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EFFECT OF GUANOSINE 3′:5′-MONOPHOSPHATE ON THE HYDROOSMOTIC ACTIVITY OF ADENOSINE 3′:5′-MONOPHOSPHATE IN TOAD URINARY BLADDER

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SUMMARY

Guanosine 3′:5′-monophosphate has a slight hydroosmotic effect on toad urinary bladder. Furthermore, this nucleotide strongly inhibits the responses to 3′:5′-adenosine monophosphate and oxytocin. The response to an increase in medium tonicity is not modified by the guanosine nucleotide. A role for guanosine 3′:5′-monophosphate in the regulation of water permeability in toad urinary bladder is proposed.

INTRODUCTION

It is widely accepted that the increase in water permeability induced by antidiuretic hormone in epithelial barriers is mediated by adenosine 3′:5′-monophosphate (cyclic AMP)[1]. The possibility that other cyclic compounds, such as guanosine 3′:5′-monophosphate (cyclic GMP) and inosine 3′:5′-monophosphate (cyclic IMP) may also act as mediators has been suggested[2–3], but no clear evidence has been reported. Cyclic GMP is the only 3′:5′-nucleotide, other than cyclic AMP, occurring in nature and it has been found in all mammalian tissues studied and in many lower phila[4]. It has been reported for toad urinary bladder that cyclic GMP induces an increase in short circuit current similar to that produced by cyclic AMP[2]. However, this sodium transport effect has not been confirmed[5]. Furthermore, a lack of effect of the guanyl derivative on water permeability of bladders from different amphibia has been observed[2–5]. We decided to further investigate the effects of cyclic GMP on water permeability in toad urinary bladder.

In contrast with previous reports[2–5] we observed a slight hydroosmotic action of cyclic GMP and a strong inhibition of the responses to oxytocin, cyclic AMP and theophylline. Cyclic GMP did not interfere with the hydroosmotic response to medium hypertonicity.

Sacs of toads (*Bufo arenarum*, Hensel) bladders were made as described by Bentley[6] and immersed in Ringer solution containing 112 mM NaCl, 5 mM KCl,

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1 mM $CaCl_2$ and 2.5 ml $NaHCO_3$ (pH 8.1). The sacs were filled with saline made hypotonic by reducing the NaCl concentration to 10%. Net water flux was measured gravimetrically over different time periods[6]. The hydroosmotic effects of 10 mM cyclic AMP, (Sigma Chemical Co., St. Louis, Mo , USA, 95% A-9501) and 10 mM cyclic GMP, (Sigma Chemical Co. 95% G-6129) are showed in Table I. It can be observed that the response to cyclic GMP represents only 20–25% of the response to cyclic AMP. The difference with the basal level is only significant during the first period of 20 min after the addition of the nucleotide. It must be emphasized that in the previous negative reports on the hydroosmotic effects of cyclic GMP, the maximal concentrations employed were 5 mM (ref. 2) and 3.35 mM (ref. 5).

We tested cyclic AMP as commercially provided, with no further purification, thus the observations reported do not differ from those of previous reports[2–5] as regards the presence of possible impurities in the cyclic GMP. However, the purity of cyclic GMP employed previously was similar to that used here (in one case the purity of the cyclic GMP was confirmed chromotagraphically[5]). Finally, the use of different species remains as a possible cause of the observed differences. The response reported here to 10 mM of cyclic GMP only represents 10–20% of the usual response to a maximal stimulation with oxytocin. A more interesting effect than this slight cyclic GMP effect on water permeability was seen in the strong interaction of cyclic GMP with oxytocin (Pitocin, a gift from Parke Davies, Co.), cyclic AMP and theophylline (B.D.H. Chemicals Ltd, Poole, England). The results are summarized in Table II. It can be observed that cyclic GMP strongly inhibited the response to oxytocin, cyclic AMP and cyclic AMP *plus* theophylline. Conversely, no relationship was observed between cyclic GMP and medium hypertonicity.

Since the concentration of cyclic GMP used is high, the specificity of its action could be questioned. To test this point, the effects of 5′-GMP (Sigma Chemical Co., G-8377) on the response to oxytocin was also studied. 5′-GMP has no significant effect on water movement (Table I) and has no inhibitory effect at all on the response to oxytocin (Table II).

In spite of the fact that the involvement of cyclic AMP in the response induced by antidiuretic hormone is clearly established, the chain of events occuring between hormone primary action and water permeability is not yet completely clear. The responses to cyclic AMP and oxytocin can be dissociated in different ways. When the epithelial layer of the frog urinary bladder is isolated from supporting tissues, the response to oxytocin is slightly increased but the response to cyclic AMP is reduced by 60–80%[7]. This dissociation can be also induced pharmacologically: probenecid (that has no significant effect on water permeability) reduces the response to oxytocin by 80% and strongly potentiates the response to cyclic AMP and theophylline[8]. Furthermore, chloropropamide potentiates the effects of vasopressin and theophylline, and inhibits the response to exogenous cyclic AMP[9]. To explain these observations, the existence of separate pools for cyclic AMP in the tissue was postulated and it was assumed that these agents (microdissection, probenecid, chloropropamide) modify the movements between the two cyclic AMP pools. The results presented here and those reported on cyclic IMP action[3] allow us to consider the possibility that the conversion between different nucleotides can act in regulating water permeability. Ferguson and Price[3] demonstrated the possibility of conversion

TABLE I

COMPARISON OF THE EFFECT OF CYCLIC AMP AND CYCLIC GMP ON THE HYDROOSMOTIC RESPONSE OF ISOLATED TOAD (*BUFO ARENARUN*, HENSEL) URINARY BLADDERS

Cyclic AMP and cyclic GMP were tested in paired hemibladders. The nucleotides were added at the end of the initial period. The differences between the responses to cyclic AMP and cyclic GMP were significant ($P < 0.001$) in both test periods.

	n	*Hydroosmotic response in* $\mu l/cm^2 \cdot 20$ *min*				
		Initial (I)	*First test period (FTP)*	*Second test period (STP)*	*FTP–I*	*STP–I*
Cyclic AMP (10 mM)	6	4.1 ± 0.2	17.9 ± 3.0	19.6 ± 5.5	13.7 ± 3.2 $P < 0.001$	15.4 ± 5.6 $P < 0.05$
Cyclic GMP (10 mM)	6	3.4 ± 0.4	9.1 ± 1.4	6.3 ± 1.7	5.7 ± 1.5 $P < 0.02$	2.7 ± 1.6 $P > 0.1$
5′-GMP (10 mM)	6	3.0 ± 0.3	3.1 ± 0.4	3.4 ± 0.4		

TABLE II

EFFECTS OF DIFFERENT AGENTS ON WATER PERMEABILITY IN THE ISOLATED TOAD URINARY BLADDER AND THEIR INTERACTIONS WITH CYCLIC GMP

	n	*Hydroosmotic response** ($\mu l/cm^2 \cdot 40$ *min*)	*Mean difference (paired hemibladders)*	*P*
Oxytocin (10^{-7} M)	10	59.0 ± 3.3		
Oxytocin (10^{-7} M) + cyclic GMP (10^{-2} M)	10	16.9 ± 3.5	-42.1 ± 4.7	< 0.001
Oxytocin (10^{-7} M)	6	55.0 ± 7.2		
Oxytocin (10^{-7} M) + 5′-GMP (10^{-2} M)	6	69.8 ± 4.0	14.8 ± 8.5	> 0.1
Cyclic AMP (10^{-2} M)	6	39.5 ± 5.2		
Cyclic AMP (10^{-2} M) + cyclic GMP (10^{-2} M)	6	7.5 ± 1.1	-32.0 ± 5.6	< 0.005
Cyclic AMP (10^{-2} M) + theophylline (10^{-2} M)	6	60.9 ± 7.6		
Cyclic AMP (10^{-2} M) + theophylline (10^{-2} M) + cyclic GMP (10^{-2} M)	6	14.8 ± 3.2	-46.1 ± 5.1	< 0.001
Sucrose (220 mM)	6	20.6 ± 2.8		
Sucrose (220 mM) + cyclic GMP (10^{-2} M)	6	25.9 ± 7.4	5.3 ± 4.9	> 0.2

* Tabulated values are the differences between the initial and test period (both of 40 min). The tested agents were added at the end of the initial period.

between cyclic AMP and cyclic IMP in toad urinary bladder and that this nucleotide has a hydroosmotic effect as important as that of cyclic AMP.

Anderson *et al.*[10] have observed that cyclic GMP is a competitive inhibitor of cyclic AMP for the cyclic AMP receptor in *Escherichia coli*. In this system, the final event is to promote the transcription of the galactose operon, regulating protein synthesis. On the other hand, cyclic GMP is almost as potent as cyclic AMP in stimulating glucose production by the isolated perfused rat liver[4]. This indicates that the interaction between cyclic AMP and cyclic GMP depends on the type of cellular function involved. Jard and Bastide[11] have demonstrated the existence of a cyclic AMP-dependent protein kinase in toad urinary bladder, but the final mechanism regulating water permeability is still unknown.

The hypertonicity of the serosal bathing fluid also increases the permeability to water in toad urinary bladder[12]. Strong relationships between the increase in medium tonicity and agents know to modify either production or destruction of cyclic AMP have been reported[13]. However, lowering cyclic AMP levels by norepinephrine or prostaglandin E1 slows the response to hypertonicity, without reversing it once it has developed and it was therefore suggested that a high level of cyclic AMP is a facilitating, but not a necessary, condition to the response to hypertonicity[13–15]. The lack of interaction with cyclic GMP observed here gives additional support to the idea that the response to hypertonicity is only partially dependent on cyclic AMP levels and that this stimulus could be acting at a point posterior to cyclic AMP formation.

The existence of a feed-back system modulating adenylate cyclase activity and activated by cellular hypoosmolarity has also been recently proposed[16]. The release of endogenous prostaglandin E1 would be the biochemical support for this system[17]. The whole mechanism controlling water permeability now appears to be very complex and the existence of different control steps becomes possible. It seems logical to propose the study of a regulatory function for cyclic GMP in the system.

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