

Some Effects of Aminoglutethimide on Water and Electrolyte Metabolism in the Female Rat

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Abstract

Aminoglutethimide phosphate (AG) was injected subcutaneously at a dosage level of 100 mg/kg body wt/day into Charles River strain female rats of approximately 160 g. Control rats were treated under the same conditions, but were injected with distilled water. Comparisons were made at 1 and 3 days.

Urine volumes were compared between water-fasted controls and treated animals. Water consumption was compared between nephrectomized controls and treated animals. Water consumption and urine output were compared between intact controls and treated animals allowed to drink *ad libitum*.

Urine, tissue, and plasma sodium and potassium levels were compared in treated and control animals, both intact and adrenalectomized.

Chronic administration of AG to intact rats induced a diabetes-insipidus-like state and many animals doubled their 24-hour water exchange. Aminoglutethimide phosphate elicited polydipsia in the absence of the adrenals or the kidneys, suggesting that polyuria was secondary to increased water intake in the complete absence of adrenal cortical hormones.

Sodium and potassium concentrations in urine, tissue and plasma of intact animals treated with AG resembled those in adrenalectomized non-treated animals, while treatment in adrenalectomized animals appeared to produce an additive effect. Such results may be interpreted as indicating that the effects of AG are mediated only in part by the adrenal cortex.

Introduction

Aminoglutethimide phosphate (AG) [α -(*p*-aminophenyl) α -ethylglutarimide] has been used clinically, and appears to have value in the treatment of generalized seizure when administered in dosages of 250-500 mg three times a day (1). Kahnt and Neher (4) in a continuing study of the effect of large numbers of drugs on beef adrenal homogenates *in vitro* found that aminoglutethimide (AG), among others, inhibited steroid synthesis. It is now generally agreed that the primary activity of AG is to inhibit the synthesis of corticoids at some step(s) between the conversion of cholesterol to pregnenolone, the latter being a precursor of all steroid hormones (2, 3). It has been shown, in rats, that AG markedly decreased the output of corticosterone (3).

The purpose of this investigation was to determine the effects of aminoglutethimide on water exchange and sodium and potassium levels in body fluids and tissues of intact and adrenalectomized animals. We expected this study to answer the question of whether AG induces physiological changes characteristic of adrenal dysfunction and whether or not its effects on water and electrolyte metabolism are brought about by direct action or by mediation through changes induced in adrenal cortical function.

Materials and Methods

Three groups of 12 rats each were used for the first phase of the experiment. Half of the animals were given daily subcutaneous injections

of AG (100 mg/kg body wt). Controls were treated the same, but were given injections of distilled water.

The first group was fasted for 24 hours then placed in metabolism cages without water or food. Urine volumes were collected in graduate cylinders for 48 hours. The urine output of controls and treated animals was compared.

The second group was placed in metabolism cages and allowed to drink *ad libitum*. The amount of water consumed, the amount of urine excreted, and the amount of weight gained were measured daily for 72 hours.

The ureters were clamped off in the third group. They were then put in cages and allowed to drink *ad libitum*. Water consumption was measured at 24 hours.

In the second phase of the work, 1- and 3-day electrolyte levels were checked both in intact and adrenalectomized controls, and in treated animals receiving daily subcutaneous injections of AG (100 mg/kg body wt). Serum, urine, and tissue electrolytes were done by flame photometry using procedures outlined in the *Coleman Flame Photometry Manual*. Tissue electrolytes were done using the procedure for urinary electrolytes, and employing 1 g samples of gastrocnemius muscle homogenized in 10 ml of distilled water.

The data were statistically evaluated using the Student's "t" test. A "P" value of less than 0.01 was considered significant.

Results

Aminogluthethimide had no effect on the 24- and 48-hour urine volume of water-fasted rats. In animals drinking *ad libitum*, it did,

TABLE 1. *Effects of aminogluthethimide on urine volume in water fasted rats, and those drinking ad libitum (numbers in parenthesis indicate number of cases and results are expressed as mean $\pm$ SE).*

Mean Body Weight in Grams	Treatment 100mg/ kg/day	Milliliters Urine			
		Water Fasted		Drinking <i>ad libitum</i>	
		24 hr	48 hr	24 hr	48 hr
170	AG**	4.0 $\pm$.4	6.1 $\pm$.7	25 $\pm$ 6	41 $\pm$ 11
		(12)	(12)	(12)	(12)
		P>.05	P>.05	P<.01	P<.01
165	none	4.0 $\pm$.6	5.6 $\pm$.9	11 $\pm$ 1	20 $\pm$ 2
		(12)	(12)	(12)	(12)

*Aminogluthethimide.

TABLE 2. *The effects of aminogluthethimide on water consumption in intact and nephrectomized animals drinking ad libitum.*

Mean Body Weight in Grams	Treatment 100mg/ kg/day	Milliliters Water Consumed		
		Intact		Nephrectomized
		24 hr	48 hr	24 hr
150	AG	43±4 (12) P<.001	73±20 (12) P<.001	30±2.4 (12) P<.01
150	none	20±2 (12)	37±5 (12)	20±1.5 (12)

however, cause significant increases in urine volume at both 24 and 48 hours (Table 1).

Nephrectomized and intact animals treated with AG exhibited significant increases in 24-hour water intake compared to controls (Table 2).

Aminogluthethimide significantly reduced the concentration of plasma sodium, in intact animals, at 1 day. Intact or adrenalectomized controls showed no changes in plasma sodium, whereas the intact and adrenalectomized treated animals exhibited about the same sodium decrease at 1 day (Table 3).

Intact animals treated for 3 days showed a decrease in sodium equal to that of the intact animals treated for 1 day, and also equal to the 3-day adrenalectomized non-treated rats. The adrenalectomized

TABLE 3. *The effects of aminogluthethimide on plasma sodium and potassium levels in the intact and adrenalectomized animals.*

Mean Body Weight in Grams	Treatment 100mg/ kg/day	Milliequivalents/Liter			
		Intact		Adrenalectomized	
		Na	K	Na	K
175	AG (1 day)	128±.8 (12) P<.01	8.4±.2 (12) P<.01	126±1 (12) P<.01	8.0±.5 (12) P<.01
177	none (1 day)	143±.6	6.9±.2 (12)	141±.9 (12)	4.5±.3 (12)
165	AG (3 days)	128±1 P<.01	8.4±.2 P<.01	115±6 P<.01	9.5±.7 P<.01
165	none (3 days)	144±.7 (12)	6.5±.3 (12)	126±.8 (12)	8.4±.4 (12)

treated animals, however, showed an even greater decrease at 3 days (Table 3).

The plasma potassium of intact and adrenalectomized animals treated with AG was elevated at 1 day, and the potassium level of the adrenalectomized non-treated animals was somewhat lower than in intact controls, but not significantly so. The potassium levels of the intact animals treated for 3 days was the same as in the 1-day intact treated animals. The 3-day adrenalectomized non-treated animals showed an elevation of plasma potassium. The plasma potassium of adrenalectomized treated animals was elevated (Table 3).

Total urinary sodium was markedly reduced in animals treated with AG for 1 day. One-day adrenalectomized controls also showed a reduction in urine sodium. At 3 days the values for the intact treated animals were slightly elevated. The adrenalectomized animals, treated and controls, showed a reduction in sodium (Table 4).

TABLE 4. *The effects of aminoglutethimide on urine output of sodium and potassium in the intact and adrenalectomized animals.*

Mean Body Weight in Grams	Treatment 100mg/ kg/day	Milliequivalents/day			
		Intact		Adrenalectomized	
		Na	K	Na	K
177	AG (1 day)	.17±.02	.2±.04	.17±.02	.18±.02
		(12)	(12)	(12)	(12)
		P<.01	P<.02	P<.02	P<.03
175	none (1 day)	.46±.02	.14±.02	.195±.01	.19±.01
		(12)	(12)	(12)	(12)
165	AG (3 days)	.46±.05	.23±.03	.15±.01	.21±.01
		(12)	(12)	(12)	(12)
		P>.05	P>.05	P<.01	P<.02
165	none (3 days)	.47±.01	.21±.02	.1±.01	.23±.01
		(12)	(12)	(12)	(12)

Urinary potassium in intact animals treated for 1 day with AG was elevated. The potassium levels in adrenalectomized treated and control animals were equal to that in the intact treated animals (Table 4).

At 3 days the potassium output in the intact treated and non-treated animals was the same. The potassium level in the 3-day adrenalectomized treated was elevated (Table 4).

The sodium concentration in muscle tissue of intact treated animals was elevated at 1 day. The 1-day adrenalectomized treated animals showed a level approximately equal to that in the 1-day intact treated

TABLE 5. *The effects of aminoglutethimide on tissue sodium and potassium in the intact and adrenalectomized animals.*

Mean Body Weight in Grams	Treatment 100mg/ kg/day	Milliequivalents/100g			
		Intact		Adrenalectomized	
		Na	K	Na	K
175	AG (1 day)	4.4±.5	44±10	5.0±.4	30±2
		(12)	(12)	(12)	(12)
		P<.01	P<.01	P<.02	P<.05
177	none (1 day)	2.7±.2	61±1	3.9±.2	36±10
		(12)	(12)	(12)	(12)
165	AG (3 days)	2.3±.4	74±10	4.9±.04	21±1
		(12)	(12)	(12)	(12)
		P<.05	P<.05	P<.01	P<.01
165	none (3 days)	2.4±.3	73±50	2.9±.3	38±5
		(12)	(12)	(12)	(12)

animals. At 3 days the intact non-treated, intact treated, and the adrenalectomized non-treated rats exhibited equal tissue sodium levels. The 3-day adrenalectomized treated animals demonstrated an increase in tissue sodium (Table 5). Intact or adrenalectomized animals treated with AG for 1 day showed a reduction in tissue potassium which, at 3 days, remained low in adrenalectomized rats, but was near the control value in intact rats (Table 5).

Discussion

The data show that aminoglutethimide has a significant effect on water intake. Treated rats allowed to drink *ad libitum*, consumed significantly more water, excreted more urine, and, over short periods, retained more water than did their controls. Treatment induced water retention during the first 24-hour period after injection, but subsequent injections failed to exacerbate this condition. Also, when treated rats were deprived of water the urine volume was not increased over the control value, thus indicating that a primary effect of the drug was on thirst rather than on excretory mechanisms. These findings are interpreted as indicating that increase in thirst was caused by a change in internal osmotic pressure which was then corrected for by drinking and establishing a new high internal fluid level; this level when reached was maintained but not increased.

Treated animals which had been nephrectomized showed an increase in fluid intake. This demonstrated that the increase in urine output found in treated animals, allowed to drink *ad libitum*, was a product of the polydipsic effect and not a cause for it.

Aminoglutethimide treatment caused an increase in plasma potassium and a concomitant decrease in plasma sodium. The adrenalectomized controls did not show a reduction in plasma sodium at 1 day, but did show this reduction at 3 days. The reason for the difference in plasma

sodium at 3 days compared to 1 day after adrenalectomy is probably because the levels of adrenal steroids in the circulation 1 day after adrenalectomy had not been sufficiently depleted to permit a lowering of plasma sodium.

In the intact treated animals, regardless of the length of treatment, there was a drop in plasma sodium equal to the levels in adrenalectomized treated animals.

The plasma potassium levels in 1- or 3-day treated, intact or adrenalectomized, animals were inversely related to the sodium levels. The potassium levels in the adrenalectomized controls were unchanged at 1 day. The potassium level in the intact and adrenalectomized treated animals at 1 day was increased.

As was expected, the plasma of 3-day adrenalectomized non-treated animals was low in sodium. The plasma sodium in the intact animal treated for 3 days was reduced to a level equal to that in the adrenalectomized controls. The adrenalectomized animals treated for 3 days showed a greater reduction in plasma sodium than did any of the other animals regardless of operative and treatment procedures. This is indicative that AG has direct effects on blood electrolyte levels independently of any action mediated via the adrenal cortex.

The urine sodium output was depressed in intact animals treated for 1 day and in 1-day adrenalectomized non-treated animals. These results could be interpreted as indicating that at 1 day neither the adrenalectomized non-treated nor the intact treated animals had complete adrenal insufficiency. At 3 days the adrenalectomized rats did show the expected urinary loss of sodium, whereas adrenalectomized treated animals at either 1 or 3 days showed a marked decrease in sodium output. This is further evidence of an effect of AG which is independent of adrenal function.

The variations and errors in urine potassium determinations were such that possible correlation with adrenal involvement could not be made. However, the drug did influence potassium excretion in that it increased it in the intact animals at 1 day and in the adrenalectomized animals at 1 day.

The tissue sodium levels in the intact treated and adrenalectomized treated rats were increased at 1 day. Such increases in tissue sodium lend credence to the concept that rapid osmotic shifts occur in the drug-induced polydipsia. The action of aminoglutethimide could cause a shift of sodium into the tissue and this in turn could set up an osmotic gradient between the plasma and the tissue. The increase in thirst could be concomitant with shifts in plasma and tissue sodium since hypothalamic thirst centers are regulated by the osmotic balance between the tissue and plasma fluids (5). The lowering of plasma sodium levels, along with increase in tissue sodium levels would tend to cause water to shift into tissues thus causing increase in tissue water and decrease in plasma water. A concomitant decrease in urinary sodium levels, and an increase in water intake would be expected.

The extra-adrenal effects of AG on urinary and tissue sodium and potassium, in the intact animals were not clearly evident at 3 days. Following treatment for 3 days the intact rats tended to exhibit more

clearly the traditional symptoms of adrenal insufficiency. This would be expected since AG does not block the effects of ACTH on adrenal cell function, morphology, and growth (4), and in intact animals the inhibition of glucocorticoid synthesis allows compensatory hypersecretion of ACTH and leads to adrenal hypertrophy (3). The increased production of ACTH would tend to cause a compensatory reaction and a change in the urine and tissue sodium and potassium.

The way in which aminoglutethimide affects molecular processes in adrenal steroid synthesis is rapidly being clarified. However, its effects, such as its influences on extra-adrenal action on water and electrolyte metabolism and on sex organs and thyroid function, are as yet poorly understood. Whether it has one site of action which is common to all these organs is not known, but it is possible that the drug acts at one locus necessary to the function of several organs.

Summary

1. A distinct relationship exists between the effects of aminoglutethimide and water consumption.

2. Aminoglutethimide produced a polydipsic effect which is then followed by a polyuria.

3. Injections of aminoglutethimide caused adrenal insufficiency-like effects.

4. Aminoglutethimide appeared to cause shifts between blood and muscle in sodium and potassium, independently of the adrenals, setting up an osmotic gradient which caused alterations in urine output of sodium and potassium.

5. The presence of the adrenals appeared to cause a compensation which decreased the effects of aminoglutethimide on water and electrolyte metabolism.

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