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ORIGINAL INVESTIGATION

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Decreased μ-opioid receptor binding in the globus pallidus of rats treated with chronic haloperidol

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Abstract *Rationale*: Chronic neuroleptic treatment produces a movement disorder in rats characterized by vacuous chewing movements (VCMs). Neuroleptics also produce a variety of changes in opioid neurotransmission in several regions of the basal ganglia. Rats with the VCM syndrome show elevated mRNA for enkephalin in striatopallidal neurons, suggesting a possible role for enkephalin in the pathophysiology of VCMs. *Objective*: This study investigated the role of μ-opioid receptor density in the basal ganglia on the expression of VCMs. *Methods*: Rats were treated with haloperidol for 24 weeks and withdrawn for 9 weeks. Mu (μ) receptors were labeled with [^{3}H]-DAMGO. *Results*: Haloperidol treatment produced a significant reduction in μ-receptor binding in the globus pallidus (P<0.05). There was, however, no relationship between μ-opioid receptor density and VCMs in this or any other region of the basal ganglia. *Conclusion*: These results replicate prior findings of a neuroleptic-induced reduction in [^{3}H]-DAMGO binding in the globus pallidus. The lack of association between VCMs and [^{3}H]-DAMGO binding in the globus pallidus or any other region suggests that prior reports of enkephalinergic mRNA changes in the striatum are not accompanied by compensatory changes in postsynaptic neurons.

Key words Autoradiography · μ-Opioid receptor · [^{3}H]-DAMGO · Haloperidol · VCM · Rat

Introduction

Tardive dyskinesia (TD) remains a significant clinical issue that continues to limit the use of neuroleptic medication (Egan et al. 1997; Casey 1999). The neurobiology of TD has been studied using a rodent model. Chronic administration of neuroleptic agents produces a movement disorder in rats characterized by vacuous chewing movements (VCMs), similar in some respects to the orobuccal dyskinesias seen in TD (Waddington et al. 1990; Egan et al. 1996a, 1996b). In rats with the VCM syndrome, there is a selective elevation in the level of striatal mRNA for enkephalin, suggesting increased activity of the striatopallidal pathway (Egan et al. 1994). Based on one model of basal ganglia function (Gerfen et al. 1991), increased activation of this pathway would be expected to increase both enkephalinergic and γ-aminobutyric acid-type (GABAergic) neurotransmission in the globus pallidus. In this case, complementary alterations in receptor density might also occur. One prior study (Sasaki et al. 1996) found a 20% reduction in μ-opioid receptor binding in the globus pallidus, suggesting that neuroleptics chronically downregulate at least the enkephalinergic component of striatopallidal neurotransmission. However, they found no association between [^{3}H]-DAMGO binding in rats and VCMs. Interpretation of this study is confounded by the limited behavioral testing, the small cohort size, and the lack of Bonferroni correction for testing multiple regions (Sasaki et al. 1996). The purpose of the current study was to assess the role of μ receptors in the VCM syndrome, focusing on the globus pallidus, ventral pallidum, striatum, and nucleus accumbens using a larger cohort of rats well characterized on behavioral measures over a prolonged period of treatment with haloperidol, followed by a 9-week withdrawal period. Because TD is typically exacerbated by neuroleptic withdrawal, we selected a prolonged withdrawal period specifically to look for changes that could underlying tardive movement disorders. We hypothesized that, in addition to the neuroleptic-induced changes in the globus pallidus and ventral pallidum seen by Sasaki et al. (1996), we would also find differences between rats with and without persistent VCMs.

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Methods

Drug treatment and animal ratings

Eighty-seven drug-naive, male Sprague-Dawley rats initially weighing 125–150 g were treated with haloperidol decanoate (n=70) or equivalent volume of vehicle (n=18), according to methods previously described (Egan et al. 1994, 1996a, 1996b). VCM ratings were conducted every 3 weeks throughout treatment (24 weeks) and withdrawal (9 weeks). Following the final rating session, haloperidol levels were measured in the cerebellum, as previously described (Egan et al. 1994). Briefly, tissue was thawed, homogenized in 1.5 ml deionized water and buffer with an internal standard, concentrated, analyzed using high-pressure liquid chromatography, and corrected for total protein concentration. Rats in the haloperidol-treated group were classified post-hoc with or without the VCM syndrome based on previously established behavioral criteria (Egan et al. 1994). Rats were classified as having the VCM syndrome if they had seven or more VCMs per 2-min rating session (2 SDs above the mean for the vehicle-treated group) for four of the last five rating sessions. Rats were classified as not having the VCM syndrome if they had no more than one rating of seven or above during the last five rating sessions. In total, there were 9 +VCM rats, 10 –VCM rats, and 17 vehicle rats. Brain tissue from other rats was not processed further. All animal use procedures were in strict accordance with the NIH *Guide for the Care and Use of Laboratory Animals*.

Receptor autoradiography

Frozen coronal sections (20-µm thick) were thaw-mounted onto gelatin-subbed slides, and stored at –70°C. Mu-opioid receptors were labeled with [^{3}H]-D-Ala^2, *N*-MePhe^4, Gly-ol^5-enkephalin ([^{3}H]-DAMGO; 54 Ci/mmol, NEN Life Science, Boston, Mass.) using a protocol adapted from Tempel et al. (1987). Brain sections were incubated at room temperature for 1 h in buffer containing 4 nM [^{3}H]-DAMGO for total binding. Nonspecific binding was determined with 4 nM [^{3}H]-DAMGO in the presence of 100 µM cold naloxone (RBI, Natick, Mass.). Slides were exposed to tritium-sensitive [^{3}H]-Hyperfilm along with calibrated [^{3}H]-microscale standards (Amersham Life Science, England) for 4 weeks at 4°C. Film images were digitized using a NIH Image (Wayne Rasband, NIMH), and the mean optical densities were measured in areas of interest traced with a hand-held cursor, as previously described (Egan et al. 1994). Rostral striatal sections extended from 1.70 mm to 0.70 mm anterior to bregma; mid striatum from 0.26 mm to 0.40 mm; posterior striatum from 0.80 mm to 1.40 mm; and midbrain sections from 4.52 mm to 6.04 mm (Paxinos and Watson 1986). The data were then converted to picomoles per milligram of wet tissue. Specific binding was taken from the total binding values since nonspecific binding was at background levels.

Statistics

Behavioral data were analyzed using two repeated-measures analyses of variance (rmANOVA) with one between and one within factor (time). Comparisons between groups were performed using Fisher's PLSD post-hoc analysis. The first rmANOVA used treatment (vehicle or haloperidol) as the between factor, while the sec-

Table 1 [^{3}H]-DAMGO (D-Ala^2, *N*-MePhe^4, Gly-ol^5-enkephalin) binding in rat brain after chronic haloperidol treatment. Values are means ± SEM in pmol/g tissue

Brain region	Vehicle	ALL hal	*P* value*	–VCM	+VCM	*P* value**
Anterior striatum			0.2918			0.4830
Dorsolateral	82.097±5.710	67.909±4.792	0.0636	72.194±6.667	56.725±7.056	0.1997
Dorsomedial	67.707±3.846	58.640±3.030	0.0689	59.996±4.218	54.556±4.229	0.4878
Ventrolateral	97.319±5.796	87.106±6.512	0.2615	93.670±8.463	69.140±10.173	0.0776
Ventromedial	69.427±2.684	64.115±2.980	0.2048	66.533±4.074	58.378±4.482	0.1988
Nucleus accumbens			0.1861			0.3279
Core	72.950±2.906	70.800±6.313	0.7681	71.384±12.145	65.488±5.839	0.6245
Dorsomedial shell	94.790±4.864	82.449±3.461	0.0496	87.100±3.317	75.257±5.193	0.2147
Ventromedial shell	58.904±2.473	62.752±5.176	0.5241	53.964±2.122	62.141±11.391	0.2677
Lateral shell	84.427±2.789	76.748±4.169	0.1503	76.782±7.782	76.055±6.192	0.9349
Mid striatum			0.2725			0.3184
Dorsolateral	40.294±2.668	40.569±1.347	0.9206	42.966±1.919	39.411±2.425	0.3100
Dorsomedial	40.000±1.887	41.095±1.327	0.6592	43.324±1.854	41.059±2.576	0.4702
Ventrolateral	47.503±1.802	48.141±1.503	0.8150	50.866±1.542	47.028±2.822	0.2292
Ventromedial	43.445±0.977	42.818±1.096	0.7435	44.058±1.131	43.101±2.045	0.6573
Ventral pallidum	42.624±1.772	39.963±1.040	0.1961	42.527±1.175	39.772±1.713	0.3705
Posterior striatum			0.0123			0.0420
Dorsolateral	36.627±1.009	35.783±0.872	0.5319	36.462±1.218	34.535±1.585	0.3699
Dorsomedial	36.779±0.700	35.658±0.752	0.3003	36.474±0.935	35.281±1.773	0.4843
Ventrolateral	49.930±1.859	47.717±1.646	0.3819	48.946±2.122	45.272±2.924	0.3486
Ventromedial	41.585±0.952	42.588±1.162	0.5314	44.621±1.901	40.646±1.810	0.1095
Globus pallidus	38.297±0.883	35.577±0.897	0.0435	35.693±1.149	36.366±1.798	0.7364
Midbrain			0.8430			0.8591
Substantia nigra compacta	38.039±2.855	40.671±1.939	0.4344	38.641±4.826	42.270±2.955	0.5137
Substantia nigra reticulata	23.947±3.040	25.755±1.783	0.5863	24.117±4.404	27.421±2.474	0.5442
Ventral tegmental area	41.940±2.566	41.287±1.477	0.8141	37.235±2.226	42.440±2.283	0.2359

* MANOVA for entire region and Fisher's post-hoc *t*-test; Hal vs Veh
** MANOVA for entire region and Fisher's post-hoc *t*-test; +VCM vs –VCM

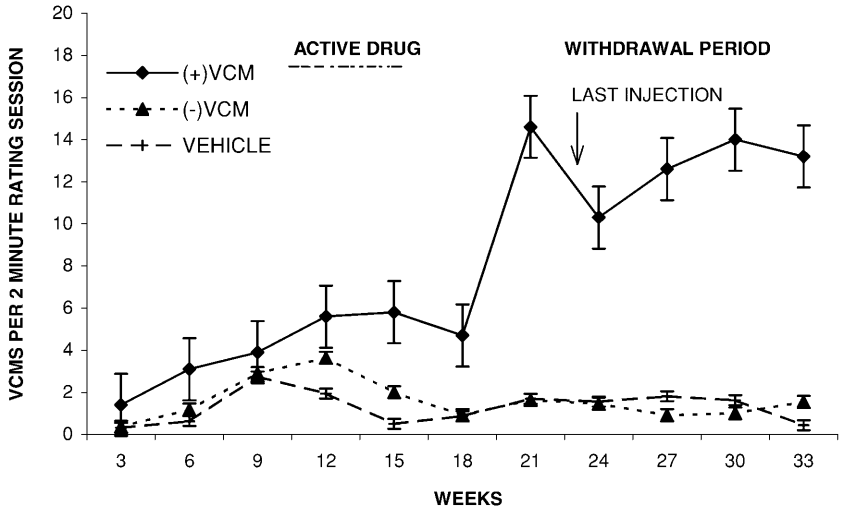

Fig. 1 Vacuous chewing movements (VCMs) in rats with chronic haloperidol. Mean VCM ratings (±SEM) in rats treated every 3 weeks for 24 weeks with haloperidol decanoate (i.m.) and withdrawn for 9 weeks. Ratings are given for groups of haloperidol-treated rats that did (+VCM) or did not (–VCM) develop persistent VCMs, in addition to the placebo group. Ratings for the +VCM were higher than for the –VCM and the vehicle groups

ond used group (groups: +VCM, –VCM, vehicle) as the between factor. This second rmANOVA was to demonstrate that the post-hoc group assignment (+VCM, –VCM) distinguished behaviorally separate groups. Autoradiography data were first analyzed at each coronal level by a multivariate analysis of variance analysis (MANOVA). Data were analyzed for main effects of drug treatment and then VCM grouping. Specific brain subregions measured at each respective coronal level are defined in Table 1 . If a given MANOVA was statistically significant, an individual ANOVA test for individual brain regions within a respective coronal level was performed. For those regions with significant ANOVAs, post-hoc comparisons were conducted to test for differences between group means.

Results

Behavioral measures

A significant effect of drug treatment was observed (F=13.312, df=1, 45, P=0.001); the effect of time (F=9.051, df=11, P<0.0001) and a treatment by time interaction (F=3.018, df=11, P=0.001) were also significant. Haloperidol gradually increased VCM ratings above those in the vehicle group over time. In a second analysis, haloperidol-treated animals were assigned to +VCM and –VCM groups. Using group as a main effect, significant effects were seen for VCM status (F=36.690, df=2, 34, P<0.0001); time (F=10.271, df=11, P<0.0001) and group by time interaction (F=6.640, df=22, P<0.0001). Mean ratings for the +VCM group were elevated relative to vehicle and –VCM groups (P<0.0001 for both; Fig. 1). No differences were seen between –VCM and control groups.

Receptor binding

Table 1 summarizes the results of [^{3}H]-DAMGO binding in all brain regions examined. MANOVA revealed a significant treatment effect at the level of the caudal striatum; [^{3}H]-DAMGO binding was reduced in haloperidol-treated rats relative to the vehicle control group (F=3.626, df=5, 27, P=0.01). Individual ANOVAs for each region defined at the level of the caudal striatum revealed a significant decrease of μ-opioid receptor binding in haloperidol versus vehicle rats in the globus pallidus (F=4.431, df=1, 31, P<0.04). All other analysis for effect of drug treatment and VCM grouping were not significant. In particular, no significant effects of group (i.e., +VCM, –VCM, vehicle) were seen in the striatum or nucleus accumbens. Finally, there were no differences in haloperidol brain levels between +VCM (13.9±3.4 ng/mg protein) and –VCM groups (10.4±1.4 ng/mg protein) following the extended withdrawal period.

Discussion

This study shows that long-term haloperidol treatment leads to a significant decrease in μ-opioid receptor binding in the globus pallidus. These changes persist during an extended 9-week withdrawal period, when brain haloperidol levels reach relatively low levels. However, there does not appear to be any relationship between reduced μ-opioid receptor levels and the persistence of VCMs. These findings are in agreement with those of Sasaki et al. (1996). Furthermore, no VCM-related changes in μ-opioid receptor levels were found in the striatum or nucleus accumbens. These data are consistent with the notion that neuroleptic treatment induces a long-term increase in striatopallidal activation and compensatory downregulation of postsynaptic receptors in the globus pallidus, but do not indicate a role for these alterations in the persistence of the VCM syndrome or, by extension, TD.

The findings from this study are consistent with a number of prior observations. For example, several authors have reported increased enkephalin mRNA and peptide levels in the striatum following both acute and chronic neuroleptic treatment (Egan et al. 1994). Chen et

al. (1994) found a similar increase in proenkephalin mRNA after irreversible blockade of D_2 dopamine receptors. Changes consistent with increased enkephalinergic striatopallidal neurotransmission have also been observed in a primary target area, the globus pallidus. For example, Delfs et al. (1994) found a decrease in mRNA encoding μ-opioid receptors in the globus pallidus in response to neuroleptic treatment, consistent with our binding results. Moreover, Auchus et al. (1992) found an increase in met-enkephalin-like immunoreactivity in the globus pallidus of haloperidol-treated rats, suggesting increased presynaptic release from striatopallidal neurons. Similar changes have also been reported in striatopallidal GABAergic neurotranmission, including increased GABA release (Chapman and See 1996) and downregulation of GABA receptors (Sasaki et al. 1997) in the globus pallidus. Alternatively, it is possible that neuroleptic-induced changes in the globus pallidus are due to local effects and are not secondary to striatopallidal activation.

We previously noted that expression of preproenkephalin mRNA was particularly elevated in rats with severe, persistent VCMs during an extended (8 month) withdrawal period (Egan et al. 1994). In this study, however, we did not find the hypothesized complementary changes in μ-receptor binding in any region. The apparent 14% difference between +VCM and –VCM groups in the nucleus accumbens, for example, were not significantly different. It is possible that a larger cohort may have yielded significant differences. The current study had sufficient power to detect a 20% difference in receptor level in most regions. Notwithstanding power issues, elevated striatal enkephalin mRNA in +VCM rats without an accompanying downregulation in μ-opioid receptors suggests that the molecular changes underlying the VCM syndrome, and possibly TD, may occur within the enkephalin-expressing GABA-ergic neurons of the striatum and not in downstream pallidal neurons.

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