“Exploring the Relationship of pH in Schizophrenic and Control Subjects Postmortem Brain Tissue”

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Abstract

The purpose of this study was to measure the pH in schizophrenic patients relative to controls in postmortem cerebellum brain tissue. Several previous studies have found a decreased pH between the two diagnoses (Lipska et al., 2006; Torrey et al., 2005). However, other studies have found no significant difference between pH and diagnosis (Stan et al., 2006). Our goal for this study was to replicate previous studies to determine whether there are any significant differences in the diagnoses in question using our own postmortem collection. Comparisons were made between schizophrenia cohorts and normal controls in a diagnosis by pH analysis using an independent sample t-test, controlling for variable such as age, race, gender, and post-mortem interval. Our analyses revealed a marginally statistical significant difference between the mean in the schizophrenic subjects pH levels (M = 6.37, SD = .26) and the control subjects pH levels (M = 6.47, SD = .37), ($t = -1.68$, $df = 98$, $p = .096$). Chi-square analyses indicated that there is no statistically significant relationship between subjects sex and diagnosis (x^2 (1, N=100) = 0.04, p = .834), and no statistically significant relationship between subjects race and diagnosis (x^2 (3, N=100) = 0.97, p = .809). The range of pH values reported in this series is inconsistent with previously published results stating that there is no difference between schizophrenic and control subjects. Our study suggests that brain pH does in fact appear to be marginally influenced by brain disease.

1. Introduction

Schizophrenia is a chronic, severe, and disabling brain disorder that affects men and women equally across all ethnic groups around the world. Approximately 1% of the world's population suffer from this illness – which is roughly 68 million people (NIMH, 2014). Individuals with the illness suffer from three distinct and debilitating types of symptoms: positive, negative, and cognitive symptoms. Positive symptoms can include hallucinations, delusions, thought disorders, and movement disorders. Negative symptoms present as abnormal emotional and social behaviors, and may involve violent outbursts. Cognitive symptoms cause impaired memory and focus capabilities. Scientists believe schizophrenia is caused by interactions between our genes and the environment. A variety of medication treatments are administered to help relieve some symptoms of schizophrenia, but most people will cope with a majority of the symptoms throughout the rest of their lives. Due to our lack of viable treatments, there is a high prevalence of suicide in schizophrenic patients. Approximately 5% to 6% of individuals with schizophrenia die by suicide – which is about 3.5 million people (DSM V, 2014).

Postmortem human brain tissue is used for the study of many different brain diseases such as schizophrenia. Postmortem brain research has have become an increasingly essential element of the effort to understand the neurobiology of psychiatric disorders (Lewis, 2002). Past research has demonstrated that major psychiatric disorders are diseases of the brain, and some of these disorders, such as schizophrenia, are associated with altered brain anatomy (Lewis, 2002). Research facilities, like the Lieber Institute of Brain Development, are working to develop novel treatments and new research tools to understand the underlying cause of schizophrenia with the hope of future prevention.

A number of previous postmortem studies have found decreased pH in brains of patients with schizophrenia (Stan et al., 2006). Factors such as pH and other various variables can present unknown relative effects in molecular biology of mental illness. The cause of decreased pH is unknown, it could be due to differences in the manner of death, premortem acidosis (agonal state immediately prior to death), or medication induced alterations (Halim et al., 2008).Observed changes from results in postmortem studies may be indicative of primary effects of the disease, the secondary effects of disease, or other confounding factors (Halim et al., 2008).

A study conducted by Mexal et al., (2006) tells us that pH has been shown to have a large effect on postmortem brain gene expression patterns and that pH represents one of the most important control parameters in human postmortem studies in schizophrenia. Mexal (2006) also discovered that prolonged agonal conditions result in increased lactic acid which reduces brain pH. Research conducted by Kingbury et al., (2005) demonstrated that pH provides a means to screen postmortem brains for potentially viable preserved MRNA and suggested pH as a way to match in case control studies on neurodegenerative diseases.

In the past, lack of replication of basic biological findings in brains of people with schizophrenia across laboratories hampered progress (Lewis, 2002). It is for this reason we choose to replicate similar studies that had been conducted previously to .Ultimately we would like to advance our knowledge of any potential effects that pH may have on transcripts of interest, RNA integrity, housekeeping genes, and so forth. All of which may play a part in understanding the molecular basis of schizophrenia. If pH is, in fact, decreased in postmortem schizophrenic subjects relative to controls, the cause for the lower pH is unknown. This study attempts to evaluate whether pH is altered by the schizophrenia disease or if it is due to other confounding factors.

2. Materials and Methods

2.1 Subjects

We measured pH tissue parameters in 100 postmortem cases diagnosed with schizophrenia (n=50) and controls (n=50). We analyzed tissue samples taken from 100 individuals aged 2 years to 97 years, with the mean age of 45 ± 20 years. The demographic characteristics of the cases and parameters used to assess case tissue quality are detailed by psychiatric diagnosis in table 1. For each case, brief psychiatric narratives were compiled summarizing the demographic/clinical/medical/death information utilizing as many sources as possible (i.e. subject psychiatric records, police reports, neuropathology reports, medical examiner information, toxicology screenings, and family interviews). Postmortem clinical diagnosis was established for each case by independent reviews from two board-certified psychiatrists, who arrived at consensus based on DSM-IV criterion lifetime diagnoses. For cases in which the two psychiatrists did not agree on a diagnosis, a third psychiatrist was brought in to help reach diagnostic agreement. Normal control cases were defined by having no history of any neurological or neuropsychiatric disease/disorder or mental illness. Schizophrenic cases were defined by following the DSM-IV criterion as stipulated previously.

Table 1 - Demographic Characteristics Grouped by Diagnosis

Diagnosis	Number of Cases	Age	Gender		Race			
			M	F	C	AA	A	H
Control	50	45 ± 20	33	17	20	18	5	7
Schizo	50	45 ± 20	32	18	24	17	3	6

2.2 Brain Sample /Tissue Retrieval

Human brain specimens were collected from the National Institute of Mental Health (NIMH), and through the Offices of the Chief Medical Examiner of Maryland, after autopsy, and through voluntary tissue donations. Tissue donations and informed consent to study brain tissue was obtained from the surviving next-of-kin for all cases. A telephone interview with the next-of-kin to gather basic demographic information and medical, substance use, and psychiatric history was conducted in house. Each brain specimen was anonymized through assignment of a unique alphanumeric identifier, and cases were stripped of all identifiable personal information within our database.

Human brains were removed from the skull, placed in cryo storage containers, and transported on wet ice. Following neuropathological examination, each brain was hemisected, cut into coronal slabs, as well as sagittal slicing for sampling the cerebellum. A block of lateral superior cerebellar tissue was cut to be pulverized for future experimentation. After sectioning, brain slabs and cerebellum tissue blocks were snap-frozen on brass plates, cooled on dry ice, and stored at -80 degress celcius until tissue was needed.

3.3 Measurement of Tissue pH

A portion of the cerebellum of each diagnosis (schizophrenia and control) was used for pH measurements. Cerebellum tissue from each brain specimen was dissected and then pulverized over small grade dry ice. Following pulverization, 500mg of cerebellum tissue was weighed out and placed in ice cold 5ml Eppendorf tubes with 3ml of distilled H20 and a vortex bead. Samples were homogenized using a Diagger Vortex Genie 2 (a hand held tissue homogenizer). The purpose of homogenization is to break separate the tissue and break it down into a more liquefied form for the pH meter and DNA analysis. The pH was measured on a

Mettler Toledo Five Easy Plus pH Meter, equipped with a glass pH electrode using a 3 point calibration at pH 4.0, pH 7.0, and pH 10.0. pH was configured for each sample and the glass electrode was rinsed twice with dH20 between each sample. After each set of samples was run the glass electrode was left to soak overnight in a pepsin/HCI (5% pepsin in 0.1 mol/L HCI) solution in order to prevent any potential buildup of proteins from pulverized tissues.

3. Results

Table 2 - Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
pH	Equal variances assumed	3.254	.074	-1.679	98	.096	-.10760	.06409	-.23479	.01959
	Equal variances not assumed			-1.679	89.066	.097	-.10760	.06409	-.23495	.01975

An independent samples t-test was conducted to examine whether there was a significant difference between schizophrenic and control cerebellum tissue in relation to their pH levels. The test revealed a marginally statistical significant difference between the mean in the schizophrenic subjects pH levels (M = 6.37, SD = .26) and the control subjects pH levels (M = 6.47, SD = .37), (t = -1.68, df = 98, p = .096) (See table 2). A second independent sample t-test was run to examine whether there was a relationship between post-mortem interval (PMI) and diagnoses. Analyses indicated that there was no significant relationship between PMI and diagnosis (M = 36.16, SD = 19.64), (t = 1.621, df = 98, p = .108). An additional independent sample t-test was run to examine whether there was a relationship between age and diagnoses. The results demonstrated that there was no significant relationship between age and diagnosis (M = 50.45, SD = 21.93), (t = 2.63, df = 98, p = .010).

A chi-square test for independence was conducted to determine if there was a relationship between the subject's sex and their diagnosis. The analyses demonstrated that there is no statistically significant relationship between subjects sex and diagnosis (x^2 (1, N=100) = 0.04, p = .834). A second chi-square test for independence was conducted to determine if there was a relationship between the subject's race and their diagnosis. The results indicate that there is no statistically significant relationship between subjects race and diagnosis (x^2 (3, N=100) = 0.97, p = .809).

4. Conclusion

The range of pH values reported in this series is inconsistent with previously published results stating that there is no difference between schizophrenic and control subjects. Our study suggests that brain pH does, in fact, appear to be marginally influenced by brain disease. With the knowledge that pH does vary by diagnosis between schizophrenia and control we are now able to utilize this information in future studies to attempt to determine the underlying cause for these differences.

Our next step in this study will be to test for pH differences in schizophrenic and control subjects across different brain regions (dorsolateral prefrontal cortex, medial frontal cortex, deep temporal cortex). This will help us to determine whether pH is consistent across all brain regions. A challenge we address in studying psychiatric illness through the use of postmortem brain tissue is attempting to disentangle the primary nature of the disease process. In the future we will look to see if pH is a consequence of having the schizophrenia disease itself for years, or whether it is from the impact of pharmacological agents used to manage the illness. We will also attempt to determine if pH difference are attributable to substance abuse, or if it is a molecular genetic contributor of schizophrenia.

5. Limitations

The great risk of poorly designed or controlled postmortem studies is that the resultant errant finding may persist untested or unchallenged for an extended period of time given the relatively small number of postmortem studies conducted and availability of specimens for replication testing (Lewis, 2002). All specimens used in this study were postmortem, and thus are confounded in some way. A significant limitation for our study is that little of the human postmortem tissue which becomes available to our research facility is obtained with a full detailed account of the subjects history which may be critical in determining suitability of tissue for inclusion in studies (Kingsbury et al., 1995).The integrity of brain structure may be affected by factors that are operative prior to death. For example, previous data strongly suggest that smoking status is associated with lower pH in the human brain after death (Lipska et al., 2006). Another problem researcher's face is identifying quality in postmortem specimens. PMI is frequently used as a measure of postmortem tissue quality; the shorter the PMI, the better the quality of the tissue. Assessment of PMI, which is the elapsed time between death and the freezing or immersion of brain tissue in fixative, can be difficult to accurately determine. Development of generally accepted and sound measures of quality control in postmortem specimens is still being attempted. We do not fully understand the full effects of quality measures such as PMI and agonal state on specimens, and whether the cut off measures we apply are appropriate for each particular study. We also have to question whether the proposed study can accurately produce reliable results in available subjects with so many unanswered questions remaining about what affects the postmortem human brain both in pre-mortem and peri-mortem. Numerous premortem, antemortem, and postmortem factors can influence the quality of human brain tissue, and the relative importance of confounding factors for specifics are often not

known. Evidence has accumulated to show that antemortem events in the brain, such as hypoxia (deprivation of oxygen), exert an important influence on a number of neurochemical parameters and terminal events (Kingsbury et al., 1995).

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