Effect of Aminoglutethimide on Norepinephrine Turnover in the Rat Heart[1] (37227)

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Stimulation of adrenergic nerves results in an increased synthesis of norepinephrine (NE). This has been demonstrated by electrical stimulation of the rat salivary gland (1), rat heart (2), guinea-pig vas deferens (3), and the cat adrenal gland (4). In addition, synthesis of NE in organs such as the heart has been reported increased following exercise and exposure to cold (5).

Imms and Jones (6) showed that adrenalectomized rats excreted significantly elevated amounts of catecholamines in urine, suggesting an increased sympathetic nerve activity. These investigators also showed that adrenalectomized rats have lowered blood pressure and proposed that this lowered blood pressure might trigger a partially compensating increase in sympathetic nerve activity, which would then give rise to the observed increase in urinary catecholamines (7).

Subsequently, increases in NE synthesis in adrenalectomized animals have been demonstrated in the rat brain (8) and in the rat heart (9, 10). In substantial agreement with this hypothesis were the findings of Landsberg and Axelrod (9) and Landsberg *et al.* (11) that hypophysectomy also resulted in increased synthesis of NE in the rat heart, thus implicating the adrenal cortex. Additional evidence implicating the adrenal cortex was the observation that adrenal steroids administered to adrenalectomized rats prevented the increase in NE turnover (10, 12).

It has also been reported that adrenal demedullectomy results in an increase in the turnover of NE in the heart (13, 14). These data would tend to suggest that: (a) removal of either the adrenal cortex or the adrenal medulla results in increased turnover, (b) removal of the adrenal medulla, not the cortex, is the cause of the increased turnover, or (c) adrenal demedullectomy might sufficiently compromise the functioning of the adrenal cortex so that in reality the increased NE turnover observed following demedullectomy might be due to adrenal cortical insufficiency.

Aminoglutethimide has been shown to inhibit steroidogenesis in the adrenal cortex by interference with cholesterol side chain cleavage in the conversion of cholesterol to pregnenolone, thus inhibiting synthesis of all adrenal steroids of biological activity (15). This action would tend to make aminoglutethimide a valuable tool in enabling one to selectivity inhibit adrenal cortical steroidogenesis without significantly disturbing the functioning of the adrenal medulla.

The purpose of this study, then, was to inhibit selectively the adrenal cortex with aminoglutethimide and thus to determine the extent of involvement, if any, of the adrenal cortex *per se* in the control of synthesis of NE in the rat heart.

Methods. Male Sprague–Dawley rats weighing between 200 and 400 g were used in this experiment. All animals were maintained on water and Purina Laboratory Chow with salt spools provided in all cages at the appropriate times. Aminoglutethimide phosphate (Elipten phosphate, Ciba) was dissolved in water (100 mg/ml) immediately before sc injection. Two doses of aminoglutethimide were tested, 100 mg/kg/day and 200 mg/kg/day, with the latter being given in two equal doses each day. The lower dose was ineffective in altering NE turnover so only the results obtained with the 200

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mg/kg/day dose is included in the present report. The drug was administered for 3, 6, and 10 days prior to measuring NE turnover. Control animals were given water sc.

Two methods were utilized to determine NE turnover. The first involved measuring the decline in specific activity of ^{3}H-NE in the heart after its iv administration. This method was used to measure NE turnover following treatment with aminoglutethimide for 3 and 6 days. The second method measured the decline of cardiac NE after the inhibition of tyrosine hydroxylase with α-methyl-*p*-tyrosine. This method was utilized for measuring NE turnover in animals treated with aminoglutethimide for 10 days. It has previously been demonstrated that both methods give similar results concerning turnover measurements (1, 10).

In the ^{3}H-NE studies, all animals were given tracer doses of 1-^{3}H-NE (10 μCi/kg; sp act 4.1–5.8 Ci/mmole) via the tail vein 2 hr after the final dose of aminoglutethimide was given. The animals were killed 1 and 24 hr later by decapitation, hearts dissected out, washed in iced saline, blotted, weighed, minced with scissors, then homogenized in 20 ml of 5% trichloroacetic acid with the Ultra-Turax homogenizer, and filtered under suction. The extracts were then absorbed on alumina columns according to the precedures described by Euler and Lishajko (16). Aliquots of eluates obtained from the alumina columns were taken for fluorometric analysis according to the automated trihydroxyindole procedure of Robinson and Watts (17). For scintillation counting, 1-ml samples of the original filtrate and eluate and 2-ml samples of the column effluent were placed in 10 ml of a 2:1 solution of toluene–Triton X-100 (Packard Instrument Co.) containing 5.5 g of 2,5-diphenyloxazole (PPO) and 150 mg of 1,4-bis[2-(5 phenyloxazolyl)]-benzene (PO POP) per liter. All samples were counted in a Packard Tri-Carb liquid scintillation spectrometer. Counting efficiency was determined by external standardization and ranged from 15 to 18%.

In the experiments utilizing α-methyl-*p*-tyrosine (αMPT), animals were injected with 200 mg/kg αMPT iv and with 100 mg/kg iv 4 hr later. Animals were killed at 2, 4, 6, and 8 hr after the αMPT, hearts removed, and endogenous NE levels measured as described above. Recoveries were carried out by adding known amounts of cold NE to the initial tissue, and carried through the entire procedure. Recovery of NE was 80%.

Because aminoglutethimide has been reported to increase the activity of phenylethanolamine-*N*-methyl transferase (18), the effect of aminoglutethimide on adrenal medullary production of catecholamines was examined. Rats were treated with aminoglutethimide for 10 days, and the daily urinary excretion of epinephrine and NE was measured. Rats were placed in individual metabolism cages, and the urine was collected into beakers which contained 1 *N* HCl to prevent oxidation of the catecholamines. Twenty-four-hour urine samples were collected, filtered under suction, and the catecholamines measured according to the procedures described above for the heart tissue.

All statistical analyses were carried out using the Student's *t* test.

Results. The effect of aminoglutethimide injected in a dose of 200 mg/kg/day for 3 or 6 days on the disappearance of ^{3}H-NE from the rat heart is depicted by Fig. 1. There was no significant difference in the 1-hr values of ^{3}H-NE in the control group compared to those from aminoglutethimide treated animals following 200 mg/kg for 3 or 6 days. Likewise, there was no difference in the 24-hr ^{3}H-NE value in the rats treated with aminoglutethimide for 3 days compared to control rats. However, the 24-hr value in hearts of animals treated for 6 days was significantly lower than the control value ($p < 0.01$), suggesting an increase in turnover of myocardial NE in the animals treated for 6 days with aminoglutethimide.

Figure 2 shows a more complete measurement of myocardial NE turnover. This figure shows the effect of aminoglutethimide injected in a dose of 200 mg/kg/day for 10 days on the turnover of NE in the heart. The results indicate that there is a highly significant increase in the turnover of NE in aminoglutethimide-treated animals compared to animals receiving vehicle control.

Table I shows the effect of daily treatment of aminoglutethimide on endogenous NE

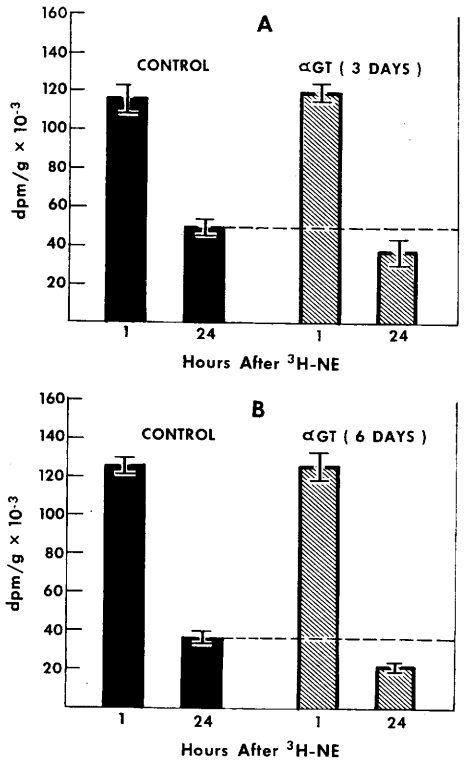

FIG. 1. Panel A, ^{3}H-NE values in the rat heart at 1 and 24 hr after the iv administration of 10 μCi/kg 1-^{3}H-NE (sp act 4.1–5.8 Ci/mmole) in control rats (black bars) and rats treated with aminoglutethimide in a dose of 200 mg/kg for 3 days (speckled bars) ± SEM. Panel B depicts similar values in another group oi control rats and rats treated with aminoglutethimide (200 mg/kg/day) for 6 days.

levels in the heart. No significant changes are seen.

The effect of daily treatment of aminoglutethimide (200 mg/kg) on the urinary excretion of epinephrine and NE is shown in Fig. 3. This treatment did not significantly alter the excretion of either amine when compared to vehicle injected controls.

Discussion. The results of this study indicate that inhibition of adrenal steroidogenesis with aminoglutethimide (*1*) does not change endogenous NE levels in the rat heart, (*2*) does not influence cardiac ^{3}H-NE uptake in the rat, (*3*) does not alter the urinary excretion of epinephrine or NE, but does result in an increase in turnover of cardiac NE when injected for 6 or 10 days with 200 mg/kg/day. Similar observations

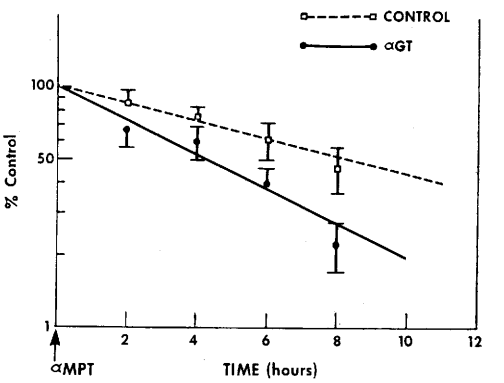

FIG. 2. The decrease in NE levels in the rat heart following the administration of α-methyl-*p*-tyrosine (200 mg/kg iv) at zero time and again 4 hr later (100 mg/kg iv) in (□- - -□) control rats and (●——●) rats treated for 10 days with aminoglutethimide (200 mg/kg/day). Data is plotted as per cent of control (zero time NE levels) vs time in hours ± SEM (I).

have been made following surgical adrenalectomy and hypophysectomy (9, 10). Adrenal cortical insufficiency is a common denominator of all three of these experiments.

Results from the present study indicate that the increased cardiac NE turnover is not seen before 6 days. This agrees well with Javoy *et al.* (8), who did not observe a significant increase in turnover of NE in the rat brain until 6 days after adrenalectomy. Further, it has been demonstrated that blood pressure was not significantly lowered in rats until the third day after adrenalectomy (12). This data is compatible with the idea that the increased NE turnover following adrenalectomy may be related to increased sympathetic nerve activity secondary to a de-

TABLE I. Effect of Aminoglutethimide on Endogenous Norepinephrine Content of the Rat Heart.

Item	Dose	Length of treatment (days)	Heart NE level (μg/g + SEM)
Vehicle	0.2 ml/day	3	0.55 ± 0.04
αGT[a]	200 mg/kg	3	0.52 ± 0.02
Vehicle	0.2 ml/day	6	0.59 ± 0.03
αGT	200 mg/kg	6	0.57 ± 0.07
Vehicle	0.2 ml/day	10	0.55 ± 0.05
αGT	200 mg/kg	10	0.57 ± 0.04

[a] αGT, aminoglutethimide.

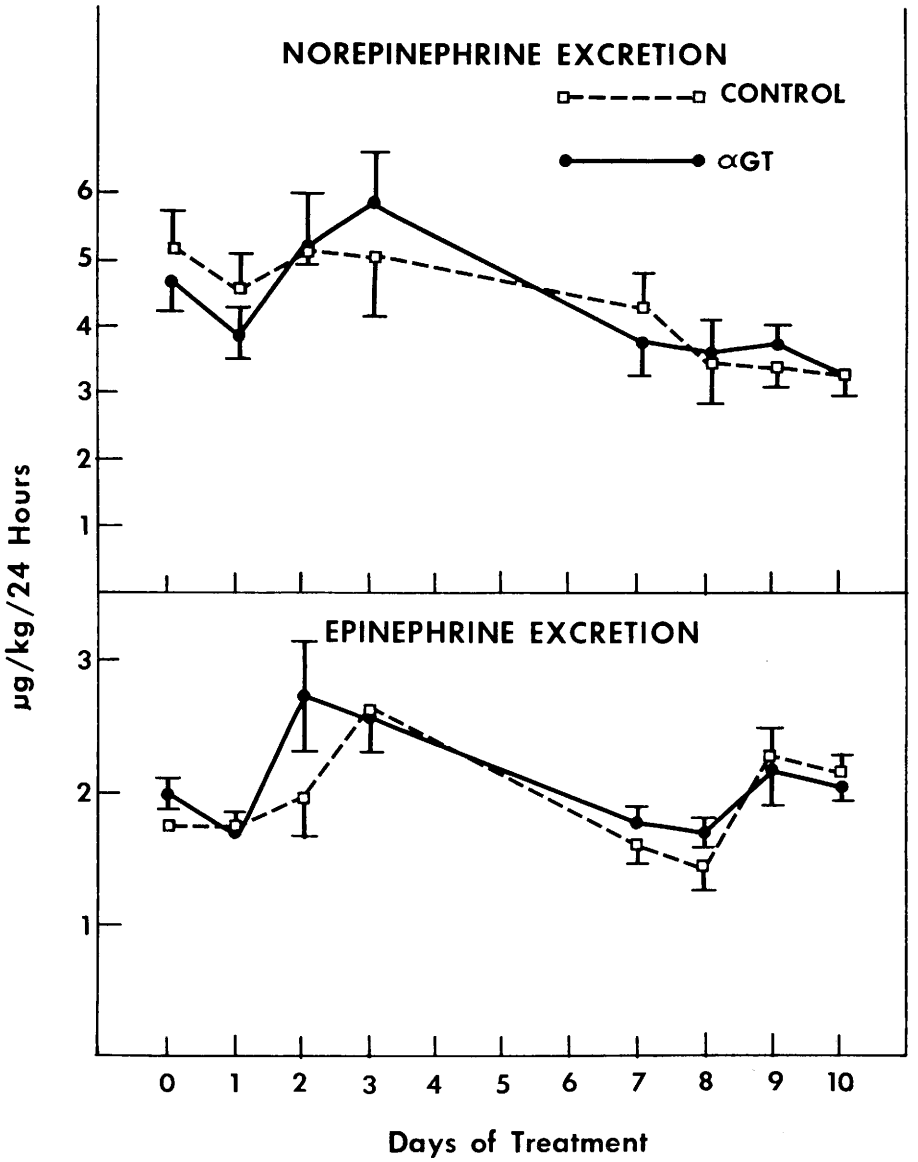

FIG. 3. The urinary excretion of NE (top panel) and epinephrine (bottom panel) in µg/kg/24 hr in (□- - -□) control rats and (●——●) rats treated with aminoglutethimide [200 mg/kg/day ± SEM (I)].

creased blood pressure (7). In support of this hypothesis is the observation that ganglionic blockers prevent the increased turnover of NE in both adrenalectomy and hypophysectomy (9, 10).

Additional support of an adrenal cortical involvement contributing to the increased turnover comes from the work of Westfall and Osada (10), who demonstrated that replacement of adrenal steroids prevented the increase in NE turnover associated with surgical adrenalectomy. It has also been demonstrated that administration of ACTH after hypophysectomy substantially decreased the expected increase in NE turnover resulting from this operation (9).

There is, however, evidence against the hypothesis of Imms and Jones (6). Dailey

and Westfall (12) demonstrated that, while replacement of either glucocorticoid or mineralocorticoid alone was sufficient to prevent the fall in blood pressure seen after surgical adrenalectomy, only a mineralocorticoid was able to prevent the increase in NE turnover. This, then, is a clear dissociation of the blood pressure-NE turnover relationship. A decreased blood pressure level, then, may not be an essential antecedent of the increased NE turnover seen in surgical adrenalectomy.

The purpose of the present study was to determine if aminoglutethimide inhibition of the adrenal cortex was sufficient to bring about increased turnover of NE in the heart in order to evaluate the importance of the adrenal cortex in exerting some influence on NE turnover. Such information becomes especially important in view of reports that adrenal demedullectomy was also sufficient to cause an increase in NE turnover (13, 14). These reports suggest that the adrenal medulla, by releasing catecholamines into the circulation, aids in maintaining levels of catecholamines elsewhere. Thus, when this source of catecholamines is removed, they postulate that local synthesis may increase in order to maintain normal levels of catecholamines.

In attempting to rule out damage to the adrenal cortex after demedullectomy, it has been pointed out that there are normal glucocorticoid levels following demedullectomy (13, 19). However, in view of the work of Dailey and Westfall (12), it would appear to be necessary to demonstrate, in addition, that mineralocorticoid levels are also normal in demedullectomy, as mineralocorticoid seems to be the more important of the two adrenal steroids as far as an interaction with the turnover of NE in the rat heart is concerned. This becomes especially important since these investigators have shown that an extremely close relationship exists between mineralocorticoid and monoamine oxidase activity. Cardiac monoamine oxidase activity is found to be increased in adrenalectomy. Of the adrenal steroids, only mineralocorticoid prevents this increase in monoamine oxidase activity; in addition, only mineralocorticoid prevents the increased turnover of NE in adrenalectomy.

The present study succeeded in demonstrating an increased turnover of NE in the rat heart on inhibition of adrenal steroidogenesis with aminoglutethimide. This adds to the evidence that there is an interaction between the adrenal cortex and NE turnover. In regard to aminoglutethimide, however, it has been demonstrated that this drug also inhibits to some extent the organification of iodine in the thyroid gland (20). Since thyroidectomy also causes an increased turnover of NE in the rat heart (9), it becomes apparent that aminoglutethimide may influence NE turnover by two routes. It is not possible to completely rule out a direct effect of aminoglutethimide on NE turnover. Since the acute administration of aminoglutethimide had no effect on turnover, however, and the increase in NE turnover was gradual and seen only after chronic administration of aminoglutethimide, it is suggested that the effect is indirect via the inhibition of glucocorticoid synthesis. Further work with aminoglutethimide must take into account these possibilities.

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