Volume 257, number 2, 348-350 FEB 07790 November 1989

GPC phosphodiesterase and phosphomonoesterase activities of renal cortex and medulla of control, antidiuresis and diuresis rats

Julian N. Kanfer and Douglas G. McCartney

Department of Biochemistry and Molecular Biology, University of Manitoba, Winnipeg, Manitoba, Canada

Received 25 July 1989

Glycerylphosphorylcholine (GPC) concentration was reported to be elevated in renal medulla of experimental animals deprived of water. The activities of GPC phosphodiesterases were similar in homogenates and membrane subfractions of renal cortex prepared from control, diuresis and antidiuresis rats. There were no differences in these preparations' ability to hydrolyze phosphorylcholine. In contrast, there was a nearly 50% reduction of non-specific phosphomonoesterase activity, using *p*-nitrophenylphosphate as substrate and membrane subfractions prepared from the antidiuresis animals. It is suggested that as a consequence, a pathway for the formation from L-α-glycerylphosphate is activated.

Glycerylphosphorylcholine; Renal medulla; Antidiuresis; Non-specific phosphomonoesterase; Phosphodiesterase

1. INTRODUCTION

Glycerylphosphorylcholine (GPC) is generally believed to be a metabolite derived from phosphatidylcholine as a result of phospholipase and lysophospholipase hydrolytic activities. Rat renal medulla homogenates efficiently release free fatty acids from lecithin without the accumulation of lysophosphatidylcholine [1]. Rodent tissues possess at least 2 phosphodiesterases for which GPC is a substrate. GPC choline phosphodiesterase activity (EC 3.1.4.2) liberates free choline and L-α-glycerylphosphate and its presence has been well documented in several tissues including kidney [2,3]. The existence of an additional hydrolytic activity, originally detected in brain tissue, GPC phosphorylcholine phosphodiesterase (EC 3.1.4.38) produces phosphorylcholine and glyceryl from GPC [4].

GPC concentration was found to be 80 times greater in the canine renal medulla than the renal cortex [5] and the GPC choline phosphodiesterase activity of a cortex homogenate was estimated to be 4-fold greater than that of a medulla homogenate [6]. The GPC concentration was increased 1.5-fold in the renal medulla of rabbits deprived of water for 24 h [7] and it was suggested that GPC may contribute to the maintenance of the intracellular osmotic balance of this tissue. The level of free choline was low in both the medulla and cortex while phosphorylcholine was undetectable.

It seemed appropriate to determine if there were any alterations in the activities of both GPC phosphodiesterases in the renal medulla and cortex of control, antidiuresis and sucrose-induced diuresis rats. The phosphomonoesterase activities towards *p*-nitrophenylphosphate and phosphorylcholine were also estimated.

Correspondence address: J.N. Kanfer, Department of Biochemistry and Molecular Biology, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba R3E 0W3, Canada

2. MATERIALS AND METHODS

[^{3}H]Choline-labelled phosphatidylcholine was purchased from NEN (Boston, MA, USA) and diluted with non-radioactive phosphatidylcholine to a final specific activity of about 3×10^5 cpm/μmol. This was saponified to provide [^{3}H]choline-labelled GPC according to a published method [8]. Phosphoryl[^{14}C]-choline was purchased from NEN (Boston, MA, USA) and diluted with the non-radioactive compound to a specific activity of about 75×10^5 cpm/μmol. *p*-Nitrophenylphosphorylcholine was purchased from Sigma (St. Louis, MO, USA).

Groups of 4-6 adult rats were either allowed access to water ad libitum as the control group, or deprived of water for 24-48 h as the antidiuresis group, or allowed access to 5% sucrose ad libitum for 24-48 h as the diuresis group. The kidneys were removed, the medulla and cortex separated and pooled. Crude membrane subfractions were prepared from 10% homogenates in 0.32 M sucrose by centrifugation at $6000 \times g$ for 10 min to obtain the P_1 pellet, at $17\,000 \times g$ for 10 min to obtain the P_2 pellet, and at $100\,000 \times g$ for 1 h to obtain the P_3 pellet. Duplicate samples of these homogenates and subcellular fractions containing about 10 μg protein were incubated with 2 mM [^{3}H]-GPC, 50 mM glycine pH 9.0 buffer in a final volume of 125 μl for 1 h. [^{3}H]Choline release from [^{3}H]GPC was estimated directly according to the procedure of Fonum [9] and phospho[^{3}H]choline after alkaline phosphatase treatment of the incubation mixtures to release free choline. The GPC phosphodiesterase activities were performed on 6 or 7 independent experiments and the values represent the averages of these results. Phosphorylcholine phosphomonoesterase activity was determined on duplicate samples of these homogenates and subcellular fractions containing about 10 μg protein and were incubated in 50 mM glycine buffer at pH 9.0 with 4 mM phosphoryl-[^{14}C]choline in a final volume of 125 μl for 1 h and the [^{14}C]choline release estimated according to a published procedure [9]. This activity was performed on 2 independent experiments and the values are the

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

average of these results. Non-specific phosphomonoesterase activity was determined on triplicate samples of these homogenates and subcellular fractions containing about 20 μg protein in incubations containing 10 mM *p*-nitrophenylphosphate in 50 mM glycine buffer pH 9.0 in a final volume of 125 μl for 1 h at 37°C. The release of *p*-nitrophenol was measured spectrophotometrically at 420 nm according to a published procedure [10]. This enzyme activity was determined on 3 independent experiments and the values presented are averages of these results. Proteins were estimated by a classical procedure [11].

3. RESULTS

3.1. *GPC phosphodiesterases*

GPC phosphodiesterase activities were 2- to 3-fold greater in the renal cortex samples than for the corresponding renal medulla samples. The activities of the P_2 and P_3 pellets were from 1.5- to 2.5-fold greater than the whole homogenates. GPC choline phosphodiesterase activity was 2- to 3-fold greater than the GPC phosphorylcholine phosphodiesterase activity for all samples (table 1). There were no apparent differences between any of the samples obtained from the control group as compared to the corresponding samples from the antidiuresis or the sucrose-induced diuresis groups except for the P_1 pellet of the control and the sucrose diuresis group. Levamesole, a phosphomonoesterase inhibitor, did not affect these GPC phosphodiesterase activities.

3.2. *Phosphoryl[^{14}]choline hydrolysis*

The capacity to liberate free choline from phosphorylcholine is equivalent for both the renal cortex and renal medulla. There is a 2- to 2.5-fold higher activity in the P_2 and P_3 pellets compared to the original homogenates. There are no differences in the corresponding activity between the two experimental groups and the controls (table 2).

3.3. p-*Nitrophenylphosphate hydrolysis*

There is little difference in non-specific phosphomonoesterase activity between the renal cortex and renal medulla samples. This activity is increased approximately 2-fold in the P_2 pellet and 3-fold in the P_3 pellet compared to the activity of the original homogenates. There is an approximate 45% reduction in this

Table 1

GPC phosphodiesterase activities of renal cortex and medulla homogenates and membrane fractions from control, andidiuresis and sucrose-induced diuresis rats[a]

	GPC choline phospho-diesterase	GPC phosphoryl-choline phospho-diesterase
Control, cortex		
Whole homogenate	2.7 ± 0.64	0.775 ± 0.327
P_1 pellet	2.4 ± 0.41	0.750 ± 0.218
P_2 pellet	5.05 ± 1.38	1.46 ± 0.49
P_3 pellet	5.8 ± 0.57	2.08 ± 0.81
Control, medulla		
Whole homogenate	1.07 ± 0.53	0.36 ± 0.18
P_1 pellet	0.58 ± 0.23	0.24 ± 0.074
P_2 pellet	2.58 ± 1.5	0.8 ± 0.5
P_3 pellet	2.86 ± 0.99	1.45 ± 0.7
Antidiuresis, cortex		
Whole homogenate	2.7 ± 0.44	0.99 ± 0.4
P_2 pellet	2.36 ± 0.64	1.02 ± 0.65
P_2 pellet	5.13 ± 0.95	1.9 ± 0.84
P_3 pellet	5.34 ± 1.21	1.9 ± 0.59
Antidiuresis, medulla		
Whole homogenate	1.02 ± 0.62	0.54 ± 0.35
P_1 pellet	0.6 ± 0.36	0.20 ± 0.09
P_2 pellet	2.58 ± 1.83	0.96 ± 0.35
P_3 pellet	2.75 ± 1.55	0.849 ± 0.48
Sucrose-diuresis, cortex		
Whole homogenate	2.4 ± 0.56	1.01 ± 0.42
P_1 pellet	2.81 ± 1.22	1.35 ± 0.55
P_2 pellet	5.31 ± 1.91	1.26 ± 0.33
P_3 pellet	5.21 ± 1.3	1.04 ± 0.42
Sucrose-diuresis, medulla		
Whole homogenate	1.01 ± 0.59	0.29 ± 0.15
P_1 pellet	0.94 ± 0.7	0.086 ± 0.037
P_2 pellet	3.01 ± 2.16	0.47 ± 0.35
P_3 pellet	2.08 ± 0.942	0.51 ± 0.33

[a] Activities expressed as μmol/mg protein per h

Table 2

Phosphomonoesterase activities of renal cortex and medulla homogenates and membrane fractions from control, antidiuresis and sucrose-induced diuresis rats[a]

	p-Nitrophenol released[b]	Choline released[c]
Control, cortex		
Whole homogenate	5.67 ± 1.23	7.9 ± 0.02
P_1 pellet	6.07	5.28 ± 2.7
P_2 pellet	11.68 ± 0.69	13.73 ± 1.72
P_3 pellet	18.1 ± 0.32	21.75 ± 4.52
Control, medulla		
Whole homogenate	4.53 ± 0.73	5.6 ± 0.77
P_1 pellet	2.73	2.78 ± 1.42
P_2 pellet	6.17 ± 2.7	12.04 ± 0.53
P_3 pellet	19.67 ± 0.17	17.0 ± 3.15
Antidiuresis, cortex		
Whole homogenate	5.83 ± 0.98	7.61 ± 2.2
P_1 pellet	5.87	6.43 ± 2.46
P_2 pellet	11.04 ± 3.07	13.0 ± 2.6
P_3 pellet	16.32 ± 2.4	19.8 ± 3.1
Antidiuresis, medulla		
Whole homogenate	3.14 ± 0.47	5.59 ± 0.9
P_1 pellet	1.51	3.04 ± 0.65
P_2 pellet	6.11 ± 1.33	10.33 ± 2.3
P_3 pellet	10.14 ± 2.99	13.33 ± 5.33
Sucrose-diuresis, cortex		
Whole homogenate	5.65 ± 0.67	8.08
P_1 pellet	8.98	12.29
P_2 pellet	9.94 ± 2.11	12.13
P_3 pellet	17.74 ± 1.59	18.43
Sucrose-diuresis, medulla		
Whole homogenate	4.36 ± 0.83	7.17
P_1 pellet	4.25	7.12
P_2 pellet	9.71 ± 4.27	17.18
P_3 pellet	14.74 ± 5.86	16.07

[a] Activities expressed as μmol/mg protein per h
[b] *p*-Nitrophenylphosphate as substrate
[c] Phosphoryl[^{14}]choline as substrate

phosphomonoesterase activity of the renal medulla homogenates and subfractions from the antidiuresis group compared to the controls. The level of significance for these was greater than 0.025, 0.001 and 0.005 for the whole homogenate, the P_2 pellet and the P_3 pellet, respectively. There are no differences in the corresponding samples obtained from the renal cortex.

4. DISCUSSION

Previous estimates of in vitro hydrolysis of GPC by renal tissue homogenates have only examined the enzyme responsible for choline release. Information on phosphorylcholine release had not been available. This may have been due to lack of appreciation of this enzymatic activity in kidney since its original description indicated its exclusive localization in brain tissue. In addition, it would be difficult to quantitate using a non-radioactive substrate. Estimations of GPC choline phosphodiesterase activity of renal tissue were based upon specific enzymatic quantitation of L-α-glycerylphosphate, the co-product [3]. Rat cortical homogenates incubated with GPC were reported to produce equimolar amounts of glyceryl, inorganic phosphate and choline [12] with an activity of about 180 nmol/mg protein per h. This level of activity is approximately 16-fold lower than that presented in this report (table 1).

The differences of the GPC concentrations in renal cortex and medulla [7,12] cannot be explained due to a remarkable difference in these hydrolytic activities. Similarly, the observed elevation of GPC concentration in the medulla during antidiuresis [7,12] does not affect either of the GPC phosphodiesterase activities (table 1). The products of GPC phosphorylcholine diesterase activity are glyceryl and phosphorylcholine. This phosphoester has been reported to be absent from the cortex, the medulla, the serum and urine of rats suggesting that this compound does not accumulate in kidney tissue and must be rapidly hydrolyzed [7]. There are no marked differences in phosphorylcholine phosphomonodiesterase activity of medulla or cortex in either the control or experimental groups (table 2).

There was an appropriate 50% reduction of non-specific alkaline phosphatase activity of the homogenate and the membrane pellets from the medulla, but not cortex, of the antidiuresis animals. Alkaline phosphatase has been thought to hydrolyze a variety of phosphomonoesters, including phosphorylcholine [13]. This assumption appears incorrect since there was no reduction in the ability of medulla samples to hydrolyze phosphorylcholine but a 50% reduction in *p*-nitrophenylphosphate hydrolysis. L-α-glycerylphosphate, a product of GPC choline phosphodiesterase activity, is generally accepted to be a substrate for alkaline phosphatase. Perhaps there is an accumulation of this phosphomonoester as a consequence of reduced alkaline phosphatase activity. This could possibly cause a diversion of the normal catabolic pathway for α-glycerylphosphate to the formation of GPC by a CDP-choline, *sn*-glyceryl-3 phosphate choline phosphotransferase [14]. This in turn might contribute to the observed GPC elevation in the medulla of antidiuresis animals [7].

Acknowledgement: Supported by grants of the Medical Research Council of Canada.

REFERENCES

[1] Limas, C. and Limas, C.J. (1983) Biochim. Biophys. Acta 753, 314–323.
[2] Dawson, R.M.C. (1955) Biochem. J. 59, 5–8.
[3] Baldwin, J.J. and Cornatzer, W.E. (1969) Biochim. Biophys. Acta 164, 195–204.
[4] Abra, R.M. and Quinn, P.J. (1975) Biochim. Biophys. Acta 380, 436–444.
[5] Shimassek, H., Kohl, D. and Bucher, T. (1959) Biochem. Z. 331, 87–97.
[6] Ullrich, K.J. (1959) Biochem. Z. 331, 98–102.
[7] Bagnasco, S., Balaban, R., Fales, H.M., Yang, Y.-M. and Burg, M. (1986) J. Biol. Chem. 261, 5872–5877.
[8] Spanner, S. and Ansell, G.B. (1987) Neurochem. Res. 12, 203–206.
[9] Fonum, F. (1968) Biochem. J. 109, 389–398.
[10] McComb, R.B., Bowers, G.N., jr and Posen, R. (1979) Alkaline Phosphatase, Plenum Press, New York.
[11] Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.S. (1957) J. Biol. Chem. 193, 265–275.
[12] Wirthensohn, G., Beck, F.-X. and Guder, W.G. (1987) Pflugers Arch. 409, 411–415.
[13] Pelech, S.L. and Vance, D.E. (1984) Biochim. Biophys. Acta 779, 217–251.
[14] Infante, J.P. (1987) FEBS Lett. 214, 149–152.