Lipid rafts, G proteins and the etiology of and treatment for depression: progress toward a depression biomarker

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'...rather than a wholesale disruption of caveolae or lipid rafts, some specific lipid raft anchor of $G_{s\alpha}$ is modified in depression or by antidepressant treatment.'

Despite decades of research, the cellular target(s) of chronic antidepressant treatment remain unknown. For the most part, antidepressant treatments, whether drug or direct stimulation, require repeated administration for approximately a month before achieving clinical benefit, while cognitive therapy typically requires 12–16 weeks not including follow-up visits. Many antidepressant drugs are suggested to increase synaptic monoamine content as they prevent the reuptake or breakdown of monoamines by presynaptic nerve terminals. These actions are contemporaneous with acute drug exposure and it is difficult to correlate these actions with the required chronic treatment. We hypothesize that chronic antidepressant drug treatment reorganizes the synaptic membrane and modifies neurotransmitter signaling and that these effects are relevant to the biology and treatment of depression.

Receptors for monoamine neurotransmitters are often G protein-coupled, and those that are coupled to G proteins, activate adenylyl cyclase and generate cAMP. Extensive studies on the role of antidepressant treatment and the $G_{s\alpha}$–adenylyl cyclase signaling cascade have been reviewed previously [1]. Both *in vivo* and *in vitro* studies have demonstrated that chronic, but not acute, antidepressant treatment with a variety of antidepressant compounds leads to an increase in cellular cAMP as well as increased physical coupling between $G_{s\alpha}$ and adenylyl cyclase. Several mechanisms proposed for chronic antidepressant action are consistent with a long-term increase in cAMP production [1]. Closely related to this is the regulation of cAMP response element binding protein (CREB) levels in depression and by antidepressant treatment. A comprehensive review by Blendy indicates that there is evidence for both increased and decreased levels of CREB and pCREB following chronic treatment with certain antidepressants [2], and a later study discovered that CREB-deficient mice display a 'depression-resistant' phenotype [3].

A concurrent area of study has been on the role of antidepressant treatment on hippocampal neurogenesis. This remains controversial since there are studies suggesting both the importance of neurogenesis in antidepressant action and those stating that antidepressants work independently of neurogenesis. A review by Sahay and Hen covers both the neurogenesis-dependent and the neurogenesis-independent mechanisms leading to the behavioral effects seen by chronic treatment with various antidepressants [4]. It should be noted that while several brain regions are involved in both depression and antidepressant responsiveness, neurogenesis is restricted to the hippocampus. This is one of many details that remains to be explained about neurogenesis and the relationship of that phenomenon to antidepressant action.

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The localization of G proteins to specific membrane domains such as caveolae and lipid rafts has generated interest as to how these cholesterol and sphingolipid-rich, detergent-resistant membrane domains modulate G-protein targeting and function [5–9]. A recent study by Allen *et al.* suggests that $G_{s\alpha}$ is targeted to lipid rafts during the process of desensitization and, as such, lipid rafts represent a membrane domain of diminished $G_{s\alpha}$–adenylyl cyclase signaling. Conversely, intact caveolae membrane domains appear essential for signaling via certain $G_{q\alpha}$-coupled receptors [10]. Thus, lipid raft/caveolae membrane domains appear to be important regulatory domains for neurotransmitter signaling (see [11] for a review) and this has direct clinical relevance in that lipid rafts/caveolae have been implicated in a number of diseases of the nervous system [12].

Using a fluorescent version of $G_{s\alpha}$, we demonstrated that $G_{s\alpha}$ is internalized through lipid rafts, and the $G_{s\alpha}$ that is sequestered in lipid rafts is not

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in the pool of $G_{s\alpha}$ that activates adenylyl cyclase [7]. Rybin *et al.* have demonstrated that cholesterol depletion increases adenylyl cyclase signaling in cardiac myocytes [13], and we have recently seen the same phenomenon in C6 glioma cells where cholesterol was modified or where lipid rafts/caveolae were disrupted genetically [Allen *et al.*, Unpublished Data]. This suggests that, rather than a wholesale disruption of caveolae or lipid rafts, some specific lipid raft anchor of $G_{s\alpha}$ is modified in depression or by antidepressant treatment.

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One focus of our research has been on the effects of chronic antidepressant treatment on the localization of $G_{s\alpha}$ to both Triton X-100 (TX-100)-resistant raft-enriched and TX-100-soluble nonraft membrane domains in cultured cells and in the human brain. These studies have demonstrated that $G_{s\alpha}$ is localized to TX-100-resistant membrane domains and the tips of elongated processes of C6 glioma cells, and this localization is altered after chronic antidepressant treatment [14–16]. Similarly, we have observed an altered distribution of $G_{s\alpha}$ in the TX-100-resistant and the TX-100-soluble membrane domains in the prefrontal cortex and cerebellum of depressed suicide subjects compared with control subjects [17]. The distribution of other G proteins, as well as a number of other raft-associated proteins, is not altered in depression or by antidepressant treatment [15,17]. The TX-100-soluble $G_{s\alpha}$ is more likely to interact with adenylyl cyclase because the TX-100-resistant membrane regions serve as inhibitory domains for $G_{s\alpha}$ signaling [7,11] and they are much more rigid cholesterol and sphingolipid-rich membrane structures [18]. It should be noted that antidepressant drugs appear to concentrate in lipid rafts [19].

Dysfunctional cAMP signaling represents a consistent finding in human cerebral cortex tissue from suicide subjects [20–25]. Some of these studies demonstrate a consistent decrease in forskolin-stimulated adenylyl cyclase activity without a change in expression or concentration of $G_{s\alpha}$ protein [20,25], while other studies have demonstrated a small increase in $G_{s\alpha}$ protein and mRNA levels in subjects with major depression [21,23]. Downstream in the cAMP signaling pathway, decreased PKA activity was observed in these same suicide subjects [22,24].

Many studies have employed cells from human blood to model the biochemistry of the brain. Recent studies using human platelets suggest that adenylyl cyclase may be attenuated in depression [26–30]. The most recent of these studies demonstrated markedly lower levels of basal, forskolin-, cesium fluoride- and Gpp(NH)p-stimulated platelet adenylyl cyclase activity in subjects with a history of major depression compared with control subjects [30]. This suggests diminished coupling between $G_{s\alpha}$ and adenylyl cyclase, consistent with our postmortem results. Furthermore, in both depression and response to antidepressants, we observe a redistribution of $G_{s\alpha}$ without any major change in the content of that protein. This suggests that a reordering of membrane domains likely accompanies both depression and chronic antidepressant therapy and is consistent with the extended time of treatment required for therapeutic effectiveness.

Curiously, our findings demonstrated increased lipid raft localization of $G_{s\alpha}$ in the cerebellum in depression. However, recent behavioral, imaging and biochemical evidence suggests that the cerebellum may be involved in the etiology of major depression. The cerebellum has neuronal connections to brainstem nuclei that supply the limbic system and prefrontal cortex with various monoamines, including serotonin and norepinephrine [31]. In fact, subjects with cerebellar lesions have displayed characteristics such as passivity, blunting of emotion and disinhibition of restraint similar to the depressed and manic states characteristic of mood disorders [32]. Furthermore, the cerebellum is rich in the $G_{s\alpha}$-coupled receptors, CRF-1 and -2. The CRF system has been implicated in the relationship between early-life stress and depression [33,34]. Finally, caveolin-1 (the major structural protein component of caveolae) has been identified in the cerebellum of mice, and caveolin-1-knockout mice exhibit neurological abnormalities associated with cortico-striatal, hippocampal and cerebellar regions [35].

'...antidepressants may exert their observed effects on cAMP signaling by liberating $G_{s\alpha}$ from TX-100-resistant membrane domains.'

In summary, previous studies in both rats and C6 glioma cells demonstrated that chronic antidepressant treatment liberates $G_{s\alpha}$, but not $G_{i\alpha}$ or $G_{o\alpha}$, from the inhibitory TX-100-resistant membrane raft domains [14–16], increasing its

association with adenylyl cyclase [16]. Coupled with postmortem human brain data, these observations demonstrate that the increased localization of $G_{s\alpha}$ in the TX-100-resistant membrane domains of depressed individuals may prevent $G_{s\alpha}$ from associating with and activating adenylyl cyclase, thus preventing the propagation of the cAMP signal. These findings are supported by recent evidence suggesting that antidepressant drugs concentrate in raft-like plasma membrane domains [19]. The intercalation of these drugs may physically inhibit the localization of $G_{s\alpha}$ to the raft domains. Thus, it appears that antidepressants may exert their observed effects on cAMP signaling by liberating $G_{s\alpha}$ from TX-100-resistant membrane domains, where it accumulates during the course of depression. Furthermore, the ratio of TX-100-soluble to TX-100-resistant $G_{s\alpha}$ may prove to be a useful biomarker for human depression and a rapid (3–4 days) harbinger of effective response to antidepressant therapy. Finally, revelation of new mechanisms for depression and new targets for antidepressant drugs may lead to the development of novel classes of therapeutic agents.

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