The role of myo-inositol in multiple sclerosis

GB YOUNG,* WJ HADER,† M HISCOCK,† KG WARREN,‡ D LOGAN§

From the University of Western Ontario, London, Ontario,* The University of Saskatchewan, Saskatoon, Saskatchewan,† The University of Alberta, Edmonton, Alberta,‡ and York University, Downsview, Ontario,§ Canada

SUMMARY Myo-inositol was given orally to nine multiple sclerosis patients and nine healthy control subjects. Pattern reversal evoked potential testing was used to assess its effect. The principal positive wave increased in amplitude, duration and area in a dose-dependent manner in the multiple sclerosis group compared with controls. Cerebrospinal fluid concentrations of myo-inositol in multiple sclerosis and controls were similar. The significance of these observations is discussed in relation to recent discoveries in inositol phospholipid function.

Myo-inositol and inositol phospholipids are components of plasma membrane and myelin.¹ They have achieved recent recognition for roles in metabolic regulation of nervous tissue² and in receptor physiology.³⁻⁹ In a preliminary report Holm¹⁰ showed that 27% of patients with multiple sclerosis appeared to metabolise myo-inositol abnormally: when myo-inositol was added to their sera a large change in redox potential occurred compared with controls. Altered myo-inositol metabolism in diabetes mellitus has also been proposed as a cause of altered nerve conduction.¹¹ We decided to pursue a possible role of myo-inositol in multiple sclerosis by: (1) examining the electrophysiological effects of orally-administered myo-inositol in multiple sclerosis patients and control subjects and (2) measuring myo-inositol in the cerebrospinal fluid (CSF) in multiple sclerosis and control patients.

Methods

The experiment was divided into two parts: (A) measurement of the effects of myo-inositol on pattern reversal evoked potentials in multiple sclerosis patients and controls and (B) determination of myo-inositol concentrations in the CSF in multiple sclerosis patients and controls.

(A) Myo-inositol and pattern reversal evoked potentials

Nine patients with clinically definite multiple sclerosis (using Schumacher criteria¹²) were matched within 3 years for age and for sex with nine healthy volunteers. Patients ranged in age from 22-44 years. Six were female and three were male. Subjects in both groups had visual acuities of 20/30 or

better. We tested all subjects in an identical manner with the same ambient lighting and at approximately the same time of day for each subject using a Nicolet CA 1000 Clinical Averager System. The reversing black and white checker board pattern was one metre from the subject's eye, with each square subtending 27 minutes of arc. Luminance was 700 cd/m² for white and 0.7 cd/m² for dark squares. The pattern reversed at 1.88 times/second. Each eye was tested separately twice at each testing session, with 100 repetitions per determination. Bandpass filter settings were 5-100 Hz. Scalp disk electrodes were used with O₂-C₂ derivation; impedances were 2-4 k ohms. Printout was on an X-Y plotter.

The major positive wave was clearly identified in each case. N₁, P₂ and N₂ peaks were identified (fig 1). Latency was from the time from the stimulus trigger to the peak of the P₂ wave. Amplitude was defined as the voltage difference between the N₁ and P₂ peaks. Duration was the N₂-N₁ peak to peak time difference. When values differed slightly in the 2 tests per determination, the values were averaged. The area "under the curve" was approximated by joining N₁, N₂ and P₂ points with straight lines and determining the area of the triangle so formed.

Each subject had four testing sessions. In Trial 1 the subject took no medication. Trial 2 testing was performed after 500 mg of myo-inositol was taken daily by mouth for 2 weeks. Trial 3 was performed after 1000 mg daily for two weeks and Trial 4 after no medication for two weeks. Myo-inositol was given in gelatin capsules containing 500 mg. A single dose was given each morning; pattern reversal evoked potential testing was performed between 3-6 hours after the last dose.

Statistical treatment of Part A utilised two sequential analyses of variance (ANOVAs) which were performed on each of the four dependent variables, that is latency, amplitude, duration and area subtended by the N₁ P₂ N₂ complex. In each case the first analysis was a 2 × 2 × 2 ANOVA in which the respective independent variables were group (multiple sclerosis versus control), eye (left versus right) and Trial (1 versus 4). The purpose of this preliminary analysis was to detect any significant change between the initial medication

Address for reprint requests: Dr G Bryan Young, Victoria Hospital, South Street Campus, London, Ontario, Canada, N6A 4G5.

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Fig 1 Two superimposed PREP responses are plotted with amplitude, latency and duration determination.

Fig 2 Mean latency values for multiple sclerosis patients and controls for the four Trials are shown. Vertical lines represent standard errors of measurement. The two groups differed significantly from each other but no effect from myo-inositol was found.

Table 1 Multiple sclerosis patients

Patient/age (yr)	Trial	Latency (ms)		Amplitude (µV)		Duration (ms)		Area (µV·ms)	
		Left eye	Right eye	Left eye	Right eye	Left eye	Right eye	Left eye	Right eye
A 33	1	134.00	135.00	2.70	4.05	57.00	66.50	76.95	159.60
	2	134.00	133.00	7.00	4.80	61.50	58.00	215.25	139.20
	3	137.00	132.00	7.95	6.50	61.00	63.00	242.48	204.75
	4	137.50	134.00	5.95	5.00	64.00	64.00	190.40	160.00
B 44	1	92.00	94.00	7.30	8.00	50.50	48.00	184.33	192.00
	2	92.00	94.00	6.50	5.60	54.00	51.00	175.50	142.80
	3	94.00	92.50	6.85	6.35	48.00	43.00	164.40	136.53
	4	91.00	94.00	5.95	5.85	49.00	49.50	145.78	144.79
C 31	1	102.00	128.00	3.75	1.50	55.50	39.50	104.07	29.63
	2	101.50	121.00	2.90	3.11	57.00	58.00	82.65	90.19
	3	101.00	121.00	4.20	3.60	55.00	59.00	115.50	106.20
	4	103.50	124.00	2.96	1.96	46.50	50.00	68.82	49.00
D 36	1	101.50	98.50	4.25	5.75	44.50	52.50	94.56	150.94
	2	103.00	101.00	5.31	5.21	52.00	50.00	138.06	130.25
	3	95.00	100.00	4.00	5.40	42.00	42.50	84.00	114.75
	4	101.50	99.50	4.70	4.45	54.00	40.00	126.90	89.00
E 29	1	96.00	95.00	8.50	15.70	50.00	48.00	212.50	376.80
	2	95.00	97.00	8.70	9.50	48.00	46.00	208.50	218.50
	3	95.00	96.50	10.70	10.90	46.00	50.00	246.10	272.50
	4	96.50	95.50	10.60	10.20	56.00	41.50	296.80	211.65
F 30	1	141.00	152.50	4.55	7.55	84.50	80.00	296.80	211.65
	2	143.50	150.50	5.80	5.80	82.50	90.00	239.25	261.00
	3	141.00	147.00	6.10	7.25	82.50	89.00	251.63	322.63
	4	101.00	108.00	4.25	4.10	70.00	67.00	148.75	137.35
G 26	1	127.50	147.50	2.20	2.10	56.00	60.00	61.60	63.00
	2	130.50	146.00	2.80	3.60	73.00	56.00	102.20	100.80
	3	129.75	145.00	2.83	3.30	59.00	65.00	83.49	107.25
	4	129.00	142.00	2.90	3.25	60.00	68.00	87.00	110.50
H 39	1	126.00	114.50	4.20	3.90	57.50	48.50	120.75	94.58
	2	125.00	114.00	7.05	7.50	62.50	55.00	220.31	206.25
	3	126.00	114.00	7.40	7.25	66.50	52.00	246.05	188.50
	4	120.00	110.00	4.30	6.75	49.00	60.50	105.35	204.19
I 21	1	111.00	120.00	4.00	4.21	27.00	58.00	54.00	122.09
	2	110.00	120.00	8.00	8.80	44.00	54.50	176.00	239.80
	3	108.50	118.25	7.20	6.70	53.00	53.25	190.80	178.39
	4	110.00	125.50	5.95	4.85	53.50	52.00	159.16	126.10

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— free trial (Trial 1) and the subsequent trial without medication (Trial 4). If Trials 1 and 4 proved to be comparable, the respective scores were averaged and the average was treated as the subject's score for 0 mg of myo-inositol. The second analysis for each dependent variable was a $2 \times 2 \times 3$ ANOVA with group, eye and myo-inositol dose as the respective independent variables. In this ANOVA the three levels of the dose factor were 0 mg (Trials 1 and 4), 500 mg (Trial 2) and 1000 mg (Trial 3). Trend analyses of the dose factor were conducted so that a statistically significant effect could be attributed to either its linear or quadratic component.

(B) *CSF inositol determination*

We collected CSF from 55 multiple sclerosis patients (mean age 36 years, SD 10 years; 20 males and 35 females) and 21 patients who did not have multiple sclerosis (mean age 37 years, SD 13 years; 10 males and 11 females). Most of the non-multiple sclerosis patients were undergoing myelography for lumbar or cervical disk disease. Other diagnoses included: migraine (1), benign intracranial hypertension (1),

hysterical paralysis (2), Guillain-Barré syndrome (1), motor neuron disease (2), cerebral infarction (2), encephalitis (2) Bell's palsy (1) and atypical facial pain (1).

Samples of CSF (0.5–0.7 ml) were diluted to 3.0 ml with distilled water and deproteinised by adding 0.15 ml of trichloroacetic acid (100 g/100 ml). Protein was precipitated by centrifugation and the supernatant was adjusted to pH 7–8 by treatment with Dowex 1-x8 (OH) ion-exchange resin. Aliquots of the supernatant solutions were placed in glass vials and dried overnight at 55–60°C. Dried samples were dissolved in dimethylsulfoxide (0.1 ml) and 0.4 ml of silylating reagent (7.0 ml pyridine, 2.0 ml hexamethyldisilazene and 1.0 ml trimethylchlorosilane) was added. The reaction was allowed to proceed at 22°C for 20 minutes. The by-products in the reaction mixture were removed by centrifugation. 5–10 µl aliquots of reaction mixture were then chromatographed on a glass column using a nitrogen carrier (45 ml/min) in a Tracor Gas Chromatograph model 560 with a hydrogen flame detector. Temperatures of inlet, detector and outlet were 205, 210 and 220°C respectively.

Table 2 *Control subjects*

Patient/age (yr)	Trial	Latency (ms)		Amplitude (µV)		Duration (ms)		Area (µV-ms)	
		Left eye	Right eye	Left eye	Right eye	Left eye	Right eye	Left eye	Right eye
J 47	1	96.00	96.00	7.15	6.05	38.00	45.00	135.85	136.13
	2	94.00	96.00	6.30	5.70	39.50	44.00	124.43	125.40
	3	95.50	95.58	4.45	4.80	51.00	40.50	113.48	97.20
	4	94.00	95.00	5.85	5.80	56.00	56.00	163.80	162.40
K 30	1	95.50	93.00	17.00	14.40	53.00	47.50	450.50	342.00
	2	94.50	93.00	16.60	16.35	46.50	45.50	385.95	371.96
	3	91.00	94.00	13.60	13.90	50.00	48.50	340.00	337.08
	4	92.00	93.50	13.60	12.70	50.00	50.00	340.00	317.50
L 27	1	88.50	95.50	7.60	4.10	42.00	48.50	159.60	99.43
	2	86.00	83.00	6.50	6.65	41.00	39.00	133.25	129.68
	3	87.00	87.00	6.40	7.95	40.00	41.00	128.00	162.98
	4	90.50	88.00	4.80	6.45	43.00	45.50	103.20	146.74
M 30	1	87.00	88.00	3.20	4.75	53.00	53.00	84.80	125.89
	2	87.00	90.50	2.25	5.55	41.00	44.00	46.13	122.10
	3	91.00	92.50	6.10	4.55	42.00	37.50	128.10	85.31
	4	90.00	90.00	5.40	6.30	45.00	46.00	121.50	144.90
N 22	1	94.50	94.00	5.80	5.70	68.50	74.00	198.65	210.90
	2	92.00	94.00	3.85	4.00	58.00	67.00	111.65	134.00
	3	91.00	90.00	4.45	4.35	66.00	55.00	146.85	119.63
	4	91.00	93.00	4.70	4.85	63.00	67.00	148.05	162.48
O 25	1	89.50	91.00	4.35	5.20	62.00	59.50	134.85	154.70
	2	89.50	90.00	4.80	4.45	60.00	65.00	144.00	144.63
	3	90.50	90.00	4.55	4.85	57.00	56.00	129.68	135.80
	4	91.00	89.00	7.00	7.35	60.00	60.00	210.00	220.50
P 36	1	90.50	93.00	7.35	8.45	41.00	44.50	150.68	188.01
	2	96.00	95.00	9.20	8.45	41.00	46.00	188.60	194.35
	3	94.00	95.50	6.85	9.95	41.50	43.00	142.14	213.93
	4	94.00	95.00	9.40	9.20	40.50	50.50	190.35	232.30
Q 34	1	93.00	91.00	2.40	3.28	35.00	46.00	42.00	75.44
	2	91.00	90.00	3.10	2.43	49.50	64.00	76.73	77.76
	3	92.50	94.50	3.10	1.95	34.50	46.00	53.48	44.85
	4	91.00	91.00	3.65	3.40	44.00	61.50	80.30	104.55
R 27	1	94.50	93.00	8.80	10.40	57.00	61.00	250.80	317.20
	2	94.00	95.00	9.00	8.20	55.00	57.00	247.50	233.70
	3	93.50	94.00	9.00	9.80	63.00	54.50	283.50	267.05
	4	96.00	95.00	8.20	8.50	59.00	55.00	241.90	233.75

Column temperature was programmed from 152–190°C. The retention time for the inositol derivative was 28 minutes. Quantitation was obtained by comparing peak areas obtained from samples with peak areas of known concentration of the following polyol mixtures: erythritol, ribitol, L-arabitol, D-sorbitol and myo-inositol. Recovery of polyols was 90–95% estimated by comparing the recovery of the standard mixture silylated directly with a sample prepared in the same way as CSF samples. Since the isomer myo-inositol comprises almost all of the inositol fraction² the total inositol concentration is equivalent to myo-inositol concentration.

We compared CSF myo-inositol levels of the 55 multiple sclerosis patients with those of the 21 control patients using the *t* test for two independent means. Paired 1-tailed *t* tests were performed on CSF myo-inositol from seven of the nine multiple sclerosis patients from Part A and seven age-matched (within 3 years) and sex-matched non-multiple sclerosis patients from Part B.

Results

(A) *Myo-inositol and pattern reversal evoked potentials* (see tables 1 and 2 for raw data)

Fig 3 (a) shows mean PREP amplitude for multiple sclerosis patients and controls for the four Trials. Vertical lines represent standard errors of measurement; (b) shows the difference in mean amplitude between multiple sclerosis and control groups for three myo-inositol dosages. Trials 1 and 4 were pooled to give the value for "0 mg myo-inositol". The amplitude difference between multiple sclerosis and controls is linearly related to myo-inositol dosage.

Fig 4 (a) shows mean duration for multiple sclerosis and control groups for the four Trials. Vertical lines represent standard errors of measurement; (b) shows the difference in mean duration between multiple sclerosis and control groups for three myo-inositol dosage regimes. Trials 1 and 4 were pooled to give the value for "0 mg myo-inositol". The difference between the two groups is linearly related to dose of myo-inositol.

No change occurred in visual acuity in control subjects or multiple sclerosis patients.

Latency Mean P_2 latencies for multiple sclerosis and control groups in the four trials are shown in fig 2. Means for the multiple sclerosis group exceeded those for the control group by 20–26 ms on each trial. An analysis of variance on Trials 1 and 4 was performed to determine whether the two groups differed in the unmedicated state. The design was a $2 \times 2 \times 2$ analysis with Group (multiple sclerosis vs Control), eye and Trial (Trial 1 vs Trial 4) as design factors. The analysis of variance yielded only a significant main effect for group, $F(1, 16) = 17.52$, $p < 0.001$, reflecting the greater mean latency for multiple sclerosis patients. The main effects for Trials and the Group $\times$ Trial interaction did not approach statistical significance ($p > 0.25$).

Since latencies on Trials 1 and 4 were equivalent, these data were averaged to provide an estimate of latency in the unmedicated state. An analysis of variance was then performed to evaluate the effect of 0

(Trials 1 and 4 pooled), 500 (Trial 2) and 1000 (Trial 3) mg of myo-inositol on P_2 latency. The only significant effect was the main effect for group, $F(1, 16) = 16.22$, $p = 0.011$, which again reflects the greater latency in the multiple sclerosis group. There was neither a significant dose effect, $F < 1$, nor a significant Group $\times$ Dose interaction, $F(2, 32)$, $p > 0.35$. Thus the latencies are not significantly altered by 500 or 1000 mg doses of myo-inositol.

Amplitude Mean P_2 amplitudes for the multiple sclerosis and control groups on each of the four Trials are shown in fig 3(a). Amplitude data were analysed in the same manner as the latency data. Preliminary analysis from the medication-free conditions (Trials 1 and 4) yielded no significant difference between groups ($p > 0.20$). In addition there was no significant Trial effect or Group $\times$ Trial interaction ($F < 1$).

An analysis of variance was performed to assess the effects of 0 (Trial 1 and 4 pooled), 500 (Trial 2) and 1000 (Trial 3) mg of myo-inositol. Although the main

Fig 5 (a) shows mean area of $N_1 P_1 N_2$ complex in multiple sclerosis and control groups for the four Trials. Vertical lines represent standard errors of measurement; (b) shows the difference in mean area between multiple sclerosis and control groups for the three doses of myo-inositol. Trials 1 and 4 were pooled to give the value for "0 mg myo-inositol". The difference in area is linearly related to myo-inositol dosage.

effects for group $F < 1$, and for dose $F(2, 32) = 1.03$ $p > 0.35$ were both nonsignificant, there was a significant group $\times$ dose interaction, $F(2, 32) = 3.30$, $p = 0.05$. Moreover trend analyses showed that the interaction is attributable to the linear component, $F(1, 16) = 7.90$, $p = 0.01$. The amplitude in the multiple sclerosis group increased compared with controls, the differential increase being related to dose in a linear manner [see fig 3(b)].

Duration The N_2-N_1 interval in ms for each group on each of the four Trials is shown in fig 4(a). As was the case with amplitude data, a preliminary analysis of duration from Trials 1 and 4 failed to show any differences. After Trials 1 and 4 were pooled, an analysis of variance was performed to ascertain the effects of myo-inositol dose. Neither the main effect for groups, $F(1, 16) = 2.13$, $p > 0.15$, nor the main effect for dose, $F < 1$ reached an acceptable level of statistical significance. Again there was a significant Group $\times$ Trials interaction, $F(2, 32) = 4.35$, $p < 0.025$, which can be attributed to the linear component, $F(1, 16) = 8.55$, $p = 0.01$. Myo-inositol enhanced the difference in duration by increasing the mean duration in the multiple sclerosis group and by decreasing it in the control group. The difference in duration between the two groups varied as a linear function of dose from 0–1000 mg. See fig 4(b).

Area under the curve The mean for multiple sclerosis and control groups and for each of the four trials is shown in fig 5(a). Although the mean area for the control group is greater than for the multiple sclerosis patients by about 40 μV -ms units in the medication-free Trials (1 and 4), the disparity in the two medication conditions is in the opposite direction; multiple sclerosis patients show the greater mean area by 5 units in the 500 mg Trial 2 and by 26 units in the 1000 mg Trial 3.

A preliminary analysis of variance on Trials 1 and 4 showed no significant Group $\times$ Trial interaction or effect for group or Trial. With Trials 1 and 4 pooled, on analysis there was a significant Group $\times$ Dose interaction, $F(2, 32) = 7.65$, $p < 0.005$, which reflects an increase in area for the multiple sclerosis group and a decrease in area for the control group. The group $\times$ dose interaction is largely attributable to the linear component, $F(1, 16) = 11.51$, $p < 0.005$. The medication-related relative increase in area for the multiple sclerosis group varied linearly with dose (fig 5b).

(B) CSF inositol determination

The mean levels of myo-inositol in the CSF of 55 multiple sclerosis and 21 control patients were similar (175 and 188 $\mu mol/l$ respectively). Variation within each group was marked (standard deviations for multiple sclerosis and control groups were 96 and

126 $\mu mol/l$ respectively). A paired 1-tailed t test using seven of the nine multiple sclerosis patients who were in Part A of the study, with seven age-sex matched control non-multiple sclerosis patients did not show a significant difference between the two groups ($p > 0.19$).

Discussion

Based on its topography¹³ the P_2 wave is likely generated in the visual cortex by local synaptic activity in response to visual stimulation. The P_2 latency is often delayed in multiple sclerosis patients probably because of conduction slowing due to demyelination.¹⁴ We also found a significant delay in mean P_2 latency in multiple sclerosis patients compared with control subjects. Myo-inositol administration was not associated with any alteration of the latency in either group, indicating conduction velocity was not affected.

Our main finding is that multiple sclerosis patients and control subjects differ significantly in the alteration of mean values of P_2 amplitude, duration and area associated with myo-inositol administration. Furthermore the differences between the two groups are dose-dependent in a linear manner. It appears to us these findings reflect a differential response to myo-inositol on either conduction or on synaptic activity.

Although conduction velocity does not appear to be increased, reversal of conduction block in some demyelinated axons in the visual pathway could produce an enhanced P_2 response. In view of the acute reversal of its effect, it seems unlikely the administered myo-inositol altered the physical structure of the axon to correct the transmembrane capacitance and resistance¹⁵ or impedance mismatch^{16,17} causing the conduction block. Hawthorne and Kai¹ proposed phosphoinositides play a role in ion channel gating. Although this has never been proven, a mechanism involving turning off sodium influx¹⁸ or blocking potassium channel activation¹⁵ may overcome conduction block.

Proposing a synaptic effect of myo-inositol raises the controversial topic of altered synaptic activity in multiple sclerosis.^{19–26} It is still unsettled as to whether there is a “synaptic blocking factor” in multiple sclerosis, although there is some evidence for altered synaptic function produced by multiple sclerosis sera.^{19–22,24,25} Eisen *et al*²⁶ also found altered neuromuscular transmission in multiple sclerosis.

Recent biochemical studies have revealed a prominent role for inositol phospholipids in the function of numerous types of receptors.^{3,5,7} When specific post-synaptic receptors are activated, phospholipase C hydrolyses phosphoinositides. One product, inositol triphosphate, is thought to function as a “second

messenger" in those systems which do not use cyclic adenosine monophosphate (cyclic AMP).^{5,6,8} Inositol triphosphate increases intracellular ionised calcium concentration by causing its release from storage sites and probably by opening membrane channels for calcium. Calcium then activates various cellular enzyme systems. Thus there is an "amplifying effect" of inositol phospholipids on the function of certain neurotransmitters.^{5,7} The administration of extra inositol could augment this second messenger function. Such an effect might be more pronounced if the system is malfunctioning from partial synaptic blockade. It is also of interest that phosphatidylinositol phosphodiesterase which cleaves phosphatidylinositol is inhibited by blood plasma, serum and blood lipoproteins.²⁷ Such substances could have access to receptor sites if the blood-brain barrier were not intact.

We found no significant differences in CSF myo-inositol concentrations in multiple sclerosis and control patients. Our values are similar to those reported by Servo *et al*²⁸ using a gas-liquid chromatographic assay and Garcia-Bunuel *et al*²⁹ using an enzymatic assay. Neither study had more than 6 multiple sclerosis patients. Two with "acute multiple sclerosis" had slightly elevated CSF myo-inositol levels,²⁹ the others did not differ from controls. It is possible the inositol was released into the CSF by myelin destruction in the acute cases. It is uncertain whether CSF levels reflect tissue levels of myo-inositol, but we have no evidence for deficiency of myo-inositol in multiple sclerosis.

Administered myo-inositol appears to have a differential pharmacological effect on pattern reversal evoked potential responses in multiple sclerosis patients and healthy controls. It is unclear whether this has any therapeutic implications. Although we noted no convincing clinical improvements during or following our study, this could be better assessed in a clinical study.

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