Scientific Research

The Relationship between Impaired Methylnicotinate Response and Oxidative Stress in Schizophrenia

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Abstract

MNA response applied methylnicotinate (MNA) results in an arachidonic acid and cyclooxygenase-dependent vasodilatatory response which is diminished in patients with schizophrenia. This observation has been suggested to form the basis of a diagnostic test for the illness although the potential utility of such a procedure is diminished since the underlying mechanism is unclear. In this study we sought to discover if reduced MNA response in schizophrenia is related to increased oxidative stress *i.e.* whether or not the two measures are negatively correlated with each other. MNA response was assessed visually in 17 patients with schizophrenia and 16 healthy controls and compared to the extent of oxidative stress in each participant assessed by quantifying the lipid peroxidation product ethane in breath. Serum vitamin E, a lipid soluble antioxidant, concentrations was also assessed. While MNA response was correlated with breath ethane concentrations, the expected relationship between the two measures was not observed. Instead a positive relationship between them suggests that some patients with schizophrenia have impaired fatty acid utilization leading to both diminished lipid peroxidation and cyclo-oygenation. This was not related to vitamin E concentrations, however, suggesting that lipid soluble anti-oxidant availability did not underlie our findings. Our data shed further light on the mechanism of impaired MNA response in schizophrenia and support the notion that this occurs consequent to a change in lipid metabolism.

Keywords

Ethane, Breath, Fatty Acid, Schizophrenia, Niacin

1. Introduction

Schizophrenia is a common mental illness affecting approximately 1% of the world's population [1]. The dis-

order is characterized by so-called positive symptoms such as delusions and hallucinations, and negative symptoms which include flattened affect and avolition [1]. Schizophrenia is likely caused by the interplay of genetics and the environment leading to altered brain chemistry and/or structure [1], however the major mechanism(s) responsible are unclear. As such objective biologically based diagnostic tests for the disorder are unavailable. It has been long been argued, however, that an altered response to the B-vitamin niacin (nicotinic acid; NA) could form the basis of such a test for schizophrenia [2]-[4]. When administered orally at sufficient doses NA causes in the relaxation of smooth muscle cells in the wall of blood vessels resulting in skin erythema ("flushing") and, in some cases, significant hypotension [5]. NA has this effect by binding to rGPR109b, a G-protein coupled receptor present on Langerhan cells residing in the skin and mucosa, resulting in the release of free fatty acids from membrane phospholipid molecules via the activation of the enzyme phospholipase A2 (PLA2) [5] [6]. One type of fatty acid, arachidonic acid, is further metabolized by cyclooxygenase and prostaglandin D2 synthase to form prostaglandin D2 (PGD2). PGD2 then diffuses from the Langerhan cell and acts via a second receptor present of the capillary wall to produce vasodilatation [5]. It has been known for some time that patients with schizophrenia have a lesser or absent vasodilatatory response to oral NA assessed either visually or by measuring skin temperature [2] [3]. Further insight into the phenomenon was gained by using an ester of NA, methyl nicotinate (MNA). MNA, unlike NA, can traverse the skin allowing it to be administered topically to produce a localized vasodilatatory rather than the subjectively unpleasant systemic response NA [4]. In a similar manner to NA the response to MNA is reduced in patients with schizophrenia, whether measured by the visual or mechanized estimation of erythema, or by direct measurement of blood flow, findings which has been replicated many times [7]-[12]. By using multiple simultaneous doses of MNA investigators have concluded that patients with schizophrenia have an altered MNA dose response [10], namely an increased EC50 for MNA, meaning that patients require a higher dose to give the same response as healthy controls. Reduced MNA and NA vasodilatatory response in schizophrenia has been associated with clinical features of the illness however the field remains phenomenological in nature given that where in the multi-step and multi-cell NA pathway a deficit(s) lies is unclear. While a genetic linkage between MNA response and the membrane synthetic enzyme fatty acid CoA ligase in the healthy population does indicate an involvement of fatty-acid dependent signaling in the upstream Langerhan cell, precisely what makes patients with schizophrenia less responsive to NA eludes investigators [13] although interest in the phenomenon remains [14] [15].

Such a lack of understanding of the mechanism of impaired MNA response has reduced the likelihood that the procedure can be used as an objective non-invasive diagnostic test [4]. One hypothesis, however, is that the bioavailability of the fatty acids required for the vasodilatatory response is decreased due to their increased utilization in other reactions with oxygen free radicals, otherwise known oxidative stress, thereby reducing the pool of fatty acids available to initiate the MNA response. Free radicals, formed during all oxidative metabolism, can react with cellular constituents including fatty acids, DNA and proteins resulting in damage to cells [16]. To reduce this deleterious effect the cell has various anti-oxidant chemicals and enzymatic means to detoxify free radicals, the resulting balance between their production and removal being termed the level of oxidative stress [16].

Many investigators have shown elevated oxidative stress in schizophrenia using a variety of chemical assays [17]-[20]. This includes our own findings which utilized the measurement of breath ethane and pentane to assess oxidative stress, volatiles biomarkers which are generated in the reaction of free radicals with omega-3 and omega-6 fatty acids respectively [21] [22]. We have previously shown both elevated levels of breath ethane and pentane, and reduced MNA response in patients with schizophrenia [11] [21]. In a subset of participants, however, data for all measurements are available giving us the opportunity to compare the two non-invasive measures, specifically to test the specific hypothesis that reduced MNA response is associated with elevated oxidative stress as estimated by measuring breath ethane concentrations. Furthermore, whether reduced MNA response is associated with lower concentrations of the lipid-soluble anti-oxidant vitamin E (alpha-tocopherol), an occurrence which would be expected to raise oxidative stress, is also investigated.

2. Methods

2.1. Participants

Subjects, both male and female, aged between 18 and 54, were recruited to the study over a 1-year period (Table 1). Patients with schizophrenia all met DSM-IV criteria [23] for the illness and were recruited either during admission to hospital for an acute exacerbation of their illness, or from the community, where it was

Table 1. Participant characteristics.

Measure	Participant group	
	Healthy control	Schizophrenia
n[a]	16	17
Age (years)	34 ± 9[b]	34 ± 10[b]
Male/Female	6/10	9/8
PANSS[c]	NA	81 ± 14[b]
BPRS[d]	NA	27 ± 13[b]
Breath ethane (PPBV[e])	0.9 ± 0.7[b]	2.7 ± 2.1[b,g]
VNR$_{20}$[f]	30 ± 11[b]	12 ± 7[b,h]
Serum vitamin E (μmol/l)	24 ± 7[b]	29 ± 9[b]

[a]number of participants; [b]values are mean ± standard deviation; [c]positive and negative syndrome scale; [d]brief psychiatric rating scale; [e]parts per billion by volume; [f]volumetric niacin ratio over 20 minutes. The two groups were compared using a series of t-tests: g : $P < 0.01$, h : $P < 0.001$.

noted that they were symptomatic but not yet requiring hospitalisation. Patients with schizophrenia were in receipt of a variety of psychotropic medications: typical antipsychotics (droperidol (9), chlorpromazine (3), thioridazine (2), zuclopenthixol (3), flupentixol (2), sulpiride (1)) atypical antipsychotics (clozapine (5), amisulpiride (1), olanzapine (4), risperidone (1)), selective serotonin reuptake inhibitors (sertaline (2), paroxetine (3), fluoxetine (1)), benzodiazepines (lorazepam (6), temazepam (1), diazepam (1)). Healthy volunteers were recruited by advertisement in a local hospital and in a local office. All healthy volunteers had no current physical health problems (as screened using a general health questionnaire), and had no current or past history of mental illness, this being determined using an interview and the Brief Symptom Inventory [24]. The study had the approval of the Highland Health Board Ethics committee (UK) and each subject gave written consent after receiving a full explanation of the study. Patients with schizophrenia were assessed using the Brief Psychiatric Rating Scale (BPRS) [25] and the Positive and Negative Symptom Scale (PANSS) [26]. Data were also collected on non-psychotropic current medication. Any subject taking non-steroidal anti-inflammatory drugs within 48 hours of tested were excluded since these compounds interfere with the methyl nicotinate response.

2.2. Methyl Nicotinate Response

The measurement of methylnicotinate response was performed as per our previous publications [4] [11]. Briefly aqueous solutions 1, 10 and 100 mM MNA, along with a vehicle control containing no MNA, were applied to the forearms of participants in the form of a paper "patch" applied for 1 minute. The resulting erythema was rated every 5 minutes over a 20 minute time period using a 4 point scale using visual inspection by a rater blinded to the diagnosis. The approximate 'area under the curve' is then calculated to produce the final score known as the volumetric niacin response over 20 minutes (VNR$_{20}$) as described [8].

2.3. Breath Analysis

The measurement of breath ethane was performed exactly as described in our previous publications [21] [22]. Briefly, the final 130 ml of the full exhalation of the lungs is collected and applied to a solid sorbent to store and concentrate the trace gas compounds present in breath. The sorbent is then heated to release the gases which are then separated by gas chromatography and ethane quantified using electron ionisation mass spectrometry.

2.3. Quantification of Serum Vitamin E Concentrations

Serum was prepared from venous blood collected from fasted subjects by allowing blood to clot at room temperature followed by centrifugation at 1000 g. Vitamin E concentration was determined by HPLC as described [27].

3. Results

We have previously reported MNA response and breath ethane concentrations in a subset of these patients [11]

[18] but summary data showing that patients with schizophrenia show reduced ($P < 0.05$) MNA-induced erythema and elevated breath ethane ($P < 0.05$) compared to healthy controls is shown in **Table 1**. Serum vitamin E concentrations, previously unreported, were unchanged. Since all three measures are available for each subject it was possible to investigate, for the first time, any relationship between them using regressional analysis (**Table 2**). MNA response was not correlated with serum vitamin E concentration but was significantly correlated with breath ethane concentrations ($P < 0.05$) in the schizophrenia group (**Figure 1**) but, contrary to the hypothesized negative correlation, the correlation was positive (Pearson correlation coefficient = 0.76) *i.e.* reduced MNA response is associated with lower breath ethane concentration rather than higher.

4. Discussion

Our data support the hypothesis that there is a relationship between impaired MNA response in schizophrenia and the level of oxidative stress in these patients as estimated using breath ethane concentrations. However a causative role for oxidative damage to cellular fatty acids in reducing MNA response is unlikely given that that apparently higher oxidative stress is associated with higher MNA response and vice versa. Instead the possibility must be considered that persons with decreased MNA response possess a reduced ability to utilize fatty acids in multiple oxygenation reactions, these being the enzymatic cyclo-oxygenase pathway essential to producing the MNA response, and to non-enzymatic reaction with reactive oxygen species. In other words the bioavailability of membrane fatty acids is compromised for these, and possibly other reactions. In such a scenario, while the average extent of oxidative stress may be elevated in schizophrenia as suggested by our own and many other studies (see Introduction), our results suggest that oxidative stress is lower or even normal (compared to healthy controls) in patients who also exhibit the greatest impairment of MNA-induced vasodilatation. This offers a potential explanation as to why, even though we [11] and many other researchers find that arachidonic acid abundance is reduced in schizophrenia [for example see reference 19], this does not correlate with reduced MNA response. Specifically, the analysis of chemically extracted lipids does not assess their biological availability for particular reactions, but merely their chemical concentration. Instead impairment on MNA response may be indicative not of less arachidonic acid but of its decreased metabolic utilization. While it could be argued that elevated activity of antioxidants could be responsible for both cyclo-oxygenase inhibition and reduced ethane production [13] [28] we found no correlation between MNA response and the serum concentration of the major lipid-soluble anti-oxidant vitamin E although it should be noted that other anti-oxidant systems need to be examined to confirm such a conclusion. Instead, other metabolic alterations may underlie our findings including the decreased release of arachidonic acid from membrane phospholipids and/or elevated rates of fatty acid reuptake, both of which could reduce MNA response [6] [12]. This in turn would be compatible with neuroscientific

Table 2. Correlation[a] between methyl nicotinic acid response (VNR20) and breath ethane and erythrocyte fatty acid concentrations.

Group	Measure	
	Breath ethane	Serum vitamin E
Healthy controls	−0.03	−0.04
Schizophrenia	0.76[b]	0.21

[a]values shown are Pearson correlation coefficients; b. $P < 0.01$.
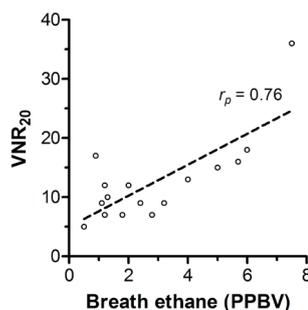

Figure 1. Relationship between methyl nicotinate response (VNR_{20}) and breath ethane concentration.

findings which suggest that impaired arachidonic acid signaling in the brain could potentiate dopaminergic neurotransmission in a manner which may heighten psychosis [29].

5. Conclusion

In summary, while a correlation was found between MNA response and oxidative stress, it is unlikely that elevated oxidative stress in schizophrenia plays any causative role. Instead, it is postulated that the bioavailability of fatty acids, in particular arachidonic acid, is reduced in patients exhibiting both impaired MNA response and a low extent of oxidative stress. Such findings shed further light on the mechanism of decreased MNA-induced vasodilatation in schizophrenia.

Acknowledgements

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