Short communication

Alterations in expression of messenger RNAs encoding two isoforms of glutamic acid decarboxylase in the globus pallidus and entopeduncular nucleus in animals symptomatic for and recovered from experimental Parkinsonism

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Abstract

Glutamic acid decarboxylase (GAD65, GAD67) mRNA expression was measured in the globus pallidus (GP) and entopeduncular nucleus (ENTO) of normal, and MPTP-lesioned cats symptomatic for and recovered from MPTP-induced Parkinsonism. In the ENTO of symptomatic cats, GAD65 and GAD67 mRNA expression were both significantly increased, while only GAD67 gene expression was increased in the GP. Levels of gene expression for both isoforms were normal in the GP and ENTO of spontaneously recovered animals. Increased expression of GAD65/67 mRNA in the ENTO corresponded with expression of Parkinsonian signs, suggesting a contribution of both isoforms to ENTO functioning and perhaps a greater contribution of GAD67 expression to GP functioning.

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The current model of basal ganglia functioning suggests that Parkinsonian motor dysfunctions can be attributed to changes in the activity of striatopallidal and striatonigral GABAergic efferents. The model implies that loss of striatal dopamine (DA) removes tonic inhibitory influences on striatopallidal neurons projecting to the external globus pallidus (GPe) and removed a tonic excitatory influence on neurons projecting to the internal globus pallidus (GPi) and substantia nigra pars reticulata (SNr). These alterations lead to inhibition of the GPe and hyperactivation of the GPi and SNr [8,1]. The hyperactivity of the GPi/SNr and the resultant enhanced inhibition of thalamocortical neurons is believed to underlie the expression of the major motor deficits associated with Parkinson’s disease (PD) [1].

Glutamic acid decarboxylase (GAD), the synthetic enzyme required for GABA synthesis, exists as two distinct isoforms, GAD67 and GAD65 [6]. Both isoforms are expressed by striatal efferents as well as GPi (entopeduncular nucleus (ENTO) in rodents and carnivores), SNr and GPe neurons [15,3], and differential expression of these two isoforms may reflect different functional states of GABAergic neurons [7]. Several studies have examined GAD mRNA expression in the pallidum in animal models of PD. GAD67 mRNA levels were increased in the GP [12,20] but not the ENTO [20] of unilateral 6-OHDA lesioned rats. In MPTP-lesioned primates, GAD67 mRNA expression was increased in both pallidal segments [21,9,11]. In unilateral 6-OHDA lesioned rats, GAD65 mRNA expression did not change in the GP or ENTO [20], while in bilateral MPTP-lesioned primates, GAD65 mRNA levels were increased in the GPi but not in the GPe [17]. These results suggest that both isoforms of the GAD enzyme play important roles in the functioning of the basal...
ganglia. However, the functional significance of changes in pallidal GAD mRNA expression after a nigrostriatal lesion remains unclear since studies have used different types of lesions (i.e. unilateral vs. bilateral), and data correlating changes in GAD mRNA expression with alterations in motor abilities associated with experimental Parkinsonism are lacking.

The feline MPTP model of PD has been well characterized by our laboratory. MPTP-lesioned cats exhibit a Parkinsonian syndrome characterized by rigidity, akinesia and reduced response to sensory stimulation [18]. However, MPTP-lesioned cats spontaneously recover gross motor functioning by 6 weeks after MPTP administration despite a continued loss of substantia nigra neurons [19], making these animals ideal for relating putative changes in pallidal GAD mRNA expression to the expression of Parkinsonian motor signs. This study examined changes in GAD65 and GAD67 mRNA expression in GP and ENTO neurons by in-situ hybridization histochemistry in normal, symptomatic and spontaneously recovered MPTP-lesioned cats.

Twelve adult male or female cats were given 7 to 10 daily injections (5 to 7.5 mg/kg, im) of MPTP–HCl dissolved in physiological saline to produce a severe Parkinsonian syndrome. MPTP injections were stopped when the animals met behavioral criteria for severe Parkinsonism according to a previously described symptom rating scale [18]. Six animals were euthanized 7–10 days after the last MPTP injection and comprised the symptomatic group. Six MPTP-lesioned animals were euthanized 6 weeks after the last MPTP injection when they had recovered gross motor functioning. Animals were considered to be recovered when their gross motor behavior was significantly improved from the symptomatic condition and total rating scores were not significantly different from normal. All animals were euthanized by sodium pentobarbital overdose. Brains were removed fresh and flash frozen in powdered dry ice. Striatal tissue blocks were cryostat sectioned at 20 μm thaw mounted onto plus slides (Fisher Scientific, Inc.) and stored at −70°C until used. Coronal sections corresponding approximately to stereotaxic coordinates AP 10.0–12.0 [2] representing the mid to caudal level of the ENTO in the cat were processed for in-situ hybridization histochemistry. This region of the ENTO was chosen for study since previous studies in rats [22] had shown caudal ENTO regions to be the motor-related subregions of this nucleus.

GAD67 and GAD65 cRNA probes were transcribed in vitro from cDNA clones encoding for human GAD67 and GAD65 cDNA (generously provided by Allan J. Tobin, UCLA) in the presence of 35S–UTP using a PROMEGA riboprobe kit. Sections were hybridized according to a previously established protocol [20] with minor alterations. Probe concentration was determined such that 1×106 cpm of labeled probe contained in 35 μl hybridization cocktail was applied to each section. Following posthybridization, slides were apposed to Kodak β-max hyperfilm for 1 week to confirm a specific hybridization signal. Slides were then dipped in Kodak NTB3 emulsion and exposed for 7 weeks. Sections were developed in Kodak D-19 developer, fixed with Kodak Rapid Fix and counterstained with cresyl violet.

Microscopic images (40×) were digitized and processed for grain counting using Scion Image for Macintosh v 1.6 and a previously published image analysis/grain counting protocol [14]. Twenty labeled cells per region, per animal were analyzed. The cresyl violet stained area of each cell was measured and the grains overlying that area were counted. Data were recorded as the number of grains per μm² of cell area.

All animals euthanized while symptomatic were similarly Parkinsonian. Animals allowed to recover regained gross motor functioning and their behavior was not significantly different from when they were normal.

Analysis of variance indicated a significant group by region main effect for GAD67 mRNA expression (F(5,30)=15.51; P<0.0001). In symptomatic cats, GAD67 mRNA expression was significantly elevated in both the ENTO (t=34.109; P<0.01) and the GP (t=35.834; P<0.01) compared to normal animals. In recovered animals GAD67 mRNA was significantly reduced compared to symptomatic animals in both the ENTO (t=9.29; P<0.05) and GP (t=27.51; P<0.01) and was not significantly different from normal. GAD67 mRNA expression was not significantly different in the GP versus the ENTO in normal animals (Fig. 1).

Analysis of variance indicated a significant group by region main effect for GAD65 mRNA expression (F(5,18)=16.09; P<0.0001). In the ENTO, there was a significant increase in GAD65 gene expression in symptomatic compared to normal animals (t=22.38; P<0.01), while a slight but statistically non-significant decrease in GAD65 gene expression was observed in the GP of these animals. In recovered animals, the level of GAD65 gene expression in the GP and ENTO was not significantly different from that in normal animals. GAD65 mRNA expression in the GP was not significantly different from expression in the ENTO of normal animals (Fig. 2).

GAD67 mRNA expression was significantly elevated in both the GP and ENTO of symptomatic MPTP-lesioned cats. Increased GAD67 mRNA expression in the ENTO of these animals may reflect hyperactivity of ENTO GABAergic neurons resulting from striatal DA denervation. These results are consistent with those from MPTP-lesioned monkeys [21] in which GAD67 gene expression was significantly increased in both the GPe and GPI of Parkinsonian animals. Given that the current model of basal ganglia functional circuitry predicts that loss of striatal DA leads to hypoactivation of the GPe, these results indicate that GAD67 mRNA expression in the GPe may be regulated by factors other than strictly striatal DAergic depletion. It has previously been suggested that...
Fig. 1. GAD67 mRNA levels expressed as grains per μm² cell area in the entopeduncular nucleus (ENTO) and globus pallidus (GP) of normal (NORM, black bars), MPTP-lesioned symptomatic (SYMP, grey bars) and recovered (REC, white bars) cats. GAD67 mRNA expression is significantly increased in both segments of the pallidum in symptomatic compared to normal cats and is indistinguishable from normal levels in recovered cats. (**) Significant difference from NORM P<0.01. (*) Significant difference from NORM P<0.05. Data are presented as mean±S.E.M.; n=6 per group.

Fig. 2. GAD65 mRNA levels expressed as grains per μm² cell area in the entopeduncular nucleus (ENTO) and globus pallidus (GP) of normal (black bars), MPTP-lesioned symptomatic (grey bars) and recovered (white bars) cats. GAD65 mRNA expression is significantly increased in the ENTO and lower (although not statistically significant) in the GP of symptomatic cats compared to normal animals. GAD65 mRNA expression is indistinguishable from normal levels in recovered cats. (**) Significant difference from NORM P<0.01. Data are presented as mean±S.E.M.; n=4 per group.

GPz GAD67 expression may be influenced by excitatory efferents from the subthalamic nucleus [21,4,13]. Subthalamic fibers are more uniformly distributed throughout the GPe compared to striatal fibers [10], and kainic acid lesions of the subthalamic nucleus prior to a 6-OHDA lesion in rats prevents the DA lesion-induced increase in GAD67 mRNA expression [5].

GAD65 mRNA expression was significantly increased in the ENTO of symptomatic animals, returned to normal levels in recovered animals. GAD65 levels in the GP were somewhat lower (although not statistically significant) in symptomatic animals and were at normal levels in recovered animals. The pattern of pallidal GAD65 mRNA expression in symptomatic parkinsonian cats is consistent with the pattern of pallidal GAD65 mRNA expression reported in 6-OHDA lesioned rats [20] and MPTP-lesioned monkeys [17]. Our results support other findings that suggest that the two GAD isoforms are regulated differentially in the two pallidal segments in response to a DAergic lesion.

In conclusion, the data presented here demonstrate that pallidal expression of both GAD65 and GAD67 mRNA is significantly altered in symptomatic MPTP-lesioned cats and returns to normal levels in recovered cats, indicating that both forms of the enzyme may be important for pallidal functioning. The current model of basal ganglia functioning predicts that in response to the loss of striatal DA, the GP is subject to excess inhibition while the ENTO is disinhibited. Results from the cat MPTP-model follow the predictions of the current model of basal ganglia functioning in that expression of Parkinsonian signs corresponds to increased GAD65/67 mRNA expression in the ENTO and recovery from MPTP-induced Parkinsonism is associated with a ‘normalization’ of GAD mRNA expression in this region. However, our data, as well as that of others [9,11,12,20,21] indicate that expression of GAD67 in the GP in response to a nigrostriatal lesion may not be simply related to changes in striatopallidal neuronal activi-
ty whereas expression of GAD65 may be more sensitive to such changes. This suggests that the functional controls of the indirect striatopallidal circuit are more complex than the current model of basal ganglia functioning describes and further supports the possible functional diversity of the two GAD isoforms. Based on our data and basic and clinical data of others [4,13,16] the model of the organization of the basal ganglia, particularly with regard to the functional regulation of the indirect circuit, may need to be re-considered.

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References