C17,20 LYASE: INHIBITION OF RAT TESTICULAR MICROSOMES AND PURIFIED LEYDIG CELLS BY AMINOGLUTETHIMIDE IN-VITRO

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Aminoglutethimide (AG) inhibits several cytochrome P450 mediated enzymes involved in steroidogenesis, such as the cholesterol side chain cleavage enzyme as well as oestrogen biosynthesis by inhibition of aromatase, for which it is used clinically in the treatment of breast cancer. Other reported sites of action of AG include the 18-hydroxylase enzyme and the 11-hydroxylase enzyme.

We have investigated the effect of AG on 17α-hydroxylase-$C_{17,20}$lyase enzyme compared with Ketoconazole (KTZ) which is a potent $C_{17,20}$lyase inhibitor.

Purified Leydig cells and microsomes were obtained from rat testes. Both Leydig Cells, and microsomes were incubated in the presence of the substrate 17-OH Progesterone, 8.25 and 1.65µmol/L respectively, together with AG (0.33 - 2000µmol/L) and KTZ (33 - 0.33µmol/L). All inhibitors were dissolved in ethanol, the concentration of which did not exceed 8% and had no effect on controls. The total volume of incubation mixtures was 300µL, and incubations were carried out in a shaking water bath at 37°C for 3 hours in an atmosphere of 95%O_2/5%CO_2. Androstenedione formation was then measured by R1A.

AG had no effect on lyase activity at the dose range of 0.33 - 660µmol/L supporting the observations of Jarman et al (1988). However AG at 825 - 2000µmol/L caused a dose related inhibition of 17,20 lyase (Table 1).

Table 1.

AGµmol/L	% max inhibition	KTZµmol/L	% max inhibition
		33	90
2000	90	16.5	88.1
1600	81.6	3.3	86.7
1090	58	1.65	78.4
825	7.1	0.33	69.05
550	1.8	0.033	39.5

An IC50 value giving the concentration required to reduce enzyme activity to 50% was determined graphically and compared with KTZ (Table 2).

AG is 1000-2000 fold less potent at inhibiting 17,20 lyase than KTZ. This inhibition may be due to a general effect on cytochrome P450 rather than a specific and selective action on 17,20 lyase, however in vivo, levels of AG in some tissues have been shown to be within this range (Pourgholami 1987), and therefore inhibition of 17-20 lyase may contribute to the therapeutic effect of AG.

Table 2 IC50 Value for AG and KTZ in Leydig cells and Microsomes

	Leydig cells [17-OH PROG] 8.25µmol/L	Microsome [17-OH PROG] 1.65µmol/L
AG	8.9×10^{-4} mol/L (n=2)	8.95×10^{-4} mol/L (n=2)
KTZ	8.76×10^{-7} mol/L (n=5)	4.7×10^{-7} mol/L (n=5)

M Jarman, O.E. Barrie, E.S. Leung and M.G. Rowlands, Anticancer Drug Design (1988) 3: 185 - 190.

M.H. Pourgholami, (1987) PhD Thesis University of Wales College of Cardiff.