New Evidence for Causal Central Mechanism of Hyperglycemia in Subarachnoid Hemorrhage Secondary to Ischemic Degenerative Disruption of Circuitry Among Insular Cortex, Nodose Ganglion, and Pancreas: Experimental Study

Mehmet Dumlu Aydın[1], Ayhan Kanat[2], Nazan Aydın[3], Abdulmecit Kantarci[4], Muhammet Ali Ayvaz[5], Halil Rakici[5], Coskun Yolas[6], Umit Kepoglu[7], Elif Demirci[8]

■ INTRODUCTION: **Although hyperglycemia is a serious complication of subarachnoid hemorrhage, its pathophysiologic mechanism based on neural circuitry has not been known.**

■ MATERIALS AND METHODS: **Twenty-five rabbits were divided into 4 groups, with 5 in the control group. The SHAM and study groups received 1 mL saline and 1 mL autologous arterial blood into the sylvian cisterna, respectively. Blood glucose values (BGVs) of all animals were recorded 3 times weekly. After 2 weeks, animals were decapitated. BGVs, the number of normal and degenerated neuron densities (DNDs) of insular cortex (IC), and nodose ganglia, degenerated islands of Reil's surfaces values, were estimated by stereologically and analyzed statistically.**

■ RESULTS: **The mean blood glucose values were measured as 101 $\pm$ 10 mg/dL in the control group (n = 5), 114 $\pm$ 11 mg/dL in the SHAM group (n = 5), and 137 $\pm$ 12 mg/dL in the subarachnoid hemorrhage (SAH) group (n = 15). The DND of the nodose ganglion was 10 $\pm$ 3/mm^3 in the control group, while it was 45 $\pm$ 7/mm^3 in the SHAM group and 1688 $\pm$ 191/mm^3 in the SAH group. The DND of the IC was 65 $\pm$ 12/mm^3 in the control group, 689 $\pm$ 112/mm^3 in the SHAM group, and 3709 $\pm$ 643/mm^3 in the SAH group. In addition, the proportion of degenerated surface areas in the islet of Langerhans was 0.3% in the control group, 6% in the SHAM group, and 28% in the SAH group.**

■ CONCLUSION: **There is an important linear relationship among the blood glucose levels, DND of the IC, and nodose ganglia and degenerated surface areas of IL following experimentally induced sylvian SAH.**

INTRODUCTION

Subarachnoid hemorrhage (SAH) remains an area of tremendous academic interest, and neurosurgeons have, and continue to, push the envelope in research within this field[1] because it is one of the most important neurosurgical diseases.[2] Neurosurgery has gone through moments of great renewal in recent decades.[3] At present, neurosurgical practice is confronted by an explosion of technology.[4,5] Despite the increased use of technology in neurosurgical practice,[6] currently, the techniques and methods of neurosurgery are at a high level.[7-9] SAH is still a potentially devastating condition[10-13] that confers high rates of morbidity and mortality.[14] During the past 3 decades, neuroscientists have gained an improved understanding of the pathophysiologic events that occur after traumatic brain injury, stroke, and subarachnoid hemorrhage.[15] Despite major improvements in surgical techniques for aneurysmal SAH, 30-day mortality from SAH has been shown to have changed little from what it was 40 years ago.[16] A better understanding of the pathophysiology of hydrocephalus after SAH would lead to better patient outcomes.[17] Understanding of the pathology after SAH continues to evolve.[18]

Key words
- Hyperglycemia
- Insular cortex
- Nodose ganglion and pancreas
- Subarachnoid hemorrhage

Abbreviations and Acronyms
DND: Degenerated neuron density
IC: Insular cortex
MRI: Magnetic resonance imaging
NG: Nodose ganglion
SAH: Subarachnoid hemorrhage
IL: islet of Langerhans

From the Departments of [1]Neurosurgery, [4]Radiology, and [8]Pathology, Medical Faculty, Ataturk University, Erzurum; [6]Department of Neurosurgery, Erzurum Regional Research Training Hospital, Erzurum; Departments of [2]Neurosurgery and [5]Gastroenterology, Recep Tayyip Erdogan University, Medical Faculty, Rize; [3]Department of Psychiatry, Bakirkoy Mental Diseases Education Hospital, Istanbul; and [7]Department of Neurosurgery, Bahcesehir University Faculty of Medicine, Istanbul, Turkey

To whom correspondence should be addressed: Ayhan Kanat, M.D.; Mehmet Dumlu Aydin, M.D. [E-mail: ayhankanat@yahoo.com; nmda11@hotmail.com]

Citation: World Neurosurg. (2017) 106:570-577.
http://dx.doi.org/10.1016/j.wneu.2017.06.176

Journal homepage: www.WORLDNEUROSURGERY.org

Available online: www.sciencedirect.com

What Is the Problem? What Is the Question?

Stress-induced hyperglycemia after acute cerebrovascular disease is common and is associated with adverse clinical outcomes.[19] The proposed mechanism for such glycemic derangement is humoral activation including catecholamine release that alters homeostasis.[19] Although a cortical site responsible for governing glucose homeostasis is presumed to exist, a specific location has never been identified.[20,21] Acute poststroke hyperglycemia has been associated with larger infarct volumes, especially insular cortex (IC) location.[21] SAH can trigger hyperglycemia.[22] Persistent hyperglycemia after SAH was associated with the development of symptomatic vasospasm.[23] A better understanding, identification, and management of modifiable risk factors for SAH are pivotal to reducing its incidence.[24] The presence of hyperglycemia following SAH, regardless of diabetes status, may be associated with increased mortality and morbidity. The rodent gustatory cortex is located in the anterior part of the IC, which is near the dorsal part of the rhinal vein and intersection of the anterior and posterior regions of the middle cerebral artery.[25] Hyperglycemia is common after stroke in diabetic and nondiabetic patients. Ischemic lesions of the vagus nerve nuclei are associated with worse early poststroke glycemic control than stroke in other locations.[26] Increased parasympathetic vagal input to the pancreas contributes to hyperinsulinemia.[27] Vagal nerve injury may also cause insulin secretion deficiency.[28] Brainstem vagovagal neurocircuits modulate insulin secretion.[29] Pancreatic vagovagal reflexes modulate blood glucose levels by insulin secretion.[30] The dorsal motor nucleus of the vagus is involved in regulation of pancreatic exocrine secretion. IC modulates vagal nerve functions[31] and is also a site with autonomic efferent projections.[32] Projection of the vagal afferents to the anterior IC has been detected in rats. Stimulation and damage to the insula have been shown to clinically and experimentally have a number of autonomic effects, including an elevation of sympathoadrenal tone and sudden cardiac death.[21] Hepatic vagal afferent nerves stimulate insulin secretion, and these nerves may be blocked by degenerative ganglion injury of vagus and IC following SAH. Therefore injury of the insula following SAH may be a good model to show the central mechanism of hyperglycemia. SAH may result in hyperglycemia in the context of sympathoadrenal dysregulation. In this experimental study, we tested the hypothesis that acute SAH-related injury of the IC may lead to hyperglycemia.

MATERIALS AND METHODS

Experiment

Twenty-five hybrid rabbits were used in this study. Animal husbandry and the study design followed the guidelines of the National Institutes of Health. The study design was approved by the Committee on Animal Research of Ataturk University. Blood glucose values of all animals were recorded before and continuation of the experimental procedures in 3 times a week. Blood samples (2 mL) were collected in a heparinized syringe and then measured a pH meter (Mettler Toledo MP 220 pH Meter, Schwarzenbach, Switzerland). Five rabbits (control group; $n = 5$) were used to determine the normal structure of the IC, vagal nerve roots, and nodose ganglia and pancreatic tissues. The remaining animals ($n = 20$) were anesthetized by subcutaneous injection of a mixture of ketamine hydrochloride (25 mg/kg), lidocaine hydrochloride (15 mg/kg), and acepromazine (1 mg/kg). After preparing the left temporal region, 5 rabbits received a 1 mL saline injection into the left sylvian cisterna for the SHAM group ($n = 5$). SAH was produced by the injection of 0.5 mL arterial blood into the sylvian cisterna taken from auricular arteries. All animals were followed up 2 weeks using the normal laboratory standards without treatment, and all of them were sacrificed at the end of the experiment.

Tissue Processing

The brains, vagal nerve root complexes, and pancreases of animals were removed and preserved in 10% formalin solution for 7 days. The specimens were embedded in paraffin blocks, and consecutive 20 sections of 5 μm of all preparations were taken for the stereologic examinations. ICs, nodose ganglia, and pancreatic tissue preparations were stained with hematoxylin-eosin (H&E) and transferase uridyl nick end labeling (TUNEL) methods.

Histologic Procedure

All preparations were analyzed by stereologic and Cavalieri methods as described previously,[33,34] and obtaining data analyzed by statistical methods. Histopathologically, cytoplasmic condensation, nuclear shrinking, cellular angulations, and pericytoplasmic halo formation secondary to cytoplasmic regression and TUNEL staining positivity were considered as the criteria of neuronal degeneration. The physical dissector method was used to evaluate the number of neurons in nodose ganglions (NGs) and IC. Data were obtained from dissector pairs, consisting of parallel sections taken at known intervals. Two labeled consecutive sections obtained from tissue samples (dissector pairs) were mounted on each slide. Twenty dissector pairs were taken in each block for analysis of neurons. A counting frame was placed on consecutive section photographs on the screen of a personal computer to count neurons. The bottom and left-hand edges of the frame were excluded for counting (exclusion) lines together with the extension lines. Other boundaries of the frame and top-right corner were considered to be inclusion points, and any particle that hit these lines or was located inside the frame counted as a dissector particle. Neurons of NG and IC were counted if they were visible in the reference section. Reference and look-up sections were reversed in order to double the number of dissector pairs without taking new sections. The average numeric density of ganglial neurons (NvGN) per mm^3 was estimated using the following formula.

$$NvGN = \sum QN/txA$$

Where ΣQN is the total number of counted neurons appearing only in the reference sections; t is the section thickness, and A is the area of the counting frame. Cavalieri volume estimation method was used to obtain the total number of neurons in each specimens. The total number of neurons was calculated by multiplication of the volume (mm^3), and the numeric density of neurons in each NG and IC was counted by stereologic methods. The relationship between the mean blood glucose values and degenerated neuron densities of the NG and IC was analyzed statistically. The Mann-Whitney U test was used to