ALTERATION OF THE CONCENTRATION OF CYCLIC NUCLEOTIDES IN THE RAT BY FUSIDIC ACID

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1. Introduction

Cyclic nucleotides (cAMP, cGMP) act as second messengers in a number of biochemical reactions which support homeostasis in the living animal [1,2]. Our recent reports show that fusidic acid, a steroidal antibiotic which inhibits in vitro protein synthesis:

(i) Stimulates the in vivo incorporation of amino acids into proteins of rat liver, kidney, brain and muscle [3].

(ii) Enhances the in vivo uptake of $^{32}P_i$ into the liver intracellular phosphate pool.

(iii) Stimulates the in vivo phosphorylation of proteins in all cell fractions of the rat liver except mitochondria [4].

(iv) Significantly increases blood glucose concentrations.

These observations suggest the involvement of cyclic nucleotides in the action of fusidic acid on protein synthesis and gluconeogenesis and/or glycogenolysis.

We have determined that fusidic acid increases the concentration of blood cAMP while simultaneously lowering the blood cGMP concentration in the intact rat. Concentrations of these cyclic nucleotides remain unchanged in liver, kidney and muscle. There is an increase in blood glucose which is positively correlated with the change in blood cAMP concentration. Alteration of kidney function through bilateral nephrectomy or ureteral ligation tended to increase blood concentrations of both cAMP and cGMP. When nephrectomized or ureter-ligated rats were treated with fusidic acid there was an increase in the concentration of cAMP and a reduction in cGMP in the blood similar to that observed in fusidic acid treated intact rats. In contrast to the failure of fusidic acid to alter tissue concentrations in intact or nephrectomized rats, fusidic acid treatment of ureter-ligated rats caused a reduction in liver and muscle cyclic nucleotide concentrations and an increase in kidney cyclic nucleotide concentrations. Alteration of kidney function failed to inhibit the stimulation of labelled amino acid incorporation into protein by fusidic acid (unpublished data, E. Z., P. Gachon, F. W. S.).

2. Materials and methods

Female Sprague-Dawley rats, weighing 165–180 g were fed Purina Rat Chow ad libitum. Bilaterial ureteral ligations, nephrectomy and sham operations of both surgical procedures were performed under ether anesthesia. Sham operations had no effect on the results of the experiments. Rats with altered kidney function were used for experiments when their blood urea concentration exceeded 225 mg/dl; 12 h post-nephrectomy and 18 h post-ureteral ligation.

Rats were injected i.p. with 0.5 ml saline or saline containing 10 mg sodium fusidate (a gift from W. O. Godtfredsen and W. von Daehme, Leo Pharmaceutical Products, Ballerup, Denmark). At arbitrarily determined times rats were decapitated as noted in fig.1 and tables 1, 2; Blood (1.98 ml) was collected into ice cold tubes containing 20 μl 0.5 M EDTA (pH 7.5 with 1 M NaOH) and vortexed, then extracted with ethanol. The ethanolic extract was evaporated to dryness in a Buchler Rotary Evapo-Mix. The dry precipitates were stored at 5°C until used for assay. Livers, kidneys and a portion of the gastrocnemius and soleus muscles from each hind limb were removed immediately, frozen between liquid N_2 pre-cooled

blocks and stored under liquid N_2 until assayed. Tissue samples were homogenized in 4 mM EDTA and deproteinized by heating in a boiling water bath for 5 min.

Cyclic nucleotides were measured with Amersham's cyclic GMP RIA KIT and cyclic AMP RIA KIT (several kits were gifts of N. Magit, Amersham, Arlington, IL).

Blood glucose and urea were measured by the glucose-oxidase–peroxidase method and the urease–Berthelot method of Boehringer-Mannhein, Indianapolis, IN, respectively.

All other chemicals and reagents were of the highest purity commercially available.

3. Results and discussion

Removal of various endocrine glands did not alter the fusidic acid-induced incorporation of labelled amino acid into protein [3]. The kidney has been shown to be one of the sites for molecular alteration of vitamin D, a sterol, in the formation of an active metabolite [5]. Similarities in molecular structure between fusidic acid and vitamin D suggest that kidney function may also play a role in the in vivo effect of fusidic acid. Surgery was timed so that blood urea concentrations in the rats were similar, after nephrectomy or ureteral ligation, just prior to fusidic acid administration. Fusidic acid did not significantly affect blood urea concentrations ($P > .05$) in intact, ureter-ligated or nephrectomized rats (table 1). In a separate experiment, blood urea concentration did not change significantly ($P > .05$) over a 60 min period after fusidic acid administration i.p. to intact rats (not shown).

In fed intact rats, fusidic acid administration resulted in an increase in blood glucose concentration which remained elevated for at least 40 min (fig.1). This increase was also observed in 24 h starved rats. (Δ + 59 mg/dl glucose, not shown.) Ureteral ligation had little effect on blood glucose concentrations, but nephrectomy reduced blood glucose by nearly 33% in fed rats (table 1). Neither of these alterations of kidney function affected the fusidic acid-induced elevation in blood glucose concentration, in fact, it was more effective (1.0-fold) in these rats than in intact rats.

The in vivo effect of fusidic acid administration on blood glucose concentration may be due to either increased glycogenolysis and/or gluconeogenesis. In the intact fed rat, 20 min post-fusidic acid aministration, blood concentration of cAMP was increased more than 2.7-fold and cGMP was reduced by more than 25% (table 1). As shown in fig.1, the change in cAMP concentration (2-fold) occurred concomitantly with the elevation in blood glucose concentration. Cyclic AMP remained elevated throughout the 60 min period. These in vivo effects of fusidic acid administration on blood cAMP concentration may be mediated via glucagon [6] or catecholamines [7,8]. However, catecholamine administration has been reported to increase blood and urinary cGMP concentration [9]. Therefore, the observed decrease in cGMP blood

Table 1
The effect of fusidic acid in vivo on the concentration of blood glucose, urea and cyclic nucleotides in the fed rat[a]

	No. rats	Glucose (mg/dl)	Urea (mg/dl)	cAMP (pmol/ml)	cGMP (pmol/ml)	cAMP/cGMP
Saline						
Intact	4	150 ± 2.8[b]	52 ± 2.3	21.4 ± 3.7	20.5 ± 2.3	1.1 ± 0.15
Ureter-ligated	4	137 ± 9.3	312 ± 13.1	34.7 ± 2.9	32.4 ± 4.3	1.1 ± 0.12
Nephrectomized	4	108 ± 2.4	234 ± 13.5	49.7 ± 8.6	30.1 ± 7.0	1.9 ± 0.43
Fusidic						
Intact	3	218 ± 39.1	58 ± 2.3	56.6 ± 10.6	14.6 ± 1.9	4.1 ± 1.19
Ureter-ligated	5	239 ± 20.8	296 ± 10.1	83.1 ± 14.7	7.1 ± 0.4	11.7 ± 1.89
Nephrectomized	4	195 ± 18.8	261 ± 6.5	77.2 ± 11.2	14.0 ± 2.2	5.6 ± 0.19

[a] Determined 20 min after fusidic acid injection
[b] Values ± standard error of the mean

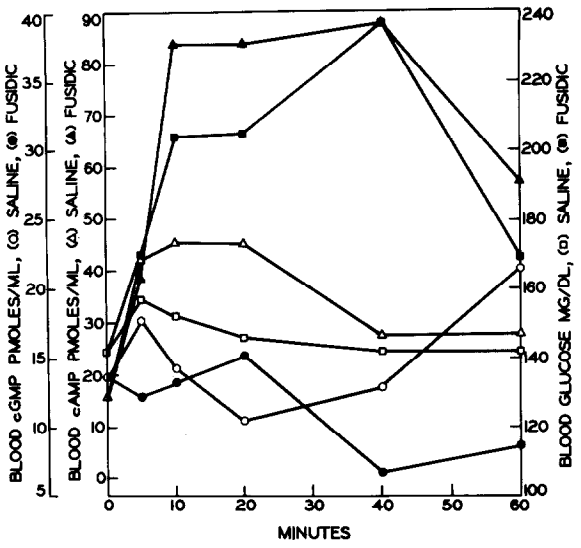

Fig.1. The effect of fusidic acid in vivo on the kinetics of blood glucose, cAMP and cGMP concentrations in the fed intact rat. Treatment, time, number of animals in parenthesis and standard error of the mean are as follows:
No injection, zero time (2): glucose ± 1.0 mg/dl; cAMP ± 3.0 pmol/ml; cGMP ± 0.1 pmol/ml.
Saline injection, after 5 min (7), 10 min (10), 20 min (4), 40 min (4) and 60 min (4) glucose ± 6.7, 6.8, 3.9, 2.7 and 17.6 mg/dl; cAMP ± 10.1, 10.1, 10.7, 5.2 and 5.5 pmol/ml; cGMP ± 2.2, 1.1, 1.8, 3.3 and 8.1 pmol/ml; respectively.
Fusidic acid injection, after 5 min (6), 10 min (8), 20 min (4), 40 min (4) and 60 min (4): glucose ± 8.3, 8.1, 22.4, 7.8 and 13.0 mg/dl; cAMP ± 4.9, 8.5, 33.6, 18.0 and 11.0 pmol/ml; cGMP ± 0.7, 0.8, 4.5, 0.6 and 3.1 pmol/ml; respectively.

concentration would suggest that fusidic acid does not act via catecholamines. While cAMP/cGMP in the blood was also increased nearly 4-fold, no significant change ($P > .05$) could be detected in liver, kidney or muscle cAMP and cGMP concentrations or ratios. Under the influence of glucagon, the liver apparently is the major source of blood cAMP [10].

Our data confirm those of [11] (perfused liver) and [12] (infused intact rat) where increases in blood concentrations of cAMP and glucose without a detectable change in liver nucleotide concentration are reported. Our data also support the findings of [13] where, under basal conditions, much of the cAMP of the liver is metabolically inert and not freely diffusable because of binding or sequestration. Our data are the first to show that steroidal molecules, other than glucocorticoids, can affect blood cAMP and glucose concentrations.

Five minutes after fusidic acid administration to the intact rat (fig.1), there was a transient reduction in blood cGMP concentration which returned to control level by 20 min. However by 40 min, blood cGMP concentration had decreased to approx. 50% of the saline control rats and remained depressed throughout the remainder of the experiment. Blood cyclic GMP concentrations in the fusidic acid-treated rats were reduced below control concentrations at 20 min in the experiment shown in table 1. The small intestine has been suggested as the source of the major portion of cGMP found in the blood [14]. The reduction in blood concentration of cGMP could be due to an inhibition in the release of cGMP from the intestine by fusidic acid. In addition, the rate of blood cGMP excretion or metabolic degradation by the kidney may have increased [10,14]. However, our results obtained from rats with altered kidney function refute this latter viewpoint (table 1) and will be discussed later.

The kidney has been shown to remove blood cAMP and cGMP via glomerular filtration [10]. This fact is supported by the increase in blood cAMP (2-fold) and cGMP (1.5-fold) after ureteral ligation and nephrectomy. Ureteral ligation also elevated cAMP concentration 1.5-fold in the liver and reduced cAMP by 50% in the kidney (table 2). There was also a 2-fold increase in cGMP concentration in both liver and kidney of the ureter-ligated rat. Only the liver concentration of cGMP was increased (1.5-fold) in the nephrectomized rat.

Neither of the alterations of kidney function suppressed the nearly 2-fold stimulation of blood cAMP concentration by the administration of fusidic acid. Fusidic acid was slightly more effective in lowering blood cGMP concentration in the nephrectomized rat than in the intact rat. However, fusidic acid was much more effective in regard to lowering blood cGMP concentrations in ureter-ligated rats. This reduction in blood cGMP concentration is a reflection of the ability of fusidic acid to inhibit tissue release of cGMP.

Kidney glomerular filtration and excretion [10] might also be increased under the influence of fusidic acid, which would account for the increase in kidney concentration of cGMP (1.7-fold) and cAMP (1.6-fold)

Table 2
The effect of fusidic acid in vivo on the concentration of cyclic nucleotides in the liver, kidney and muscle of the fed rat[a]

	No. rats	Liver			Kidney			Muscle		
		cAMP (pmol/g wet wt)	cGMP (pmol/g wet wt)	cAMP/cGMP	cAMP (pmol/g wet wt)	cGMP (pmol/g wet wt)	cAMP/cGMP	cAMP (pmol/g wet wt)	cGMP (pmol/g wet wt)	cAMP/cGMP
Saline										
Intact	4	217 ± 7.3[b]	9.8 ± 1.0	23 ± 3.4	514 ± 50.4	14.1 ± 2.1	40 ± 8.7	519 ± 38.0	26.3 ± 3.0	20 ± 2.5
Ureter-ligated	4	344 ± 14.9	20.6 ± 2.0	17 ± 0.9	256 ± 46.9	31.8 ± 3.6	8 ± 0.8	371 ± 59.7	20.2 ± 3.9	20 ± 4.1
Nephrectomized	4	378 ± 19.6	16.5 ± 1.4	24 ± 3.2	–	–	–	454 ± 29.5	26.6 ± 5.5	19 ± 4.1
Fusidic										
Intact	3	218 ± 22.8	12.9 ± 2.6	18 ± 3.6	528 ± 77.9	16.3 ± 1.4	32 ± 4.2	509 ± 41.6	24.8 ± 3.0	21 ± 0.8
Ureter-ligated	5	310 ± 40.6	13.6 ± 2.0	26 ± 5.5	392 ± 57.4	54.3 ± 10.9	9 ± 1.1	274 ± 36.3	15.6 ± 2.5	18 ± 1.4
Nephrectomized	4	386 ± 46.8	14.5 ± 1.5	28 ± 5.8	–	–	–	467 ± 30.4	24.0 ± 2.8	20 ± 3.6

[a] Determined 20 min after fusidic acid injection
[b] Values ± standard error of the mean

in the ureter-ligated rat (table 2). Alternatively, the inability to eliminate urine from the kidney might inhibit only the metabolic degradation of cyclic nucleotides. Of the tissues assayed, only muscle cAMP concentration was reduced by 25%. This increase in urinary excretion of cGMP may also have occurred when fusidic acid was administered to the intact rat, however we did not determine urinary cyclic nucleotides. If the kidney excretion rate of blood cyclic nucleotides was the only point of control of fusidic acid, then the nephrectomized rat should have exhibited an elevated blood cGMP concentration which was not the case. This shift in tissue concentrations of cyclic nucleotides in the ureter-ligated rat under the influence of fusidic acid was not observed in intact or nephrectomized rats (table 2). Apparently the kidney becomes a 'sink' for cyclic nucleotides when excretion or metabolic degradation is inhibited by fusidic acid administration to the ureter-ligated rat.

Cyclic GMP has been suggested as a stimulator of polypeptide synthesis [20], since rat liver EF-2 has guanyl cyclase activity and may be involved in the conversion of GTP to cGMP. In vitro experiments have shown that fusidic acid blocks functions dependent on EF-2 and a specific eukaryotic ribosome-independent GTPase which is distinct from EF-2 [21]. Although we have also observed an early inhibition in the incorporation of labelled leucine into isolated hepatocytes, protein synthesis is apparently released from this inhibition as the incubation time progresses (unpublished data, E.Z., F.W.S.). However, free polyribosomes isolated from livers of rats administered fusidic acid in vivo were nearly twice as active in an in vitro protein synthesizing system as those from livers of controls (unpublished data, F. W. S., A. A. Hochberg).

Several reports have indicated that steroids such as estrogen can increase cGMP concentration in the uterus which the authors attributed to a reduction in cGMP phosphodiesterase, whereas progesterone inhibits the increase in cGMP [16]. Furthermore, other steroids, such as glucocorticoids, increase urinary excretion of cGMP with a concomitant decrease in the concentration in the lung [17,18]. The effect of glucocorticoids is difficult to interpret, since in adrenalectomized rats the hepatic concentration of cGMP was decreased and skeletal muscle concentration increased while both tissues exhibited increased guanyl cyclase and cGMP phosphodiesterase activity [19].

The in vivo stimulation of the incorporation of labelled leucine into tissue proteins (anabolic processes) by fusidic acid [3] could be attributed to an increase in the cGMP concentration at the site(s) of polypeptide (protein) synthesis. This is reflected by the decrease in blood cGMP concentration. This increased incorporation is more rapid than can be accounted for by increased in vitro DNA synthesis as a result of elevated concentrations of cAMP [15]. Modification of cell membrane release or transport by fusidic acid could be a method of control which determines the level of 'active' cGMP at the site(s) of polypeptide synthesis. This would not necessarily involve detectable increases or decreases in guanyl cyclase activity or tissue cGMP concentration which may be 'physiologically unnecessary'.

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