Effect of Aminoglutethimide on Rabbit EKG and Blood Pressures

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Abstract

Aminoglutethimide (Elipten-Ciba, AG) administered IV 50 mg/kg BW to level three anesthetized New Zealand White rabbits, was found to produce parallel reductions in intraventricular and systemic pressures. Time of obtainment of maximum pressure decrease was influenced by rate of injection. Heart rate decreased in conjunction with the decrease in pressures. The absence of a reflexive increase in heart rate during the period of decreased pressures suggests a sympathetic-vagal mediated action by AG. An alternative hypothesis is suggested by AG's previously described role as an anticonvulsant: decreased muscular activity would also result in lowered blood pressures. No arrhythmia could be attributed to AG.

Introduction

Aminoglutethimide (alpha-(para-aminophenyl)-alpha-ethyl glutarimide, Elipten, AG) is a derivative of the nonbarbiturate hypnotic glutethimide and was introduced as an anticonvulsant in 1960. By 1966 the U.S. Food and Drug Administration had withdrawn it from the market following reports of unfavorable side effects during clinical usage: goitrous hypothyroidism (20), adrenal insufficiency (4, 5) and congenital pseudo-hermaphroditism (8). AG was given Investigational New Drug status in 1966 by the F.D.A. to allow continued dispension to epileptics who were dependent upon AG for remission of seizures and to allow experimental evaluation. Further studies have shown AG to be a competitive inhibitor of the mitochondrial desmolase enzyme system blocking steriodogenesis at the conversion of cholesterol at $\triangle$-5-pregnenolone (4, 26).

Recently AG has been used in treatment of cancer of the breast (4, 6, 13) and prostate (22), in treatment of tumors of the adrenals (5, 24), in treatment of primary and secondary hyperaldosteronism (5, 10, 27), and to induce abortion (9).

AG has been shown to increase urinary sodium through a reduction in aldosterone secretion (3, 4, 5, 14). Fishman et al. (5) reported a marked sodium diuresis in patients on 0.75 to 2.0 g AG/day with no concomitant change in serum electrolyte concentrations. Camacho et al. (3) reported a decrease in serum sodium and chloride and an increase in serum potassium in patients on 0.75 to 1.0 g AG/day within three days. Pollock (19) found a decrease in serum sodium and an increase in serum potassium 30 minutes after an injection of 100 mg AG/kg BW in homeotherms but not in poikilotherms. Zimmerman (28) also reported similar serum electrolyte changes with elevated tissue sodium and depressed tissue potassium in the rat. Studies on adrenalectomized rats led Zimmerman to conclude that AG caused a shift of potassium from plasma to tissue and of sodium from tissue to plasma independent of any adrenal cortex mediated action.

Hayden and Brett (7) found that AG caused an increase in the potassium efflux of rabbit red blood cells without changing sodium influx. They concluded that AG enters the RBC and may occupy site(s) in or on the membrane and hypothesized that AG may change membrane permeability or sodium-potassium ATPase activity in the membrane.

The observation by Pollock (19) that an intracardial injection of AG produced cardiac arrest in ventricular diastole followed by resumption of activity at a faster rate with stronger force, suggested a pilot study on the effect of AG on the heart. AG was found to induce tachycardia in the turtle for approximately 24 minutes with reoccurrences of tachycardia of one minute every 20 minutes. The electrocardiogram evidenced a shortened T-P (rate determined) and P-R (pacemaker location or conduction rate determined) interval with no QRST changes. No cardiac arrest was observed but other pilot studies have reported that a topical application of AG on an arrhythmic turtle heart restored rhythmicity (Brett, Personal Communication).

This study was to determine AG's effect on heart action—electrical and contractile as measured by EKG and blood pressure.

Materials and Methods

Methoxyflurane (Penthrane: Abbott Labs) was selected as the anesthetic because of its reported minimum effect on heart activity. Brown (2) and others have reported no EKG changes with Methoxyflurane. However, depression of myocardial contractility with Methoxyflurane has been reported by many investigators including Woods (1), Brown (2), and Redondo et al. (21) but is reported to be less than for Ethrane, Halothane and chloroform. Methoxyflurane does not sensitize the heart to epinephrine nor does it cause a release of catecholamines into the blood stream (16).

Induction of anesthesia was performed with diethyl ether in a closed chamber 15 minutes after subcutaneous injection of 0.05 mg/kg BW atropine (atropine methyl bromide) at the scruff of the neck to alleviate excessive salivation and mucus secretions due to ether irritation. Eye blink and muscular relaxation were used as indicators of level of anesthesia. Surgical anesthesia (level three) was maintained with Methoxyflurane administered through a tracheal cannula inserted below the larynx. Methoxyflurane vaporized by copper kettle technique was administered through non-rebreathing open circuit with pressure maintained by a Narco respirator (Narco Bio-Systems). Induction of Methoxyflurane was accomplished on a 2:1 inspiration-expiration ratio with maintenance on a 1:1 ratio. Inflation of the lungs with an average of 16 cm water pressure occurred 70 times a minute.

Systemic blood pressure was obtained by cannulation of the right femoral artery with Intramedic P50 polyethylene tubing inserted to the aorta. Left ventricular pressure was acquired by passing a cannula into the left ventricle via the right carotid through the semilunar valves. Intraventricular and systemic pressures determined by Statham P23Db transducers were recorded in conjunction with EKGs from needle elec-

trodes on a Gilson ICM-5 Polygraph at a paper speed of 25 mm/sec. After stabilization on Methoxyflurane, the vehicle was injected as a control and then 50 mg AG/kg BW was injected into the marginal ear vein and the parameters were monitored. AG was maintained in a solution of distilled water of pH 4 (HCl) at a concentration of 100 mg AG/ml. Elapsed time of injection was influenced by volume of AG solution and by rate of venous drainage.

Records were analyzed for changes in pressures, heart rate and arrhythmias. Data were subjected to statistical analyses utilizing regression analysis and Students' "t" test.

Results

Durations of the various intervals and waves of the EKGs were found to be within the normal ranges (12, 15, 25) for all rabbits observed. Injection of AG produced significant ($p>.01$) parallel reductions in intraventricular and systemic pressures (Table 1). The rate of injection influenced the time required for the pressure to reach maximum decrease. There is a general direct correlation between rate of injection and time to maximum decrease in pressure (Fig. 1). All AG

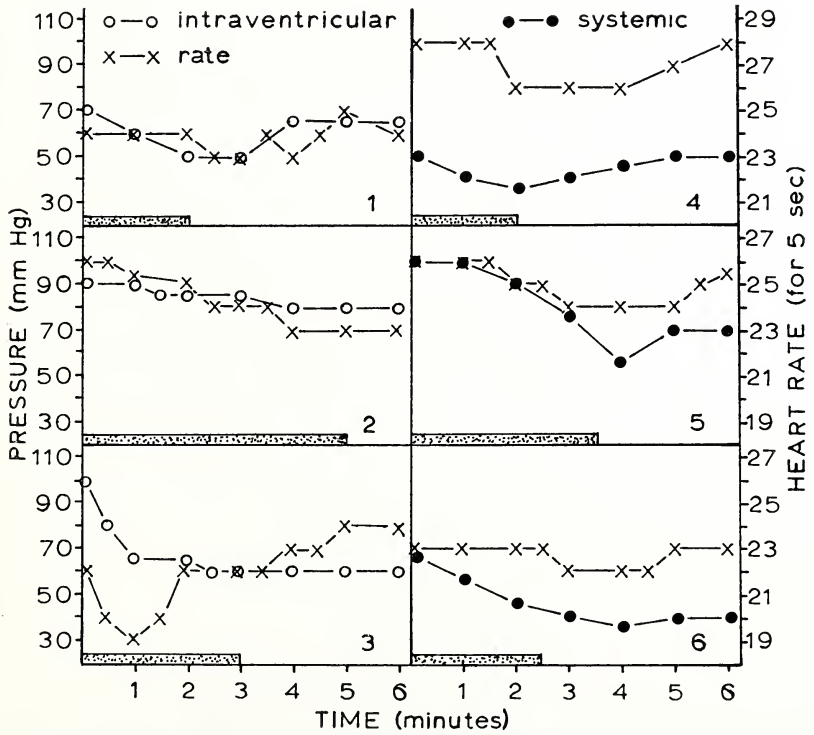

FIGURE 1. *Heart rate and intraventricular pressures for rabbits 1, 2, and 3 and systemic pressures for rabbits 4, 5, and 6 are plotted as a function of time from start of AG injection. Duration of AG injection is expressed as a bar on the time axis.*

injected rabbits showed a decrease in heart rate which lagged behind the decrease in intraventricular and systemic pressure (Fig. 1).

Both AG and control cannulated animals evidenced an arrhythmia in the form of premature ventricular beats; one rabbit also developed paroxysmal ventricular tachycardia (Fig. 2).

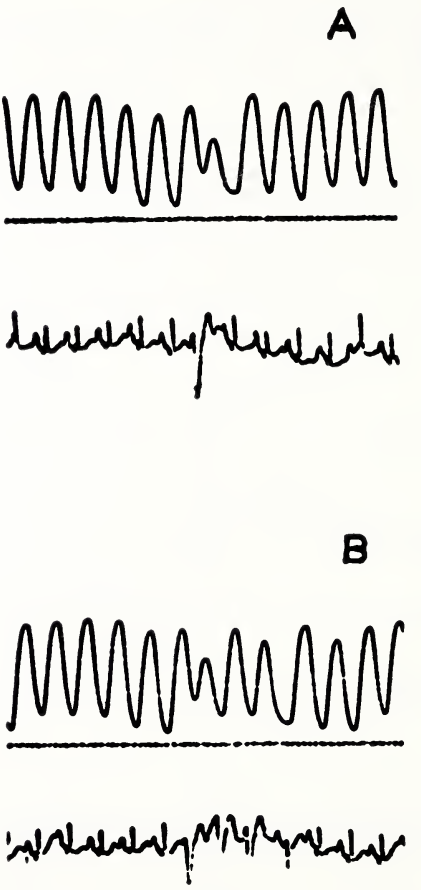

FIGURE 2. *EKG (Lead II) strip A is an example of a premature ventricular systole in an anesthetized, cannulated animal. Tracing B shows a series of premature ventricular systoles—paroxysmal ventricular tachycardia. Premature beats are due to an enhanced pacemaker. Intraventricular pressure appears as the upper recording on both strips.*

TABLE 1. *Intraventricular (I) and systemic (S) pressures for anesthetized, cannulated rabbits injected IV with 50 mg AG/kg BW.*

1	I	70/5	60/10	50/10	50/15	65/10	65/40	65/40
(2 min)	S	70/50	50/40	50/45	50/40	50/40	50/40	50/40
2	I	90/0	90/0	85/0	85/0	80/0	80/0	80/0
(5 min)	S	85/60	85/60	80/55	80/55	80/50	80/50	80/60
3	I	100/20	65/10	65/20	60/20	60/20	60/20	60/20
(3 min)	S	90/80	50/40	40/35	40/35	40/35	40/35	40/35
4	I							
(2 min)	S	50/40	40/30	35/30	40/35	45/40	50/35	50/40
5	I							
(3.5 min)	S	100/75	100/70	90/65	75/45	55/35	70/45	70/45
6	I							
(2.5 min)	S	65/45	55/40	45/40	40/35	35/30	40/35	40/35

Discussion

Blood pressure in the functioning organism is determined by blood volume, vascular resistance and cardiac output. In the present study, blood volume was considered to be a constant. Under constant vascular resistance, intraventricular pressure is an index of myocardial contractility. With changes in vascular resistance, intraventricular pressure loses its value as an index to contractility. A constant myocardial contractile level with a decrease in vascular resistance would be observed as a decrease in intraventricular and systemic pressure due to a decreased column of blood that the heart would be ejecting against. Lowered myocardial contractility with a constant vascular resistance also would be demonstrated as a decrease in intraventricular pressure. AG reduces both intraventricular and systemic pressures which could be due to a decrease in vascular resistance and/or myocardial contractility.

The effects of the anesthetic on the physiological parameters of the organism always present a problem when one is attempting to analyze the effects of a specific drug. Methoxyflurane was selected because of its minimal effect on heart action. Methoxyflurane produces it's negative inotrophic effect on heart muscle by blocking glycolysis prior to the phosphofructose kinase step and by some other mechanism (17, 18). Paradise and Ko (18) suggest a variety of action sites in the heart for Methoxyflurane. In the present study, Methoxyflurane's depressant action, evidenced by intraventricular recordings, was allowed to stabilize before injection of AG; therefore, the additional decrease in pressure upon AG injection indicates an AG or AG-Methoxyflurane mediated action.

AG appears to have a triform action on the cardiovascular system of the anesthetized rabbit. These actions may or may not be independent of one another—decreased intraventricular pressure, decreased systemic pressure and decreased heart rate. Two mechanisms may be proposed to explain these actions and the time factor involved in them. AG may affect the ionic stability of the cell or it may affect sympathetic and/or vagal tone.

Increases in K^+ efflux from rabbit RBCs has been shown to occur with AG. Hayden and Brett (7) have suggested that this may be due

to AG's effect on ATPase inhibition or to a change in membrane permeability since AG does not increase Na^+ influx. The decrease in muscular activity observed upon injection of AG could result from K^+ efflux from muscular cells. With a decrease in muscular activity, vasodilation would result from relaxation of the vascular bed. Blood with an increased K^+ content would pool in the tissues, increasing dilation. Intraventricular pressure would decrease due to both a reduction in venous return to the heart and to a proposed loss in contractility.

Sympathetic control influences muscle relaxation, pacemaker activity, speed of conduction through the AV node, and cellular metabolism. Catecholamines mediate sympathetic control with norepinephrine having the major influence in the heart. A decrease in sympathetic tone would produce a decrease in intraventricular pressure through several mechanisms: lengthening of time-to-peak tension, decreased rate of tension development, increased total twitch duration, decreased Ca^{++} flux, and decreased cyclic AMP formation and activity (11). The chronotrophic effect of catecholamines is due to increased deactivation of outward K^+ currents which increases the rate of diastolic depolarization of pacemaker cells (11).

A decrease in sympathetic tone is indicated by the results obtained. Sympathetic control is important in maintaining blood pressure, heart rate and myocardial contractility. The anesthetized animal tends to use sympathetic means to restore blood pressure levels (23). That the heart rate did not increase with a decrease in systemic pressure (Fig. 1), indicates that the baroreceptor reflex was unable to function suggesting impaired sympathetic activity. Sympathetic stimulation also is important in maintaining vasoconstriction; a decrease in sympathetic tone would decrease vascular resistance, thus lowering systemic pressure.

Another alternate hypothesis involves vagal stimulation. Increased vagal activity would explain the decreased heart rate and the failure of the sympathetics to check vagal action. To elucidate the action AG has on the sympathetics and vagus, studies should be performed on sympathectomized (reserpine or 6-hydroxydopamine) and vagotomized animals.

Premature ventricular systoles, evidenced in both Methoxyflurane and AG-Methoxyflurane animals (Fig. 2) are the result of enhanced pacemaker activity at ectopic foci. No P wave was observed, indicating an ectopic focus not in the atria. The intraventricular septum is susceptible to induced ectopic foci formation due to mechanical stimulation. This suggests that the premature ventricular systoles present in cannulated animals could be due to the cannula stimulating the septum.

Acknowledgments

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Literature Cited

1. BAGWELL, E. E., E. F. WOODS. 1962. Cardiovascular effects of Methoxyflurane anesthesia. Anesthesiol. 23:53-57.
2. BROWN, B. JR. 1971. A comparative study of the effect of five general anesthetics on myocardial contractility. Anesthesiol. 34:236-247.
3. CAMACHO, A. M., R. CASH, A. J. BROUGH, and R. S. WILROY. 1967. Inhibition of adrenal steroidogenesis by Aminoglutethimide and the mechanism of action. JAMA 202:20-26.
4. CASH, R., A. J. BROUGH, M. COHEN, and P. SATOH. 1967. Aminoglutethimide (Elipten Ciba) as an inhibitor of adrenal steroidgenesis: mechanism of action and therapeutic trial. J. Clin. Endocr. & Metab. 27:1239-1248.
5. FISHMAN, L. M., C. W. LIDDLE, D. P. ISLAND, N. FLEISCHER, and O. KUCHEL. 1967. Effects of Aminoglutethimide on adrenal function in man. J. Clin. Endocr. & Metab. 27:481-490.
6. HALL, T., J. BARLOW, C. GRIFFITHS, and Z. SABA. 1969. Treatment of metastatic breast cancer with Aminoglutethimide. Clin. Res. 17:402.
7. HAYDEN, M. A., and W. J. BRETT. 1971. The effect of Aminoglutethimide on membrane permeability in rabbit and human erythrocytes. Proc. Indiana Acad. 80:510-516.
8. IFFY, L., J. S. ANSELL, J. S. BRYANT, and W. L. HERRMANN. 1965. Nonadrenal female pseudohermaphroditism: an unusual case of fetal masculinization. Obstet. & Gynec. 26:59-65.
9. JANNE, O., U. PASTORE, H. TIMONEN, and R. VIHKO. 1974. Plasma and urinary steroids in pregnant women during para-aminoglutethimide treatment. Contraception 9:239-247.
10. KUCHEL, O., K. HORKY, and I. GREGOROVA. 1970. The treatment of secondary hyperaldosteronism with oedema by aminoglutethimide. Pharm. Clin. 2:138-142.
11. LANGER, G., and A. BRADY (eds.). 1974. The Mammalian Myocardium. Wiley & Sons, New York, N.Y. 310 p.
12. LEVINE, H. D. 1942. Spontaneous changes in the normal rabbit electrocardiogram. Amer. Heart J. 24:209-214.
13. LYNNE, C., D. GROWER, and M. H. LESSOF. 1971. The effects of aminoglutethimide and prednisolone on steroid secretions in cases of carcinoma of the breast. J. Endocr. 49:xxi-xxii.
14. MACHENO-RICO, E., O. KUCHEL, W., NOWACZYNSKI, K. SETH, C. SASAKI, K. DAWSON, and J. GENEST. 1973. A dissociated effect of Aminoglutethimide on the mineral corticoid secretion in man. Metab. 22:123-132.
15. NELSON, C. V., W. C. WAGGONER, and P. R. GASTONQUAY. 1964. High fidelity electrocardiograms of normal rabbits. Amer. J. Physiol. 205:1107-1111.
16. NORTH, W. C., and C. R. STEPHEN. 1966. A second look at Methoxyflurane: four years' experience. Anes. and Anal. 45:117-125.
17. PARADISE, R. R., and G. KRISHNA. 1972. The effect of pyruvate on human atrial muscle contractility depressed by Methoxyflurane and by pentobarbitol. Anesthesiol. 36:364-368.
18. PARADISE, R. R., and K. KO. 1973. Multiple mechanisms of action of halothane and Methoxyflurane on force of contraction of isolated rat atria. Anesthesiol. 39:278-284.
19. POLLOCK, G. P. 1969. A comparative study of some effects of aminoglutethimide phosphate on serum potassium and sodium. Proc. Indiana Acad. 78:468-471.
20. RALLISON, M. I., L. F. KUMAGAI, and F. H. TYLER. 1967. Goitrous hypothyroidism induced by an anticonvulsant compound. J. Clin. Endocr. & Metab. 27:265-272.
21. REDONDO, J., L. NAVAKOVIC, F. OLIVARI, and M. PENNA. 1971. The effect of Methoxyflurane on myocardial contractility and reactivity. Anesthesiol. 34:450-457.
22. ROBINSON, R. G., and B. S. THOMAS. 1971. Effect of hormonal therapy on plasma testosterone levels in prostatic carcinoma. Brit. Med. J. 4:391-394.

23. RUCH, T., and H. PATTON (eds.). 1974. Physiology and Biophysics. W. B. Saunders Co., Philadelphia, Pa. Vol. 2, 546 p.

24. SCHTEINGART, E. D., R. CASH, and J. W. CONN. 1966. Aminoglutethimide and metastatic adrenal cancer. Maintained reversal (six months) of Cushing's syndrome. JAMA **198**:1007-1010.

25. SZABUNIEWICZ, M., H. D. HIGHTOWER, and J. R. KYZAR. 1971. The electrocardiogram and spatiocardiogram of the rabbit. Can. J. Comp. Med. **35**:107-114.

26. WILROY, R. S., A. M. CAMACHO, and R. CASH. 1967. Inhibition of adrenal steroidogenesis by aminoglutethimide and mechanism for action. JAMA **202**:114-120.

27. WOODS, J. W., G. W. LIDDLE, E. G. STANT, A. M. MICHELAKIS, and A. B. BRILL. 1969. Effect of an adrenal inhibitor in hypertensive patients with suppressed renin. Arch. Intern. Med. **123**:366-370.

28. ZIMMERMAN, R. E. 1968. Some effects of aminoglutethimide on water and electrolyte metabolism in the female rat. Unpublished M.A. Thesis, Indiana State University.