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*PERSPECTIVE*

# Constitutive Trafficking—More Than Just Running in Circles?

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**ABSTRACT**

The CB1 cannabinoid receptors are among the most highly expressed G protein coupled receptors (GPCRs) in the brain. Their activation has been associated with a wide range of behaviors, including cognition, pain perception, drug addiction, and memory consolidation, and they have been pharmaceutically targeted for pain therapies, smoking cessation, and appetite control. The CB1 receptor has been challenging to study at the molecular level given the hydrophobic nature of its lipid-based agonists and difficulties expressing the recombinant cDNA in cellular cultures. Early transfection studies in cell cultures revealed predominantly intracellular localization of the CB1 receptor and it was not clear whether this was the "normal" distribution or whether this was some artifact of the cellular model systems. However, studies of the endogenously expressed CB1 receptors using specific antibodies have shown that the there is usually a distinct intracellular, vesicular localization of the CB1 receptor. Confocal microscopy analysis reveals that the intracellular CB1 receptors are localized to vesicles, which implies that the receptors are "trafficking" or undergoing a continuous cycle of internalization and membrane relocalization. The article by McDonald et al. (p. 976) in this issue of *Molecular Pharmacology* addresses whether this constitutive trafficking is related to the activation state of the receptor and whether it plays a role in axonal versus somatodendritic receptor localization.

The canonical life of a GPCR is to reside at the cell surface and, upon activation, to become phosphorylated, desensitized, internalized, and then either degraded or recycled. While internalized, the receptor may also take part in activating signaling cascades such as those first identified for the $\beta_2$-adrenergic receptor and more recently for the angiotensin II 1A receptor (Luttrell et al., 1999; Ahn et al., 2003). It is not clear what benefit might exist for a receptor that is constitutively internalized in the absence of agonist stimulation. However, it is clear that constitutive internalization may have a great impact on receptor function in vivo as evidenced by the point mutation in the conserved DRY motif within the vasopressin receptor, which produces a constitutively internalized and therefore unresponsive and nonfunctional receptor and is an underlying cause of nephrogenic diabetes insipidus (Barak et al., 2001).

The CB1 receptors constitutively internalize in their native state as evidenced by immunocytochemical staining both in cell cultures and in neurons (McIntosh et al., 1998, Pettit et al., 1998). The apparently constant state of receptor trafficking is not unique to cannabinoid receptors; other GPCRs undergo such constant trafficking, including protease activated receptor 1, serotonin 2C receptors, and melanocortin-4 receptor (Paing et al., 2002; Marion et al., 2004; Mohammad et al., 2006). Constitutive endocytosis has been associated with constitutive activation states of the GPCR, and this fits well within the canonical model of GPCR activation-dependent trafficking. Constitutive activation can be demonstrated by use of an inverse agonist, which will further decrease the basal signaling levels of the receptor and, in cases where constitutive activation results in receptor endocytosis, the inverse agonist promotes a redistribution of the receptor to the cellular surface (Barak et al., 2001; Marion et al., 2004). Whether it is due to constitutive activation or some other as-yet unappreciated receptor function, the question remains as to what benefit underlies such an energy-consuming process as that involved in the constant trafficking of a membrane-bound GPCR. In the article by McDonald et al (2007), the constitutive endocytosis, but not the activation state of the receptor, is reported to underlie the highly polarized membrane distribution of the CB1 in axons relative to somatodendritic receptor expression profiles.

Initial studies examining the constitutive activation/endo-

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**ABBREVIATIONS:** AM281, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3-carboxamide.

cytosis profiles of the CB1 receptor were performed in transfected human embryonic kidney 293 cell culture systems (Leterrier et al., 2004). Here they found that the intracellular pool of CB1 receptors was derived from membrane-bound populations that were not representative of newly synthesized receptor pools. An inverse agonist, AM281, promoted the diminishment of intracellular receptor fluorescence and an increase in surface receptor fluorescence intensities, suggesting that the constitutive endocytosis of the receptors was due to constitutive activity of the receptor, in that "reversing" the receptor activation state also reversed the trafficking.

Just as there are technical advantages to studying receptor trafficking in cellular model systems, there are definite limitations as well. Constitutive activity of the CB1 was shown in both transfected CB1 receptors in cell culture systems as well as in neurons (Pertwee, 2005). Leterrier et al. (2006) expanded their studies to examine CB1 receptor trafficking and constitutive activity in cultured hippocampal neurons. They showed that the endogenous CB1 receptor is found in endocytic vesicles in the somatodendritic neuronal regions but is localized to cell surface on axons. Treatment with the AM281 inverse agonist prompted the redistribution from intracellular vesicles to somatodendritic cell membranes, yet did not alter the distribution of axonal CB1 receptors. Using overexpression of dominant-negative trafficking proteins to block endocytotic processes also resulted in a redistribution of the intracellular CB1 receptors to the somatodendritic cell surface, yet, in this case, produced a concomitant decrease in axonal distribution. Kinetic analysis of trafficking suggests that CB1 receptors first transiently appear in the cell body membranes and that the endocytic trafficking may be somehow involved in the ultimate redistribution of the receptors to the axon. Because somatodendritic trafficking is more dynamic relative to axonal receptor trafficking, the inverse agonism that rapidly effects vesicular distributions in the cell body may not affect the overall axonal distributions. According to this model, the dominant-negative inhibitors of endocytosis would chronically block endocytosis, thereby impairing the ultimate distribution of the CB1 receptor from the cell body to the axonal membranes. Taken together, the Leterrier studies propose a model in which a constitutively active CB1 receptor is constitutively internalized from the somatodendritic cell membranes and subsequently trafficked to the axonal membranes, and the process depends upon the constitutive activity of the receptor (Leterrier et al., 2004, 2006).

The current study by McDonald et al. (2007), appearing in this issue of *Molecular Pharmacology*, proposes a different model following similar studies also performed in hippocampal neuronal cultures. The authors report that constitutive endocytosis, independent of the activation state, underlies the domain polarity of CB1 receptor distribution in neurons. In their study, the inverse agonist AM281 fails to prevent receptor endocytosis, and no relocation to the somatodendritic cellular membrane is seen. Transfected mutant CB1 receptors that were previously determined to lack constitutive activity were also found to continue to internalize in the absence of agonist stimulation. As with the previous study, the authors do not detect alterations in axonal receptor distribution after the AM281 treatment. Overexpression of a dominant-negative inhibitor of receptor endocytosis (mutant dynamin K44A) did prevent receptor internalization in the somatodendritic cell membranes, which results in an increase in cell surface expression in the cell body. Compared with axonal expression levels, which did not decrease in the most recent study, the shift in polarity may be influenced primarily by an increase in cell body surface localization over an unchanging axonal population of receptors. Although the earlier study (Leterrier et al., 2006) reports that constitutive receptor activity underlies constitutive internalization, which is an essential step in ultimate axonal receptor distribution, the article presented in this issue suggests that the trafficking is actually independent of the activation state of the receptor.

Ultimately, for the CB1 receptors and for other constitutively endocytosing GPCRs, the purpose served by this constant energy-consuming trafficking is unclear. It is intriguing that the axonal receptor population seems to be spared of this cycling compared with the cell body, and studies on this distinction between the domains may give insight into the molecular components determining the constitutive trafficking in the somatodendritic compartments—regardless of whether such trafficking is due to agonist-independent activation. It is attractive to imagine that there is a purpose behind the movement; otherwise, it would seem to be thermodynamically unfavorable for the cell. However, whether the purpose of constitutive receptor internalization is to remove constitutively activated receptors, transport receptors from one cellular domain to another, or even to engage in a currently unanticipated signaling cascade remains to be determined and will likely require tools that are beyond the current experimental approaches. The CB1 receptors remain a viable target for pharmaceutical therapeutics for a number of conditions, and with antagonist therapies already being developed, the constitutive activity as well as the constitutive endocytosis must be considered although the purpose remains not fully understood.

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