Brain Research 879 (2000) 1–6

Research report

Opiate receptor avidity is increased in rhesus monkeys following unilateral optic tract lesion combined with transections of corpus callosum and hippocampal and anterior commissures

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Abstract

Opiate receptor avidity \( (B'_{\text{max}}/K_i) \) was measured in four rhesus monkeys following unilateral lesioning of the optic tract combined with transection of the corpus callosum and the hippocampal and anterior commissures depriving one hemisphere of visual input (Tract and Split), two animals with transection of commissures only (Split), and nine healthy monkeys with positron emission tomography (PET) and 6-deoxy-6-[\( ^{18} \)F]fluoronaltroxetine (cyclofoxy, CF), a \( \mu \)- and \( \kappa \)-opiate receptor antagonist. Opiate receptor avidity was found to be significantly higher in the Tract and Split animals, only, bilaterally, throughout the lateral cortex and in the cingulate and posterior putamen (41–117%). Ipsilateral changes were consistently greater than those contralateral, but this asymmetry was of statistical significance only in the parietal and occipital cortices. Cyclofoxy avidity was decreased in the medial cortex of both the Tract and Split animals (−25%). The results suggest that opiate pathways undergo extensive alteration in response to changes in brain functional activities brought about through hemispheric visual deprivation. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Neurotransmitters, modulators, transporters, and receptors

Topic: Opioids: anatomy, physiology and behavior

Keywords: Neurodegeneration; 6-Deoxy-6-\( ^{18} \)F]fluoronaltroxetine; Positron emission tomography; Cortex; Visual system

1. Introduction

Decreases in opiate receptor avidity as quantified with the positron-emitting opiate antagonist 6-deoxy-6-\( ^{18} \)F]fluoronaltroxetine (cyclofoxy, CF) occur in Alzheimer’s disease [3] and following the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioning of rhesus monkeys [4,5]. While, it is likely that these changes reflect adaptive responses to alteration in function in the MPTP-lesioned animals, it is not clear in the Alzheimer’s patients whether the avidity changes reflect primarily the direct effects of neurodegeneration or an adaptational response. To examine the question of functional vs. direct opiate receptor avidity changes in response to neurodegeneration we elected to examine opiate receptor avidity in animals with lesions of the visual system. The visual system has provided exceptionally useful models with which to study structural and biochemical neuronal plasticity in the context of development and of altered function [6,14,25].

2. Materials and methods

2.1. Subjects

Fifteen monkeys (Macaca mulatta), four that had received unilateral transection of the optic tract combined with transection of the corpus callosum and hippocampal...
and anterior commissures (Tract and Split), two that had received transection of the corpus callosum and commissures only (Split) and nine normal animals were PET (positron emission tomography) scanned using cyclofoxy as the tracer. The lesioned animals were scanned 3 to 5 years after their surgery.

2.2. Surgical procedures

All animals were initially sedated with ketamine (ketamine HCl, 10 mg/kg) and surgical level of anesthesia achieved with isoflurane gas. All neurosurgical procedures were carried out under aseptic conditions with the aid of an operating microscope. Intravenous fluids were provided throughout the surgical procedure. The animal was draped in a heating blanket and its vital signs (heart and respiration) monitored continually. The head was secured in a head holder and commissurotomy performed by a dorsal approach. The cerebral midline was exposed through a unilateral bone flap and reflection of a dural flap. The corpus callosum was exposed with gentle retraction of the medial wall of one hemisphere. Using a small glass pipette together with suction, the corpus callosum throughout its rostral-caudal extent was transected. At a level immediately caudal to the descending columns of the fornix the anterior commissure was visualized through the third ventricle and transected. Upon completion of the commissurotomy the dura was replaced over the brain and the bone flap sewn in position using nylon sutures.

The optic tract was visualized through an orbitofrontal approach. A small craniotomy was made over the prefrontal cortex, the dura reflected and the frontal lobe gently retracted to view the ventral medial portion of the brain. Using a glass pipette the optic tract was transected immediately behind the optic chiasm. The wound was closed in anatomical layers using absorbable suture. Prophylactic doses of antibiotics were administered and continued for 2 weeks postoperative. In general the monkeys recovered without incident.

2.3. PET scan procedure

On the day of scan, each monkey was intubated and had venous and arterial lines inserted under barbiturate anesthesia. During the scan each animal was maintained under light anesthesia with isoflurane and positioned prone in a stereotaxic frame. 62.5% of the tracer dose (2-5 mCi, specific activity=3.09±1.74 Ci/m mole) of CF was administered as a bolus while the rest of the dose was administered as a continuous infusion to achieve constant radioactivity levels in all brain regions between 60 and 90 min after tracer injection. Thirty coronal slices were obtained through the head with a Scanditronix 2048-15B scanner (in plane resolution of 5.5-6.5 mm full-width half-maximum and an axial resolution of 5-6 mm.). Scanning protocol, methods for cyclofoxy synthesis, infusion, and metabolite analysis, have been previously described [1,4,8].

2.4. Data analysis

Pixel by pixel $V_T$ (volume of distribution) images were generated by a non-linear parameter estimation algorithm [1]. The algorithm is used to generate estimates of three parameters based on a one-compartment model and the measured plasma CF input curve: $K_i$ (ml plasma/min/ml tissue) the CF influx constant from plasma to tissue, $k_2$ (min⁻¹) the CF efflux constant from tissue to plasma, and $V_s$ the plasma volume (ml plasma/ml/ml tissue). From these estimated parameters, the total volume of distribution ($V_T$) was calculated from the ratio of $K_i$ to $k_2$. Based on the assumptions of the model, this value is equal to the concentration ratio at equilibrium between tissue and plasma and includes tracer in the tissue that is free, non-specifically bound, and specifically bound to receptor. Another interpretation of $V_T$ is the volume of plasma which contains an equivalent amount of tracer as 1 ml of tissue, thus the units of ml plasma/ml tissue.

To estimate the binding potential or avidity of opiate receptors (the specific volume of distribution, $V_s$, roughly equivalent to the estimate of Unoccupied Receptor Density/the Dissociation Constant of the Receptor, i.e., $B_{max} / K_D$, also referred to as CF avidity, an estimate of nonspecific binding ($V_{NS}$) is required ($V_s = V_T - V_{NS}$). In practice, this is obtained from the volume of distribution of a region with little or no specific opiate receptor binding which in the monkey is the cerebellum and in humans is the primary occipital cortex. The general use in PET tracer studies of the avidity measure, $V_s$, instead of receptor density ($B_{max}$), is the result of not being able to accurately estimate receptor density based on a single injection of tracer. The single injection PET study is roughly equivalent to an in vitro study in which the binding of only a single low (relative to the $K_D$) concentration of radioactively labeled ligand to tissue is measured. The use of a region without specific binding in the PET study for determination of $V_{NS}$ is roughly equivalent to the in vitro binding of labeled ligand to tissue in the presence of a high concentration of unlabeled ligand. Tissues that bind more labeled ligand in vitro after subtracting nonspecific binding have a higher binding potential either as a result of increased receptor density, decreased $K_D$ or a combination of both. In the in vivo instance, differences in $V_s$ can also arise as a result of changes in endogenous ligand concentrations leading to more or less competition with the labeled tracer for the receptor.

To obtain localized values for the volumes of distribution, regions of interest (ROIs) were placed individually for each animal, under visual guidance, through neuroanatomical matching to a standard template designed specifically to use in PET studies of rhesus monkeys.
[4,5,8] and by comparison with each animal’s MRI scan and an atlas of the rhesus monkey brain in coronal planes. All the ROIs were placed by a single investigator experienced in nonhuman primate neuroanatomy and the use of the template.

2.5. Statistical analysis

Where homologous left and right hemispheric regions could be evaluated, the effect of the Tract and Split on opiate receptor avidity was determined using two-way ANOVARs (repeated measures) with GROUP (normal, lesion) as the between-subject factor and SIDE (ipsilateral, contralateral) as the within-subject factor. In the presence of a significant statistical interaction effect (GROUP × SIDE), the effect of lesion was assessed separately for the ipsilateral and contralateral sides by independent t-test.

Because there were only two split animals, a separate ROI statistical analysis using the independent t-test for comparison to normal controls was used in an exploratory attempt to determine the likelihood that any significant changes observed in the Tract and Split animals were likely to be the result of the transection of the commissural fibers, only, rather than the combination of optic tract lesion and transection of commissural fibers.

3. Results

3.1. Tract and Split

As previously reported [4] and illustrated in Fig. 1, the in vivo pattern of CF binding in monkey brain closely resembles that reported for the pattern of in vitro naloxone binding [26]. Aside from the medial frontal cortex, increases in opiate receptor avidity were seen in all the cortical regions (occipital, parietal, temporal, frontal, central) of the Tract and Split animals examined compared to healthy controls (Table 1). The largest effects were observed in the occipital and parietal cortices where there

![Fig. 1. Three posterior coronal PET slices, representative of those used in the ROI analysis of the CF data, are displayed. The upper slice was taken from the scan of a normal rhesus and the bottom two slices from the scans of two of the Tract and Split animals, one lesioned on the left and one lesioned on the right. Total volume of distribution for each pixel is color coded in ascending order (blue, green, yellow, red). Scale is from 0 to 55 ml plasma/ml tissue). As is illustrated, the posterior cortices of the Tract and Split animals have higher Vₜₐₜₜ (opiate receptor avidities, Table 1) than healthy animals in both contralateral and ipsilateral hemispheres, with highest avidities observed ipsilateral to their lesions.](image-url)
Table 1

<table>
<thead>
<tr>
<th>ROI</th>
<th>Normal Controls</th>
<th>Tract and Split</th>
<th>% Differences in ( V_c )</th>
<th>Effect of lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (s.d.)</td>
<td>Mean (s.d.)</td>
<td>Mean (s.d.)</td>
<td>Mean (s.d.)</td>
</tr>
<tr>
<td><strong>Cortical structures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Medial frontal</td>
<td>14.39 (2.24)</td>
<td>10.65 (1.93)</td>
<td>-26.0%</td>
<td>8.3 (0.015)</td>
</tr>
<tr>
<td>*Orbital</td>
<td>12.04 (1.69)</td>
<td>11.16 (2.07)</td>
<td>41.4%</td>
<td>50.2%</td>
</tr>
<tr>
<td>*Frontal</td>
<td>10.96 (1.70)</td>
<td>10.78 (1.55)</td>
<td>50.5%</td>
<td>51.9%</td>
</tr>
<tr>
<td>*Central</td>
<td>6.59 (1.29)</td>
<td>6.52 (1.29)</td>
<td>68.4%</td>
<td>66.4%</td>
</tr>
<tr>
<td>*Temporal</td>
<td>11.77 (1.74)</td>
<td>10.92 (2.21)</td>
<td>58.3%</td>
<td>52.0%</td>
</tr>
<tr>
<td>#Parietal</td>
<td>7.37 (1.82)</td>
<td>7.52 (1.32)</td>
<td>77.5%</td>
<td>51.3%</td>
</tr>
<tr>
<td>#Occipital</td>
<td>3.81 (1.42)</td>
<td>3.75 (1.26)</td>
<td>116.5%</td>
<td>66.7%</td>
</tr>
<tr>
<td><strong>Limbic and paralimbic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Cingulate</td>
<td>12.21 (1.60)</td>
<td>15.79 (3.05)</td>
<td>29.1%</td>
<td>8.1 (0.016)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>9.87 (2.31)</td>
<td>10.38 (2.67)</td>
<td>36.6%</td>
<td>21.7%</td>
</tr>
<tr>
<td>Amygdala</td>
<td>23.91 (2.77)</td>
<td>24.09 (3.44)</td>
<td>7.0%</td>
<td>8.3%</td>
</tr>
<tr>
<td><strong>Basal ganglia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate</td>
<td>29.61 (4.19)</td>
<td>29.12 (4.03)</td>
<td>5.7%</td>
<td>8.3%</td>
</tr>
<tr>
<td>Ant. putamen</td>
<td>23.75 (2.83)</td>
<td>23.26 (2.29)</td>
<td>8.2%</td>
<td>6.0%</td>
</tr>
<tr>
<td>*Post. putamen</td>
<td>14.32 (2.35)</td>
<td>14.51 (2.19)</td>
<td>43.4%</td>
<td>44.0%</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>17.63 (2.70)</td>
<td>16.81 (4.67)</td>
<td>24.5%</td>
<td>22.7%</td>
</tr>
<tr>
<td><strong>Subcortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial thalamus</td>
<td>29.65 (3.95)</td>
<td>27.30 (4.82)</td>
<td>-7.9%</td>
<td>0.86 (NS)</td>
</tr>
<tr>
<td>Thalamus</td>
<td>25.67 (3.45)</td>
<td>24.24 (3.19)</td>
<td>9.0%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>29.07 (3.91)</td>
<td>25.6 (7.46)</td>
<td>-11.9%</td>
<td>1.27 (NS)</td>
</tr>
</tbody>
</table>

*Means and standard deviations for the specific distribution volumes (ml plasma/ml tissue) of the regions of interest (ROIs) and the total distribution volumes for the cerebellum (non-specific CF avidity) and muscle (background) for the normal controls and Tract and Split (unilateral optic tract lesion combined with transection of callosal fibers) monkeys are tabulated with standard deviations in parentheses. Where there were homologous left and right ROIs, the effect of lesion was determined as the \( \bar{F} \) as in Cohen et al. [1,11] value and its corresponding P-value for the main effect of the two-way repeated measures analysis of variance (ANOVAR) with GROUP (i.e. lesion or normal as the grouping factor) and SIDE (ipsilateral vs. contralateral) as the within-group factor. When the interaction, i.e. GROUP \( \times \) SIDE was significant, paired comparisons between the ipsilateral sides of the normal and lesioned animals, and between the contralateral sides of the normal and lesioned animals were made by the least significant difference method (LSD). Where statistical analyses were not determined for homologous left and right ROIs, e.g. the hypothalamus, means and standard deviations are tabulated as ‘Medial’. In these instances the F-values and corresponding P-values are based on one-way ANOVAs. Statistical significance, observed either by ANOVA or ANOVAR for the effect of GROUP, i.e. lesion, is indicated by a ‘*’ and a significant interaction by a ‘#’ placed next to the name of the ROI.

were significant interactions between LESION and SIDE (Occipital: \( F_{1,11} = 17.0, P < 0.002 \); Parietal: \( F_{1,11} = 17.9, P < 0.002 \)). Independent t-tests, confirmed the presence of higher CF avidity on both the ipsilateral and contralateral sides of the Tract and Split animals (parietal: ipsilateral, \( t = 5.16, P < 0.0004 \); contralateral, \( t = 4.04, P < 0.002 \); occipital: ipsilateral, \( t = 5.24, P < 0.0003 \); contralateral, \( t = 3.3, P < 0.008 \)). Thus, the significant interactions were the result of larger increases on the ipsilateral compared to the contralateral sides. For example, compared to the healthy controls the increase in the ipsilateral occipital cortex of the Tract and Split animals was nearly twice (116.5%) that observed in the contralateral occipital cortex (66.7%).

Of the limbic and subcortical structures examined, increases in CF avidity were found in the cingulate and posterior putamen. There were no significant changes in the volumes of distribution of CF in the opiate-poor regions of the cerebellum (\( V_c = 10.6 \pm 1.8 \) for the normal controls and \( 8.5 \pm 1.3 \) for the Tract and Split animals, \( P = \text{NS} \)) and of an area of neck and shoulder muscle (\( V_c = 6.8 \pm 1.3 \) for the normal controls and \( 6.3 \pm 1.1 \) for the Tract and Split animals, \( P = \text{NS} \)). Thus, the differences in specific CF distribution volumes (\( V_c \)) in the opiate receptor-rich brain areas are unlikely to be the result of changes in the nonspecific uptake of CF.

3.2. Split

To determine whether any of the above differences in the Tract and Split animals might have arisen as a result of the transection of the callosal fibers rather than the result of the unilateral optic tract lesion or the combination, the same ROIs in the healthy animals were compared to those in the Split animals. Only, the medial cortex had a
4. Discussion

4.1. Possible causes of opiate responses to lesions

The likely causes of a reduction in CF avidity in a region, as was observed in the medial frontal cortex in the Tract and Split animals, are axonal (Wallerian) degeneration and transneuronal degeneration. The location of the medial region together with the observation of similar changes in the Split animals are consistent with either of the above mechanisms. Thus, the lowering of CF avidity in the medial frontal cortex was likely to have been the result of the transection of the callosal fibers. In those brain regions, however, in which higher opiate receptor avidity was found in the Tract and Split animals, and not in the Split animals, another mechanism must be at work. In these areas, if cutting the callosal fibers had any effect these were likely to have been the result of an interaction with the unilateral optic tract lesion, i.e., through eliminating many of the pathways that might distribute visual information from the ‘seeing’ cortex to the visually deprived cortex. A similar conclusion was drawn by other investigators with respect to the effect of callosal transection on the hypometabolic regional pattern observed in their Tract and Split animals prepared by a nearly identical method [13].

If opiate pathways were responsive to changes in regional functional activities, an explanation would be apparent not only for the widespread, and bilateral nature of the increases in CF avidity observed in the Tract and Split animals, but also for the nonhypothesized changes in opiate avidity found in five patients with Huntington’s Disease (decreases in thalamus and anterior cingulate and increases in the prefrontal cortex) studied with 11C-diprenorphine (a partial, subtype, non-specific opiate receptor agonist) [23], and the bilateral CF avidity decreases previously observed in unilaterally-lesioned MPTP monkeys [5]. In the latter animals, although dopamine loss is primarily restricted to one side of the brain changes in functional basal ganglia functional activities are likely to be bilateral in nature.

Consistent with a change in functional activities inducing CF avidity changes are the anatomical studies of connectivity and the electrophysiological, regional metabolic and blood flow studies in normal as well as lesioned animals that have established that the areas of the brain contributing to and essential for many visual system functions are extensive [6,12–14,28]. Areas of the brain thought to contribute to visual function include frontal, temporal and parietal cortex, and cingulate as well as some areas of the subcortex including the posteroventral putamen, all areas showing increases in CF avidity in response to the optic lesion.

4.2. Specificity of opiate responses

The likelihood that the CF avidity changes are non-specific in nature is made less likely by prior findings in unilaterally- [5] and bilaterally-lesioned [4] MPTP animals. In response to these lesions of the dopamine system, the CF avidity of the amygdala, thalamus, caudate and anterior putamen were decreased compared to the healthy controls. These same brain areas were found to be unaffected in the current study. Thus, despite the extentiveness and the size and bilateral nature of the CF avidity increases observed in the Tract and Split monkeys, they are likely to be specific responses to hemispherically selective visual deprivation.

Consistent with this working hypothesis are studies in the rat following unilateral orbital enucleation where selective neurotransmitter receptor density changes, on the order of 20%, have been noted to occur at synapses both primary and secondary to the lesion and with different time dependencies [2]. For example, while GABA_A and 5-HT_1 (serotonin) receptors were reduced in the visually deprived cortex, 5-HT_2 and cholinergic receptors were not. The reductions in GABA_A and 5-HT_1 receptors were associated with the reductions in cerebral metabolic activities in the visual cortex. Analogous adaptations may occur in the primate [10,11], as reorganization of pathways and adaptive changes in input from other brain areas would be expected to be involved in the adjustment of the brain to various forms of deprivation.

4.3. Nature of opiate response measured

While the current study demonstrates a response in the opiate system it does not address the specific nature of the response. Because CF binds to both μ- and κ-opiate receptors, both receptors contribute to the V_sh measurement of cyclofoxy avidity. Thus, it is uncertain whether one or both opiate receptor subtype avidities are affected in the Tract and Split animals. Moreover, as up-regulation and down-regulation can occur in opiate receptors in response to occupancy [18,20,21], the regional effects observed in the Tract and Split animals are likely to reflect both the direct and indirect effects of the lesions on the μ- and κ-opiate receptors, and the direct and indirect effects of an alteration in endogenous opiate receptor concentrations in the synapse. That endogenous opiate concentrations can be
altered in response to brain insults has experimental support [17,19,22].

Because µ- and κ-opiate receptors are likely to have distinct functional roles [7,9,15,24,27] and may respond differentially to lesions [16], the current findings provide a reason to conduct studies designed to determine the effects of lesioning on opiate subtype avidity and to determine whether avidity changes reflect alterations in density or receptor occupation or both. Clarifying the underlying processes responsible for opiate receptor avidity alteration following unilateral optic tract lesion combined with transection of callosal fibers, may clarify the role the opiate pathways play in the brain’s response to alterations in regional function as occurs under a variety of conditions.

Acknowledgements

The authors gratefully acknowledge the invaluable contributions of Drs Ronald Blasberg, Michael Channing, Nancy Ostrowski, Candace Pert and Kenner Rice to the development of the opiate receptor antagonist 6-deoxy-6-β-[18F]fluoronaltroxetine.

References


