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EFFECT OF GAMMA-GUANIDINOBUTYRAMIDE ON THE ACTIVITY OF UREA CYCLE ENZYMES IN THE LIVER OF THE RAT

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Abstract—γ-Guanidinobutyramide has been shown to reduce blood urea levels in experimental animals and humans by an extra-renal mechanism. The effect of this compound on the activity of urea cycle enzymes in rat liver has been investigated by *in vitro* and *in vivo* studies. Evidence of *in vitro* inhibition of argininosuccinate synthetase activity has been obtained. It is, however, unlikely that the reduction of blood urea observed after therapeutic exhibition of the drug is due to urea cycle enzyme inhibition.

GAMMA-GUANIDINOBUTYRAMIDE (γGB), a compound of low toxicity with structural similarities to arginine has been shown to lower elevated blood urea levels in diabetics by an extrarenal mechanism.[1] It has also been shown to lower blood urea in experimental animals,[2] and to decrease urea formation in rat liver slices.[3] The mechanism by which these effects are produced is quite unknown. The experiments reported here investigate the possibility that the compound inhibits one or more of the enzymic steps involved in urea synthesis, which together constitute the urea cycle (Fig. 1).

(1) Ammonia + Bicarbonate + 2ATP $\xrightarrow[\text{kinase}]{\text{Carbamate}}$ Carbamoyl phosphate + 2ADP + Inorganic phosphate

(2) Carbamoyl phosphate + Ornithine $\xrightarrow[\text{Carbamoyltransferase}]{\text{Ornithine}}$ Citrulline + inorganic phosphate

(3) Citrulline + Aspartate + ATP $\xrightarrow[\text{synthetase}]{\text{Argininosuccinate}}$ Argininosuccinate + AMP + inorganic pyrophosphate

(4) Argininosuccinate $\xrightarrow[\text{lyase}]{\text{Argininosuccinate}}$ Arginine + Fumarate

(5) Arginine + H_2O $\xrightarrow{\text{Arginase}}$ Urea + Ornithine

FIG. 1. Simplified urea cycle.

METHODS

Commercial reagents were used, with the exception of α-isonitrosopropiophenone which was synthesized by the method of Claisen and Manasse.[4]

Except in the case of arginase (EC 3.5.3.1), the activity of the urea cycle enzymes

were measured by the methods of Brown and Cohen[5] as modified by McClean and Gurney.[6] The enzymes were measured in the supernatant fraction from a 10% (w/v) rat liver homogenate prepared in 0·1 (w/v) cetyl trimethyl ammonium bromide solution, the homogenate being centrifuged at 4000 *g* for 15 min at 5°. Arginase activities were determined by the method of Folley and Greenbaum,[7] the initial homogenate being further diluted 1:10 with a manganese saline solution (8 g $MnSO_4.4H_2O$/l. of 0·9% saline), and activated at 37° for 30 min before assay. Carbamate kinase (EC 2.7.2.2) and ornithine carbamyl transferase (EC 2.1.3.3) were measured by the rate of citrulline formation using diacetyl monoxime for colour development.[8]

The remaining three enzymes, argininosuccinate synthetase, (EC 6.3.4.5), argininosuccinate lyase (EC 4.3.2.1) and arginase were measured by the rate of urea formation, using a colour reaction with α-isonitrosopropiophenone.[9]

All enzymes were measured at 37° and results are expressed as micromoles of product formed per gram of liver (wet weight) per hour under the conditions of the assay.

Individual results recorded are mean values derived from duplicate or triplicate assays with appropriate enzyme and reagent blanks.

The effect of the compound was studied both *in vitro* and *in vivo*.

In vitro *experiments*. The enzyme activities were measured in the supernatant fraction from a 10% w/v cetyl-trimethyl ammonium bromide homogenate of adult rat liver, in the presence of γGB. A 10^{-1} M solution of the hydrochloride was prepared in phosphate buffer (pH 7·0). This solution was added to the assay tubes in amounts such that final concentrations of 1×10^{-2} M and 4×10^{-2} M were produced. Control assays were carried out without the addition of the compound and the results compared. It had been shown by others[10] that concentrations of 5×10^{-2} M would depress urea formation in rat liver slices.

In vivo *experiments*. (a) In a first experiment a group of six male rats (100–200 g) were given γGB hydrochloride 100 mg daily by gastric tube, the dose being made up in 0·05 M phosphate buffer, pH 7·0 and contained in 1 ml. Two control animals were given phosphate buffer solution, containing no compound, daily by gastric tube. Two of the test animals were killed 3 hr after the first dose of the compound, two after the third daily dose and two after the fifth dose. The control animals were also sacrificed on the fifth day. The livers were removed immediately after death and homogenates prepared without delay. The urea cycle enzymes were assayed. (b) In a second experiment two groups of five female rats (100–200 g) were used. Five control animals were given an intraperitoneal injection of 0·05 M phosphate buffer, pH 7·0. Five test animals were given a similar injection containing 100 mg of γGB hydrochloride. Both groups were killed 2 hr later. The total carcass weights were noted (control group 1220 g, test group 1192 g), the livers were removed and all five urea cycle enzymes determined on each animal. (c) In a third experiment, γGB was added to the drinking water of a group of ten female rats (100–200 g). A preliminary study suggested that the daily water intake of such rats was ca. 30 ml per animal; 150 mg of the compound per 30 ml was added to the drinking water. Since spillage and other losses made accurate measurement of the daily intake of each animal impracticable it was hoped that this would ensure a dose of ca. 100 mg/day. After 1 week, the test animals and a group of ten control animals, maintained on a similar diet but with a normal water supply, were killed and the liver argininosuccinate synthetase activity measured.

TABLE 1. *IN VITRO* STUDIES ON THE EFFECT OF γ-GUANIDINOBUTYRAMIDE ON UREA CYCLE ENZYME ACTIVITIES IN ADULT RAT LIVER HOMOGENATES

Enzymes	No. of experiments	Without added γGB	Added γGB 1×10^{-2} M	Added γGB 4×10^{-2} M
Carbamate	3	422	378	284
kinase		406	336	220
(CK)		354	320	263
Ornithine				
Carbamoyl	2	11,000	10,400	9520
Transferase		9860	9320	8600
(OCT)				
Argininosuccinate		68	65	51
Synthetase	4	82	78	61
(ASS)		73	70	60
		80	75	67
Argininosuccinate		169	169	160
Lyase	2	111	111	93
(ASL)				
Arginase	3	70,000	68,200	67,000
(ARG)		45,000	41,800	44,300
		50,800	50,800	57,800

All results expressed as units/g liver (wet wt.)/hr.

TABLE 2. THE EFFECT OF ORAL ADMINISTRATION OF γ-GUANIDINOBUTYRAMIDE (100 mg daily) ON THE ACTIVITY OF UREA CYCLE ENZYMES IN ADULT RAT LIVER

Group	Animal no.	CK	OCT	ASS	ASL	ARG
	1	503	17,100	67	170	154,000
Control	2	549	17,300	85	179	147,800
	mean	526	17,200	76	175	150,900
γGB	1	440	13,200	44	208	121,200
Single dose	2	>500	11,300	43	217	88,200
	mean		12,250	44	213	104,700
γGB	1	278	9600	44	184	100,000
daily for 3	2	499	13,800	63	163	128,200
days	mean	388	11,700	54	174	114,100
γGB	1	546	17,700	37	148	154,000
daily for 5	2	518	18,300	62	150	147,200
days	mean	532	18,000	49	149	150,600

All results expressed as units/g liver (wet wt.)/hr.
Abbreviations as in Table 1.

RESULTS

The results of these studies are shown in the accompanying tables. The *in vitro* studies (Table 1) show a dose-related inhibition of argininosuccinate synthetase activity by γGB, the decrease in activity produced by a 4×10^{-2} M concentration of the compound being highly significant ($t = 8{\cdot}4$ $P < 0{\cdot}01$). There is also a possible decrease in the activity of both carbamate kinase and ornithine carbamoyl transferase ($P < 0{\cdot}05 > 0{\cdot}02$).

TABLE 3. THE EFFECT OF γ-GUANIDINOBUTYRAMIDE 100 mg BY INTRA-PERITONEAL INJECTION ON THE ACTIVITY OF UREA CYCLE ENZYMES IN ADULT RAT LIVER

Enzyme	Control animals mean ± S.E.M.	Test animals mean ± S.E.M.
CPK	280 ± 26·9	302 ± 18·9
OCT	11,960 ± 697	13,160 ± 852
ASS	96 ± 10·6	86 ± 1·4
ASL	173 ± 17·3	192 ± 8·8
ARG	54,500 ± 13,800	102,900 ± 11,643

Abbreviations as in Table 1.
Enzyme activities expressed as units/g wet wt.

Neither of the first two *in vivo* studies (Tables 2 and 3) show statistically significant changes in enzyme activities. However, the results of the initial experiment with oral administration of the compound are rather suggestive of inhibition of argininosuccinate synthetase activity. The third experiment was undertaken to investigate the possibility further, but the results (Table 4) show no difference between the activity of the control and test animals.

TABLE 4. THE EFFECT OF ADDITION OF γ-GUANIDINOBUTYRAMIDE TO THE DRINKING WATER OF RATS ON HEPATIC ARGININO-SUCCINATE SYNTHETASE ACTIVITY

	ASS Activity mean ± S.E.M.
10 Control animals	74·6 ± 6·9
10 Test animals	73·9 ± 5·2

Enzyme activities expressed as units/g wet wt.
Abbreviations as in Table 1.

DISCUSSION

The demonstration of *in vitro* inhibition of three enzymes of the urea cycle, including argininosuccinate synthetase which catalyses the rate-limiting step of the cycle, by a compound known to reduce blood urea levels, is interesting.

However, it is unlikely that the level of the drug in body fluids produced during therapy would approach these demonstrated to produce *in vitro* inhibition. Moreover, even if the degree of inhibition observed in these experiments did occur during therapy, at least 50 per cent of the normal activity of the rate-limiting enzymes would persist. It can be calculated on theoretical grounds that this is adequate to support normal production of urea in any physiological situation. Kennan and Cohen[11] suggested that in human beings only one-fifth of the potential synthetic ability of the liver was used for the daily synthesis of urea. This is supported by the fact that normal levels of blood urea have been consistently reported in patients with argininosuccinic aciduria, an inborn metabolic error involving decreased activity of argininosuccinate lyase; the liver of a patient with this condition which we examined[12] showed approximately 3 per cent of normal activity.

It is therefore concluded that the reduction in blood urea which follows the exhibition of γGB is due to causes other than inhibition of the enzymic steps involved in urea synthesis.

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