

Ankit Shah and Muhammad Shahid, two Rutgers seniors headed to medical school next year, have more in common than their cell biology and neuroscience major.

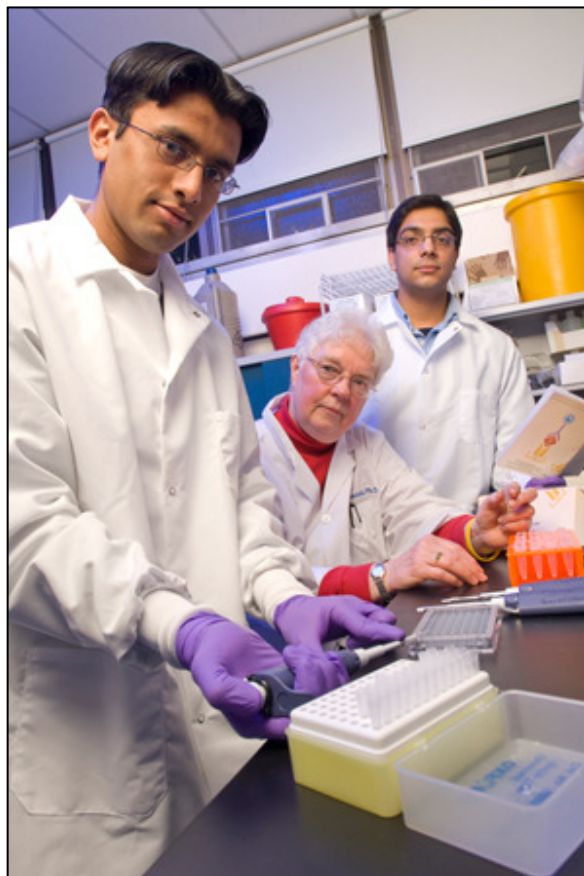
Both have been conducting research at the Center of Alcohol Studies for the past three years on osteocalcin, a protein synthesized in bone, and its relationship to the body's response to stress. With Assistant Research Professor Patricia Buckendahl, the two have worked with "knockout" mice, lab specimens in which the gene that regulates the production of osteocalcin has been deleted. Their projects were funded in part by the Aresty Research Center for Undergraduates.

Jersey City native Shah injected the knockout mice and a control group with alcohol. "The reason we injected mice with ethanol prior to stressing them was to see if the alcohol could relieve some of the symptoms of stress typically seen in humans and animals. Particularly, we wanted to see if the interaction of alcohol and the absence of osteocalcin could further relieve the symptom of stress," Ankit said.

He then subjected both groups to stress by restraining them for two hours. The knockout mice showed changes that would lead to lower production of epinephrine and norepinephrine, two hormones that help the body cope with stress. Shah concluded that "apparently, the absence of osteocalcin protein affects the ability to respond to stress." Since the protein is made in bone, it is possible that "leading a stressful life can lead to brittle bones."

Shahid, a Plainsboro resident who said he came to Rutgers because of the extensive research opportunities available, also administered stress to mice that were missing osteocalcin and found decreased levels of stress-coping hormones. He then injected the same mice with osteocalcin and discovered that replacing the protein reversed the changes observed by Shah, supporting Shah's finding that osteocalcin is tied to the stress response.

Buckendahl, the principal investigator on both projects, has seven undergraduates working in her lab, all doing related work on alcohol consumption and osteocalcin. "I don't have any graduate students, I



don't have any lab technicians. It's just me and these outstanding undergraduates," Buckendahl said. "Somebody took the time to head me in the direction of research, and now it's payback time. I'm very dedicated to the concept of undergraduates in research."

Ankit Shah's research abstract:

"Alcohol Consumption and Stress Response in the Absence of Osteocalcin"

Introduction

Osteocalcin (OC), a Gla-containing extracellular calcium-binding protein, is synthesized almost exclusively by osteoblasts. Although most OC is deposited with mineral during bone formation, a consistent amount is released directly to circulation (pOC). pOC is influenced not only by osteoblastic activity, but also by stimuli that increase stress-responsive hormones, including glucocorticoids (GC) and catecholamines. Ethanol (EtOH) consumption is often increased following stressful experiences. Stress hormone elevation as well as EtOH consumption may alter synthesis and secretion of OC.

For the present experiments, we compared (1) EtOH consumption and (2) stress response in wild-type C57BL/6 mice (WT), heterozygous (Het), and OC null mutants (KO) bred by crossing KO males donated by Drs. Karsenty and Ducy, Baylor Medical Center⁶, with wild-type C57BL/6 females. F2 progeny of the resulting Het mice have been used for these experiments. Note that KO mice have no OC in plasma or bone.

We evaluated EtOH preference for and consumption of increasing concentrations of ethanol from 2 to 13 or 15% EtOH, w/v, in two studies. The first was under mildly stressful conditions while the second served as a control setting. From these mice, we harvested adrenals for determination of EtOH effects on the catecholamine synthetic pathway, namely TH, dopamine beta-hydroxylase (DBH, synthesizes norepinephrine from dopamine), and phenylethanolamine N-methyl transferase (PNMT, synthesizes epinephrine from norepinephrine). TH is the rate limiting enzyme of this pathway. We hypothesize that OC might exert feedback control of EtOH consumption.

Two stress studies were conducted to evaluate differences in the activation of catecholamine synthesizing pathways in the presence or absence of OC. Mice were restrained for two hours and killed immediately (Stress Experiment One) or three hours after release (Stress Experiment Two).

Results and Conclusion

KO mice had significantly greater preference for and consumption of EtOH during Study 1 where they were stressed. Study 2 showed no significant differences in consumption between the two genotypes until 15% EtOH was reached.

The elevated level of adrenomedullary gene expression in KO in study 2 confirms previous findings that EtOH is a stressful stimulus.

Catecholamine synthetic pathways were attenuated in the absence of OC as seen in two sets of EtOH consumption and WMR experiments.

Stress significantly increased DBH gene expression in both genotypes, but was attenuated in KO mice relative to WT. Gene expression of PNMT followed similar patterns. Non-stressed controls did not differ.

Accumulated data suggest that response to a wide variety of very different stimuli (restraint, EtOH consumption as well as touch, light level, heat) is altered in the absence of OC. This appears to be associated with decreased sympathoadrenal function.

Photograph by Nick Romanenko