Of Stress and Alcoholism, Of Mice and Men

by John J. Medina, PhD

Several columns ago ("Schizophrenia, DISC1, and Animal Models," *Psychiatric Times,* April 2008, page 22), I earnestly cautioned against the temptation to apply behavioral data from laboratory animals directly to the human experience.

I noted that the human cortex is the size of a baby blanket, whereas the mouse cortex is the size of a postage stamp. I explained that animal research historically works best when it acts as a guiding "flashlight" for human research, illuminating biological processes in which human-based investigations might reasonably succeed. I gave one example of such research that explored the role of the "disrupted in schizophrenia 1" (DISC1) gene in schizophrenia and promised to give more. The subject of this column is a payment on that promise.

This month I will examine the relationship between alcohol use disorder, stress, and a neuropeptide called substance P (SP). The data that led directly to research with human subjects came from the mouse-based genetic manipulation of a gene called neurokinin-1 receptor (NK1R), the receptor for SP. To understand this research thread, I will need to review some basic biology behind a class of biochemicals called tachykinins, of which SP is its most famous member. I begin, however, with an attempt to understand the relationship between the experience of stress, relapse rates in alcohol-dependent populations, and how mouse research ended up helping a cohort of stressed-out patients.

Stress, relapse, and alcohol dependence

Of the many frustrating aspects of treating patients with alcohol dependence, the uncomfortably high relapse rate must rank highest on the list. There are many reasons for relapse, which can roughly be categorized into extrinsic and intrinsic trigger points. One of the best-characterized extrinsic triggers of relapse is environmental alcohol-associated cues. One of the best characterized intrinsic triggers is the patient's experience of stress—especially if the patient is in a stress-susceptible population.

The relationship between stress and alcohol dependence has been studied extensively in animal models. Increases in alcohol consumption due to laboratory-induced alcohol dependence are always accompanied by an increased sensitivity to environmental stressors. The relationship has been characterized biochemically. Alcohol dependence can induce changes in the extrahypothalamic regions of the brain by a specific upregulation of corticotropin-releasing hormone (CRH). The hypothalamus synthesizes many neuroactive molecules in response to aversive stimuli, including vasopressin and CRH. CRH is sent to the pituitary, activating downstream signals that eventually result in the arousal of the hypothalamic-pituitary-adrenal axis.

Of the many frustrating aspects of treating patients with alcohol dependence, the uncomfortably high relapse rate must rank highest on the list.

The relationship between stress and alcohol dependence is so strong that the neural systems mediating stress have been investigated as possible pharmacological targets. One promising lead involves the biochemistry behind SP, the neurotransmitter known to be involved in stress responses and drug reward. Another lead involves the investigation of the role the insular cortex (insula) plays in mediating these associating behaviors. The insula is a region that lies just beneath the central sulcus, under the operculi. This region has been shown to be involved in the subjective experience of cravings of many kinds and has been recently shown to b e involved in the maintenance of addictive behaviors. Research in these systems, including ligand-receptor interactions and activation of the insula, form the bulk of this column.

Tachykinins

SP is the most important member of a family of excitatory neuropeptides collectively called tachykinins (**Figure**). Synthesized by glial and neuronal cells in the central and peripheral nervous system, SP binds to the receptor NK1R. Together, both ligand and receptor are expressed in the hypothalamus, amygdala, and nucleus accumbens. That expression profile turns out to be important because these tissues are not only involved in mediating stress but they are also involved in mediating many rewarding responses related to addiction-forming behaviors.

Studies have shown just how powerful a role this system plays in stress responses—at least in mice. The researchers employed a technique known as genetic knockouts, a genetic engineering technology that deletes a specific gene early in development but does not otherwise impede normal growth. Mice can be created that are either heterozygous for the knocked-out gene (only one of the pair are affected) or homozygous. If the gene for the SP receptor is knocked out in mice, their responses to stress are greatly diminished. These data were confirmed with the development and administration of specific antagonists to the receptor (pharmacologically blocking the ability of SP to exert its effects on nerve cells). Using genetically unmodified animals, the same stress-dampening behaviors were observed when compared with controls.

Clearly, the responses to stress were being affected by interfering with SP-mediated biochemistries. What about rewarding behaviors in the genetically modified animals? Consistent with SP's job description, these knockout mice demonstrated noticeably reduced rewarding behaviors in response to various drugs. The mice exhibited greatly inhibited response curves in opiate self-administration tests, for example. They also demonstrated a loss of conditioned place preference for opiates. Not only were these animals not responding to aversive stimuli, they were also not responding to normal rewarding cues.

Do these data indicate anything about alcohol-related behaviors? To conduct these experiments thoroughly, one would need to induce—or at least attempt to induce—alcohol-dependent behaviors in the genetically modified mice. There are established protocols for creating such behaviors in wild-type animals. What would happen if these wild types were compared with the genetically manipulated mice in standard alcohol-administration experiments?

The short answer is that the altered mice did not behave at all like the unmodified controls. Although the knockout mice had constant availability to alcohol, as did the controls, they displayed remarkably lower consumption levels. This diminution was shown even under conditions in which the concentrations of alcohol were high (3% to 15%). In these conditions, the pharmacological effects of ingesting alcohol typically trump other consumptive motivations, such as its taste. The animals were not buying it. They never established an alcohol dependency.

Interestingly, these reduced behaviors only showed up in the presence of the homozygous knockouts and not in the heterozygous group. If just one receptor gene was still active, the alcohol-associated behaviors typically observed in the wild-type animals returned with a vengeance in the heterozygous knockouts. Not only did these results serve as a convenient internal control, they also showed the great responsiveness of the system to alcohol.

What about humans?

The most obvious question to be answered relates to human behaviors: What role might the SP-NK1R system play in alcohol dependency in people? You cannot perform knockout experiments in humans, but there are other ways of assessing NK1R's role in people. There is an NK1R antagonist that functions as if it were

(Please see Stress and Alcoholism, *page 20)*

Stress and Alcoholism

Continued from page 18

dropped from research heaven, and it goes by the tongue-twisting name of LY686017. This antagonist is brain-penetrant, well tolerated, orally available, and, most fortunate of all, displays a high affinity for human NK1R. Several of these antagonists have been developed, including GR205171.

Since the experimental question really revolved around stress and alcoholism, it was important to establish the effects of these antagonists on stress responses in typical human populations. Sure enough, administration of GR205171 was shown to reduce social anxiety. It also inhibited measurable brain responses to social stressors, as measured by the Trier Social Stress Test (TSST).

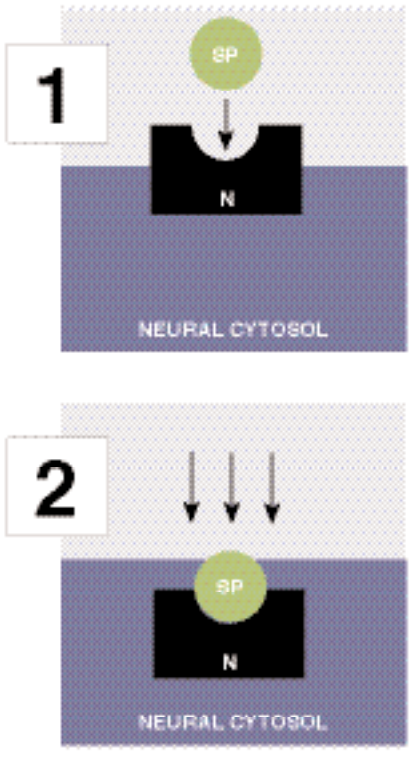
But what about the effects in patients with alcohol dependency, especially in those with high-stress backgrounds? A group of researchers from academic and industry-related laboratories decided to find out. Enrollees in the experimental group (n = 25) had to have 3 measurable traits. First, they must have received a formal diagnosis of alcoholism and to have presented with alcohol-related problems as the primary complaint. Second, they had to score above 39 on the Spielberger Trait Anxiety Inventory, a measure that reliably detects the overt presence of anxiety and stress. Third, they had to have ingested alcohol within the past 30 days. All patients were hospitalized throughout the study; some were treated with withdrawal therapies if needed before experimental administration.

Using a combination of behavioral tests in the presence of the antagonists and even a little brain imaging, the re-

Figure

Tachykinin receptors and their antagonists

Substance P (SP) is the archetypal tachykinin, a family of neuroactive peptides that mediate a wide variety of processes in neural tissues. SP exerts its biological effects by binding to the tachykinin receptor neurokinin-1 (NK1R) in the multistep process shown below. These effects can be blocked by the administration of well-tolerated, high-receptor affinity antagonists such as LY686017.



Receptor binding

As an endogenous ligand, SP binds to NK1R (N). Although drawn as a simple rectangle, the actual receptor is a serpentine, heterotrimeric G-coupled receptor consisting of 3 transmembrane domains.

Internalization

Via a process known as endocytosis, the cell in which the binding occurs engulfs the occupied receptor. The molecules are thus internalized into the cytosolic compartment of the nerve cell.

Signal transduction

Once the receptor is internalized, a cascade of internal signaling processes is initiated in the nerve cell, which results in the activation of specific biochemical signals. Depending on the neural (and tissue) type, these signals can range from cell-growth stimulation to excitatory neurotransmission processes. Most relevant to the mental health community, this binding has been shown to mediate depression-related behavior and feelings of anxiety.

Antagonist mechanism

The administration of specific SP antagonists (A) has been shown to destabilize the SP-N complex inside the nerve cell. The result is that the complex cannot be maintained within the cytosol, and its intracellular effects are blunted. SP no longer functions normally.

searchers hit pay dirt. First, patients who had received LY686017 showed remarkably suppressed alcohol cravings. This was measured by the Alcohol Urge Questionnaire, which was given to each patient, and by the Clinical Global Impression Scale, a weekly inventory that was given to an impartial observer and shown to be a reliable indicator of patient behavior. As expected, the cravings declined over time.

Second, the researchers subjected the patients to a simulated real-life challenge using cues associated with high-relapse probabilities. The investigators used the TSST and an alcohol-cue challenge protocol. Patients who were treated with LY686017 showed reduced craving response, even in these more robustly tempting real-world situations, which was consistent with the results of the previous inventories. Treated patients also showed an inhibited cortisol response to the challenge compared with controls. Clearly, LY686017 had an effect in these higher-risk situations. It also had effects on stress.

Third, these patients were also assessed using functional MRI during craving experiences. The idea was to allow researchers to evaluate what was occurring in the brains of the treated patients—particularly within the insula—following a presentation of negative and positive emotional stimuli. This was done by visually presenting the patient with pictures in the International Affective Picture System (a standardized visual cue presentation), mixed with pictures of nonalcoholic and alcohol-related beverages, during imaging.

Once again, positive results were obtained in the LY686017-treated patients. They exhibited much less activation to the negative images in brain regions associated with emotional responses to visual stimuli (such as ventral putamen, caudate nucleus, and several temporal areas). The most dramatic difference occurred in the activation of the insula, however. Treated groups showed markedly suppressed blood-oxygen-level–dependent signals compared with controls. Given the role the insula plays in addictive behaviors, this is an intriguing finding. Curiously, the group also demonstrated greater brain activation to the positive images in these same experiments.

Something interesting was clearly happening in patients whose NK1R responses had been blocked. In a word, at least in the short-term, they were getting better.

Conclusions

There are great reasons to be excited about these data. For the first time, the manipulation of a ligand-receptor system that is involved in mediating rewarding behaviors and stress has been shown to greatly reduce cravings for alcohol. These data were demonstrated in the most important and arguably toughest populations—stressed persons in the throes of alcohol addiction.

Among other things, the results suggest powerful new directions in the manufacture of medications that are capable of ameliorating the consumptive aspects of alcohol dependencies. Neural processes involved in behavioral stress might make terrific targets for pharmacological-based interventions and address the issue near its behavioral headwaters—alcoholic cravings.

There are, of course, cautions to be made in the interpretation of these data, including some potential genetic confounders. Some people are born with genetic backgrounds that make them potentially more sensitive to stress. There are also people born with predilections toward addictions of many kinds, including alcoholism. How these genetic backgrounds are related to each other and what that relationship has to do with the NK1R-blocking studies is not known. Indeed, alcohol dependence itself can certainly produce stress, but if you are more stress-sensitive, does that add an additional variable to these data?

The typical nature/nurture and chicken-and-egg issues need to be addressed in future experiments. Stratifying individuals as to the types of stress they experience and doing so with larger trials throughout will be required to definitively establish the relationship between NK1R, stress, and alcohol dependency.

These cautions do nothing to dampen the power of the findings or the enthusiasm for future experiments. The data illuminate the powerful role continued research with animals has in the study of behavioral issues of importance to mental health professionals who are interested in human motivations. Because of the mice data, there are now clear links between gene, cell, and behavior in this otherwise murky world of alcoholic relapse and stress in humans. Such robust findings are relatively rare in the never-ending quest to characterize the distance between genes and behaviors. Whatever else the data present, you have to admit that these tiny little mammals make quite a flashlight.

Dr Medina is a developmental molecular biologist and private consultant, with research

Book Review