μ Opioid Receptor Coupling to G\textsubscript{i/o} Proteins Increases during Postnatal Development in Rat Brain

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Received December 13, 2004; accepted April 27, 2005

ABSTRACT

μ Opioid receptors are densely expressed within rat striatum and are concentrated in anatomically discrete patches called striosomes. The density of striosomal μ receptors remains relatively constant during postnatal development, but little is known about their functional maturation. We examined the extent of G protein coupling by μ opioid receptors in rat brain during development, focusing on striosomes within the striatum because of receptor density. The μ receptors were quantified using \[^{3}H\]D-Ala\textsubscript{2},N-Me-Phe\textsubscript{4},Gly\textsubscript{5}-ol-\textasciitilde-\textendashenkephalin (DAMGO) autoradiography. Adjacent sections were analyzed for DAMGO-stimulated guanosine 5'-O-(3'-\[^{35}S\]thio)triphosphate (\[^{35}S\]GTP\textsubscript{S}) binding to assess μ receptor activation of G\textsubscript{i/o} proteins. Striosomal μ receptor expression increased only slightly between postnatal day 5 and adult. In contrast, μ receptor-stimulated \[^{35}S\]GTP\textsubscript{S} binding increased from 0.13 to 2.6 fmol/mg tissue over the same period, a 20-fold difference. The ratio of specific DAMGO-stimulated \[^{35}S\]GTP\textsubscript{S} binding to \[^{3}H\]DAMGO binding, representing the relative number of G proteins activated per receptor, increased 19-fold between postnatal day 5 and adult. Similar patterns were observed throughout the striatum and other brain regions such as the nucleus accumbens, although the extent of change varied from region to region. These data indicate that μ opioid receptors exhibit enhanced function in the adult rat brain compared with the neonate. These data also suggest that this increase in G protein coupling is developmentally regulated and that in the developing rat brain the density of μ opioid receptor expression may not necessarily correlate with receptor activation of G proteins.

μ Opioid receptors (MORs) are heptahelical receptors expressed by neurons of the central, peripheral, and enteric nervous systems. Nearly all of the biochemical responses to MOR activation discovered thus far involve signal transfer through G proteins, heterotrimeric proteins whose activity is regulated by guanine nucleotide binding. Agonist-occupied MOR activation discovered thus far involve signal transfer from region to region. These data indicate that μ opioid receptor expression may not necessarily correlate with receptor activation of G proteins.

This research was supported by the National Institutes of Health Grant DA016346 and the University of Nebraska Medical Center. This work was submitted to the University of Nebraska as partial fulfillment of the requirements for the degree of Doctor of Philosophy. Portions of this work were presented previously in abstract form: Talbot JN, Happe HK, and Murrin LC (2002) Mu opioid receptor/G protein coupling efficiency increases in developing rat striatum. Pharmacologist 44:A134.

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Article, publication date, and citation information can be found at http://jpet.aspetjournals.org. doi:10.1124/jpet.104.082156.

ABBREVIATIONS: MOR, μ opioid receptor; \[^{35}S\]GTP\textsubscript{S}, 5'-O-(3'-\[^{35}S\]thio)triphosphate; CNS, central nervous system; DAMGO, D-Ala\textsubscript{2},N-Me-Phe\textsubscript{4},Gly\textsubscript{5}-ol-\textasciitilde-\textendashenkephalin; PND, postnatal day; Ad, adult; RGS, regulators of G protein signaling; GPCR, G protein-coupled receptor.
activated by specific receptors (Traynor and Nahorski, 1995; Harrison and Traynor, 2003). Combining the \[^{35}S\]GTP\(\gamma\)S assay with autoradiography allows G protein activation by specific receptors to be anatomically defined and quantified (Sim et al., 1995; Happe et al., 2000). These techniques have been used to evaluate the coupling of MORs to G proteins in the CNS as well as the relationship between receptor binding and receptor-stimulated G protein activation. In the rat brain, MOR agonists stimulate \[^{35}S\]GTP\(\gamma\)S binding to varying degrees in different brain regions (Sim et al., 1996a; Tsuji et al., 1998). Childers and colleagues further evaluated MOR/G protein coupling by determining the efficiency of G protein activation by MORs. MOR-stimulated \[^{35}S\]GTP\(\gamma\)S binding was directly compared to MOR density (\[^{3}H\]naloxone binding), resulting in an “amplification factor” or the relative number of G proteins activated per receptor (Sim et al., 1996b; Maher et al., 2000). The relative MOR/G protein coupling efficiency varied significantly between brain regions (Maher et al., 2000). These studies suggested that signal transfer from MORs to G proteins is regulated in a tissue-specific manner in the CNS.

For some systems, receptor expression during development seems to parallel G protein activation, as might be expected. For example, during postnatal development, increased \(\alpha_2\) adrenergic receptor expression in the rat forebrain parallels increases in \(\alpha_2\) adrenergic receptor-stimulated \[^{35}S\]GTP\(\gamma\)S binding (Happe et al., 1999). Also in the rat, cannabinoid receptor expression corresponds with agonist-stimulated \[^{35}S\]GTP\(\gamma\)S binding during embryonic development (Berendro et al., 1998). However, a number of studies suggest disparate G protein coupling by MORs during development. In several embryonic mouse brain regions, including the striatum, radioligand binding to MORs is observed before MOR agonist-stimulated \[^{35}S\]GTP\(\gamma\)S binding can be detected (Nitsche and Pintar, 2003). In addition, sensitivity of MORs to the GTP analog 5'-guanylyl-imidophosphate in postnatal day 27 rats is twice that of animals at day 10, suggesting weaker MOR/G protein coupling in neonatal animals (Windh and Kuhn, 1995). These data suggest that during development MOR binding may not necessarily correlate with MOR-activated G proteins. The objective of the current study was to determine whether MOR/G protein coupling changes during postnatal development. We used receptor autoradiography and the \[^{35}S\]GTP\(\gamma\)S autoradiographic assay to show that \(G_{i/o}\) protein activation by MORs increased dramatically during postnatal development despite very small changes in MOR density, indicating MORs exhibit increasing function with advancing development. These data suggest that MOR-stimulated G protein coupling is a developmentally regulated process and that in the developing rat brain MOR expression may not necessarily correlate with MOR function.

Materials and Methods

Materials. \[^{35}S\]GTP\(\gamma\)S (1250 Ci/\(\mu\)mol) was obtained from Perkin-Elmer Life and Analytical Sciences (Boston, MA). \[^{3}H\]DAMGO (68 Ci/\(\mu\)mol) was purchased from Amersham Biosciences, Inc. (Piscataway, NJ). DAMGO, naloxone hydrochloride, and GTP\(\gamma\)S were purchased from Sigma-Aldrich (St. Louis, MO). GDP was purchased from U.S. Biochemical Corp. (Cleveland, OH). All other chemicals were research grade.

Animals and Tissue Preparation. Sprague-Dawley rats (Sasco, Kingston, NY) were bred in our colony. Litters were culled to nine pups and monitored for normal growth by body weight. Brains were collected at postnatal day (PND) 5, 10, 15, 21, and 30, and from adults (Ad) (determined by weight, 250 ± 25 g), rapidly frozen on dry ice, and stored wrapped in Parafilm and foil at −80°C. Tissue sections were cut from brains at 16 \(\mu\)m using a cryostat, thaw-mounted onto subbed slides, and stored at −20°C until use. Coronal sections centered on the rostral, medial, and caudal regions of the striatum were used (plates 12–31; Paxinos and Watson, 1986). Procedures were in strict accordance with The National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local Institutional Animal Care and Use Committee.

Agonist-Stimulated \[^{35}S\]GTP\(\gamma\)S Binding Assay. The \[^{35}S\]GTP\(\gamma\)S assay was carried out as described previously (Sim et al., 1995; Happe et al., 2001). Briefly, tissue sections were rehydrated in assay buffer (50 mM glycolline, 3 mM MgCl\(_2\), 1 mM EGTA, and 100 mM NaCl, pH 7.45) for 10 min. Sections were then immersed in assay buffer containing 2 mM GDP for 15 min, followed by incubation for 210 min in assay buffer containing 2 mM GDP, 0.25 mM dithiothreitol, and 0.1 nM \[^{35}S\]GTP\(\gamma\)S. Agonist-stimulated and basal \[^{35}S\]GTP\(\gamma\)S binding were measured in the presence (stimulated) and absence of 3 \(\mu\)M DAMGO, a MOR-specific agonist. DAMGO-stimulated \[^{35}S\]GTP\(\gamma\)S binding was also measured in the presence 5 \(\mu\)M naloxone to determine background. Nonspecific \[^{35}S\]GTP\(\gamma\)S binding was determined in the absence of agonist and the presence of 1 \(\mu\)M unlabeled GTP\(\gamma\)S. All incubations were performed at room temperature (25°C).

Immediately after incubations, tissue sections were washed twice (5 min each) in ice-cold 50 mM glycolline buffer, pH 7.45, containing 0.25 mM dithiothreitol and dipped once in ice-cold water. Sections were dried under a stream of cool air for 30 min and left at room temperature overnight. Sections were apposed to Hyperfilm-\(\beta\)MAX (Amersham Biosciences, Inc.) for 1 day to 1 week and were quantified by densitometric analysis with commercial \(^{14}C\) standards (American Radiolabeled Chemicals, St. Louis, MO) that had been individually calibrated to \(^{35}S\) tissue standards (Miller and Zahniser, 1987). Data are expressed as femtomoles per milligram of tissue.

\[^{3}H\]DAMGO Autoradiography. The \(\mu\) opioid receptors were analyzed with \[^{3}H\]DAMGO quantitative autoradiography using tissue sections adjacent to those used in the DAMGO-stimulated \[^{35}S\]GTP\(\gamma\)S binding assay. Tissue sections were incubated at room temperature with 3 nM \[^{3}H\]DAMGO in 50 mM Tris-HCl, pH 7.45, for 45 min. Nonspecific binding was determined by the addition of 5 \(\mu\)M naloxone. After incubations, sections were washed twice (5 min each) in ice-cold 50 mM Tris-HCl and briefly dipped once in ice-cold water. Sections were then treated identically to those used in the \[^{3}H\]DAMGO binding assay except Hyperfilm-\(^{3}H\) autoradiographic film (Amersham Biosciences, Inc.) was used with previously calibrated commercial tritium standards.

Data Analysis. Films were developed using standard methods and analyzed using the MCID-M5 system (Imaging Research, St. Catherines, ON, Canada). Four sets of serial sections from rostral, medial, and caudal striatal regions of three to four rat brains were assayed at each developmental age. Striosomes were identified from surrounding matrix by the much greater density of MOR (4- to 5-fold). For each section, \[^{3}H\]DAMGO binding levels were measured in the matrix and in four striosomes. Corresponding areas in adjacent tissue sections were measured for \[^{35}S\]GTP\(\gamma\)S binding. Basal values of \[^{35}S\]GTP\(\gamma\)S binding were subtracted from DAMGO-stimulated binding, as described previously (Happe et al., 2000).

Results

In preliminary studies, we noticed an apparent discrepancy between MOR expression (\[^{3}H\]DAMGO autoradiography) and activation of G proteins by MORs (DAMGO-stimulated \[^{35}S\]GTP\(\gamma\)S binding) during postnatal development. In the current study, we examined in detail the relationship
between these two. To anatomically compare MOR levels with MOR coupling to G_{i/o} proteins in the developing rat brain, adjacent tissue sections from PND5, 10, 15, 21, and 30, and adult were used for either DAMGO-stimulated \[^{35}S\]GTP\(_{\gamma}\)S binding or \[^{3}H\]DAMGO binding followed by quantitative autoradiographic analysis. High levels of \[^{3}H\]DAMGO binding were found in the cingulate cortex, amygdala, thalamic nuclei, core and shell regions of the nucleus accumbens, CA1 region of the hippocampus, and in striosomes of the caudate-putamen (Fig. 1), in agreement with previous studies (Atweh and Kuhar, 1977; Quirion et al., 1983). DAMGO, a MOR-specific agonist, significantly stimulated \[^{35}S\]GTP\(_{\gamma}\)S binding in these same regions in adjacent brain sections. In adults, there was a high degree of anatomical correlation between \[^{3}H\]DAMGO binding and DAMGO-stimulated \[^{35}S\]GTP\(_{\gamma}\)S binding, with both being greatest in striosomes. On the other hand, the correlation was less precise at the early developmental ages studied (PND5–PND15). At these earlier ages, \[^{3}H\]DAMGO binding was similar in density to that observed in the adult, whereas DAMGO-stimulated \[^{35}S\]GTP\(_{\gamma}\)S binding was much weaker at the early ages compared with the adult. This was most apparent in the striosomal regions of the striatum, anatomically distinct areas of particularly robust MOR expression (Pert et al., 1976; Atweh and Kuhar, 1977; Herkenham and Pert, 1981), and so we focused our detailed studies on this area.

We used densitometric analysis to quantify MOR levels in striosomes from rostral areas of the striatum during development. \[^{3}H\]DAMGO binding increased from 83 ± 1 fmol/mg tissue at PND5 to 112 ± 6 fmol/mg tissue at PND10 but did not change significantly from PND10 through adulthood (Fig. 2). The modest increase in \[^{3}H\]DAMGO binding observed between PND5 and PND10 is in agreement with previous autoradiographic studies indicating maximum levels of MOR density in striosomes are reached before the second postnatal week (Kent et al., 1981). However, no significant changes in \[^{3}H\]DAMGO binding were observed after PND10.

By contrast, specific DAMGO-stimulated \[^{35}S\]GTP\(_{\gamma}\)S binding increased dramatically over the same time period, from 0.25 ± 0.01 fmol/mg tissue at PND5 to 3.10 ± 0.28 fmol/mg tissue in the adult, whereas basal (unstimulated) \[^{35}S\]GTP\(_{\gamma}\)S binding increased from 0.12 ± 0.01 to 0.71 ± 0.20 fmol/mg tissue (Fig. 3A). Specific DAMGO-stimulated \[^{35}S\]GTP\(_{\gamma}\)S binding (basal subtracted; Fig. 3B) showed a 20-fold increase (0.13 ± 0.01 to 2.63 ± 0.05 fmol/mg tissue). The period between PND10 and PND15 saw the largest increase in \[^{35}S\]GTP\(_{\gamma}\)S binding with incremental increases occurring at each age measured thereafter. These data were also analyzed as the ratio of specific DAMGO-stimulated \[^{35}S\]GTP\(_{\gamma}\)S binding to \[^{3}H\]DAMGO binding. This ratio represents the relative amount of G_{i/o} protein activated per receptor, a reflection of the G protein coupling efficiency of MORs under our assay conditions. In PND5 rats, the striosomal MOR coupling efficiency was 0.15 ± 0.01 compared with 2.95 ± 0.46 in the adult, a 19-fold increase (Fig. 4).

This same pattern of increasing MOR coupling efficiency during development was also found in striosomes in the medial and caudal regions of the striatum. Striosomal \[^{3}H\]DAMGO binding remained relatively constant from PND5 to adulthood in both the medial and caudal striatum (Table 1). Striosomal DAMGO-stimulated \[^{35}S\]GTP\(_{\gamma}\)S binding levels were comparable in the rostral, medial, and caudal regions of the striatum at PND5 (0.13 ± 0.01, 0.19 ± 0.03, and 0.22 ± 0.05 fmol/mg tissue, respectively). However, greater differences were manifest between these same regions during the later stages of postnatal development. In
In several brain regions, including striosomes, MOR expression reached adult levels early in postnatal development, yet MOR coupling to G proteins (and presumably to their associated signal transduction pathways) did not reach maximum levels until adulthood. These data suggest there is a disparity between MOR expression and receptor activation of G proteins during development. Changes in MOR/G protein coupling seem to be the result of the increased activation of G proteins by MORs rather than changes in either receptor density or agonist affinity for MORs. During postnatal development of the rat brain, DAMGO has been shown to bind with the same affinity to a single high-affinity population of MORs (Spain et al., 1985). DAMGO labels the agonist high-affinity state of MORs, the state that activates G proteins upon agonist stimulation. A previous study has shown a parallel pattern of opioid receptor development in striosomes using the antagonist [3H]naloxone, which would label the total receptor population (Kent et al., 1981). We did not see the drop in MOR density during the second postnatal week found by Kent and colleagues. This may reflect a dif-

Discussion

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Fig. 2. Specific [3H]DAMGO binding in striosomes of rostral striatum in rat brains during postnatal development. Tissue sections harvested at postnatal day 5, 10, 15, 21, and 30 and Ad were incubated with 3 nM [3H]DAMGO in assay buffer (50 mM Tris-HCl, pH 7.45). Naloxone (5 μM) was used to determine nonspecific binding. Data are presented as femtomoles of [3H]DAMGO bound per milligram of tissue and are the means ± S.E.M. of four striosomes from each of four tissue sections from three to four rat brains. In B, each data set is significantly different from the rest (p < 0.05) using analysis of variance followed by the Tukey-Kramer multiple comparisons test except for day 5 versus day 10, day 15 versus day 21, day 21 versus day 30, and day 30 versus Ad.

Fig. 3. [35S]GTPγS binding in striosomes of rostral striatum in rat brains during postnatal development. Tissue sections harvested at postnatal day 5, 10, 15, 21, and 30 and Ad were incubated with [35S]GTPγS in the presence (stimulated) and absence (basal) of 3 μM DAMGO in assay buffer. GTPγS (1 μM) was added to determine nonspecific binding. Data are expressed as femtomoles of [35S]GTPγS bound per milligram of tissue: basal and DAMGO-stimulated (A) and specific (basal subtracted) DAMGO-stimulated (B). Data are the means ± S.E.M. of four striosomes from each of four tissue sections from each of three to four rat brains. In A, each data set is significantly different from the rest (p < 0.01; Student’s t test).
Our findings are in agreement with other reports suggesting disparate coupling of MOR to G proteins during development. Sensitivity of MOR to the GTP analog 5'-azidoanilido[32P]GTP is unaltered by DOR-selective and KOR-selective inhibitors (Sim et al., 1996a).

Several factors could be regulating this maturation of MOR signaling through G proteins. Our data indicate that changes in MOR levels are responsible for little, if any, of the increase in G protein coupling. It is possible that G proteins themselves may regulate MOR signaling during development and a thorough examination of G protein levels, by immunohistochemistry for example, will be necessary to determine this. Our data (Fig. 3A; Table 1) indicate that basal [35S]GTPγS binding increased significantly during development, suggesting increased G protein expression. This increase in G protein levels could contribute, at least in part, to increased coupling between MORs and G proteins. MORs activate multiple members of the family of G proteins, including Gox1, Gox2, Gox3, Gox4, and Gox5 (Chan et al., 1995), and it has been suggested that MORs activate each with varied efficacy and potency. For example, MORs exhibit preferential coupling to Gox5 in SH-SY5Y cells as measured by α-azidoanilido[32P]GTP affinity labeling (Laugwitz et al., 1993). In addition, in Chinese hamster ovary cells, MOR agonists stimulated incorporation of α-azidoanilido[32P]GTP into Gox1 and Gox5 with greater potency than Gox2, whereas the rank order of maximal labeling (efficacy) was Gox5 > Gox4 > Gox3 (Chakrabarti et al., 1995). In addition, MORs fused to Gox1 stimulated [35S]GTPγS binding more efficiently than MORs fused to Gox2 (Massotte et al., 2002). These data suggest that MORs exhibit preferential coupling to different Go subunits. Therefore, it is possible that MOR signal transduction may be altered by the complement of Go subunits available for coupling. In this sense, the G proteins themselves may regulate MOR signal transduction through the developmentally

**TABLE 1**

Developmental changes in MOR levels and function in striosomes of the medial and caudal striatal regions

<table>
<thead>
<tr>
<th>Striatal Region</th>
<th>Postnatal Age</th>
<th>[3H]DAMGO Binding</th>
<th>[3H]GTPγS Binding</th>
<th>Coupling Ratio</th>
<th>Fold Increase in Coupling Ratio (Compared with Day 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medial</strong></td>
<td></td>
<td>[3H]DAMGO Binding</td>
<td>[3H]GTPγS Binding</td>
<td>Coupling Ratio</td>
<td>Fold Increase in Coupling Ratio (Compared with Day 5)</td>
</tr>
<tr>
<td>Day 5</td>
<td>61.6 ± 3.1</td>
<td>0.13 ± 0.02</td>
<td>0.19 ± 0.03</td>
<td>0.32 ± 0.07</td>
<td>1.0</td>
</tr>
<tr>
<td>Day 10</td>
<td>76.7 ± 4.4</td>
<td>0.24 ± 0.02</td>
<td>0.31 ± 0.12</td>
<td>0.39 ± 0.12</td>
<td>1.2</td>
</tr>
<tr>
<td>Day 15</td>
<td>74.4 ± 18</td>
<td>0.47 ± 0.1</td>
<td>0.70 ± 0.31</td>
<td>0.80 ± 0.13</td>
<td>2.5</td>
</tr>
<tr>
<td>Day 21</td>
<td>68.0 ± 2.7</td>
<td>0.59 ± 0.03</td>
<td>1.22 ± 0.27</td>
<td>1.81 ± 0.35</td>
<td>5.7</td>
</tr>
<tr>
<td>Day 30</td>
<td>77.0 ± 7.4</td>
<td>0.61 ± 0.02</td>
<td>1.47 ± 0.06</td>
<td>1.97 ± 0.12</td>
<td>6.2</td>
</tr>
<tr>
<td>Ad</td>
<td>59.1 ± 9.6</td>
<td>0.62 ± 0.04</td>
<td>1.61 ± 0.25</td>
<td>2.34 ± 0.28</td>
<td>7.4</td>
</tr>
<tr>
<td><strong>Caudal</strong></td>
<td></td>
<td>[3H]DAMGO Binding</td>
<td>[3H]GTPγS Binding</td>
<td>Coupling Ratio</td>
<td>Fold Increase in Coupling Ratio (Compared with Day 5)</td>
</tr>
<tr>
<td>Day 5</td>
<td>55.5 ± 4.4</td>
<td>0.16 ± 0.01</td>
<td>0.22 ± 0.05</td>
<td>0.38 ± 0.05</td>
<td>1.0</td>
</tr>
<tr>
<td>Day 10</td>
<td>60.5 ± 7.8</td>
<td>0.27 ± 0.02</td>
<td>0.31 ± 0.13</td>
<td>0.44 ± 0.15</td>
<td>1.1</td>
</tr>
<tr>
<td>Day 15</td>
<td>65.3 ± 12.2</td>
<td>0.48 ± 0.05</td>
<td>0.65 ± 0.08</td>
<td>1.01 ± 0.15</td>
<td>1.1</td>
</tr>
<tr>
<td>Day 21</td>
<td>41.3 ± 1.1</td>
<td>0.50 ± 0.01</td>
<td>0.89 ± 0.22</td>
<td>2.20 ± 0.46</td>
<td>5.7</td>
</tr>
<tr>
<td>Day 30</td>
<td>49.2 ± 1.3</td>
<td>0.66 ± 0.08</td>
<td>1.23 ± 0.19</td>
<td>2.59 ± 0.43</td>
<td>6.8</td>
</tr>
<tr>
<td>Ad</td>
<td>40.6 ± 6.1</td>
<td>0.74 ± 0.2</td>
<td>0.77 ± 0.25</td>
<td>2.01 ± 0.76</td>
<td>5.3</td>
</tr>
</tbody>
</table>
timed and subunit-specific expression of G proteins in specific regions of the CNS. Surprisingly, little has been reported regarding the developmental expression of specific G\(\alpha_i\) subunits in specific anatomical areas. One study measuring G\(\alpha_{16}\) expression in several regions of the rat brain, including hippocampus, thalamus, and the cortex, demonstrated that during postnatal development levels of G\(\alpha_{11}\), G\(\alpha_{12}\), and G\(\alpha_{16}\) increase; G\(\alpha_{12}\) remains unchanged; and G\(\alpha_{16}\) decreases (Ilnyatovych et al., 2002). These data suggest that G\(\alpha_i\) subunit expression changes during development in a subunit-specific manner. Because MORs activate individual G\(\alpha_{16}\) subunits with varying efficiencies, it also will be necessary to determine not only the relative levels of expression of G proteins during development but also the relative contribution of each G\(\alpha_{16}\) subunit to the signal generated by MORs.

The contribution of pertussis toxin-insensitive G proteins, such as G\(\alpha_i\), to the DAMGO-stimulated [\(^{35}\)S]GTP\(\gamma\)S binding is not known. The expression of G\(\alpha_i\) relative to G\(\alpha_{16}\) in the brain suggests that its contribution may be less than that of other G proteins (Friberg et al., 1998), especially in light of the observation that both basal and stimulated [\(^{35}\)S]GTP\(\gamma\)S binding is unaltered in mice lacking G\(\alpha_i\) (Laitinen, 2004).

Additional factors besides G proteins could serve as regulators of MOR/G protein coupling during development. It is well established that regulators of G protein signaling (RGS) proteins negatively regulate GPCR signaling by accelerating the GTPase activity of the G\(\alpha_i\) subunit (Hollinger and Hepler, 2002). Several members of the RGS protein family have been shown to negatively regulate MOR signaling in vitro and in vivo (Potenza et al., 1999; Zachariou et al., 2003). In particular, expression of RGS2 in the rat striatum seems to be highest during the early postnatal period (PND2 and PND10), with a dramatic decrease in expression between PND10 and PND18 (Ingi and Aoki, 2002), a period that roughly parallels the large increases in MOR-stimulated [\(^{35}\)S]GTP\(\gamma\)S binding observed in the rat striatum. Although RGS proteins are obvious and otherwise attractive candidate regulators of G protein action, they are unlikely to explain our current findings due to the hydrolysis-resistant and therefore “RGS-insensitive” nature of GTP\(\gamma\)S.

The nonvisual arrestins regulate GPCR signaling by promoting uncoupling of the receptor from its cognate G proteins and subsequent desensitization (Claing et al., 2002). In the striatum, arrestin 2 (\(\beta\)-arrestin 1) increases throughout postnatal development, whereas arrestin 3 (\(\beta\)-arrestin 2) decreases slightly (Gurevich et al., 2002). These data suggest that the influence of arrestin 2 on MOR signaling, from a purely kinetic standpoint, should increase throughout development, resulting in less rather than more G protein signaling by MORs. Therefore, arrestin 2 is an unlikely mediator of developmental increases in MOR-stimulated G protein activation. Decreasing arrestin 3 expression could enhance G protein activation, although the contribution of the small decrease in arrestin 3 reported by Gurevich and colleagues relative to the near 20-fold increase in MOR/G protein coupling during development is unknown. Thus, the exact identity of the regulator(s) of these phenomena remains unclear.

With the advent of proteomic technology, it is possible to identify protein binding partners for receptors such as the MOR. In many instances, regulators of GPCRs have been identified that alter receptor signaling and, in so doing, provide yet another level of signal transduction regulation (Brady and Limbird, 2002). Several recent reports using yeast two-hybrid methodology describe proteins that functionally interact with MOR, including phospholipase D\(\alpha\) (Koch et al., 2003), filamin A (Onoprishvili et al., 2003), and periplakin (Feng et al., 2003), and that modulate either G
protein signaling or receptor internalization. Proteins such as these may be differentially expressed during development in such a manner as to regulate MOR signal transduction. The developing rat brain seems be an ideal tissue in which to identify such proteins, given the nearly 20-fold difference in activation of G proteins by MORs in neonate versus adult animals. Such studies could yield valuable information regarding the developmental differences in MOR signaling as well as pointing to as yet unidentified regulators of MOR signal transduction.

References


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