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The Effect of Aminoguanidine Treatment, With or Without Food Restriction on the Liver, Stomach and Small Intestine of the Rat

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Abstract—A comparative investigation has been made into the effects of aminoguanidine treatment (60 mg kg^{-1} day^{-1}) with or without dietary restriction (i.e. 50% reduction in food intake) on the protein, RNA and DNA composition of the liver, small intestine and stomach of young rats (0·1 kg body weight). After 3 weeks of dietary restriction the wet weights of the liver and small intestine decreased by 56 and 52%, respectively. There were significant reductions (approx 50%) in total hepatic and intestinal protein, RNA and DNA. Changes in ratios of RNA/protein, RNA/DNA and protein/DNA were only significant for intestinal RNA/DNA, where a 15% reduction was observed. In contrast, stomach wet weight and total protein content were unaltered by dietary deprivation. Stomach RNA and DNA contents were reduced by only 18–21%, and the protein/DNA ratio increased by 22%. Similar responses of liver, small intestine and stomach to dietary deprivation were observed in aminoguanidine-treated rats. Aminoguanidine-treatment of rats on an unrestricted diet for three weeks had no effect on the wet weights, total protein, RNA or DNA contents of the liver, stomach or small intestine. In dietary-restricted rats, the liver and stomach were unaffected by the treatment. However, aminoguanidine treatment of dietary-restricted rats caused significant increases in the amounts of intestinal protein, RNA and DNA by approximately 15%. The treatment abolished the dietary restriction-induced decrease in total intestinal DNA/body weight. The wet weights of the lung, diaphragm, kidney, spleen and testes of both fed and dietary-restricted rats were also unaffected by aminoguanidine. It was concluded that the stomach is protected against the deleterious effects of nutritional deprivation; the data derived from the small intestine of dietary-restricted rats suggests aminoguanidine may have some potential therapeutic properties as a trophic agent.

Although there is an abundance of literature on the effects of nutritional deprivation on individual tissues of the hepato-gastrointestinal tract, there is a paucity of information on the relative ways in which individual tissues might respond. In this paper, a systematic investigation is reported into the comparative effects of chronic (i.e. 3 weeks) nutritional deprivation on the liver, stomach and small intestine of the laboratory rat.

We also investigated the potential therapeutic effects of aminoguanidine hydrochloride on the response to nutritional deprivation, as well as its actions in rats with free access to food. Aminoguanidine has been shown to promote tissue growth in rats as well as to reduce nitrogen excretion in cancer cachexia in man (Baylin et al 1975). However, since the original studies by Baylin et al (1975), the anabolic effects of aminoguanidine have not been substantiated. Mennigen et al (1989) suggested that in rat small intestine, aminoguanidine might alter mucosal proliferation, but those authors were unable to substantiate that in studies on resected small intestine. Sugiyama et al (1985) also showed that aminoguanidine retarded liver growth in chick embryos, and other studies suggested that this occurred by a reduction in protein and DNA synthesis (Sugiyama et al 1980). We have therefore determined whether similar perturbations in normal biochemistry occurred in normal and dietary restricted rats. To assess these effects we measured protein, RNA and DNA composition in the liver, stomach and small intestine.

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Materials and Methods

Treatment of animals

Male Wistar rats (Bantin and Kingman Ltd, Aldebrough, Hull, UK) were obtained at 53–64 g and maintained on a standard, laboratory chow ("Diet LAD2", Labsure, Manea, Cambridgeshire, UK) until they weighed 72–85 g. They were then ranked and divided into four groups of equal mean body weight (mean ± s.e.m., n = 6); Group 1, controls (78 ± 2 g) with unrestricted access to food; Group 2, aminoguanidine-treated (78 ± 2 g) with unrestricted access to food; Group 3, dietary-restricted controls (78 ± 2 g); Group 4, dietary-restricted, aminoguanidine-treated (78 ± 2 g).

Dietary restriction was imposed by feeding rats 50% of the diet consumed by the control group, which was measured daily. Aminoguanidine hydrochloride was added to the drinking water at an initial concentration sufficient to achieve an intake of 50–60 mg kg^{-1} day^{-1}, which was the same dosage as used by Baylin et al (1975). The aminoguanidine solution was freshly prepared each day.

At the end of three weeks, rats were killed, and the liver rapidly dissected, blotted, weighed and immediately frozen in liquid nitrogen. The stomach and entire small intestine were also dissected, flushed with ice-cold (0–4°C) 0·15 M NaCl, blotted, weighed and also frozen in liquid nitrogen. All samples were stored at −70°C until analysis.

Tissue analysis

All processing was carried out at 0–4°C, unless stated otherwise. Tissues were homogenized in water and a portion equivalent to 200–400 mg precipitated in 10 mL 0·2 M

perchloric acid. After centrifugation (2000–3000 *g*, 15 min), acid supernatants were discarded and the pellet washed twice in 10 mL 0·2 M perchloric acid. Protein pellets were solubilized in 0·3 M NaOH and incubated for 1 h at 37°C. A portion was removed for estimation of protein as described by Gornall et al (1949), and DNA was determined by a modification of the method described by Downs & Wilfinger (1983). After re-precipitation of the protein in 0·4 M perchloric acid, RNA was measured in acid supernatants by the method of Munro & Fleck (1969). To ensure strict compatibility of the data, the liver, stomach and small intestine were processed and assayed in parallel.

Statistics

All data are presented as mean ± s.e.m. of 6 observations, in each group except the dietary-restricted controls where n = 5. Differences between means were assessed by Student's *t*-test, using the pooled estimate of variances. Significance was indicated at $P < 0.05$.

Results

Body weights and food intake

The final body weights of the rats were as follows (mean ± s.e.m., n = 6); Group 1, 252 ± 5 g; Group 2, 250 ± 6 g; Group 3, 156 ± 1 g; Group 4, 157 ± 1 g. Food intake in control and aminoguanidine-treated rats were virtually identical. For example after 1 week, daily food intake was 23·1 ± 0·8 and 23·1 ± 0·5 g in control and aminoguanidine-treated rats, respectively. Similar results were obtained for those other days (days 8–21) on which food intake was measured. By virtue of the experimental design, there was also no difference in dietary intake between rats which were dietary-restricted and those dietary-restricted with concomitant aminoguanidine treatment.

Effect of aminoguanidine treatment with and without dietary-restriction on the weights of lung, diaphragm, kidney, spleen and testes

Table 1 shows the responses of various tissues to dietary deprivation, and demonstrates varying degrees of susceptibilities. The greatest change in tissue weight was observed for spleen (44% decrease in wet weight) and the testes were least affected (16% decrease in wet weight). In general these changes were negated when expressed per unit body weight and in the case of the lung and testes there was an apparent increase. Similar results of dietary deprivation were observed in aminoguanidine-treated rats. However, aminoguanidine treatment itself had no significant effect on any of the tissue weights.

The response to dietary restriction without aminoguanidine treatment

Dietary restriction caused the wet weight of the liver and small intestine to fall by 56 and 52%, respectively (Table 2). When expressed relative to body weight, the magnitude of the effect was reduced slightly, i.e. a 30 and 22% decline in liver and intestine weight, respectively. In contrast, the weight of the stomach was unaltered by dietary deprivation and its weight relative to body weight apparently increased by 65% (Table 2).

Hepatic protein concentration (mg (g tissue wet wt)$^{-1}$) increased as a consequence of dietary deprivation (16%, $P < 0.001$), although the concentration of protein in the other two tissues was unaffected. The total hepatic protein content decreased by 50%. A similar decrease in the total protein content was observed for the small intestine, although the protein content of the stomach was unaltered by dietary deprivation (Table 3).

Table 4 shows that the concentration of hepatic RNA increased by 17% in response to dietary deprivation but total hepatic RNA content decreased by 49%. Total RNA content in the small intestine and stomach also decreased, by 58 and 21%, respectively.

The changes in DNA were similar to perturbations in RNA, i.e. hepatic DNA concentration increased by 22%, and total DNA in liver, small intestine and stomach fell by 47, 51 and 18%, respectively (Table 5).

Derived variables of protein, RNA and DNA are displayed in Table 6. In all three tissues the capacity for protein synthesis, indicated by the RNA/protein ratio, was unaltered by dietary deprivation. However, when RNA was expressed in relation to DNA, effectively representing the amount of

Table 1. The effect of aminoguanidine treatment with or without dietary restriction on the weight of various tissues of the rat.

	Without dietary restriction		With dietary restriction	
	Control	+Aminoguanidine	Control	+Aminoguanidine
	Tissue wet weight (g)			
Lung	1·49 ± 0·06	1·60 ± 0·08	1·10 ± 0·03*	1·08 ± 0·03
Diaphragm	0·53 ± 0·04	0·52 ± 0·04	0·39 ± 0·03**	0·36 ± 0·03
Kidney	2·40 ± 0·06	2·39 ± 0·10	1·49 ± 0·02***	1·43 ± 0·03
Spleen	0·70 ± 0·04	0·70 ± 0·02	0·39 ± 0·03***	0·40 ± 0·02
Testes	2·74 ± 0·14	2·67 ± 0·12	2·29 ± 0·09*	2·68 ± 0·23
	Tissue weight/body weight (g kg^{-1})			
Lung	5·91 ± 0·21	6·39 ± 0·24	7·06 ± 0·17***	6·85 ± 0·19
Diaphragm	2·11 ± 0·15	2·05 ± 0·14	2·48 ± 0·17	2·30 ± 0·18
Kidney	9·54 ± 0·19	9·54 ± 0·20	9·55 ± 0·13	9·12 ± 0·26
Spleen	2·78 ± 0·16	2·79 ± 0·20	2·51 ± 0·18	2·52 ± 0·11
Testes	10·90 ± 0·50	10·80 ± 0·60	14·70 ± 0·60***	17·00 ± 1·40

All data are presented as mean ± s.e.m. of 5–6 observations. Differences between means were assessed by Student's *t*-test, using the pooled estimate of variance from all 4 groups. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with animals without dietary restriction.

Table 2. The effect of aminoguanidine treatment, with or without dietary restriction, on the weight of the liver, small intestine and stomach of the rat.

	Without dietary restriction		With dietary restriction	
	Control	+Aminoguanidine	Control	+Aminoguanidine
	Tissue wet weight (g)			
Liver	14·03±0·36	13·94±0·40	6·11±0·14*	5·96±0·09
Small intestine	8·05±0·45	7·60±0·35	3·87±0·15*	3·82±0·15
Stomach	1·09±0·04	1·04±0·03	1·11±0·08	1·06±0·03
	Tissue weight/body weight (g kg^{-1})			
Liver	55·79±1·88	55·83±1·23	39·11±0·92*	38·00±0·72
Small intestine	31·90±1·46	30·43±1·16	24·75±0·94*	24·34±0·84
Stomach	4·34±0·19	4·16±0·16	7·14±0·56*	6·77±0·17

* $P<0·001$ compared with animals without dietary restriction.

Table 3. The effect of aminoguanidine treatment, with or without dietary restriction, on the protein composition of liver, small intestine and stomach.

	Without dietary restriction		With dietary restriction	
	Control	+Aminoguanidine	Control	+Aminoguanidine
	Protein concn (mg (g wet wt)$^{-1}$)			
Liver	148±2	149±3	172±4**	171±1
Small intestine	69±2	70±3	67±3	74±1†
Stomach	102±5	106±4	104±11	103±3
	Total protein content (mg)			
Liver	2080±30	2080±50	1050±20**	1030±20
Small intestine	558±45	528±31	257±12**	283±12
Stomach	111±4	110±4	110±3	109±3
	Total protein content/body weight (mg kg^{-1})			
Liver	8240±220	8320±60	6740±140**	6550±150
Small intestine	2210±150	2110±100	1640±70*	1800±70
Stomach	440±14	438±7	699±16**	695±16

* $P<0·01$, ** $P<0·001$ compared with animals without dietary restriction. † $P<0·05$, compared with animals without aminoguanidine.

Table 4. The effect of aminoguanidine treatment, with or without dietary restriction, on the RNA composition of liver, small intestine and stomach.

	Without dietary restriction		With dietary restriction	
	Control	+Aminoguanidine	Control	+Aminoguanidine
	RNA concn (mg (g wet wt)$^{-1}$)			
Liver	6·82±0·11	6·93±0·10	7·96±0·17**	7·52±0·09
Small intestine	4·67±0·26	4·60±0·17	4·11±0·32	4·82±0·14†
Stomach	4·40±0·58	4·15±0·22	3·41±0·35	3·44±0·22
	Total RNA content (mg)			
Liver	95·5±2·0	96·5±2·8	48·5±0·8**	46·6±1·0
Small intestine	37·3±2·3	35·1±2·5	15·7±1·1**	18·5±1·1
Stomach	4·7±0·5	4·3±0·2	3·7±0·2*	3·7±0·3
	Total RNA content/body weight (mg kg^{-1})			
Liver	380±10	386±5	311±5**	297±8
Small intestine	148±7	140±9	101±8**	118±7
Stomach	19±2	17±1	23±1*	23±2

* $P<0·05$, ** $P<0·001$ compared with animals without dietary restriction. † $P<0·05$ compared with animals without aminoguanidine.

protein synthetic machinery per cell, small but significant decreases (i.e. 15%, $P<0·05$) were observed in the small intestine only. The DNA-unit (protein/DNA ratio or apparent cell size) was increased in the stomach (22%, $P<0·01$) but was unaltered in either the liver or small intestine.

In general, when data for protein, RNA and DNA were expressed relative to body weight, the magnitude of the effects on hepatic and small intestinal protein, RNA and DNA were reduced (Tables 3–5). In contrast, significant increases in stomach protein (59%, $P<0·001$), RNA (21%, $P<0·05$) and DNA (33%, $P<0·001$) were observed when data were expressed relative to body weight.

Table 5. The effect of aminoguanidine treatment, with or without dietary restriction, on the DNA composition of liver, small intestine and stomach.

	Without dietary restriction		With dietary restriction	
	Control	+Aminoguanidine	Control	+Aminoguanidine
	DNA concn (mg (g wet wt)$^{-1}$)			
Liver	3·15±0·11	3·19±0·08	3·84±0·11***	3·79±0·05
Small intestine	3·48±0·21	3·38±0·18	3·59±0·20	4·19±0·10†
Stomach	4·18±0·28	4·40±0·30	3·53±0·45	3·40±0·09
	Total DNA content (mg)			
Liver	44·1±1·6	44·5±2·0	23·4±0·6***	22·6±0·6
Small intestine	28·1±2·5	25·7±1·7	13·7±0·5***	31·6±0·8
Stomach	4·5±0·2	4·5±0·3	3·7±0·2*	3·6±0·2
	Total DNA content/body weight (mg kg^{-1})			
Liver	175±6	177±4	150±4**	144±4
Small intestine	111±8	102±5	88±3*	102±4
Stomach	18±1	18±1	24±1***	23±1

* $P<0·05$, ** $P<0·01$, *** $P<0·001$ compared with animals without dietary restriction. † $P<0·05$ compared with animals without aminoguanidine.

Table 6. The effect of aminoguanidine treatment, with or without dietary restriction, on derived parameters in liver, small intestine and stomach.

	Without dietary restriction		With dietary restriction	
	Control	+Aminoguanidine	Control	+Aminoguanidine
	RNA/protein (mg g^{-1})			
Liver	46·0±0·7	46·4±0·5	46·4±0·7	46·1±0·5
Small intestine	67·6±3·0	66·5±2·1	61·6±5·3	65·3±2·6
Stomach	42·4±4·1	39·4±2·3	33·9±2·0	34·2±2·7
	RNA/DNA (mg mg^{-1})			
Liver	2·18±0·07	2·18±0·04	2·08±0·04	2·07±0·03
Small intestine	1·35±0·05	1·37±0·05	1·15±0·09*	1·15±0·04
Stomach	1·04±0·09	0·95±0·04	1·01±0·05	1·03±0·05
	Protein/DNA (mg mg^{-1})			
Liver	47·3±1·3	46·9±1·0	44·8±0·8	45·0±0·6
Small intestine	20·0±0·7	20·6±0·3	18·7±0·5	17·7±0·3
Stomach	24·6±0·9	24·5±1·4	29·9±1·4**	30·5±1·2

* $P<0·05$, ** $P<0·01$ compared with animals without dietary restriction.

Effect of dietary deprivation in aminoguanidine-treated rats

Effects of dietary deprivation on the liver and stomach in aminoguanidine-treated animals were similar, both in qualitative and quantitative terms, to the effects of dietary deprivation in control rats; no overt abatement or exacerbation of the nutritional response was observed in aminoguanidine-treated groups. However, in the small intestine the magnitude of the dietary restriction-induced effects on protein, RNA and DNA composition was reduced. In the case of the DNA/body weight, no effect of dietary restriction was observed, whilst a significant decline was observed in control rats in response to dietary restriction.

Effect of aminoguanidine treatment in control and dietary restricted rats

Aminoguanidine treatment of rats with free access to food was without effect on the liver, small intestine or stomach. In dietary restricted rats, aminoguanidine treatment increased the intestinal concentrations of protein (10%, $P<0·05$), RNA (17%, $P<0·05$) and DNA (17%, $P<0·05$). The concomitant increases in the total amounts of protein, RNA and DNA (10, 18 and 17%, respectively) did not achieve statistical significance ($P>0·05$): this probably represented a type II statistical error. For future reference and comparative information we have therefore also quoted confidence limits of the data. The 95% confidence limits of the mean changes were as follows: for the changes in total protein content, +2 to −22%; for the changes in total RNA content, +1 to −36%; for the changes in total DNA content, +2 to −31%.

Discussion

Comparative studies into the effects of experimental malnutrition on the tissues of the gastrointestinal tract are relatively limited, although extensive studies have been carried out into the response of individual tissues. For example, McNurlan & Garlick (1981) investigated the response of the liver and small intestine to dietary protein deprivation, but examination of the stomach was not included. We hypothesized that different regions of the intestinal tract might respond to relatively chronic limitations in nutritional supply with varying sensitivities. In this study we examined the effects of reducing food intake by 50% on the composition of protein, RNA, DNA and derived

parameters (RNA/DNA, RNA/protein and protein/DNA ratios) in the liver, small intestine and stomach of young rats. These variables have been useful in describing gross biochemical changes when nutritional or nitrogen supply is limited and basically ascertain if protein or nucleic acid contents are sub-optimal for normal tissue maturation (Waterlow et al 1978). For example, perturbations in RNA will determine if anabolic or catabolic processes are taking place (Henshaw et al 1971; Waterlow et al 1978).

The rationale for carrying out the above nutritional deprivation studies concomitantly with aminoguanidine treatment was based on the original observations by Baylin et al (1975) which showed that aminoguanidine treatment could increase liver and body weight. Furthermore, aminoguanidine was preferentially concentrated in the intestine and liver, by 488 and 147%, respectively, compared with plasma. The cellular uptake of aminoguanidine may thus be tissue-specific, as none was detectable in the brain, and in contrast, levels in the kidney were approximately 8 times that in plasma (Baylin et al 1975). In the study of Baylin et al (1975), aminoguanidine uptake by the intestine caused histaminase activity to be markedly reduced (by 93%), compared with the aminoguanidine-induced reduction in hepatic histaminase activity of only 23%. If the potential anabolic effects of aminoguanidine are due to its properties as a diamine oxidase inhibitor, then it is possible to speculate that the cyto-proliferative activities of aminoguanidine would be greater in the small intestine, i.e. the site of greater inhibition of histaminase. Thus aminoguanidine might be useful in selectively maintaining tissue nitrogen content in the small intestine during nutritional compromise.

Effect of nutritional deprivation

The results in Tables 2–6 showed that the liver and small intestine were exceedingly susceptible to chronic nutritional deprivation. The decreases in tissue mass, protein, RNA and DNA were entirely consistent with previous publications in this field, (for a review see Waterlow et al 1978). The reductions in liver and intestine protein contents would have contributed to the generalized enhanced nitrogen excretion observed in malnutrition, whilst RNA changes reflect adaptive reductions in the capacity or potential for hepatic or intestinal protein synthesis. This has also been supported by isotope studies, which have clearly shown that rates of hepatic and intestinal protein synthesis are reduced as a consequence of food restriction (McNurlan et al 1979). Meanwhile the reductions in DNA would suggest macromorphological dysfunction in response to starvation, and possibly the number of cells per organ.

The most remarkable finding of our study was that there were virtually no effects of chronic dietary deprivation on the stomach. Protein content in the stomach was maintained despite a small reduction in RNA and DNA contents. It is difficult to propose a mechanism to explain this, but the observations would suggest that if protein synthesis was reduced (indicated by the reduction in stomach RNA content) then protein degradation would also have to decrease. Evidence to support the contention that protein synthesis in the stomach is sensitive to nutritional supply is not unequivocable as 1 or 2 days of starvation does not alter the fractional rate of stomach protein synthesis, although synthesis rates in liver and small intestine do decline (Preedy et al 1988). However, it is important to distinguish between cessation of food intake and a partial reduction in dietary ingestion. It was possible that the presence of food matter was a stimulus for maintaining protein content of the stomach. However, this does not explain why intestinal protein content was reduced; ingested material passing from the stomach also has to go through the lumen of the small intestine, albeit in different stages of digestion. Other rat tissues also displayed a patterned response: the testes were relatively unaffected by dietary deprivation, when compared with the diaphragm, lung, kidney and spleen. Nevertheless, the slight reduction in testicular weight did achieve statistical significance, whereas the stomach weight showed no change.

Effect of aminoguanidine

This study showed that the diamine oxidase inhibitor, aminoguanidine, had no effect on the liver or stomach. However, intestinal protein, RNA and DNA concentrations increased in the dietary-restricted group. Total intestinal contents of protein, RNA and DNA also appeared to increase, by 10–18%, but these changes did not achieve statistical significance, probably reflecting a type II error. Nevertheless, aminoguanidine completely abolished the food restriction-induced decline in intestinal DNA relative to body weight. These observations suggest that aminoguanidine may have potential therapeutic properties, especially in situations where there is a need to induce a trophic response in the small intestine. Recently, Erdman et al (1989) showed that aminoguanidine was able to increase mucosal DNA and protein composition in rats with 80% jejuno-ileal resection, but no comparable studies appear to have been carried out in normal rats receiving aminoguanidine alone. These observations suggest that diamine oxidase may be a regulatory factor in mucosal growth, or alternatively might cause changes in protein and DNA indirectly by some other mechanism. Mennigen et al (1989) also examined the effects of aminoguanidine on the small intestinal mucosa, but showed that aminoguanidine did not affect mucosal proliferation.

Although the above studies indicated a possible therapeutic role of aminoguanidine, directly contrasting evidence has been obtained by other groups. For example, Sugiyama et al (1985) showed aminoguanidine reduced the growth of the liver and induced hypoplasia in the gall bladder of chick embryos. Subsequent studies with labelled amino acids and thymidine, suggested that this may have been due to a reduction in hepatic DNA and protein synthesis. Although incorporation of labelled uridine into RNA was studied, no significant effects were observed on the whole chick embryo, which presumably largely comprised of muscle, bone and skin (Sugiyama et al 1980). In contrast, the addition of aminoguanidine to in-vitro systems was shown to inhibit hepatic RNA synthesis with concomitant perturbations in nucleolar ultra-structure (Nishiyama & Kurebe 1980), as opposed to a lack of effect on RNA synthesis in chick liver in-vivo (Sugiyama et al 1980). Overall the data suggests that aminoguanidine may be selective not only towards individual nucleic acids, i.e. DNA, but also towards specific organs and model systems. There is an obvious requirement for further investigation and characterization of the pharmacological effects of aminoguanidine.

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