EFFECT OF HORMONES ON CONTENT OF PURINE NUCLEOSIDE CYCLIC MONOPHOSPHATES IN PERFUSED RAT LIVER

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1. Introduction

Many short-term responses to extra-cellular stimuli are apparently mediated by changes in the content of cyclic-AMP in cells [1]. However, a group of hormones can exert a rapid catabolic effect on liver glycogen metabolism, which is not mediated by cyclic-AMP, and does not involve activation of cyclic-AMP-dependent protein kinase [2]. This was first demonstrated by a study of α-adrenergic mechanisms [3] and the lack of a role for cyclic-AMP in α-adrenergic effects has consistently emerged in subsequent investigations [4–7]. A further hormone which stimulates glycogen breakdown is vasopressin [8] which also causes no increase in hepatic content of cyclic-AMP [9]. Both vasopressin and angiotensin II can increase the amount of available glycogen phosphorylase *a* in liver [10,11] but without associated activation of assayable protein kinase or phosphorylase kinase [11].

The above hormones can exert catabolic effects on lipid metabolism [2] and on other processes in liver, so it is important to gain further insight into their mechanism of action and their effects on nucleotide levels in liver. As angiotensin II does not produce a stable activation of phosphorylase kinase in liver [11] it should follow that this hormone does not increase liver cyclic-AMP content. Also, oxytocin, which can inhibit hepatic glycogen synthesis [12], would be expected to act on the same receptor as vasopressin and, therefore, not to alter the cyclic-AMP content. These predictions are confirmed here.

Another purine nucleoside cyclic monophosphate which may be implicated in hormone response mechanisms is cyclic-GMP. The effects of the above-described hormones on the contents of cyclic-AMP and cyclic-GMP in perfused rat liver are described here. They were determined by radioimmunoassay, which offers the most specific and precise method for analysis of these nucleotides. The contents of these cyclic nucleotides in livers perfused with insulin and acetyl choline are also reported.

2. Experimental

Livers of fed male 200 g Sprague-Dawley rats were perfused with Krebs-Ringer-bicarbonate saline containing rat erythrocytes (haematocrit 12%), bovine serum albumin (2.5% w/v), and glucose which remained steady at about 10 mM [8]. After 40 min, hormones were added as a single dose; after a further short period, livers were rapidly freeze-clamped. Effluent perfusate was sampled over about 30 s, with a mean point at 5 min or 10 min after hormone addition; cells were immediately deposited by brief centrifugation and plasma was removed.

Tissue samples were extracted with 10 vol. 10% trichloroacetic acid (TCA) and plasma samples with equal vol. TCA. Precipitated protein was removed by centrifugation. Extracts were stored frozen for not more than 10 days before analysis of purine nucleoside cyclic monophosphates by radioimmunoassay [13–15].

Extracts were washed with water-saturated diethyl ether to remove the TCA. Cyclic-AMP was measured

directly in these extracts as in [14]. Cyclic-GMP in extracts was concentrated 5-fold and purified before assay, by elution from a column of AG 1-X2 resin, Cl^- form, with 300 mM HCl, as in [15]. Standard curves were constructed using pure nucleotides in amounts from 10^{-13}–10^{-11} mol in the assay. Standard amounts of nucleotides added to extracts were recovered quantitatively (90–110%). Treatment of extracts with beef heart phosphodiesterase (Boehringer Corp. Ltd.) destroyed all exogenous cyclic nucleotides added and more than 85% of endogenous immunoreactive material in both assays. The residual activity detected after phosphodiesterase treatment was near the lower limits of the assay procedures.

3. Results and discussion

The hepatic contents of cyclic-AMP and cyclic-GMP, in response to various hormones, are given in table 1. The values in control liver and perfusate are of the same order as those reported in other studies.

Perfusate cyclic nucleotide concentrations were measured as these should provide a sensitive index of small changes in tissue content [16]. In agreement with this expectation, glucagon produced an approx. 10-fold increase in tissue cyclic-AMP content but a 100-fold rise in the concentration in perfusate.

Further hormones were investigated which exert a glycogenolytic effect on liver at the concentrations employed in these perfusions [8–13]. Vasopressin did not increase cyclic-AMP in liver or perfusate, confirming previous measurements [9], and it also did not affect cyclic-GMP. Angiotensin II and oxytocin did not alter the concentration of cyclic-AMP or cyclic-GMP in liver or perfusate (table 1).

Of the two agents tested which can exert anabolic effects on liver metabolism, neither insulin nor acetyl choline brought about any change in the cyclic-AMP or cyclic-GMP content of liver or perfusate. This contrasts with their reported action on liver slices [17].

Although these findings are in one sense negative, they offer a powerful insight into the mechanisms of action on liver of the above hormones (other than glucagon); thus the data show that none of the multiple effects which these hormones exert on liver can be mediated by either purine nucleoside cyclic monophosphate.

The group of hormones which can exert catabolic effects on liver metabolism, not mediated by cyclic-AMP or cyclic-GMP, currently comprises catecholamines (α-adrenergic mechanism [3–7]), vasopressin, oxytocin and angiotensin II. Their mechanism of action may involve Ca^{2+} [2,4,6,11,18–20] but details still have to be clarified.

Table 1
Contents of purine nucleoside cyclic monophosphates in perfused liver

Hormone	Min after Hormone	Content of nucleotide			
		Liver (pmol/g)		Plasma (nM)	
		Cyclic AMP	Cyclic GMP	Cyclic AMP	Cyclic GMP
Control	–	890 ± 70 (8)	23 ± 3 (9)	8.6 ± 2.3 (9)	1.0 ± 0.2 (7)
Glucagon (10^{-7} M)	10	8500 ± 380 (3)	13 ± 3 (3)	880 ± 370 (3)	1.3 ± 0.2 (3)
Vasopressin (5 mU/ml)	10	710 ± 20 (4)	17 ± 2 (3)	7.8 ± 2.7 (5)	0.8 ± 0.2 (5)
Oxytocin (1 U/ml)	5	940 ± 30 (3)	18 ± 2 (3)	7.0 ± 2.0 (3)	0.7 ± 0.1 (3)
Angiotensin II (4 ng/ml)	5	880 ± 80 (4)	24 ± 4 (4)	5.0 ± 2.0 (3)	0.6 ± 0.1 (3)
Acetyl choline (20 μg/ml)	10	780 ± 50 (5)	22 ± 2 (5)	6.2 ± 2.3 (5)	1.3 ± 0.2 (4)
Insulin (10 mU/ml)	10	710 ± 20 (5)	17 ± 2 (5)	12.7 ± 7.8 (3)	0.8 ± 0.1 (3)
Vasopressin (mU/ml)[a]	10	980 (2)	–	1.5 (2)	0.9 (2)

[a] Average values from two perfusions of mouse liver

Livers from fed male Sprague-Dawley rats (200 g) were perfused, and at 40 min, hormones were added as a single dose. Control perfusions received no addition. Liver and perfusate plasma were analysed for cyclic-AMP and cyclic-GMP by radio immunoassay. Results are means ± S.E.M. of the number of measurements in parentheses

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