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Short communication

Dopamine D_3 receptor density elevation in aged Fischer-344 × Brown-Norway (F1) rats

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Abstract

The density of dopamine D_3 receptors was determined in young (4-month-old) and aged (37-month-old) Fischer-344 × Brown-Norway (F1) male rats using the putative D_3 receptor-preferring agonist, [^{3}H](+)-7-hydroxy-2-(*N,N*-di-*n*-propylamino)tetralin ([^{3}H](+)-7-OH-DPAT). In the presence of the non-hydrolyzable GTP analog, 5'-guanylylimidodiphosphate (Gpp(NH)p), the density of dopamine D_3 receptors in the striatum and nucleus accumbens was significantly increased (29–102%, respectively) in aged Fischer-344 × Brown-Norway (F1) rats compared to young adults. These findings suggest that dopaminergic activity in aged rats is compromised by increased D_3 receptor density, resulting in altered striatal/nucleus accumbens function via presynaptic or postsynaptic modifications.

Keywords: Striatum; Nucleus accumbens; Basal forebrain

1. Introduction

The aging process produces widespread alterations in dopaminergic receptors which result in profound cognitive and motor changes. In general, D_2 dopamine receptor density is decreased in most regions in an age-related fashion (Sakata et al., 1992). The mechanism for this dopamine receptor loss is unclear, but may involve receptor loss due to cell death and/or decreased protein synthesis capabilities. Although Sakata et al. (1992) reported no change in D_2 receptor mRNA expression, it was suggested that an alteration in post-transcriptional processing occurred during the aging process. Other investigators have reported that expression of D_2 receptor mRNA is reduced in aged animals (Valerio et al., 1994). Therefore, reductions in D_2 receptor density might be due to decreased production of receptor protein or the formation of non-functional receptor variants following errors in post-transcriptional processing.

Few studies have addressed age-related changes in the dopamine D_3 receptor, a member of the novel 'D_2-like' receptor group. The D_3 receptor has been cloned and characterized (Sokoloff et al., 1990; Bouthenet et al., 1991). The distribution of the D_3 receptor protein was initially reported to be restricted primarily to mesolimbic areas (Sokoloff et al., 1990; Bouthenet et al., 1991), but was subsequently demonstrated to constitute a small number of 'D_2-like' receptor binding sites in the striatum (Wallace and Booze, 1995). Valerio et al. (1994) reported that the expression of D_3 receptor mRNA in aged rats was reduced in the olfactory tubercle, a region with a dense D_3 receptor population, but was unchanged in the striatum. Schmauss et al. (1993) demonstrated that mRNA expression for the D_3 receptor is reduced in parietal and motor cortices of schizophrenic and Alzheimer's disease (mean age > 70 years old) patients. The D_3 receptor has also been implicated as a potential locus for the treatment of neuropsychiatric disorders (Sokoloff et al., 1990), thus the D_3 receptor is a candidate for therapeutic treatment of neuropsychiatric disorders associated with the aging process. Additional studies investigating the function of the D_3 receptor during the aging process are critical for greater understanding of deficits associated with the aging process and disease.

2. Materials and methods

2.1. Animals

Adult (4-month-old) and aged (37-month-old) male Fischer-344 × Brown-Norway (F1) hybrid rats were main-

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tained on a 12-h photoperiod with food and water available ad libitum. This hybrid strain of rat is currently the model of choice for aging studies due to their increased longevity and lack of age-related pathology (Sprott, 1991). Animals were killed by rapid decapitation, brain tissue was removed and subsequently frozen in liquid nitrogen. Tissues (striatum and nucleus accumbens, including olfactory tubercle) were stored at −80°C until analysis.

2.2. Tissue preparation and receptor binding

Striatal and nucleus accumbens (containing olfactory tubercles) tissue homogenates were prepared as previously described (Wallace and Booze, 1995). In brief, binding to the dopamine D_3 receptor was performed by addition of tissue homogenate (900 μl; 250–300 v/w) to 50 μl [^{3}H](+)-7-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin ([^{3}H](+)-7-OH-DPAT; 10 nM; Amersham, Arlington, IL, USA; 155 Ci/mmol) and 50 μl of buffer (total binding) or 1 μM dopamine (non-specific binding). The density of D_3 sites labeled by 10 nM [^{3}H](+)-7-OH-DPAT would represent > 90% of the total density of D_3 receptors based on fractional occupancy ($[^*L]/K_d + [^*L]$). To prevent the labeling of high-affinity D_2 sites by [^{3}H](+)-7-OH-DPAT, 10 μM Gpp(NH)p was included in the assay buffer. Other investigators have reported that both 0.2 mM (Gonzalez and Sibley, 1995) and 0.1 mM (Levesque et al., 1992) Gpp(NH)p effectively abolish [^{3}H]7-OH-DPAT binding to high-affinity D_2 receptors. We have determined that 10 mM Gpp(NH)p and 0.3 mM GTP also prevent labeling of high-affinity D_2 receptors (data not shown). Binding was performed in the presence of 0.5 μM carbetapentane to prevent [^{3}H](+)-7-OH-DPAT binding to σ_1 receptors (Wallace and Booze, 1995). Incubations were carried out to equilibrium (90 min) at room temperature (21 ± 2°C) and terminated by rapid filtration onto GF/B filters (presoaked for 2 h in 0.3% polyethyleneimine) under reduced pressure by a Brandel Tissue Harvester. Radioligand bound was determined by scintillation spectrophotometry (efficiency of 45%).

2.3. Chemicals and statistics

Carbetapentane and (±)-7-OH-DPAT were obtained from Research Biochemicals (Natick, MA, USA). All other chemicals were purchased from either Sigma Chemicals (St. Louis, MO, USA) or Fisher Scientific (Springfield, NJ, USA). Data were analyzed as fmol/mg protein using two-way (age × region) ANOVA.

3. Results

In 4-month-old rats, the density of D_3 receptors labeled by 10 nM [^{3}H](+)-7-OH-DPAT in the presence of 10 μM Gpp(NH)p exhibited significant [$F(1,20) = 8.37$; $P < 0.01$]

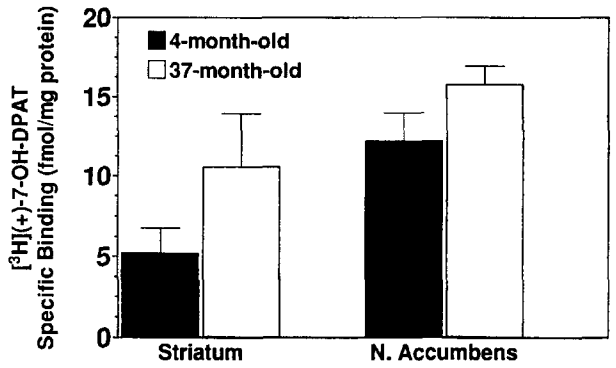

Fig. 1. The density of D_3 receptors ($n = 4$) was determined with 10 nM [^{3}H](+)-7-OH-DPAT in the presence of 10 μM Gpp(NH)p to prevent the labeling of high-affinity D_2 receptors. Binding was initiated by the addition of radioligand and allowed to proceed at room temperature until equilibrium (90 min). D_3 receptor density was significantly upregulated in 37-month-old rats compared to 4-month-old rats in both the striatum and nucleus accumbens. The distribution of D_3 receptors demonstrated significant effects of region as well as age, but no interaction between age and region was observed.

regional variation and was in excellent agreement with the values reported by Levesque et al. (1992). In 4-month-old animals, the density of D_3 receptors in the nucleus accumbens was 2.33-fold greater than the D_3 receptor density in the striatum (Fig. 1). In 37-month-old animals, this difference was reduced to 1.49-fold greater in the nucleus accumbens compared to the striatum. There was a significant effect of age since the density of D_3 receptors was elevated [$F(1,20) = 4.48$; $P < 0.05$] in both the striatum (102%) and nucleus accumbens (29%) in 37-month-old rats compared to 4-month-old rats (Fig. 1). The density of dopamine D_2 receptors was unchanged in both the striatum and nucleus accumbens from aged rats (data not shown).

4. Discussion

In the present study, the observation of significant upregulation of D_3 receptors in both the striatum and nucleus accumbens in 37-month-old rats is a striking contrast to the age-related downregulation observed in D_2 receptor density (Sakata et al., 1992). The D_3 dopamine receptor exhibits the characteristics of a presynaptic autoreceptor in the striatum (Meller et al., 1993) and nucleus accumbens (Gilbert et al., 1995). These findings suggest that the D_3 receptor may have an important function both in motor control (striatum) and limbic (nucleus accumbens) response. Alterations in D_3 receptor density or function might have an important role in the motor and cognitive decline associated with the aging process.

Preliminary studies suggest the D_3 receptor mRNA expression in the cortex may be reduced in disorders associated with the aging process (Schmauss et al., 1993), although D_3 receptor mRNA expression in the striatum is

unchanged (Valerio et al., 1994). Therefore, elevated D_3 receptor binding in the striatum and nucleus would represent an increased functional D_3 receptor in these regions. Our findings suggest a unique function for the D_3 receptor during the aging process, perhaps serving to compensate for the loss of D_2 receptors in the striatum. Collectively, these data support a hypothesis for age-related alterations in dopamine D_3 receptors in both the striatum and nucleus accumbens. Development of therapeutic compounds which selectively modulate the D_3 receptor may result in uniquely efficacious treatments for the cognitive and motor dysfunction commonly observed during the aging process.

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