Transducing Emotionality: The Role of Adenylyl Cyclases

Boris Tabakoff and Paula L. Hoffman

Various psychiatric disorders, including depression, anxiety, and other mood disorders, as well as responses to addictive drugs, have been linked to malfunctions of receptors coupled to the cyclic adenosine monophosphate (AMP) second-messenger signaling system and/or malfunction of downstream mediators such as protein kinase A (PKA) and the transcription factor cyclic AMP response element–binding protein. What has generally been largely ignored are the enzymes that generate cyclic AMP, the family of adenylyl cyclases (ACs). There are nine isoforms of membrane-bound adenylyl cyclase, and one soluble isoform of the enzyme (1). The activation of the membrane-bound isoforms is regulated by receptors via heterotrimeric G proteins (e.g., Gsα); however, various isoforms display additional patterns of regulation by Giα, Gβγ, and other factors such as Ca^{2+}, calmodulin, and protein kinases. The AC family has been classified into four categories, based on differential patterns of regulation (e.g., Ca^{2+}/calmodulin stimulation, Gβγ stimulation, Giα inhibition, Ca^{2+} inhibition, etc.). There are also other regulators of specific isoforms of adenylyl cyclase, including RGS2 (regulator of G protein signaling 2); Snapin, a member of the SNAP-25/Snare complex; Ric-8, a guanine nucleotide exchange protein; and the PKA scaffolding protein, AKAP 79 (1). Although the expression patterns (tissue and brain regional localization) of the various AC isoforms play a role in dictating their specificity in affecting physiological processes, clearly the diverse regulatory control of ACs places them in a position to integrate signaling that is initiated by activation of cellular receptors. For instance, the group II ACs, which include AC2, AC4, and AC7, are conditionally activated by Gβγ: when enzyme activity is stimulated through receptor-coupled Gsα, the activity can be enhanced 5- to 10-fold by Gβγ, although Gβγ has no effect alone. Therefore, when Gβγ is released as a result of activation of Gi/Go-coupled receptors, AC2 and AC7 are synergistically stimulated in the presence of Gsα, facilitating cross talk between G protein–coupled receptors. For example, depending on receptor colocalization, activation of D1 (Gsα-coupled) and 5-hydroxytryptamine$_2$ (Giα-coupled) receptors can result in synergistic activation of AC7 via the interaction of Gsα and Gβγ with this isoform, but a similar activation of D1 and 5-hydroxytryptamine$_2$ receptors in a cell expressing AC5 would produce an inhibition of activated AC5 by Giα. AC7 activity is also modulated by phosphorylation via the δ isoform of protein kinase C (2). Overall, the specific regulation of AC isoforms can modulate the levels and possibly cellular localization of cyclic AMP, such that the production of and response to cyclic AMP in a given cell are fine-tuned for modulation of downstream signaling pathways (1).

All of the AC isoforms are expressed in brain, some localized to particular regions, and some more widespread (1). Given their key roles in neurotransmitter signaling, leading to modification of neuronal activity, they have been investigated with respect to psychiatric disorders. The paper by Joeyen-Waldorf *et al.* (3) focuses on the AC7 isoform as a player in the genetic vulnerability to major depression. In this translational study, the authors utilize the animal model of a serotonin transporter knockout mouse, which displays an increased emotionality phenotype as an adult. This phenotype is thought to be influenced by the developmental effect of the lack of the serotonin transporter. Comparison of transcript expression levels in the amygdala that differ both between the knockout mice and wild-type littermates, and between postmortem brain samples of controls and individuals with major depression, identified 28 differentially expressed genes in common. Among these was *ADCY7*, which codes for the AC7 isoform of AC. This gene was of particular interest because of its previous association with a depressive phenotype in mice and humans (4). The authors then went on to use functional magnetic resonance imaging to show that a genetic polymorphism found in the 3′-untranslated region of *ADCY7* in humans, and which is representative of a haplotype capturing variability across the entire gene, is associated with a greater threat-related amygdala reactivity. This difference in function of the human brain was proposed to predispose the individuals with this genetic polymorphism to a subtype of depression and possibly to other mood disorders.

This work focuses attention on the importance of considering polymorphisms that affect the AC signaling molecules as potential genetic mediators of psychiatric disorders. Such polymorphisms may alter the function of particular AC isoforms or, as is likely in the current instance, may affect the expression levels of the ACs, leading to differences in activity that affect the function of neural circuitry. Such polymorphisms add a layer of variation in receptor-initiated, cyclic AMP–mediated cellular signaling cascades that may be able to distinguish individuals with a propensity for mental disorders. In the past, attention has more often been focused on the G protein–coupled receptors themselves. For example, the corticotropin-releasing factor (CRF) receptor has been well studied with respect to a role in anxiety and depression (e.g., [5]). Some variants in the CRF receptor gene have been suggested to interact with environmental factors and contribute to the genetic basis of these disorders. However, it should also be noted that the CRF receptor in the pituitary is coupled to AC7, and mice with genetic manipulations of AC7 display differential responses to stress and alcohol administration (6). Common polymorphisms in human *ADCY7*, influencing AC7 function or expression, are thus also likely to contribute to differential hypothalamic-pituitary-adrenal axis responses in stress-related disorders. It is of interest that female mice with different levels of AC7 in the brain drink more alcohol than wild-type mice and that a polymorphism in human *ADCY7* is also associated with alcohol dependence (7). One implication of such studies is that polymorphisms in *ADCY7* that are associated with anxiety and depression may play a role in alcohol drinking behavior and in comorbidity in certain subtypes of alcohol dependence. It has also been found that AC7 may be located presynaptically in γ-aminobutyric acid neurons in the amygdala and can mediate CRF-stimulated γ-aminobutyric acid release in this brain region (8). One can propose that this pathway may provide a mechanistic explanation of the differences in amygdala threat reactivity that Joeyen-Waldorf *et al.* (3) found to be associated with the *ADCY7* polymorphism.

The study of Joeyen-Waldorf *et al.* (3) illustrates several important issues related to candidate gene identification for complex

From the University of Colorado School of Medicine, Aurora, Colorado.
Address correspondence to Boris Tabakoff, Ph.D., University of Colorado School of Medicine, Aurora, Colorado 80045; E-mail: boris.tabakoff@ucdenver.edu.
Received Jan 31, 2012; accepted Feb 1, 2012.

0006-3223/$36.00
doi:10.1016/j.biopsych.2012.02.001

psychiatric traits. Cross-species analysis of candidate genes provides increased confidence in the role of a particular gene product in a phenotype of interest. Although for psychiatric traits, the phenotypes investigated in humans and animals are not likely to be exactly congruent, it is relevant to consider endophenotypes of the human disease that can be modeled in the experimental animals. The focus on the relevant endophenotype and ACs in this and other studies stresses the potential importance of these integrating signal transducing enzymes. Activation of a particular G protein–coupled receptor generates a rather narrow response. It is the constant integration of the activity status of several receptor types by signal transducing systems such as the ACs that generates the divergent effects of receptor stimulation and regulates the activity of the downstream effectors of cyclic AMP signaling such as PKA or cyclic AMP response element–binding protein.

The potential of the AC genes to display structural variation is well known (i.e., not only single-nucleotide polymorphisms in the coding and noncoding regions of the AC genes, as discussed in the current manuscript, but also splice variants and other deletions or insertions) and can significantly modify the function and/or expression levels of ACs. For example, AC8, which has been shown to be involved in learning and memory, is known to display three splice variants, one of which is missing the exon that controls inhibition of the enzyme by $G\beta\gamma$. The importance of various AC isoforms for psychiatric disorders is becoming more widely recognized (i.e., calcium/calmodulin-stimulated AC8 has also been implicated in stress adaptation and mood disorders [hyperactivity] [9]), and AC5 has been linked to anxiety-related behavior (10). As more is understood about the genetic variation in these enzymes that is associated with particular mental disorders, their potential as targets for therapeutic interventions for affective disorders and drug abuse/addiction may begin to be realized.

This work was supported in part by the Banbury Fund. The authors report no biomedical financial interests or potential conflicts of interest.

1. Sadana R, Dessauer CW (2009): Physiological roles for G protein-regulated adenylyl cyclase isoforms: insights from knockout and overexpression studies. *Neurosignals* 17:5–22.
2. Nelson EJ, Hellevuo K, Yoshimura M, Tabakoff B (2003): Ethanol-induced phosphorylation and potentiation of the activity of type 7 adenylyl cyclase. Involvement of protein kinase C delta. *J Biol Chem* 278:4552–4560.
3. Joeyen-Waldorf J, Nikolova YS, Edgar N, Walsh C, Kota R, Lewis DA, *et al.* (2012): Adenylate cyclase 7 is implicated in the biology of depression and modulation of affective neural circuitry. *Biol Psychiatry* 71:627–632.
4. Hines LM, Hoffman PL, Bhave S, Saba L, Kaiser A, Snell L, *et al.* (2006): A sex-specific role of type VII adenylyl cyclase in depression. *J Neurosci* 26:12609–12619.
5. Hauger RL, Risbrough V, Oakley RH, Olivares-Reyes JA, Dautzenberg FM (2009): Role of CRF receptor signaling in stress vulnerability, anxiety, and depression. *Ann N Y Acad Sci* 1179:120–143.
6. Pronko SP, Saba LM, Hoffman PL, Tabakoff B (2010): Type 7 adenylyl cyclase-mediated hypothalamic-pituitary-adrenal axis responsiveness: influence of ethanol and sex. *J Pharmacol Exp Ther* 334:44–52.
7. Desrivières S, Pronko SP, Lourdusamy A, Ducci F, Hoffman PL, Wodarz N, *et al.* (2011): Sex-specific role for adenylyl cyclase type 7 in alcohol dependence. *Biol Psychiatry* 69:1100–1108.
8. Cruz MT, Bajo M, Maragnoli ME, Tabakoff B, Siggins GR, Roberto M (2011): Type 7 adenylyl cyclase is involved in the ethanol and CRF sensitivity of GABAergic synapses in mouse central amygdala. *Front Neurosci* 4:207.
9. Razzoli M, Andreoli M, Maraia G, Di Francesco C, Arban R (2010): Functional role of Calcium-stimulated adenylyl cyclase 8 in adaptations to psychological stressors in the mouse: implications for mood disorders. *Neuroscience* 170:429–440.
10. Kim KS, Lee KW, Baek IS, Lim CM, Krishnan V, Lee JK, *et al.* (2008): Adenylyl cyclase-5 activity in the nucleus accumbens regulates anxiety-related behavior. *J Neurochem* 107:105–115.