered EEDQ affects the behaviors of adult and preweanling rats differently. In adult rats, the behavior-inducing properties of DA agonists are fully attenuated when measured 24 h after EEDQ treatment (Arnt et al., 1988; Cameron and Crocker, 1988; Meller et al., 1989; McDougall et al., 1992); whereas, DA receptor inactivation leaves the behavior of preweanling rats unaffected. More specifically, preweanling rats show normal levels of NPA-, quinpirole-, and SKF38393-induced locomotor activity, grooming, and head-down sniffing when tested 24 h after systemic administration of EEDQ (McDougall et al., 1992, 1993; Mestlin and McDougall, 1993). The reason for this age-dependent difference has not been determined.

In adult rats, EEDQ has been microinjected into various brain regions in order to examine whether D1 and/or D2 receptor stimulation contributes to the mediation of muscle tone, various unlearned behaviors, ipsilateral circling, and fixed-interval operant responding (Hamblin and Creese, 1983; Bordi et al., 1989; Cameron and Crocker, 1989; Giorgi and Biggio, 1990a,b; Lee et al., 1995; Neisewander et al., 1995; Hemsley et al., 2002; Cory-Slechta et al., 2002). For example, Neisewander et al. (1995) have shown that infusing EEDQ into the lateral CPu attenuates the SKF38393-induced grooming and oral movements of adult rats. To determine the receptor type responsible for mediating a particular behavior, DA receptors can be selectively protected from EEDQ-induced inactivation by pretreating rats with D1 (e.g., SCH23390) or D2 (e.g., sulpiride or raclopride) reversible antagonists (Meller et al., 1985). Using this technique, Cameron and Crocker (1989) reported that D2 receptors in the ventrolateral CPu are necessary for apomorphine-induced head-down sniffing. Likewise, quinpirole stimulates ipsilateral circling in EEDQ-treated adult rats, but not when D2 receptors were protected by a pretreatment injection of raclopride (Giorgi and Biggio, 1990a). In sum, administering EEDQ either systemically or via intracerebral infusion produces the same general pattern of effects in adult rats; namely, nonselective and selective DA agonists have minimal behavioral impact if DA receptors are inactivated. It is not known whether intrastriatal infusions of EEDQ affect the behavior of preweanling rats in an adult-like manner.

When considered together, previous studies typically report that reversible DA agonists and antagonists either affect preweanling and adult rats in a similar manner or they induce quantitative differences in behavioral responsiveness (i.e., a particular drug causes more intense behavioral effects at one age vs. another). One goal of the present study was to determine whether microinjecting the irreversible DA receptor antagonist EEDQ into the dorsal CPu would produce qualitatively different behavioral effects in preweanling and adult rats. Qualitative differences are of particular interest because they may result from significant ontogenetic changes in the neural mechanisms underlying behavior. To examine this issue, EEDQ or vehicle were bilaterally infused into the dorsal CPu on PD 17 or PD 84 and the full DA agonist R(−)-propylnorapomorphine hydrochloride (NPA) (0–20 μg) was microinjected into the same structure 24 h later. To ensure that NPA (0.5 μl per side) was not stimulating DA receptors outside the area of EEDQ-induced alkylation, a relatively greater volume of EEDQ (0.75 μl per side) was infused into the dorsal CPu of both age groups. A second purpose of this study was to determine whether EEDQ's behavioral effects result from the inactivation of DA receptors or some other receptor type. To accomplish this goal, separate groups of preweanling rats were pretreated with SCH23390 and sulpiride in order to protect D1 and D2 receptors, respectively, from EEDQ-induced alkylation. Homogenate ligand-binding assays were used to measure D1 and D2 receptor concentrations in the CPu, while autoradiography was used to examine the dispersal pattern of EEDQ after microinjection.

EXPERIMENTAL PROCEDURES

Animals

A total of 273 male and female Sprague–Dawley rats were used. Adult male rats ($N = 65$) were purchased from Charles River (Hollister, CA, USA) and began testing on PD 83; whereas, 208 male and female rats were born and bred at California State University, San Bernardino (CSUSB) and underwent experimental procedures beginning on PD 16. Litters were culled to 10 pups on PD 3. Rat pups were kept with the dam and littermates in large polycarbonate maternity cages ($56 \times 34 \times 22$ cm) with wire lids, while adult rats were group housed in maternity cages until time of surgery. Food and