The Effect of Aminoglutethimide on Membrane Permeability In Rabbit and Human Erythrocytes

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Abstract

Studies were conducted to determine if AG alone causes hemolysis and if AG would affect the permeability (hemolysis time) to a known hemolytic agent, *e.g.* ethylene glycol. Sodium and potassium uptake in human and rabbit erythrocytes was compared between AG treated and control blood by use of the flame photometer. Rabbit blood was exposed to AG and cell fractions were assayed by spectrophotometric and chemical means to determine whether AG enters the red blood cell and/or occupies sites in the membrane.

AG increases the hemolysis time of ethylene glycol in fresh human and rabbit red blood cells and decreases the time in human blood bank blood; increases the K^+ efflux from rabbit erythrocytes; enters the red blood cell and may occupy site(s) in or on the membrane.

Introduction

The drug aminoglutethimide phosphate (α-ethyl-α-p-aminophenyl glutarimide, Elipten, AG) has been used clinically as an anticonvulsant in the treatment of epilepsy (2, 10, 14). The motor, psychic, and sensory symptoms of epilepsy, including loss of consciousness, are based on a substrate of paroxysmal disturbances of the electrical activity of the brain (1). A thorough search of the literature reveals that the mode of action of the drug in correction of these symptoms is not known. It is of interest that the drug was not infallible in stopping convulsions when used alone, but was effective when combined with small doses of other drugs (4, 12, 13). In 1965, the drug was withdrawn from the market by the Federal Drug Administration due to side effects, namely adrenal toxicity (3) and induced goitrous hypothyroidism (16). Eversole and Zimmerman (8) and Pollock (15) observed ataxia on administration of the drug to white rats.

A primary activity of the drug is inhibition or synthesis of adrenal cortical steroid hormones (5, 6, 11). Fishman *et al.* (9), in clinical studies on man, found that the most consistent action of AG is to decrease aldosterone secretion with concomitant changes in electrolyte metabolism. Aminoglutethimide phosphate markedly decreases the output of corticosterone in the rat (6). Zimmerman (18) demonstrated that AG causes an elevation of sodium and decrease in potassium concentration in muscle tissue and the reverse in plasma with associated retention of water. This change appears to be independent of adrenal secretion. A similar AG induced change in normal serum and plasma sodium and potassium values in warm blooded animals, but not in cold blooded animals, was determined by Pollock (15). This shift of sodium from plasma to tissue in the treated animal may suggest a change in membrane permeability or transport of

sodium. Brett and Hayden (unpublished data) found that AG apparently restores normal rhythmicity to an arrhythmic turtle heart when applied topically.

The purpose of this study was to test the hypothesis that the drug AG exerts its effect on water and electrolyte balance by virtue of a direct action on the cell membrane resulting in a permeability change, and to determine if AG localized either in the plasma membrane or within the cell.

Methods and Materials

Erythrocytes were from rabbit blood collected in Alsever's solution, fresh human blood in acid citrate dextrose solution and 26-day old human blood bank blood. Hemolysis times were determined for red blood cells in distilled water, AG, ethylene glycol and AG in the presence of ethylene glycol. Solutions were unbuffered or buffered to approximately pH 4 and 7. Five drops of blood were added to 10 ml of test solution, cells were dispersed by two rapid inversions of the tube and hemolysis time was determined as having occurred when light absorption at 640 mu fell to an optical density of 0.2 using a Bausch and Lomb Spectronic 20 spectrophotometer.

Additional experiments were performed to determine if the presence of AG influenced Na increase and K decrease in erythrocytes similarly to that observed by Zimmerman (18) in rat muscle tissue. Rabbit cells were exposed to an AG concentration of 1 mg/ml by adding 1 ml of 0.9% NaCl solution containing 5 mg of AG to 4 cc of blood in Alsever's solution. Human cells were exposed to AG concentrations of 0.05, 0.5 or 5.0 mg/ml by adding 1 ml of blood in ACD solution to 1 ml of 0.9% or 0.3 M NaCl solution containing 0.1, 1.0 or 10 mg AG. Control solutions were prepared as above except for the omission of AG. Concentrations of sodium and potassium were determined using a Coleman Model 143 flame photometer.

Localization of AG in tissue was determined by fractionating rabbit red blood cells as summarized in Figure 1. AG content was assayed spectrophotometrically before and after removal of plasma proteins by gel filtration (Biogel P2) or by the method of Douglas and Nichols (7). The latter method utilizes extraction of AG into dimethyl chloride and reaction with p-dimethyl aminobenzaldehyde (Ehrlich's reagent) to yield a Schiff's base which was quantitated spectrophotometrically.

The data were statistically evaluated using the Student's "t" test and significance was determined at the 1% level.

Results

Hemolysis data (Table 1) show that AG significantly shortens the hemolysis time of a known hemolytic agent, ethylene glycol, in human blood bank cells but increases the time in fresh human and rabbit blood cells. This is not due to an increase in particle number

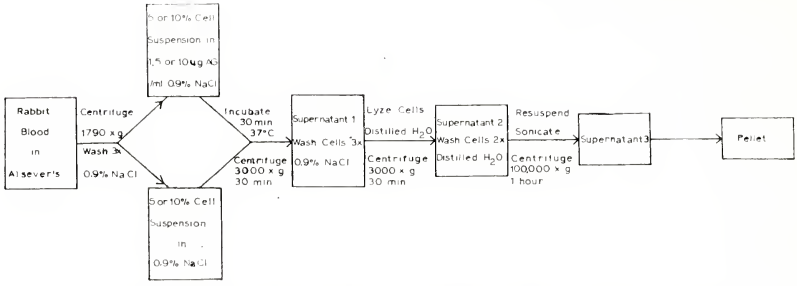

FIGURE 1. *Flow sheet for treatment and assay of rabbit blood cells exposed to aminoglutethimide.*

as indicated by the results obtained with 0.3088 M EG which has a particle number equivalent to that in the EG-AG solution. Hemolysis in a 0.3 M solution of AG alone was found to occur in an average time of 15 minutes. An increase in pH increases the hemolysis time of AG-EG in all cases except rabbit cells at pH 7; whereas, EG solutions at an unbuffered or buffered pH near 7 show a shorter hemolysis time than at pH 4 except for blood bank blood. Hemolysis time tends to be shorter in most solutions with rabbit blood cells than in human cells and in fresh human blood than in blood bank cells.

TABLE 1. *Hemolysis times in seconds for human blood bank blood (HBBB), fresh human blood (FHB) and rabbit blood (RB) obtained in test solutions at a given pH. (Numbers in parentheses indicate number of cases; results are expressed as the mean ± S.E.).*

Type of Blood	Water	0.3 M EG	0.3088 M EG	0.3 M EG + 1 mg AG/ml
SET A—Solutions not buffered				
	pH 8.00	pH 7.45	pH 7.15	pH 3.55
HBBB	11.55±.24 (6)	15.37±.42 (6)	15.48±.48 (6)	11.17±.29 (6)
FHB	-4.5[1] (10)	5.0±.06 (10)	5.01±.20 (9)	10.9±.29 (6)
RB	-5.0[1] (9)	5.45±.35 (9)	5.53±.10 (9)	6.81±.12 (9)
SET B—Solutions buffered to pH 4				
HBBB	11.13±.44 (6)	16.08±.27 (6)	15.32±.33 (6)	14.32±.16 (6)
FHB	9.14±.34 (7)	15.45±.40 (8)	15.63±.39 (8)	14.75±.23 (6)
RB	5.27±.12 (10)	10.4±.22 (10)	10.58±.24 (10)	12.77±.25 (10)
SET C—Solutions buffered to pH 7				
HBBB	11.52±.34 (6)	14.72±.32 (6)	14.57±.25 (6)	19.33±.56 (6)
FHB	9.08±.23 (5)	11.84±.16 (8)	11.73±.22 (8)	15.39±.27 (8)
RB	5.46±.12 (10)	8.21±.16 (10)	8.30±.14 (10)	10.73±.16 (10)

[1] Standard error not applicable.

AG significantly increased potassium efflux from rabbit blood cells but did not significantly increase sodium influx. Values in meq/l for controls were 157.6±1.37 (n=5) for Na and 2.2±0.0 (n=5) for

K, while for the experimentals the values were 156.4±0.51 (n=5) for Na and 2.66±0.05 (n=5) for K. A significant efflux of sodium occurred in human blood bank cells exposed to AG in 0.3 M NaCl solution but no change in potassium occurred. Values for experimentals were 225.16±0.26 (n=6) for Na and 1.6±0.0 (n=6) for K and for controls were 223.25±0.35 (n=2) for Na and 1.6±0.0 (n=2) for K.

TABLE 2. *AG content of rabbit blood fractions as measured by Schiff base reaction.*

Fraction	AG/ml recovered	
	5 ug/ml	1 ug/ml
Supernatant 1 plus washes	4.19	
Lysed cell supernatant plus washes	1.05	0.55
Sonicated cell supernatant plus pellet	0.00	0.48
Total	5.24	1.03

Analysis of fractions of rabbit red blood cells (Table 2) demonstrates that AG enters the cells and the presence of almost half of the AG in the sonicated supernatant and pellet of the 1 ug/ml treatment suggests the presence of AG in the membrane. Aminoglutethimide phosphate was found to have a main absorption peak at 237 mu which was found to be linear with concentrations from 1-10 ug AG/ml water or 0.9% NaCl solution, but the presence of plasma proteins prevented quantitative analysis of AG in experimental solutions. Figure 2 shows the spectrophotometric assay of AG after removal of plasma proteins. Both the difference in amplitude and width of the peaks between the standard and test solution indicate that although much of the AG was recovered in Supernatant 1, some of the AG remained with the cells.

Discussion

The relatively shorter hemolysis time for fresh human blood cells exposed to all solutions than for blood bank cells should be of clinical interest. One criterion used to determine storage time for blood bank blood is cell fragility as measured by hemolysis. Oxygen carrying capacity and the side effect potential of drugs could be seriously modified in blood bank blood as it approaches legal expiration date.

Hemolysis of both human and rabbit blood cells is affected by pH. Human blood has a pH of 7.35-7.45 and any pH which differs drastically from the normal pH could produce a change in the configuration of some cell component or influence the active transport system. Dissociation of AG is influenced by pH and its effect on EG penetration in human blood bank cells is increased at a lower pH.

Hemolysis results are distinctly different for rabbit cells and human cells. Rabbit blood cells have a shorter hemolysis time than

human cells in most solutions and show a longer hemolysis time for EG at all pHs upon the addition of AG. A difference in the membrane and/or transport system of human and rabbit erythrocytes exists. Na efflux is three times faster in rabbit than human cells (17). The possible deficiency of ATPase in rabbit erythrocytes has been suggested by Duggan *et al.* (17).

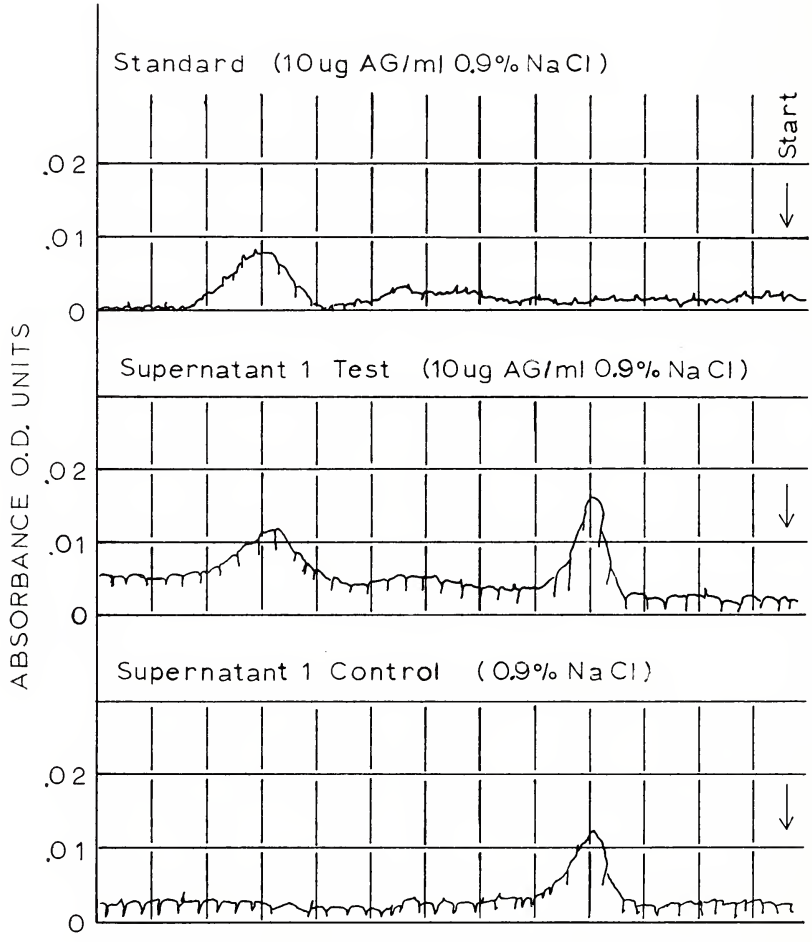

FIGURE 2. *Aminoglutethimide determination by ultraviolet absorption.*

A conspicuous difference was found between rabbit and human blood in the effect of AG on Na and K flux. The increase in K efflux in rabbit blood agrees with Pollock's (15) *in vivo* studies but the increase in plasma Na in human blood is just the reverse. The change in permeability due to storage might account for this difference. The relationship of the RBC to plasma is not the same as the

relationship of other tissue cells to plasma and care must be used in comparing results of the two types of studies. An increase in potassium efflux without a comparable sodium influx would result in an increased amplitude of the membrane resting potential. Increased resting potential could explain the basis for AG's effect on elimination of epileptic convulsions and its production of ataxia. Electrophysiological studies should clarify the relationship of AG to cation transport. Although this study indicates that AG can produce an electrolyte shift by a direct effect on the cell membrane, the possibility of AG eliciting this shift through extra-adrenal tissue cannot be eliminated.

Both chemical and spectrophotometric assays demonstrate that AG enters the red blood cell. Data obtained from the chemical test show that lysis released all the retained AG in the 5 ug treatment, but only one-half of the retained AG in the 1 ug treatment. The release of AG by sonication suggests the presence of the compound in the cell membrane; whereas, the release by lysis suggests that AG is retained within the cell rather than in the membrane. One of two explanations could serve to reconcile this apparent conflict. Disruption of the membrane during lysis of the cells in the 5 ug test may have released the AG from the membrane or incomplete release of AG from the cell may have occurred during lysis of the RBC in the 1 ug test. Future work should be designed so as to eliminate these possible discrepancies.

The results show that AG significantly decreases the hemolysis time of ethylene glycol in human blood bank blood and increases the time in fresh human and rabbit blood; increases the K efflux from rabbit erythrocytes and efflux of Na in blood bank cells; enters the red blood cell and may occupy site(s) in or on the membrane.

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