Research Submission

The Relation of Brain Ouabain-Like Compounds and Idiopathic Intracranial Hypertension

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Objectives.—To determine the relationship between levels of ouabain-like compounds (OLC) in the cerebrospinal fluid (CSF) and the occurence of idiopathic intracranial hypertension (IIH).

Background.—OLC are naturally occurring inhibitors of the sodium-potassium ATPase that are found in the CSF of mammals. Since the production of CSF is dependent upon sodium-potassium ATPase activity, and since there is evidence that the increased intracranial pressure found in the condition of IIH may be the result of increased CSF production, we hypothesized that the level of endogenous OLC would be reduced in the CSF of patients with IIH.

Methods.—CSF samples were obtained from n = 7 patients with IIH and n = 31 patients with neurological disorders other than IIH ("control" patients) who had lumbar puncture as part of their routine evaluation or treatment. The concentration of OLC in the CSF samples was measured using an established ELISA.

Results.—Patients with IIH exhibited a concentration of OLC in the CSF of 0.11 ± 0.03 ng/mL. In comparison, the concentration of OLC in CSF samples from non-IIH control patients was 0.12 ± 0.01 ng/mL. These values were not statistically different when compared with a *t*-test ($P = 0.31$). However, the concentration of OLC did negatively correlate to the opening pressure on lumbar puncture, but only in the IIH group ($r = -0.80$, $P = .03$). Furthermore, IIH patients who were newly diagnosed or who were unsuccessfully treated (n = 5 of 7 IIH patients) exhibited OLC concentrations of 0.06 ± 0.1 ng/mL, which is nearly lower than that of the control group ($P = .06$).

Conclusions.—The average concentration of OLC in IIH patients (treated and untreated) is unlikely to be distinguishable from that in non-IIH control patients with other neurological conditions. However, the concentration of OLC may be inversely related to the intracranial pressure in patients with IIH, and it may prove to be lower in the subgroup of untreated and unsuccessfully treated IIH patients.

Key words: pseudotumor cerebri, ouabain, cerebrospinal, intracranial pressure

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Idiopathic intracranial hypertension (IIH) is a disorder of elevated intracranial pressure with no identifiable cause. Symptomatically, IIH most commonly causes headache and transient visual obscurations, but it is not a benign condition since it may lead to blindness or other vision loss (ie, enlargement of the blind spot, constriction of the visual fields).[1] IIH typically develops in obese young women. Other risk factors for IIH include the use of glucocorticoids, menstrual irregularities, and pregnancy.

Such observations would suggest that abnormal steroid activity may be important in the development of IIH. However, a mechanistic link between abnormal steroid activity and IIH has not been substantiated and most endocrine evaluations of IIH patients have been unrevealing.[2] Knowing that the brain is itself a steroid-producing organ[3] some studies have even gone so far as to evaluate steroid levels within the CSF,[4,5] although no consistent relationship with IIH was demonstrated through those efforts.

One steroid found in the brain that may have a clear connection to CSF fluid dynamics is ouabain and its related compounds. Ouabain is a naturally occurring plant steroid and ouabain-like compounds (OLC) are found in mammalian tissues including brain,[6] where they are likely produced.[7] OLC, and compounds similar to them such as the drug digoxin, potently inhibit the activity of the sodium-potassium ATPase. Abnormal inhibition of the sodium-potassium ATPase by endogenous OLC has been implicated in several medical disorders including hypertension,[8] congestive heart failure,[9] and cerebral artery vasospasm following subarachnoid hemorrhage.[10]

Since sodium-potassium ATPase activity is known to be the limiting factor in the production of CSF,[11] endogenous OLC may serve to naturally inhibit the production of CSF. Conversely, loss of endogenous OLC may allow increased production of CSF, which would then increase the pressure within the brain. As a demonstration of this relationship, administration of ouabain into the brains of experimental animals has been shown to reduce CSF sodium concentration[12] and CSF production.[11,13] Furthermore, digoxin historically had been used by some as a treatment for IIH prior to the advent of carbonic anhydrase inhibitors, though its efficacy was never well substantiated.[20,21]

Table 1.—The Diagnosis of Non-IIH "Control" Patients

Diagnosis	Number of Patients
Brain tumor	6
Demyelinating disease	4
Meningitis	4
Migraine	3
Subarachnoid hemorrhage	3
Dementia or delirium	3
Fever of unknown origin	2
Autoimmune disease	2
Seizure	2
Vasculitis	1
Shunt failure	1

We hypothesized that in IIH patients the concentration of OLC in CSF would be lower than in patients with other neurological diseases. We report here the results of a correlational study testing this hypothesis.

METHODS

This protocol was approved by the Institutional Review Boards of the Northwestern Memorial Hospital and the Detroit Medical Center. Patients were enrolled into the study from the neurology and ophthalmology clinics and neurology in-patient services after providing informed consent. The only exclusion criteria for this study was the use of digoxin, which can potentially interfere with the ouabain-like compound assay, although no such exclusion was necessary. Patients with a new diagnosis or with an established diagnosis of IIH were included.

We analyzed the CSF of IIH patients for the level of endogenous OLC. CSF was withdrawn by routine lumbar puncture with the patient in the lateral reclined position. Opening pressure was measured at the time of lumbar puncture and was obtained in all patients. Routine laboratory tests (ie, cell counts, protein, and glucose levels) were performed on all CSF samples, and additional tests were performed at the discretion of the treating neurologist in a manner appropriate to the patient's condition. Management of the patient's medical condition(s) was not affected by enrollment into the study.

The concentration of OLC in IIH patients was compared against those from patients with other types
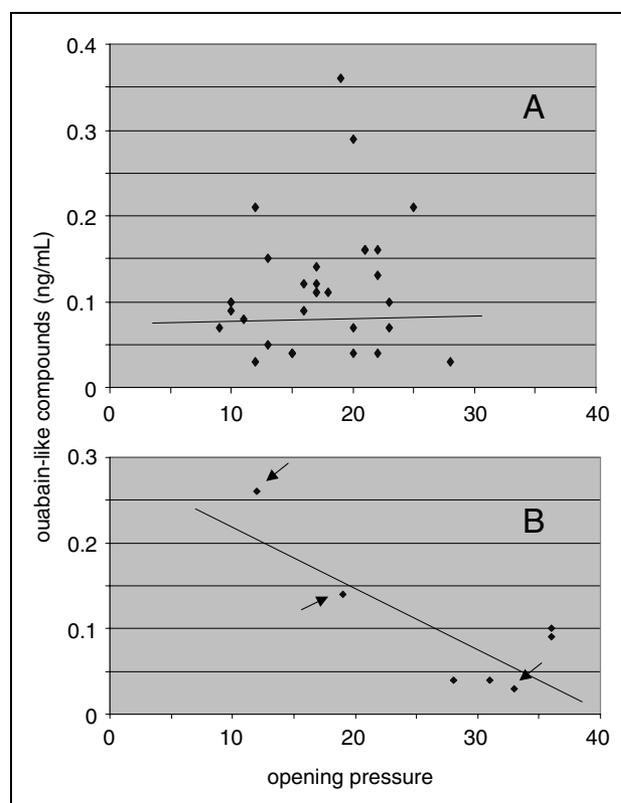
Figure.—Relationship of opening pressure on lumbar puncture with cerebrospinal fluid ouabain-like compound (OLC) levels in (A) control patients and (B) patients with idiopathic intracranial hypertension (IIH). IIH patients being treated with acetazolamide prior to their lumbar puncture are noted with arrows.

of neurological disease who were also subject to routine lumbar puncture for diagnostic purposes ("control" patients). The breakdown of the diagnoses carried by the control patients is provided in Table 1.

IIH was diagnosed according to the criteria of Ahlskog and O'Neill[14] either at the time the CSF sample was drawn for OLC measurement (ie, a new diagnosis of IIH; n = 5 patients) or else according to a previous evaluation (ie, an established diagnosis of IIH; n = 2 patients). Briefly, these criteria are (i) neurological symptoms or signs consistent with elevated intracranial pressure, (ii) opening pressure >20 cm H_2O in the reclined position as measured during lumbar puncture performed at the time of diagnosis, and (iii) normal CSF studies excluding bleeding from the trauma of lumbar puncture. More recently updated diagnostic criteria[22] have set the threshold for opening pressure in IIH at 25 cm H_2O. The n = 5 newly di-

agnosed patients in our study all achieved that level of opening pressure. However, for the remaining 2 patients with a preexisting diagnosis of IIH, we could not obtain the opening pressure on their diagnostic lumbar puncture because of HIPAA restrictions.

All patients with suspected IIH were evaluated prior to enrollment in the study for occlusions of the cerebral venous system and were found to have none. Control patients who had conditions other than IIH were diagnosed accordingly by the treating neurologist. All patients in this study were examined with lumbar puncture as part of the standard diagnostic and/or therapeutic requirements for their condition; no effort was made to enroll patients for the sole purpose of sample collection. Patients were enrolled in this study in a consecutive manner.

OLC were measured in CSF samples by means of ELISA.[15] Briefly, CSF samples or ouabain standards and rabbit antiouabain antiserum were added to each well in an ovalbumin-ouabain-coated enzyme immunoassay plate. Plates were then incubated at room temperature for 2 hours and rinsed. Antiouabain antibodies remaining bound to the ovalbumin-ouabain were reacted with goat antirabbit IgG-peroxidase conjugate (Jackson Immunolabs) for 1 hour at room temperature. After being washed, the amount of peroxidase enzyme remaining in each well was determined by the addition of 3,3′5,5- tetramethylbenzidine base substrate solution. The absorbance in each well was measured at 450 nm with the use of a microplate recorder. The concentration of OLC in each sample was calculated from the reduction in absorbance according to the ouabain standard curve. In the above assay, 0.01 ng/mL of ouabain represent the minimal detectable amount that could be reproducibly measured. The concentration of OLC is expressed as nanogram ouabain equivalents per milliliter of CSF and is not normalized to the protein concentration of the CSF sample.

The concentration of OLC in IIH and control groups was compared with one-tailed unpaired *t*-test. Linear regression analysis was also performed on the measurements of opening pressure and the concentration of OLC in each group of patients to determine any relationship therein. *P* < .05 was defined as significant. All data are displayed as the mean ± SEM.

Table 2.—Demographic and Clinical Characteristics of IIH and Non-IIH Control Patients

	IIH Patients (n = 7)	Non-IIH Control Patients (n = 31)
Age	37.0 ± 7.6	55.6 ± 3.2
Sex (# female/male)	5/2	19/12
Average height and weight	169.3 ± 3.1 cm	169.3 ± 1.4 cm
	109.5 ± 13.6 kg	68.7 ± 2.6 kg
Body mass index	37.6 ± 3.9 kg/m^2	23.8 ± 0.7 kg/m^2
Pre-existing medical conditions	n = 2 hypertension	n = 3 hypertension
	n = 1 each of migraine,	n = 2 arthritis
	empty sella syndrome,	n = 2 migraine
	asthma, hyperthyroidism	n = 2 coronary artery disease
		n = 1 each of lupus, sarcoid,
		histiocytosis, lymphoma, seizures, stroke
Oral contraceptive use	1	3
Glucocorticoid use	0	5
Vitamin A use	0	0
Active smoking	1	2

RESULTS

Comparison of the demographic and clinical characteristics of the IIH and control patients is shown in Table 2. As could be expected, IIH patients were significantly younger and heavier than control patients. Lumbar puncture opening pressures were also higher in the IIH patients (27.9 ± 3.2 cm H_2O vs 17.2 ± 0.9 cm H_2O in the control patients), although there were 2 patients in the IIH group who had been previously diagnosed and treated for IIH who had opening pressures less than 20 cm H_2O when their CSF samples were drawn. These 2 patients were subject to lumbar puncture for evaluation of unexplained, recurrent headaches. At the time of enrollment into the current study, 3 of the 7 IIH patients were taking medications specifically for their IIH (ie, acetazolamide) though only the aforementioned 2 patients had their diagnoses of IIH supported by lumbar puncture before starting acetazolamide therapy; the remaining patient had apparently been started on acetazolamide only a few days prior to diagnostic lumbar puncture.

Overall, the concentration of OLC did not appear to be different between IIH and control patients. In patients with IIH, the concentration of OLC was measured as 0.11 ± 0.03 ng/mL, whereas in control patients the concentration of OLC was measured as 0.12 ± 0.01 ng/mL. Comparison of these 2 groups with a *t*-test found them to be indistinguishable (*P* = 0.31).

As shown in the Figure, regression analysis did demonstrate a linear relationship between the concentration of OLC in CSF and the opening pressure on lumbar puncture in the IIH patients (y = −.007 × +0.29; r = −0.80, P = .03). No such relationship was observed in control patients (y = 0.002 × +.07; r = 0.16, P = 0.40). The concentration of OLC did not relate to body mass index in either group.

DISCUSSION

The underlying cause of IIH is unknown, but several mechanisms have been proposed to cause this disorder.[16-19] Of these, an overproduction of CSF seems the most likely cause for most cases of IIH (reviewed in[16]). Accordingly, we hypothesized that IIH patients would exhibit reduced concentrations of OLC in the CSF. However, comparing the concentration of OLC in the CSF of IIH patients (treated and untreated) and non-IIH control patients could not demonstrate a difference between the 2 groups. While our data are underpowered to definitively show that the concentration of OLC in the IIH and control groups are not different, it does not seem that any difference can be demonstrated between the groups without evaluation of a very large number of patients. The power of the comparison with our data was calculated to be .09, and in order to obtain a power of 0.80 it was estimated that 790 patients would be needed in each group.

If such large numbers of patients are required to demonstrate a difference between the groups, it would be unlikely that the measurement of the concentration of OLC could identify any given patient as having IIH. Certainly this type of analysis is complicated by the use of patients with multiple neurological conditions as our control group, but unfortunately this was an unavoidable limitation of our study and resources.

Despite the fact that we could not distinguish between the 2 groups by simply comparing their averaged OLC concentrations, we did observe an inverse relationship between intracranial pressure and the concentration of OLC in patients with IIH. No relationship was seen between these 2 variables in patients who did not have IIH. This observation would suggest that endogenous OLC may be relatively deficient in patients with active IIH. Indeed, elimination of the 2 patients with the preexisting diagnosis of IIH who were successfully treated with acetazolamide (ie, opening pressure <20 cm H_2O) leaves 5 patients with a new diagnosis of IIH who together have an average opening pressure of 32.8 ± 1.2 cm H_2O and an average CSF OLC concentration of 0.06 ± 0.1 ng/mL, which by itself is nearly lower than that of the non-IIH control group ($P = .06$).

Unfortunately, such analyses are severely limited by the small number of patients with IIH that we could enroll in the study. We hope, with the publication of these preliminary data, first and foremost to obtain collaborative assistance for this project. It is our goal to enroll a larger number of IIH patients into a future study that better defines the relationship between intracranial pressure and the concentration of OLC in the CSF.

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