Biochimica et Biophysica Acta, 778 (1984) 260–268
Elsevier

BBA 72293

THE ALTERATION OF MEMBRANE PROTEINS IN HUMAN ERYTHROCYTE MEMBRANES INDUCED BY QUINOLINIC ACID, AN ENDOGENOUS NEUROTOXIN

CORRELATION OF EFFECT WITH STRUCTURE

BENNETT T. FARMER, II, MAREN M. NICHOLAS and D. ALLAN BUTTERFIELD *

Department of Chemistry, University of Kentucky, Lexington, KY 40506-0055 (U.S.A.)

(Received June 4th, 1984)

Key words: Membrane protein; Neurotoxin; Quinolinic acid; Spin label; (Erythrocyte membrane)

Quinolinic acid (2,3-pyridinedicarboxylic acid), an endogenous metabolite of L-tryptophan, reportedly via the kynurenine pathway, has been previously shown to possess neurotoxic properties when injected into rat striatum (Schwarcz, R., Whetsell, W.O., Jr. and Mangano, R.M. (1983) Science 219, 316–318) and to alter the physical state of human erythrocyte membrane proteins, as judged by ESR spectroscopy (Farmer, B.T., II and Butterfield, D.A. (1984) Life Sci. 35, 501–509). Both the morphologic and ESR studies employed nicotinic acid as one comparative control and found that the effect of quinolinic acid is significantly different from that of nicotinic acid. In the present study, we report that the effects of several structural analogues and positional isomers of quinolinic acid on the ESR parameter associated with the physical state of membrane proteins in human erythrocyte membranes suggest the following conclusions concerning the structure-effect relationship of quinolinic acid: The alteration in the conformation of membrane proteins: (1) requires the presence of two carboxylic acid groups; (2) is independent of their relationship to one another on the pyridine ring; (3) is slightly dependent on the presence of the pyridine nitrogen atom but is independent of the positional relationship of the two carboxylic acid moieties to the heteroatom; and (4) seems to depend upon the presence of restricted internal motion derived from the aromaticity in these compounds.

Introduction

Much interest has arisen recently concerning the existence of endogenous neurotoxic compounds in human brain due to the observation that ibotenic acid and kainic acid, two exogenous excitatory neurotoxins [5], induce neuronal damage reminiscent of Huntington's disease when injected into mammalian brain [6]. Schwarcz et al. [3] have postulated that the hyperfunction of endogenous, excitatory (or possibly inhibitory) neurotoxins may be the basis for certain neurodegenerative disorders, of which Huntington's disease is a member. These authors have found that quinolinic acid, an endogenous metabolite of L-tryptophan, reportedly formed via the kynurenine pathway [1,2], acts as an excitatory neurotoxin when injected into rat striatum and concomitantly induces severe, axon-sparing neuronal death [3]. As an initial effort to better understand how quinolinic acid might cause neuronal death, the interaction of quinolinic acid with human erythrocyte membranes was examined to determine if this compound would affect the physical state of membrane proteins in a reasonably specific manner, employing nicotinic acid as one comparative control [4]. Quinolinic acid altered the electron spin resonance spectrum

* To whom correspondence should be addressed.

of the protein specific spin label employed ($P < 0.001$) suggesting that the physical state of membrane proteins was significantly changed by this compound [4]. Nicotinic acid had no statistically significant effect on the conformation of proteins [4]. In contrast to that of proteins, the physical state of cell surface carbohydrates and membrane lipids were unaffected by quinolinic acid as judged by ESR spectroscopy [4]. In the present study, we have further characterized the molecular interaction of quinolinic acid with erythrocyte membrane proteins by examining the structural components of this potentially neurotoxic substance.

Methods

Chemicals. The spin labels, 2,2,6,6-tetramethyl-4-maleimido-piperidine-1-oxyl (MAL-6) and 2-(3-carboxylpropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl (5-NS), were obtained from Syva. Ultraviolet and melting point analyses showed that no isomaleimide contamination was present in the MAL-6 preparation. Quinolinic acid (99% pure), 2,5-pyridinedicarboxylic acid (2,5-PDC) (98%), 2,6-PDC (99%), 3,4-PDC (97%), 3,5-PDC (98%), and picolinic acid (2-carboxylic acid pyridine) (99%) were obtained from Aldrich. L-Tryptophan, the monopotassium salt of phthalic acid (KHP), and nicotinic acid were obtained from Sigma and were used without further purification. 3-Hydroxyanthranilic acid was obtained in highest purity from Pfalz and Bauer. Dry pyridine was a gift from Dr. Laren Tolbert. Benzoic acid was obtained from Fischer Scientific and was reagent grade. $^{36}Cl^-$ was obtained from Amersham with a specific activity of 78.3 μCi/ml.

Preparation of membranes and spin labeling. Blood was drawn into heparinized tubes from healthy volunteers. Intact cells and erythrocyte membranes (ghosts) were prepared as described previously within one hour of obtaining the blood [7,8]. Noncovalent incorporation of 5-NS into the membrane lipid phase of human erythrocytes was performed according to previously detailed procedures, described in Refs. 9 and 10, except that the concentration of 5-NS was halved for this study to further reduce residual free spin label. Erythrocyte membrane lipids were isolated according to standard methods [11]. Membrane proteins in human erythrocyte ghosts were selectively spin labeled with MAL-6 as previously described [10,12]. Ghosts (1.0 ml) with a known protein content were placed in a 15-ml Corex centrifuge tube to which 10 ml of 5 mM sodium phosphate (pH 8.0) (5P8) containing MAL-6 in a weight ratio of one mg spin label to 25 mg membrane protein was added. The tubes were placed in the dark at 4°C for 16 h and subsequently washed five times in 5P8 buffer to remove excess spin label [10,12]. Recently Barber et al. [13] confirmed that our method of spin labeling erythrocyte membrane proteins in ghosts (devoid of hemoglobin) with MAL-6 gave highly reproducible ESR spectra that could be employed with confidence.

In order to determine the effect of various quinolinic acid analogues on the physical state of the membrane, 0.24 ml of spin labeled ghosts were incubated for 30 min at room temperature with 0.06 ml of these compounds at a final concentration of 0.2 mM in 5P8 buffer. The control consisted of 0.24 ml of spin labeled ghosts plus 0.06 ml 5P8 buffer similarly incubated. The pH of the final solution was within 0.1–0.15 pH units of the original ghost-buffer suspension. The ionic strength was essentially unaltered. Fresh solutions of the compounds were prepared weekly except for 3-hydroxyanthranilic acid (3-HA), which was prepared fresh on each day that ESR spectra were recorded since this compound is apparently sensitive to air oxidation. Despite this precaution the solution generally had reached a light amber color before the spectra could be recorded. It is to be noted that with the exception of the investigations of glutamate which were performed earlier [8], studies of each compound were performed at the same time with the same control, thus eliminating possible biological variation between compounds.as

ESR spectra were obtained on a Varian E-109 X-band ESR spectrometer employing an E-238 quartz aqueous sample cell and TM mode resonant cavity in a room held at constant temperature and humidity. Dry N_2 gas at a flow rate of 5SCF per hour was used to purge the resonant cavity and to maintain a constant cavity temperature for each sample. Spectrometer conditions are indicated in the figures. In all cases power and modulation broadening were avoided.

Measurement of chloride efflux. Chloride efflux

measurements on intact cells were made as described in [14] with some slight modifications. In one experiment 150 mM NaCl/5 mM sodium phosphate (pH 8.0) (phosphate-buffered saline), a high-ionic strength buffer, was used as the loading and efflux buffer. In another experiment, 5 mM NaCl/5 mM sodium phosphate/290 mM sucrose (pH 8.0) (5,5P8(S)), a low ionic strength buffer, was used as the loading and efflux buffer. Intact cells were loaded with $^{36}Cl^-$ in the appropriate buffer for 10 min at room temperature. 4.0 μl of a 50 mM solution of quinolinic acid, nicotinic acid, or KHP (pH ~ 8.0) was added per ml of erythrocyte suspension (50% hematocrit) so that the final concentration of the respective compound was 0.2 mM and the final hematocrit remained approx. 50%. Each compound was allowed to incubate for 30 min at room temperature before the cells were centrifuged and the supernatant aspirated. 4.0 μl of the loading buffer was added to the erythrocyte suspension in the control experiment. The cells were injected into the appropriate efflux buffer, which was also 0.2 mM in the respective compound being studied within that sample, to give a final hematocrit of 0.5%. Efflux measurements were carried out at 1.5 ± 0.3°C as described previously [14].

Results

Fig. 1 shows the $M_I = +1$ (low field) resonance lines of the ESR spectrum of MAL-6 attached to membrane proteins in erythrocyte ghosts. This spectrum has been described in great detail previously as being reflective of at least two different classes of spin label binding sites of erythrocyte ghost proteins: strongly and weakly immobilized [7–10,12,13,15–17]. The protein specific spin label used is chiefly bound to membrane skeletal components (60–80%) and the transmembrane protein Band 3 (10–30%) [15,17]. The ESR spectral ratio of the $M_I = +1$ weakly immobilized component of MAL-6 attached to membrane proteins in erythrocyte ghosts to that of the strongly immobilized component is a sensitive monitor of the physical state of membrane proteins [10]. Examination of paired differences of the W/S ratio between control and samples treated with various substances would likely increase the significance

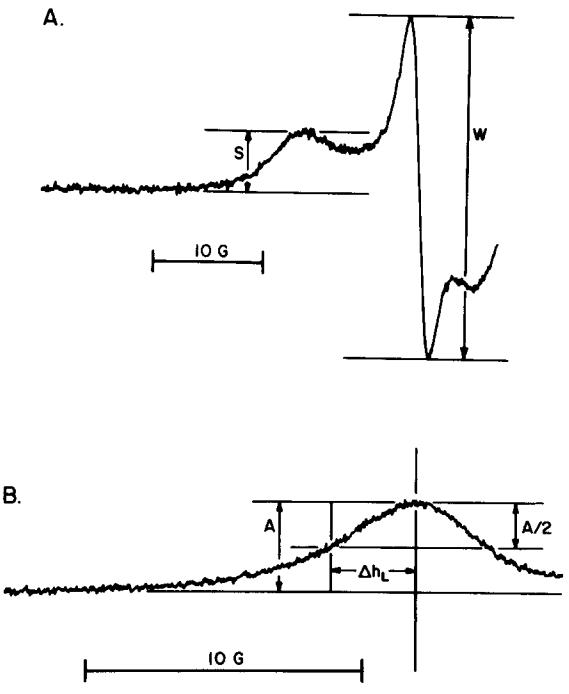


Fig. 1. (A) Typical ESR spectrum of the low field ($M_I = +1$) lines of the piperidine maleimide spin label attached to membrane proteins in human erythrocyte membranes. The amplitudes of the spin label covalently bound to strongly and weakly immobilized binding sites are indicated by S and W, respectively. Power = 14 mW, modulation amplitude = 0.32 G, scan range = 100 G, time constant = 0.25 s, scan time = 16 min. (B) Typical ESR spectrum of the low-field portion of the 5-NS population whose nitroxide z-axis (along the symmetry axis of the nigrogen p orbital containing the unpaired electron) is parallel to the applied, static magnetic field. The increase in line-width, when compared to the rigid lattice-limit spectrum, is reflective of the rate of exchange of an average 5-NS molecule between parallel and perpendicular orientations. Power = 16 mW, scan range = 40 G. All other settings are the same as in (A).

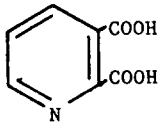
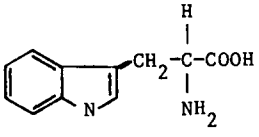
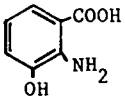
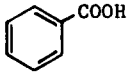
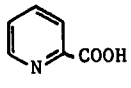
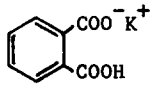
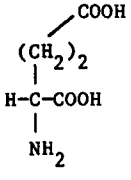
between samples by eliminating the effects of slight, possible experimental differences which lead to fluctuations in the observed values [10,12].

Several sets of experiments were performed to help determine which structural features of quinolinic acid are important in the highly significant decrease in the W/S ratio of MAL-6 labeled erythrocyte membranes caused by this compound [4]. In contrast to quinolinic acid [4], which arises from the same metabolic pathway of L-tryptophan as does 3-hydroxyanthranilic acid, the latter two compounds have no statistically significant effect

TABLE I

COMPARISON OF THE EFFECTS OF STRUCTURAL ANALOGUES OF QUINOLINIC ACID ON THE CONFORMATION OF MEMBRANE PROTEINS OF HUMAN ERYTHROCYTE GHOSTS AS JUDGED BY THE *W/S* RATIO OF THE PROTEIN-SPECIFIC SPIN LABEL, MAL-6

n.s., not significant.

Compound added to ghosts		$(W/S)_{buffer} - (W/S)_{compound}$ (mean ± S.E.)	*N*	*P*
A. 	Quinolinic acid	0.44 ±0.041	18	< 0.00001
B. 	TRP	0.034±0.11	5	n.s.
C. 	3-Hydroxy-anthranilic acid	0.31 ±0.12	4	n.s.
D. 	Pyridine	0.032±0.075	6	n.s.
E. 	Benzoic acid	0.24 ±0.094	4	< 0.1, n.s.
F. 	Picolinic acid	0.39 ±0.093	6	< 0.01
G. 	Potassium hydrogen phthalate (KHP)	0.36 ±0.13	6	< 0.05
H. 	Glutamic acid	−0.19 ±0.08	15	< 0.05

on the *W/S* ratio (Table I). Pyridine also has no effect on the *W/S* ratio, implying that the pyridine ring of quinolinic acid contributes little, if any, by itself to the effect of quinolinic acid on the conformation of membrane proteins (however, see below). As noted earlier, nicotinic acid did not cause a decrease in the *W/S* ratio of MAL-6 [4]. Benzoic acid, which differs from nicotinic acid only in having a benzene ring instead of a pyridine ring (both are aromatic, planar, and rigid), also produced no statistically significant effect on the *W/S* ratio ($P < 0.1$, Table I). Potassium hydrogen

phthalate (KHP) produced a statistically significant decrease in the W/S ratio as opposed to its monocarboxylic acid counterpart, benzoic acid. The effect of KHP, however, was not as great as that observed for quinolinic acid ($P < 0.05$), as shown in Table II. In order to investigate the specificity of the effect on membrane proteins produced by dicarboxylic acids, we reanalyzed our previously published data on the effects of glutamate [8], an aliphatic dicarboxylic amino acid, by examination of paired differences. The W/S ratio of MAL-6 bound to erythrocyte ghosts was slightly increased relative to control ($P < 0.05$) (Table I). The effect of quinolinic acid on the W/S ratio is approximately 3-fold greater than that of glutamic acid and in the opposite direction ($P < 0.0001$) (Table II).

The one anomaly in our results is the effect of picolinic acid on the W/S ratio. Although picolinic acid has only one carboxylic acid group, its effect is statistically indistinguishable from that of quinolinic acid (Table II). Since the carboxylate moiety is adjacent to the pyridine N atom in the ring for picolinic acid, we initially hypothesized that this might lead to ion-pairing, thereby rendering picolinic acid effectively neutral and more likely to interact with the lipid bilayer. In nicotinic acid, such ion-pairing would be much smaller since the carboxylate moiety is meta to the pyridine N. The results of the experiment to test this hypothesis are presented in Table III. Δh_L (Fig. 1), the half-width at half-height of the low-field line in the 5-NS spectrum, is a sensitive and convenient monitor of membrane lipid rotational motion [18]. Neither picolinic acid nor nicotinic acid altered the rotational motional characteristic of lipid alkyl chains of intact erythrocyte membranes or of liposomes composed of extracted erythrocyte membrane lipids, as judged by Δh_L of 5-NS. We previously noted that quinolinic acid also has no effect on membrane fluidity in either system [4].

Table IV presents the results of an experiment in which we compared the effect on the W/S ratio of MAL-6 induced by various ring isomers of quinolinic acid to that of quinolinic acid itself. In all cases, the various ring isomers caused a statistically significant decrease in the W/S ratio. Moreover, comparison of the means of the raw W/S ratios demonstrated that no ring isomer produced an effect statistically distinguishable from any other or from quinolinic acid.

Since one of the main physiological functions of the erythrocyte requires it to have an efficient chloride transport system and since chloride transport seems to be altered in Huntington's disease erythrocytes [14], the efflux rate constant of the exchange pathway for chloride transport in normal erythrocytes was monitored in both the presence and absence of quinolinic acid. Table V presents the results of these experiments. Initially, phosphate-buffered saline was used as the loading and efflux buffer. Since the interaction of quinolinic acid with the erythrocyte membrane may be essentially ionic [4], a low ionic strength sucrose buffer was employed in the second experiment. With the sucrose buffer, deviations from linearity of the

TABLE II

COMPARISON OF THE W/S RATIO OF MAL-6 ATTACHED TO MEMBRANE PROTEINS IN HUMAN ERYTHROCYTE GHOSTS TREATED WITH QUINOLINIC ACID AND COMPOUNDS HAVING CERTAIN IDENTICAL STRUCTURAL COMPONENTS

All studies were performed at the same time except that of the effect of glutamate on the physical state of membrane proteins. Consequently, in this case the effects of glutamic acid (Glu) relative to quinolinic acid (QA) were inferred from differences of each from their respective controls. n.s., not significant.

		$(W/S)_A - (W/S)_B$	(Mean ± S.E.)	P
A	–	B		
1. KHP	–	Quinolinic acid	0.20 ±0.074	< 0.05
2. $(5P8\text{-}QA)_{mean}$	–	$(5P8\text{-}Glu)_{mean}$	0.64	< 0.0001 [a]
3. Picolinic acid	–	Quinolinic acid	0.052 ± 0.075	n.s.

[a] Statistical significance was calculated employing a two-tailed, Student's t-test analysis on these respective means of differences in the W/S ratios of MAL-6.

TABLE III

EFFECTS OF QUINOLINIC ACID AND OTHER STRUCTURAL ANALOGUES ON THE FLUIDITY OF THE LIPID BILAYER AS MONITORED BY THE HALF-WIDTH AT HALF-HEIGHT OF THE $M_I = +1$ LINE OF THE FATTY ACID SPIN LABEL 5-NS IN ERYTHROCYTE MEMBRANES OR ISOLATED LIPOSOMES

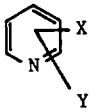
Results are reported as mean ± S.E. (gauss).

Control	Quinolinic acid	Nicotonic acid	Picolinic acid
A. Erythrocyte membranes [a]			
3.11 ± 0.039 $n = 14$	3.08 ± 0.051 [b] $n = 14$	3.08 ± 0.074 [b] $n = 8$	3.13 ± 0.066 [b] $n = 8$
B. Liposomes			
2.95 ± 0.042 $n = 13$	2.98 ± 0.055 [b] $n = 13$	2.91 ± 0.051 [b] $n = 13$	2.92 ± 0.046 [b] $n = 8$

[a] Erythrocyte membranes were labeled with 5-NS and treated as previously described [9,10].
[b] No statistical difference between treated samples and control.

TABLE IV

COMPARISON OF THE EFFECTS OF RING ISOMERS OF QUINOLINIC ACID (QA) ON THE CONFORMATION OF MEMBRANE PROTEINS OF HUMAN ERYTHROCYTE GHOSTS AS JUDGED BY THE *W/S* RATIO OF THE PROTEIN-SPECIFIC SPIN LABEL, MAL-6

Compound added to ghosts				$(W/S)_{buffer} - (W/S)_{compound}$ (mean ± S.E.)	*N*	*P* [a]
1. 5P8				0.00	–	–
2.  Isomers of QA:						
	X	Y	Name			
A.	2-COOH	3-COOH	QA	0.44 ± 0.041	18	< 0.00001
B.	2-COOH	4-COOH	2,4-PDC [b]	0.43 ± 0.047	7	< 0.0001
C.	2-COOH	5-COOH	2,5-PDC	0.53 ± 0.060	5	< 0.001
D.	2-COOH	6-COOH	2,6-PDC	0.55 ± 0.12	4	< 0.02
E.	3-COOH	4-COOH	3,4-PDC	0.42 ± 0.078	7	< 0.002
F.	3-COOH	5-COOH	3,5-PDC	0.48 ± 0.11	5	< 0.02

[a] Statistical significance was inferred employing a two-tailed Student's *t*-test of paired differences.
[b] PDC, pyridinedicarboxylic acid.

TABLE V

EFFECTS OF QUINOLINIC ACID AND TWO STRUCTURAL ANALOGUES ON THE CHLORIDE EFFLUX RATE CONSTANT IN HUMAN ERYTHROCYTES

Results are reported as mean ± S.E. (s^{-1}). A. Efflux buffer, phosphate-buffered saline, $n = 4$. B. Efflux buffer, 5 mM NaCl, 5 mM sodium phosphate, 290 mM sucrose (pH 8.0) (5,5P8(S)), $n = 3$.

Control	Quinolinic acid	Nicotinic acid	KHP
A. Phosphate-buffered saline			
0.084 ± 0.008	0.091 ± 0.007 [a]	0.080 ± 0.006 [a]	0.088 ± 0.002 [a]
B. Sucrose buffer			
0.119 ± 0.06	0.118 ± 0.009 [a]	0.106 ± 0.017 [a]	0.126 ± 0.016 [a]

[a] There is no statistical difference between any treated sample and the respective control.

first-order rate plot began to occur after 13 s. Since this experimental protocol is actually monitoring the self-exchange pathway for chloride, from which we can derive the efflux rate constant if we assume negligible re-entry of $^{36}Cl^-$ into the cell (0.5% hematocrit) [14], we ascribe these observed deviations to be low salt (5 mM) concentration of the efflux buffer. When using phosphate-buffered saline, all points were kept for determining the efflux rate constant. In neither experiment, however, were we able to demonstrate any alteration in k_{eff} when erythrocytes were treated with quinolinic acid, nicotinic acid, or KHP.

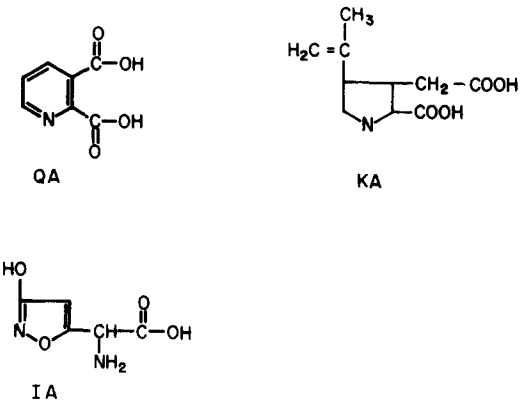


Fig. 2. Structure of the various acids referred to in this work. QA, quinolinic acid; IA, ibotenic acid; KA, kainic acid.

Discussion

The comparison of the effects on the physical state of membrane proteins caused by quinolinic acid, nicotinic acid, pyridine and KHP and benzoic acid suggests that the presence of the two carboxylate anions (at physiological pH) impart to quinolinic acid the majority of its effect. The ring N alone has no effect, as determined by pyridine, but in conjunction with two carboxylic acid substituents (KHP vs. quinolinic acid), seems to provide a slight additional effect over and above that of the two carboxylate anions. From the studies employing the various ring isomers of quinolinic acid, it can be concluded that any alteration of the substitution pattern of the two carboxylic acid groups on the pyridine ring has no effect on the overall decrease in the W/S ratio. The one other structural feature of quinolinic acid that gives it potency seems to be its aromaticity suggesting that its planarity or rigidity may be important. Glutamate, an aliphatic, motionally unrestricted dicarboxylic acid, has virtually no effect on the W/S ratio compared to that of controls [8] or if anything increases this parameter (Table I). Additionally, in support of the idea that the effect of quinolinic acid is not some general artifact produced by cyclic dicarboxylic acids, it should be noted that kainic acid (Fig. 2), an exogenous, excitatory neurotoxin, which is also a non-aromatic, cyclic dicarboxylic acid compound, produces no effect on the W/S ratio [8]. The neurotoxic mechanisms of kainic acid, moreover, are reportedly quite different than those of ibotenic acid [5], which like quinolinic acid, has an aromatic character. The interaction site(s) of quinolinic acid, its ring isomers, and KHP is (are) not known. Although the majority of the spin label is bound to sites on the cytoplasmic side of the membrane, alterations in W/S of MAL-6 have been used to differentiate binding of specific lectins on the exterior surface of erythrocyte ghosts [19,20]. Consequently, if quinolinic acid interacts with proteins at the cell surface, the sensitivity of ESR spin labeling methods is sufficient to monitor this interaction.

Quinolinic acid, under both physiological and low ionic strength, non-physiological conditions, where ionic interactions would become stronger, was shown not to affect k_{eff} of chloride, the transport protein for which is band 3 [21]. Moreover, since the presence of quinolinic acid towards normal, intact erythrocytes did not reproduce our findings of increased Cl^- efflux in Huntington's disease [14], it is possible that a mere excess of quinolinic acid may not be responsible for the pathology observed in Huntington's disease. Rather, a neuronal membrane defect (also manifested in erythrocytes) which may make some neuronal membranes more sensitive to certain endogenous compounds, i.e., neurotransmitters, or neurotoxins such as quinolinic acid, may be involved in this disorder.

There are a number of erythrocyte membrane structural and functional characteristics similar to those in neuronal membranes [22–33]. Among these similarities are: receptors for insulin [22,23], opiates [24], and perhaps acetylcholine [25], a

membrane-bound acetylcholinesterase [26], and transport systems for chloride, choline, water, glucose, Ca^{2+}, Na^{+}, and K^{+} that are reportedly identical to or homologous with those in nervous tissue [27–30]. Moreover, several erythrocyte membrane proteins have been shown to have direct counterparts in a variety of cells including neurons [31–33]. Since quinolinic acid does not affect lipid motion both in the erythrocyte membrane or in isolated liposomes (Table III), it is possible that this compound leads to neuronal death by interacting not with lipid components but with certain, perhaps specific, protein components, i.e., transport proteins or protein neurotransmitter-receptors. However, the relationship between the effect of quinolinic acid on neurons and on human erythrocytes needs to be more closely and critically studied and evaluated to be certain of this possibility. Interestingly, morphologic studies with 2,4-PDC, 3,4-PDC, and KHP, conducted as in Ref. 3, show striking neuronal loss in rat hippocampus (Butterfield, D.A., unpublished observations).

There is one anomalous effect in our study, that of picolinic acid. Although possessing only one carboxylic acid group, picolinic acid produces an effect on the W/S ratio statistically indistinguishable from quinolinic acid. This difference in picolinic acid as compared to nicotinic acid cannot be attributed to any alteration in lipid fluidity (Table III), but the molecular basis of the effect with picolinic acid remains unknown at this time.

In summary, we can conclude that the presence, but not the position, of the two carboxylic acid groups, the presence of aromaticity, and the presence of the N heteroatom impart to quinolinic acid its potential for altering the conformation of erythrocyte membrane proteins. Further experiments to characterize the interaction sites of quinolinic acid on the membrane and assess the neurotoxicity of analogues and isomers of this compound are in progress.

Acknowledgments

This work was supported in part by NIH grants AG-02759 and AG-00084 and a grant from the Alzheimer's Disease and Related Disorders Association, Inc. We thank Dr. Laren Tolbert for the gift of dry pyridine.

References

1 Rawn, J.D. (1983) Biochemistry, p. 857, Harper and Row, New York
2 Gholson, R.K., Ueda, I., Ogasawara, N. and Henderson, L.M. (1964) J. Biol. Chem. 239, 1208–1214
3 Schwarcz, R., Whetsell, W.O., Jr. and Mangano, R.M. (1983) Science 219, 316–318
4 Farmer, B.T., II and Butterfield, D.A. (1984) Life Sci. 35, 501–509
5 Schwarcz, R., Kohler, C., Fuxe, K., Hökfelt, T. and Goldstein, M. (1979) Adv. Neurol. 23, 655–668
6 Chase, T.N., Wexler, N.S. and Barbeau, A. (eds.) (1979) Huntington's Disease, Raven Press, New York
7 Butterfield, D.A. and Markesbery, W.R. (1981) Life Sci. 28, 1117–1131
8 Butterfield, D.A., Akaydin, A. and Kommor, M.L. (1981) Biochem. Biophys. Res. Commun. 102, 190–196
9 Butterfield, D.A. and Fitzpatrick, M.A. (1980) J. Magnet. Reson. 37, 159–163
10 Butterfield, D.A. (1982) Biol. Magnet. Reson. 4, 1–78
11 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509
12 Butterfield, D.A. and Markesbery, W.R. (1981) Biochem. Int. 3, 517–525
13 Barber, M.J., Rosen, G.M. and Rauckman, E.J. (1983) Biochim. Biophys. Acta 732, 126–132
14 Butterfield, D.A. and Markesbery, W.R. (1982) J. Neurol. Sci. 57, 29–39
15 Butterfield, D.A., Roses, A.D., Appel, S.H. and Chesnut, D.B. (1976) Arch. Biochem. Biophys. 177, 226–234
16 Schneider, H. and Smith, I.C.P. (1970) Biochim. Biophys. Acta 219, 73–80
17 Fung, L.W.M. and Simpson, M.J. (1979) FEBS Lett. 108, 269–273
18 Mason, R.P., Giavedoni, E.B. and Dalmasso, A.P. (1971) Biochemistry 16, 1196–1201
19 Feix, J.B., Green, L.L. and Butterfield, D.A. (1982) Life Sci. 31, 1001–1009
20 Butterfield, D.A., Farmer, B.T., II and Feix, J.B. (1983) Ann. N.Y. Acad. Sci. 414, 169–179
21 Grinstein, S., Ship, S. and Rothstein, A. (1978) Biochim. Biophys. Acta 507, 294–304
22 Baumann, G., Abramson, E.C. and Dobny, E.C. (1983) J. Cell. Biochem. 21, 233–237
23 Polychronakos, C., Guyda, H.J. and Posner, B.I. (1983) J. Clin. Endocrinol. Metab. 57, 436–438
24 Abood, L.G., Atkinson, H. and MacNeil, M. (1976) J. Neurosci. Res. 2, 427–431
25 Huestis, W.H. and McConnell, H.M. (1974) Biochem. Biophys. Res. Commun. 57, 726–732
26 Silver, A. (1974) The Biology of Cholinesterases, North Holland, Amsterdam
27 Surgenor, D.M. (1974) The Red Blood Cell, 2nd Edn., Academic Press, New York
28 Kimelberg, H.K. (1981) Biochim. Biophys. Acta 646, 179–184
29 Fambrough, D.M., Engel, A.G. and Rosenberry, T.L. (1982) Proc. Natl. Acad. Sci. USA 79, 1078–1082

30 Sastry, B.V.R. and Sadavongvivad, G. (1979) Pharmacol. Rev. 30, 65–132
31 Lazarides, E. and Nelson, W.J. (1983) Science 220, 1295–1299
32 Bennett, V. and Davis, J. (1981) Proc. Natl. Acad. Sci. USA 78, 7550–7554
33 Lazarides, E. and Nelson, W.J. (1983) Science 222, 931–933