AMINOGLUTETHIMIDE AND GLUTETHIMIDE: EFFECTS ON 18-HYDROXYCORTICOSTERONE BIOSYNTHESIS BY HUMAN AND SHEEP ADRENAIS IN VITRO

By

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ABSTRACT

The conversion of [1,2-3H]corticosterone to 18-hydroxycorticosterone in vitro was studied on human and animal adrenal tissue homogenates. Human adrenals were surgically resected from a patient with Cushing's disease. Sheep adrenal homogenates were prepared from the pooled glands of 20 animals. Incubations supplemented with a NADPH generating system were performed in order to evaluate the effect of aminoglutethimide and its closely related compound glutethimide on corticosterone 18-hydroxylation in vitro. Increasing concentrations of the two drugs were assayed on both human and animal adrenal homogenates. Aminoglutethimide was clearly found to inhibit corticosterone 18-hydroxylation in sheep adrenal homogenates as a 72.6% inhibition occurred in the presence of only 0.2 µmole of the drug. Inhibition reached 91.1% in the presence of 0.5 µmole aminoglutethimide. When added to the human incubated adrenal, a 59.4% inhibition occurred in the presence of 0.5 µmole aminoglutethimide.

Glutethimide, a sedative of wide clinical usage, was also found to inhibit corticosterone 18-hydroxylation but the inhibitory effect occurred only in the presence of much higher concentrations. In fact, 5.0 µmoles were necessary to obtain a 43.9% inhibition of 18-hydroxycorticosterone synthesis.

This study clearly demonstrates the marked inhibitory effect of aminoglutethimide on corticosterone 18-hydroxylation. Glutethimide, to a lesser extent, also inhibits 18-hydroxycorticosterone synthesis.
Aminoglutethimide\(^*\) \([\alpha(p\text{-}aminophenyl)\text{-}\alpha\text{-}ethylglutarimide]\), originally introduced as an anticonvulsant turned out to be a potent inhibitor of adrenal steroidogenesis producing adrenocortical insufficiency in humans treated with the drug (Camacho et al. 1966, 1967).

The inhibitory effect of the product on adrenal biosynthesis was first reported by Kahnt & Neher (1966) who suggested a drug interference with an early step in steroid synthesis, cholesterol 20\(\alpha\)-hydroxylation.

If the site of action of aminoglutethimide was at the onset of hormone synthesis, then a fall in the synthesis of all steroids should appear following the blockade. Paradoxically, adrenal function exploration of patients treated with aminoglutethimide seldom showed a fall in cortisol secretion whereas aldosterone secretion and excretion were regularly reduced (Fishman et al. 1967; Schteingart & Conn 1967; Philbert et al. 1968; Horky et al. 1968; Horky & Küchel 1969; Küchel et al. 1970a,b). These findings led us to assume that aminoglutethimide could also interfere with another step in the aldosterone biosynthetic pathway.

The present study was undertaken in order to investigate the effect of aminoglutethimide on corticosterone 18-hydroxylation in sheep adrenal homogenates and in human adrenals removed from a 30 year old woman with Cushing’s disease. At the same time, the question arose as to whether the chemical related compound, glutethimide, a sedative with wide clinical usage, might have similar effects on corticosterone 18-hydroxylation. The present report describes our findings.

**MATERIAL AND METHODS**

*Labelled steroids and reagents*

\([1,2-\text{\(^3\)}\text{H}]\)corticosterone \((\text{S.A.} = 40-60 \text{Ci/mmole})\) and \([4-\text{\(^{14}\)}\text{C}]\)corticosterone \((\text{S.A.} = 30-50 \text{mCi/mmole})\) were purchased from New England Nuclear Corporation. Their radiochemical purity was checked by paper chromatography before use.

\([4-\text{\(^{14}\)}\text{C}]\text{18-hydroxycorticosterone}\) was obtained from \([4-\text{\(^{14}\)}\text{C}]\)corticosterone by sheep adrenal homogenate incubation. Radiochemical purity was checked by mixing an aliquot with a trace amount of \([1,2-\text{\(^3\)}\text{H}]\text{18-hydroxycorticosterone}\). After periodic followed by chromic oxidation, the derivative isotope ratio remained constant throughout multiple paper chromatographies.

Nicotinamide adenine dinucleotide phosphate (NADP) and potassium hydroxide were purchased from Merck, fumaric acid from Sigma Corp. and sterile Earle medium from Institut Pasteur (Paris).

Aminoglutethimide and glutethimide were generously supplied by Ciba Pharmaceutical Corp.

\(^*\) Aminoglutethimide = *Elïpten®* (Ciba).
Glutethimide = *Doriden®* (Ciba).
Adrenal tissue

*Human adrenals.* – The experiments were prepared with an aliquot of left and right adrenals removed from a 30 year old woman with Cushing’s syndrome caused by an excessive pituitary secretion of ACTH (Cushing’s disease).

Adrenal resection was first investigated in the right adrenal and after a 3 week period with the left one. During these 3 weeks the patient received no treatment. The right adrenal weighed 11.0 g and the left one 13.5 g. Histological examination showed in both cases a very narrow zona glomerulosa and a marked hyperplasia of the zona fasciculata and reticularis with several pseudo-adenomatous foci.

*Sheep adrenals.* – Glands were removed from sheep (age 4–6 months), 10 min after death and brought to the laboratory in ice-filled containers.

Experimental procedure

The adrenal glands were dissected free of adipose tissue and the cortex was partly separated from the medulla. The cortical tissue was weighed and homogenized with a Potter-Elevejelm homogenizer in Earle medium buffer, pH = 7.3 (NaCl: 6.80 g; KCl: 0.40 g; CaCl2: 0.20 g; MgSO4, 7H2O: 0.20 g; NaH2PO4, 2H2O: 0.156 g; NaHCO3: 2.20 g; glucose: 1 g; phenolsulphonaphtaleine: 0.02 g; dist. H2O q.s. 1000 ml). The sodium/potassium ratio (28.6) in this buffer is almost the same as that reported by Müller (1971) as being the most favourable for aldosterone synthesis in vitro.

Each human adrenal incubation flask contained 145 mg of the left or homogenized adrenal in 6.0 ml Earle medium. Each sheep adrenal incubation contained 500 mg homogenized adrenal in 10.0 ml Earle medium. Sheep adrenal homogenates were prepared from the pooled glands of 20 animals. Incubation flasks contained a NADPH generating system i.e. NADP (1 µmol/ml) and fumaric acid (5 µmol/ml) neutralized with a 0.1 n potassium hydroxide solution (Touitou & Legrand 1970). Measured amounts of [1,2-3H]corticosterone were dispatched in incubation flasks, evaporated to dryness, then solubilized with 0.1 ml propylene glycol during the 12 h preceding incubation.

Due to the solubility of aminoglutethimide and glutethimide in organic solvents (Douglas & Nicholls 1965) ethyl acetate was used in this procedure. In the experiments, the two products were added in a 0.05 ml volume. With the same experimental conditions, some control flasks received only 0.05 ml ethyl acetate without these drugs and some were prepared without any addition at all. Approximately 2 h elapsed after removal of the adrenals. During this period of time, the glands were stored at 4°C. Aerobic incubations were carried out at 37°C in a Dübnnoff metabolic shaker for 75 min. After incubation, 15.0 ml acetone was added to stop any enzymatic reactions and precipitate protein.

In order to account for procedural losses during subsequent extraction and identification of synthesized steroid, a trace amount of [4-14C]18-hydroxy cortisol was added into the incubation flasks which were then left to stand overnight at 4°C. Incubation mediums were filtered through a small funnel with a glass cotton layer into a Büchi vessel and the filter washed several times with aqueous acetone.

Acetone was then removed by vacuum distillation with a Büchi rotavapor at a temperature below 40°C, and the final aqueous phase was partitioned with chloroform (v/v) three times. The chloroform extracts were then evaporated to dryness by vacuum distillation. In order to evaluate the total radioactivity recovered in the chloroform extracts, 100.0 ml absolute ethanol was added into vessels and an aliquot was taken for radioassay.

519
Paper chromatography

The following solvent systems were used for paper chromatography (PC) of labelled steroids and conversion products:

PC 1: dichloroethane–ethylene glycol
PC 2: toluene–propylene glycol
PC 4: benzene–formamide

Radioactive area detection on chromatograms was performed with a radiochromatogram scanner Packard model 7200.

Steroid characterization

Steroid separation was achieved by paper chromatography (PC) until $^{3}H/^{14}C$ ratio remained stable. After a first chromatography in PC 1 system for 4 h, the 18-hydroxy-corticosterone area was purified in PC 1 system for 17 h. $[4-^{14}C]$18-hydroxycorticosterone showed exactly the same polarity as the synthesized steroid in these chromatographic systems. Synthesized 18-hydroxycorticosterone was then characterized by periodic acid oxidation into a compound the polarity of which in the various chromatographic systems used (PC 3, PC 4, PC 5), was identical to that of the periodic acid oxidation derivative obtained from $[4-^{14}C]$18-hydroxycorticosterone i.e. 18-hydroxycorticosterone 20$\rightarrow$18$\gamma$-lactone. The latter compound was further oxidized with chromic acid into its 11-keto derivative i.e. 18-hydroxy-11-dehydrocorticosterone lactone, a steroid of weaker polarity (checked in PC 3, PC 4, PC 5). The isotope ratio remained constant throughout repeated chromatographies before and after periodic acid oxidation and chromic acid oxidation, thus establishing that the synthesized steroid behaved as the carbon-14 labelled steroid.

Periodic oxidations were performed on dried extracts solubilized in 0.3 ml methanol with an equal volume of 2 mg/100 ml aqueous periodic acid solution containing 2 g/106 ml pyridine and were left standing overnight at laboratory temperature. The solutions were then partitioned 3 times with 10.0 ml chloroform according to Raman et al. (1964). Chromic oxidations were performed with 1.0 ml solution of chromium trioxide (100 mg) in 5.0 ml pure acetic acid and water (1:1) for half an hour, in darkness. Extraction was then realized as previously described.

The conversion percentage was calculated on the purified periodic acid derivative on the basis of the ratio of the loss corrected radioactivity after the last steroid purification to the total radioactivity. Tritium and carbon-14 were measured in a liquid scintillation spectrometer (Packard, model Tricarb 3375). Radioactive countings are expressed as disintegrations per minute (dpm).

RESULTS

The conversion rate of corticosterone to 18-hydroxy corticosterone was thus established on two Cushing type adrenals and was found quantitatively quite similar for both glands. Incubations prepared with two such adrenals allowed us to observe in both glands a 18-hydroxy corticosterone production about 7 pmol/145 mg/75 min (Table 1).
Effect of aminoglutethimide (AG) and glutethimide (G) on the conversion of [1,2-3H]corticosterone to 18-hydroxycorticosterone (18-OHB) by human adrenal tissue. Each flask contained: [1,2-3H]corticosterone, NADP (1 µmol/ml), fumarate (5 µmol/ml), and homogenized human adrenal tissue (145 mg) in a total volume of 6.0 ml. AG and G were solubilized in ethyl acetate (Eth-Ac) and introduced in the medium in a volume of 0.05 ml. Incubation lasted for 75 min at 37°C in air.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Total radioactivity recovered DPM × 10^6</th>
<th>Per cent conversion</th>
<th>Per cent inhibition</th>
<th>Produced 18-OHB pmol/145 mg/75 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right adrenal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>15.70</td>
<td>5.20</td>
<td></td>
<td>7.43</td>
</tr>
<tr>
<td>None</td>
<td>15.61</td>
<td>5.30</td>
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<td>7.53</td>
</tr>
<tr>
<td>Left adrenal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>14.05</td>
<td>5.29</td>
<td></td>
<td>6.76</td>
</tr>
<tr>
<td>None</td>
<td>14.15</td>
<td>5.13</td>
<td></td>
<td>6.60</td>
</tr>
<tr>
<td>Eth-AC</td>
<td>14.90</td>
<td>2.04</td>
<td></td>
<td>2.78</td>
</tr>
<tr>
<td>Eth-AC</td>
<td>15.32</td>
<td>2.10</td>
<td></td>
<td>2.93</td>
</tr>
<tr>
<td>AG 0.5 µmole</td>
<td>15.94</td>
<td>0.84</td>
<td>59.4</td>
<td>1.22</td>
</tr>
<tr>
<td>AG 1.0 µmole</td>
<td>16.59</td>
<td>0.78</td>
<td>62.3</td>
<td>1.18</td>
</tr>
<tr>
<td>G 0.2 µmole</td>
<td>16.82</td>
<td>2.33</td>
<td></td>
<td>3.42</td>
</tr>
<tr>
<td>G 0.5 µmole</td>
<td>16.15</td>
<td>1.24</td>
<td>41.0</td>
<td>1.83</td>
</tr>
<tr>
<td>G 1.0 µmole</td>
<td>16.60</td>
<td>0.91</td>
<td>57.0</td>
<td>1.38</td>
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</table>

When aminoglutethimide was introduced in these incubations, an inhibition of approximately 60% of human 18-hydroxycorticosterone synthesis occurred in comparison with controls receiving 0.05 ml ethyl acetate without aminoglutethimide. This solvent volume, by itself, inhibited 60% the steroid synthesis as compared with controls receiving no ethyl acetate (Table 1). In contrast, the same volume of solvent realized only a 34% inhibition of the steroid synthesis in sheep adrenal tissue (Table 2).

Sheep adrenal tissue incubations were carried out in order to study the effect of increasing aminoglutethimide doses (0.1–0.5 µmole) on corticosterone 18-hydroxylation. An approximately 72% inhibition of 18-hydroxycorticosterone biosynthesis already occurred in the presence of 0.2 µmole aminoglutethimide. This inhibition progressively increased and reached 91% in the presence of 0.5 µmole aminoglutethimide (Table 2).

In order to look for a possible effect of glutethimide, an aminoglutethimide structural similar, we incubated using identical experimental procedures the
Table 2.
Effect of increasing aminogluthethimide (AG) concentrations on the conversion of tritiated corticosterone to 18-hydroxy corticosterone (18-OHB) by sheep adrenal homogenates. Each flask contained in a total volume of 10.0 ml: [1,2-3H]corticosterone NADP (1 \(\mu\)mol/ml), fumarate (5 \(\mu\)mol/ml) and homogenized adrenal tissue (500 mg) prepared from the pooled glands of 20 animals. AG was introduced in the medium in a volume of ethyl acetate (Eth-Ac) 0.05 ml. Incubation lasted for 75 min at 37°C in air.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Total radioactivity recovered (\text{dpm} \times 10^6)</th>
<th>Per cent conversion</th>
<th>Per cent inhibition</th>
<th>Produced 18-OHB pmol/500 mg/75 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8.27</td>
<td>12.60</td>
<td></td>
<td>9.50</td>
</tr>
<tr>
<td>Eth-Ac</td>
<td>9.11</td>
<td>7.64</td>
<td></td>
<td>6.33</td>
</tr>
<tr>
<td>AG 0.1 (\mu)mole</td>
<td>7.53</td>
<td>7.03</td>
<td>8.0</td>
<td>4.81</td>
</tr>
<tr>
<td>AG 0.2 (\mu)mole</td>
<td>7.80</td>
<td>2.09</td>
<td>72.6</td>
<td>1.48</td>
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<tr>
<td>AG 0.3 (\mu)mole</td>
<td>8.24</td>
<td>1.41</td>
<td>81.5</td>
<td>1.06</td>
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<tr>
<td>AG 0.4 (\mu)mole</td>
<td>8.74</td>
<td>1.32</td>
<td>82.7</td>
<td>1.05</td>
</tr>
<tr>
<td>AG 0.5 (\mu)mole</td>
<td>8.55</td>
<td>0.68</td>
<td>91.1</td>
<td>0.52</td>
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Table 3.
Effect of increasing glutethimide (G) concentrations on the conversion of tritiated corticosterone to 18-hydroxy corticosterone (18-OHB) by sheep adrenal glands in vitro. Experimental conditions are identical to those described in Table 2. Where indicated, G was introduced in the incubation medium in a volume of ethyl acetate (Eth-Ac) 0.05 ml. Incubation lasted for 75 min at 37°C in air.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Total radioactivity recovered (\text{dpm} \times 10^6)</th>
<th>Per cent conversion</th>
<th>Per cent inhibition</th>
<th>Produced 18-OHB pmol/500 mg/75 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eth-Ac</td>
<td>7.60</td>
<td>8.47</td>
<td></td>
<td>5.58</td>
</tr>
<tr>
<td>Eth-Ac</td>
<td>8.25</td>
<td>8.61</td>
<td></td>
<td>6.46</td>
</tr>
<tr>
<td>G 0.2 (\mu)mole</td>
<td>8.10</td>
<td>8.55</td>
<td>0</td>
<td>6.31</td>
</tr>
<tr>
<td>G 0.3 (\mu)mole</td>
<td>8.20</td>
<td>8.28</td>
<td>0</td>
<td>6.17</td>
</tr>
<tr>
<td>G 0.4 (\mu)mole</td>
<td>7.90</td>
<td>9.10</td>
<td>0</td>
<td>6.53</td>
</tr>
<tr>
<td>G 0.5 (\mu)mole</td>
<td>8.15</td>
<td>7.83</td>
<td>0</td>
<td>5.80</td>
</tr>
</tbody>
</table>

Experiment II

<table>
<thead>
<tr>
<th>Addition</th>
<th>Total radioactivity recovered (\text{dpm} \times 10^6)</th>
<th>Per cent conversion</th>
<th>Per cent inhibition</th>
<th>Produced 18-OHB pmol/500 mg/75 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eth-Ac</td>
<td>20.50</td>
<td>8.10</td>
<td></td>
<td>15.10</td>
</tr>
<tr>
<td>Eth-Ac</td>
<td>19.90</td>
<td>7.10</td>
<td></td>
<td>12.90</td>
</tr>
<tr>
<td>G 1.0 (\mu)mole</td>
<td>18.90</td>
<td>6.60</td>
<td>13.2</td>
<td>11.40</td>
</tr>
<tr>
<td>G 2.0 (\mu)mole</td>
<td>19.50</td>
<td>5.75</td>
<td>24.4</td>
<td>10.20</td>
</tr>
<tr>
<td>G 5.0 (\mu)mole</td>
<td>18.30</td>
<td>4.27</td>
<td>43.9</td>
<td>7.10</td>
</tr>
</tbody>
</table>

522
same quantities of glutethimide as previously used to put aminoglutethimide inhibitory activity in evidence. A 41 % inhibition of 18-hydroxycorticosterone synthesis occurred when 0.5 μmole glutethimide was added to the human adrenal incubation (Table 1). On the other hand, the same quantity, did not however, alter the steroid synthesis in sheep adrenals and a ten times higher concentration was required to obtain an equivalent inhibition percentage (Table 3).

**DISCUSSION**

The first observation suggesting a possible inhibition of adrenal steroidogenesis by aminoglutethimide were reported by Camacho et al. (1966, 1967). Further investigations made it possible to locate a blockade between cholesterol and Δ5-pregnenolone.

The effects of aminoglutethimide on cholesterol 20α-hydroxylation as well as on 11β-hydroxylase were then demonstrated (for review see Touitou et al. 1973a,b).

These findings contrast with an unexpectedly normal cortisol secretion rate in treated patients, whereas aldosterone secretion has always been found to be reduced, thus suggesting the possibility that aminoglutethimide might interfere with one of the final steps of aldosterone biosynthetic pathway.

The results described show that aminoglutethimide is a potent inhibitor of corticosterone 18-hydroxylation in vitro. The concentrations of aminoglutethimide we used in sheep and human adrenal homogenate incubations were chosen in order to obtain approximately the same concentrations of the compound as those found post-mortem in the adrenals of a treated patient. Since adrenal tissue from a 64 year old woman with advanced metastatic breast carcinoma treated daily with 1 g Elipten® for 4 days was found to contain 500 μg aminoglutethimide per gram of tissue (Cash et al. 1967), we performed experiments with 0.1 to 1.0 μmole aminoglutethimide.

The inhibitory effect of the drug on sheep adrenal steroid synthesis is important since it occurs with only 0.2 μmole aminoglutethimide and reaches 91 % with 0.5 μmole (Table 2). Much higher concentrations do not markedly increase the inhibition of 18-hydroxycorticosterone synthesis (Touitou & Legrand 1971).

18-Hydroxycorticosterone synthesis, as well as the effect of aminoglutethimide was studied in human adrenals removed from a 30 year old woman with Cushing’s disease. The secretion and urinary excretion of aldosterone in Cushing’s syndrome have most frequently been found to be normal or rarely increased; quantitatively analysis of steroid adrenal content in Cushing’s syndrome showed aldosterone concentrations identical to that found in normal human adrenals (for review see Glaz & Vecsei 1971). This has also been found
in vitro (Bailey et al. 1960; Dyrenfurth et al. 1960; Kumagai et al. 1964). In contrast, few data are available on the in vitro synthesis of 18-hydroxycorticosterone, the aldosterone immediate precursor, in Cushing type adrenals. Incubations prepared with two such adrenals allowed us to observe very similar corticosterone to 18-hydroxycorticosterone conversion rates, about 5.20% for both glands (Table 1).

When aminoglutethimide was added in human adrenal incubation, a potent inhibitory effect on corticosterone 18-hydroxylation occurred (Table 1). It is to be noted that the addition of 0.05 ml ethyl acetate (volume of solvent in which aminoglutethimide had been added in the incubations) to some control flasks resulted in an approximately 60% inhibition of 18-hydroxycorticosterone synthesis in human adrenal tissue (Table 1) and an approximately 40% inhibition in sheep adrenal tissue (Table 2), when compared to corresponding controls receiving no solvent.

In vivo, one of the most marked effect of aminoglutethimide on adrenocortical steroidogenesis is the inhibition of aldosterone synthesis. When given to normal subjects or in patients with Cushing's syndrome, adrenal cortex carcinoma, and primary or secondary hyperaldosteronism, it causes a rapid and considerable fall in aldosterone secretion and excretion (for review see Touitou et al. 1973a). The results we obtained with 18-hydroxylase in vitro might explain this very significant fall in aldosterone, further corroborated by typical changes in the electrolytes with increased urinary sodium and reduced urinary potassium excretion (Fishman et al. 1967; Mancheno-Rico et al. 1971).

Taking into account the inhibition of corticosterone 18-hydroxylation demonstrated in human and animal adrenals in vitro, the marked reduction of aldosterone secretion in normal subjects treated with aminoglutethimide and the increased secretion of the ACTH-dependent aldosterone precursors i.e., 11-deoxycorticosterone and 18-hydroxy-11-deoxycorticosterone deserves some discussion:

In spite of the hypersecretion of deoxycorticosterone, the secretion of aldosterone is always dramatically decreased.

The increased secretion of 18-hydroxy-11-deoxycorticosterone itself attributed to an ACTH hypersecretion, is diminished after the administration of dexamethasone, thus confirming the role of ACTH in the increased secretion of this steroid.

It should be noted that dexamethasone reduces 18-hydroxy-11-deoxycorticosterone hypersecretion within the normal values, but not below these.

This suggests that aminoglutethimide inhibits corticosterone 18-hydroxylation but not deoxycorticosterone 18-hydroxylation, perhaps because the drug does not affect the 18-hydroxylase present in the zona fasciculata. It would thus be active only on the enzyme present in the zona glomerulosa.

This hypothesis is in agreement with the data reported in vivo by Melby
et al. (1972) on the dog and by Spark et al. (1971) and Spark (1972) on a subject with hyperaldosteronism, after treatment with GPA 2282 (3-methyl-2-[3-pyridyl]-indole-methone-sulphonate). These investigators observed a reduction in 18-hydroxycorticosterone and aldosterone secretion while 18-hydro-11-deoxycorticosterone secretion remained unaltered. These findings could also be explained if the drug was only effective on the 18-hydroxylase of the zona glomerulosa.

The potent inhibitory effect of aminogluthethimide on 18-hydroxylase and its structurally close relationship with glutethimide led us to look for this kind of activity in the latter compound.

18-Hydroxycorticosterone synthesis by human adrenal tissue was also significantly inhibited by 0.5 μmole glutethimide, but this inhibition was much weaker than that observed with the same tissue in the presence of the same concentration of aminogluthethimide. In contrast, 1 μmole of either compound produced the same inhibitory effect on human adrenal homogenates (Table 1). A marked inhibition appeared in sheep adrenal homogenates though with much higher glutethimide concentrations (Table 3). However, glutethimide at therapeutic dosage, is not likely to interfere with adrenal steroidogenesis in vivo, since to our knowledge no adrenal insufficiency has ever been reported. Within 7 days, on a dose of 1 g/day, 15 healthy did not show any change in adrenal or pituitary reactivity (McMahon & Foley 1967). Yet, high concentrations of the drug were reported to inhibit adrenal cortex synthesis in the rat (Johnston et al. 1968; Daniels-Severs & Vernikos-Danellis 1973). These data and our findings regarding the in vitro inhibitory effect of glutethimide on animal and human adrenal suggest that patient intoxicated with the drug may have reduced adrenal function.

This study using sheep and human adrenal homogenates thus establishes clearly that aminogluthethimide, in addition to its effects on cholesterol 20α-hydroxylase, at the onset of steroid biosynthesis, likewise interferes with corticosterone 18-hydroxylation, one of the final steps of aldosterone biosynthesis. Glutethimide, a widely used chemical analogue, also inhibits 18-hydroxycorticosterone synthesis in vivo, though this inhibition is less and requires higher concentrations.

REFERENCES


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