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Erythrocyte Membrane Alterations in Huntington Disease: Effects of γ-Aminobutyric Acid

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The interaction of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) with erythrocyte membranes from patients with Huntington disease and normal controls has been studied by electron spin resonance. GABA affects the physical state of erythrocyte membrane proteins in control and Huntington disease differently. In addition, after exposure of spin-labeled Huntington disease erythrocyte membranes to 0.1 mM GABA, the relevant electron spin resonance parameters reflecting the physical state of membrane proteins are indistinguishable from those of untreated control membranes. These findings support the concept that this disease is associated with a generalized membrane defect.

Key words: GABA, Huntington disease, spin labeling, erythrocyte membranes, protein alterations

Huntington disease (HD), inherited in an autosomal dominant manner, is characterized clinically by progressive involuntary movements and dementia [1], pathologically by cell degeneration in the neostriatum and cerebral cortex [2], and biochemically by decreased levels of γ-aminobutyric acid (GABA) [3] and decreased activities of glutamic acid decarboxylase and choline acetyltransferase in the basal ganglia [4].

Although HD has been considered a defect of the basal ganglia, recent biophysical and biochemical experiments from our laboratory have suggested that this disease may be associated with a generalized membrane abnormality. Electron spin resonance (ESR) studies of erythrocyte membranes have demonstrated alterations in the physical state of membrane proteins in HD erythrocytes [5]. Scanning electron microscopic investigations of unmanipulated erythrocytes revealed an increased number of stomatocytes in HD compared to controls [6], with a slight exacerbation of this effect when the cells were processed in fixative of lower pH. These findings probably result from an altered response to fixation by an altered membrane in HD and support the ESR results. We have also observed the activity of the erythrocyte membrane sodium plus potassium-stimulated adenosine triphosphatase to be increased in HD [7].

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The molecular basis for the clinical manifestations of Huntington disease is unknown. One hypothesis to explain the presence of choreiform movements in HD involves the decreased amount of GABA [3], an inhibitory neurotransmitter in the basal ganglia. In the current report we present the results of ESR studies of the interaction of GABA with erythrocyte membranes from patients with Huntington disease and normal controls.

MATERIALS AND METHODS

GABA and β-alanine were purchased from Sigma Chemical Company and the spin label employed, 2,2,6,6-tetramethylpiperidin-1-oxyl-4-maleimide (MAL-6), was obtained from Syva. All other reagents were of the highest purity available.

Heparinized blood was obtained by venipuncture from twelve different individuals from eleven different families with well-documented Huntington disease. All patients had involuntary movements and/or dementia and a positive family history for HD. Twenty-five percent of the subjects were in the early stages of the disease, 42% in the mid stages, and 33% in the late stages. Seventy-five percent of the patients were ambulatory and active, and the majority were on a regular at-home diet; the others were on a regular hospital diet. One-third of the HD patients were on no medication while the remainder were on phenothiazines or a butyrophenone, or on an assortment of medication. A different, healthy, sex- and age-matched, drug-free individual with no family history of inherited neurologic disease served as a control for each HD subject.

Intact erythrocytes and erythrocyte membranes (ghosts) were prepared and the spin labeling performed using MAL-6 as previously described [5]. Magnetic resonance experiments were performed on a Magnion-Ventron MVR-9X or a Varian E-109 electron spin resonance spectrometer.

Membrane protein content was estimated according to the method of Lowry et al [8]. The GABA incubation was performed as follows: 50 μl of a solution of GABA dissolved in 5 mM sodium phosphate buffer, pH 8.0 was incubated with 200 μl of control or HD ghost membranes to a final concentration of 0.1 mM GABA for 15 minutes at room temperature and the ESR spectra recorded. Control experiments in which 200 μl of ghosts was incubated with 50 μl of the buffer without GABA were also performed. In experiments in which the ESR signal intensity of membrane-bound MAL-6 per mg membrane protein was determined, NaOH was added to a final concentration of 0.2 M. This procedure is known to convert all MAL-6 binding sites to weakly immobilized ones [10, 11]. The line shapes of the resulting spectra were the same in all such experiments. SDS-polyacrylamide gel electrophoresis of erythrocyte membranes was performed as previously described [9].

RESULTS

The spin label used in the present study is covalently bonded principally to sulfhydryl (SH) groups of membrane proteins, although fewer than 5% of the labeled sites may be amino groups [12]. The principal proteins of the erythrocyte membrane labeled by MAL-6 are thought to be the set of large (> 200,000 dalton) peripheral proteins (spectrin), the higher-molecular-weight proteins present in smaller amounts, and a group of transmembrane proteins (Band 3) [11]. A typical ESR spectrum of MAL-6 incorporated into control erythrocyte membranes is shown in Figure 1. Spectra similar to that in Figure 1 have been previously described [10–15] as having ESR parameters reflective of at least two different classes of SH group sites in the red blood cell membrane: one strongly immobilized and the other weakly immobilized. The ratio of the ESR spectral amplitude

of MAL-6 attached to weakly immobilized SH groups (W) to that of MAL-6 attached to strongly immobilized SH groups (S) is a convenient monitor of protein organizational and/or conformational changes in membranes [10–15].

The mean W/S values of control and subject experiments were compared by a two-way analysis of variance [16] (Table I). This two-tailed method of statistical analysis minimizes the effects of possible fluctuations from day to day that may occur with biological samples. P is the significance of the mean values of $(W/S)_{control}$ and $(W/S)_{subject}$ calculated from the two-way analysis of variance. $(W/S)_{HD}$ is significantly increased compared to $(W/S)_{control}$ ($P < 0.025$, Table IA), suggesting alterations in the physical state of membrane proteins in erythrocytes in Huntington disease. These data confirm our previous ESR results [5].

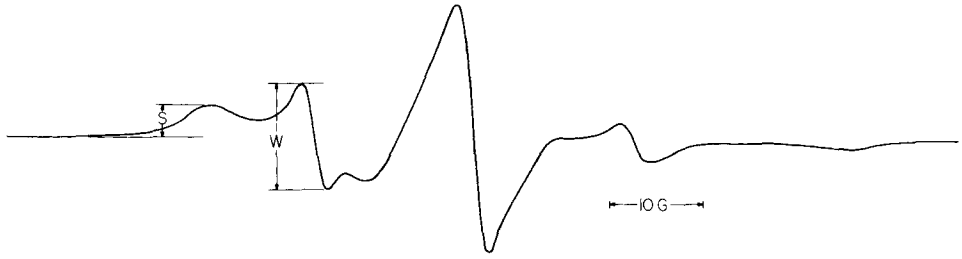

Fig. 1. Typical electron spin resonance spectrum of MAL-6 attached to membrane proteins in control erythrocytes. The ESR spectral amplitudes of MAL-6 attached to weakly immobilized sulfhydryl groups (W) and that of MAL-6 attached to strongly immobilized sulfhydryl groups (S) are indicated.

TABLE I. Effect of GABA on the W/S Ratio of ESR Spectra of MAL-6 in Erythrocyte Membranes in Normal (N) and Huntington Disease (HD)*

A) No GABA added

$(W/S)_N$	$(W/S)_{HD}$
4.13 ± 0.19	4.53 ± 0.20

$P^a < 0.025$

B) Differential effect of 0.1 mM GABA in normal and Huntington disease

$(W/S)_N$	$(W/S)_N$ + GABA	$(W/S)_{HD}$	$(W/S)_{HD}$ + GABA
4.13 ± 0.19	4.77 ± 0.26	4.53 ± 0.20	4.22 ± 0.23

$P < 0.005$ $\quad$ $0.05 < P < 0.1$

C) Equalization of MAL-6 W/S ratio in HD after addition of 0.1 mM GABA

$(W/S)_N$	$(W/S)_{HD}$ + GABA
4.13 ± 0.19	4.22 ± 0.23

$P > 0.8$

*Mean ± SEM for 12 different samples in each case are presented.

[a]P value calculated by a two-way analysis of variance [16].

GABA has a differential effect on control and HD erythrocyte membranes as reflected by the W/S ratio of MAL-6 (Table IB). This ratio is highly significantly increased in control membranes upon incubation of 0.1 mM GABA ($P < 0.005$); however, upon GABA incubation, a reduction of this ratio in Huntington disease erythrocytes compared to untreated HD membranes is suggested, although the significance is borderline ($0.1 > P > 0.05$). However, after addition of GABA to erythrocyte membranes from patients with Huntington disease, the W/S ratio of MAL-6 attached to membrane proteins is not altered from that of untreated normal controls ($P > 0.8$, Table IC), indicating that this ESR parameter has been lowered by GABA treatment (compare Table IA and IC). Moreover, the results shown in Table IC suggest that on the basis of ESR parameters GABA has made the physical state of proteins in HD erythrocyte membranes indistinguishable from that of controls.

Initial experiments in which control and HD erythrocytes were incubated with GABA followed by centrifugation at 27,000g for 30 minutes and recentrifugation of the resulting supernatant under the same conditions were performed. Aliquots of the second supernatant were subjected to SDS-polyacrylamide gel electrophoresis and ESR spectroscopy. Some of the peripheral protein, spectrin, is released into the supernatant from control membranes under these conditions as observed on gels and by the presence of a noticeable ESR signal. The W/S ratio of this signal detected at high spectrometer gain was approximately one-third that of MAL-6 attached to untreated erythrocyte ghosts, a value that is typical for spectrin [D.A.B., unpublished results]. Approximately 89% of the total MAL-6 signal intensity remained in the membrane pellet after GABA treatment. In contrast, the HD supernatant either demonstrated no spectrin ESR signal or at extremely high instrument gain settings, one of much lower intensity than that observed in control samples. In addition, under the conditions employed no HD protein was observable on SDS-polyacrylamide gels, suggesting that GABA has interacted differently with control and HD erythrocyte membranes and supporting the ESR data in Table IB and IC.

Preliminary evidence for the specificity of GABA has been obtained. Four different experiments were performed in which control and HD erythrocyte membranes were incubated with β-alanine (3-aminopropionic acid), a compound differing from GABA only in that it has one less CH_2 group. No definitive differences in the W/S ratio of MAL-6 could be demonstrated in either control or HD erythrocyte membranes upon incubation with β-alanine.

DISCUSSION

A differential effect of 0.1 mM GABA, an inhibitory neurotransmitter, on the physical state of membrane proteins in Huntington disease and control erythrocytes has been suggested in the present experiments. The relevant ESR parameter, the W/S ratio, was increased in control samples upon GABA incubation, whereas this parameter was apparently decreased in Huntington disease (compare Table IA, IB, and IC). After treatment with GABA, no statistically significant difference in the W/S ratio in Huntington disease erythrocytes compared to untreated controls could be demonstrated (Table IC). This last result suggests that GABA has caused a change in the physical state of membrane proteins in erythrocytes in HD such that the original ESR differences (Table IA) are no longer present. Our previous studies [5–7] have suggested that HD may be associated with a generalized membrane defect. If the phenomenon observed in the present experiments with erythrocytes were to occur in the basal ganglia, it would suggest that increasing GABA levels in the neostriatum in HD may cause remaining neuronal membranes to revert to a

normal physical state. This suggestion lends support for the pharmacological strategy of inhibiting GABA transaminase activity [17, 18] and replacing GABA [18] in HD. Moreover, by monitoring the effects on the physical state of membrane proteins in erythrocytes induced by various drugs potentially useful in HD therapy, the relative effectiveness and usefulness of these pharmacological agents may be discerned. Experiments employing these concepts are currently in progress.

GABA is thought to act as an inhibitory neurotransmitter by increasing Cl^- conductance [19]. In the erythrocyte the major anion transporting protein is located in Band 3 [20], a set of 100,000-mw transmembrane proteins. It may be pertinent to the present study that we recently found in HD erythrocytes an increased activity of the $Na^+ + K^+$-stimulated adenosine triphosphatase [7], the phosphorylated intermediate of which is located in Band 3 [21].

Although our initial experiments with β-alanine suggest a specific GABA effect, it is still uncertain whether there exist GABA receptors in the erythrocyte membrane. However, the discovery of acetylcholine [22] and opiate [23] receptors, and the presence of acetylcholinesterase [24] in the erythrocyte membrane lend support for such a possibility. Experiments using GABA antagonists are currently in progress to resolve the specificity of GABA interaction observed in the present study.

Spectrin, the large, filamentous, myosin-like peripheral protein located on the cytoplasmic side of the erythrocyte membrane, is thought to be important in the equilibrium conformation adopted by several membrane proteins partially by serving as an "anchor" to which these other proteins are attached [25]. The loss of some spectrin from control erythrocyte membranes exposed to 0.1 mM GABA might be expected to allow these other proteins to become more free to move, resulting in a higher W/S ratio consistent with the present results.

The molecular mechanisms of the interaction of GABA with erythrocyte membranes are as yet unclear, but the differential response of this neurotransmitter on the physical state of extraneural membranes in HD and controls lends support to the concept that Huntington disease is a diffuse membrane disease.

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