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PROBENECID: EFFECTS ON WATER PERMEABILITY IN FROG URINARY BLADDER

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SUMMARY

The action of probenecid (probenecid is an agent known to inhibit the release of cyclic AMP by pigeon erythrocytes) on the urinary bladder of *Rana esculenta* has been investigated.

Conspicuous modifications in the responses of the bladder to various compounds were observed; surprisingly, hydrosmotic responses to exogenous cyclic AMP and theophylline were strongly enhanced, although the response to neuropeptides was inhibited.

A single primary action of probenecid on cyclic AMP transfers is suggested as a tentative explanation of these results.

INTRODUCTION

Strong experimental evidence suggests a close relationship between adenosine 3′,5′-cyclic monophosphate (cyclic AMP) and the mechanism of action of antidiuretic hormone.

The hypothesis that cyclic AMP could act as an intracellular mediator of this hormone is supported by the observations that:

(1) tissue cyclic AMP levels are increased following hormonal stimulation in toad urinary bladder[1];

(2) neurohypophyseal hormones increase adenyl-cyclase activity of cellular fractions of kidney[2,3] and urinary bladder[4], and in this last tissue the peptide concentrations required for half maximal stimulation in the cell-free and intact system are parallel[5];

(3) Incubation in the presence of cyclic AMP reproduces the hormonal effects on water and sodium transport in toad bladder[4].

However, the extracellular concentrations of cyclic AMP required to produce these effects are much higher than the measured tissue levels[1]. The same discrepancy has been encountered for other tissues[7] and has been attributed to a low cellular permeability to the nucleotide[8]. Thus the biological activity of exogenous cyclic AMP raises the problem of its penetration into the cells and its transfer to a specific receptor.

The same membrane, however, may exhibit very different permeabilities to the

nucleotide in opposite directions. So, although cyclic AMP added to the media did not enter the pigeon erythrocyte, these cells were found to release the nucleotide in the media and to do so against an apparent concentration gradient[9]. This release was markedly lowered by probenecid at a concentration which did not impair the total production of cyclic AMP by the cells.

In view of these results, we investigated the effects of probenecid on the hydrosmotic response of the frog urinary bladder to exogenous cyclic AMP, to theophylline an inhibitor of cyclic AMP inactivation and to neuropeptides*. It was observed that probenecid inhibits the response to neuropeptides and paradoxically strongly increases the response to both exogenous cyclic AMP and theophylline.

MATERIAL AND METHODS

Bladders from *Rana esculenta* previously kept in tap water at 22° were mounted between two lucite chambers and the net water flux was measured and recorded as described by BOURGUET AND JARD[10]. The serosal side was bathed with a Ringer solution (NaCl, 112 mM; KCl, 5 mM; $CaCl_2$, 1 mM; $NaHCO_3$, 2.5 mM), (pH 8.1) and the mucosal side with the same solution in which the NaCl concentration was reduced to 5.6 mM.

Short-circuit current was measured by the technique of USSING AND ZERAHN[11], modified by MAETZ *et al.*[12].

In some cases (dissected hemibladders), the non-epithelial layers of the urinary bladder were eliminated by microdissection, as previously described[13].

Unless otherwise stated, test substances were added to the serosal medium at a controlled pH of 8.1. Oxytocin (syntocinon) and arginine vasotocin were donated by Sandoz, Basel. Cyclic AMP was purchased from Schwartz Bioresearch; theophylline from Serlabo, Paris. Probenecid was a gift of Theraplix Laboratories.

RESULTS

Influence on the water net flux

No effect of probenecid was observed when added to the mucosal solution (1 mM).

Added alone to the serosal solution, probenecid (10 μM to 2 mM) had no effect, but strongly modified the action of various other agents.

Action on the response to neurohypophyseal peptides. Table I shows that probenecid, at concentrations as low as 10 μM, reduces the hydrosmotic response to oxytocin.

This inhibition can be observed both after the response to oxytocin has developed (Fig. 1A) or when the hormonal stimulation occurs after a 15 min pretreatment with probenecid (Fig. 1B). This effect is also observed with maximal concentrations of oxytocin (up to 0.09 μM) and with arginine vasotocin, the natural hormone of *R. Esculenta* (Fig. 2).

Action on the response to cyclic AMP and to theophylline. Contrary to the response to oxytocin, the hydrosmotic response to cyclic AMP is strongly heightened by probenecid.

* A preliminary report[6] was given at the Biophysical Society Annual Meeting, Baltimore, Md., 1970.

Fig. 3 illustrates two series of experiments: in Part A, probenecid (1 mM) was added to the incubation medium, after the response to 5 mM cyclic AMP had developed. Part B shows that a response elicited by cyclic AMP after a pretreatment with probenecid is more than 3 times higher than controls obtained just before or after the test response. When paired values are considered (Table II) it appears that the interference is statistically significant for probenecid concentrations as low as 50 μM.

It was previously shown that in frog urinary bladder the removal of non-epithelial layers reduces the response of the epithelium to exogenous cyclic AMP to

TABLE I

INHIBITION OF THE HYDROSMOTIC RESPONSE TO OXYTOCIN (0.22 nM) BY PROBENECID

Probenecid concn. (μM)	*Control* *	*Pretreated with probenecid*	*Mean difference between paired values*	*n*
10	1.266	1.112	0.154 ± 0.021 ***	8
40	1.560	1.288	0.272 ± 0.064 **	5
100	1.291	0.945	0.346 ± 0.054 ***	8
1000	1.742	0.644	1.098 ± 0.129 ***	10

* Mean increase in water net flux ($\mu l \cdot cm^{-2} \cdot min^{-1}$) at the peak of the response.
** $P < 0.025$.
*** $P < 0.001$.

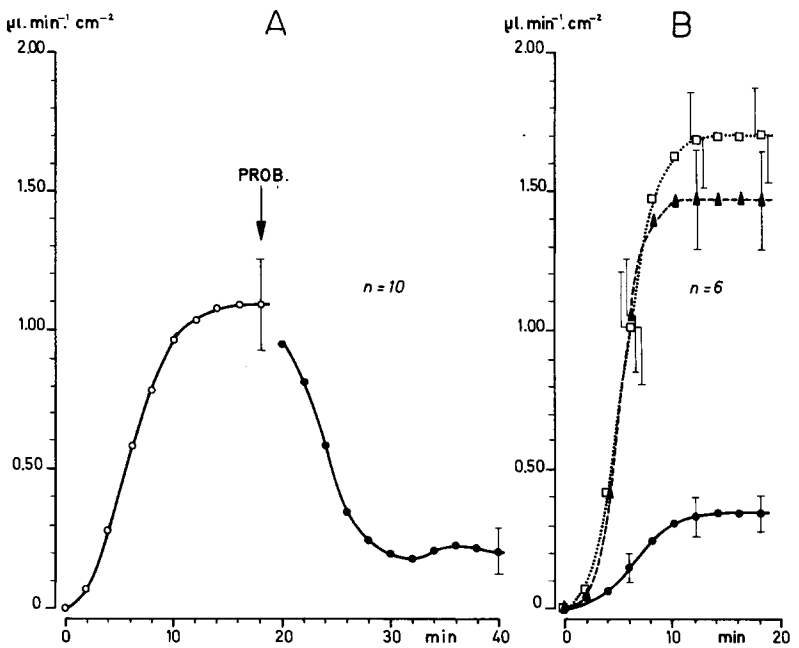

Fig. 1. Influence of probenecid (1 mM) on the hydrosmotic response to oxytocin (mean increase in water net flux. The peptide was added at time zero). A. Probenecid was added after the response had developed. B. The two upper curves represent controls obtained just before (□) or after (▲) a response in presence of probenecid (lower curve) added to the serosal side at time *minus* 15.

some 43 % of that of control hemibladder, while its reactivity to oxytocin is either slightly increased or unmodified[13].

The ability of probenecid to restore a full sensitivity of the isolated epithelium to cyclic AMP was thus investigated in a series of experiments. In this series, the response of the dissected epithelium to cyclic AMP was 66,3 % of that of the total

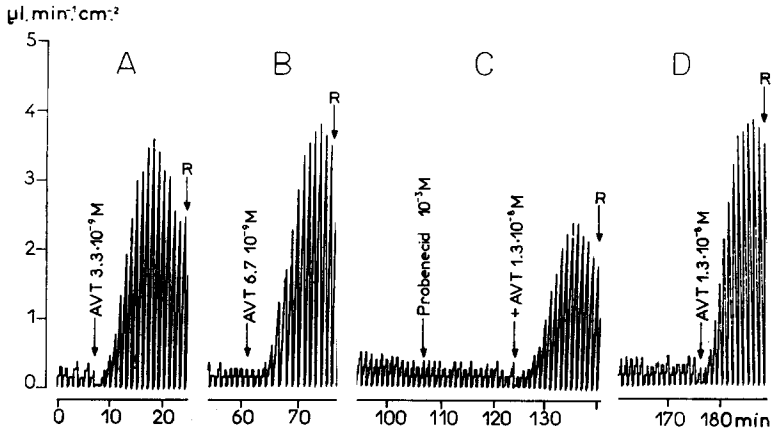

Fig. 2. Influence of probenecid on the hydrosmotic response to maximal concentrations of arginine–vasotocin (AVT). All the responses were recorded sequentially on the same hemibladder. Record was interrupted during the washout of the peptide (R). Comparison of responses A, B and D shows that a concentration of 3.3 nM can be considered as maximal for arginine–vasotocin. C shows a clear inhibition by probenecid.

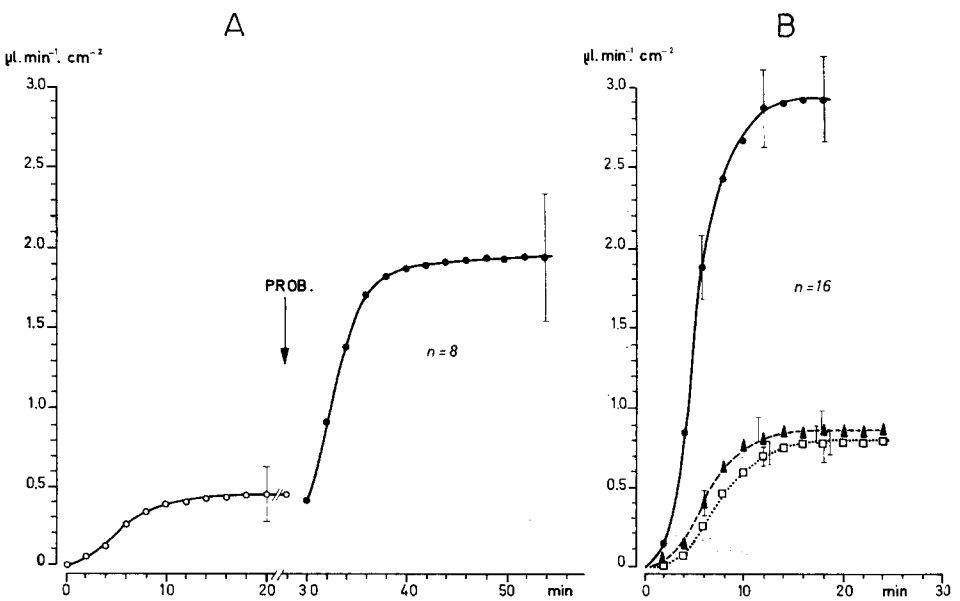

Fig. 3. Influence of probenecid (1 mM) on the hydrosmotic response to cyclic AMP. (Mean increase in water net flux. The nucleotide was added at time zero.) A. Probenecid added after the response had developed. B. The lower curves represent controls obtained just before (□) or after (▲) a response (upper curve) in presence of probenecid, added to the serosal side at time *minus* 15.

TABLE II

INFLUENCE OF PROBENECID ON THE HYDROSMOTIC RESPONSE TO CYCLIC AMP (10 mM)

Probenecid concn. (μM)	*Control* *	*Pretreated with probenecid*	*Mean difference between paired values*	*n*
10	1.330	1.408	0.078 ± 0.081	8
50	1.286	1.610	0.324 ± 0.114 **	9
100	1.535	2.078	0.543 ± 0.111 ***	7
500	0.936	2.446	1.530 ± 0.134 ***	7
1000	0.984	2.897	1.908 ± 0.295 §	10

* Mean increase in water net flux ($\mu l \cdot cm^{-2} \cdot min^{-1}$) at the peak of the response.
** $P < 0.025$.
*** $P < 0.005$.
§ $P < 0.001$.

TABLE III

EFFECT OF PROBENECID ON THE RESPONSE OF TOTAL AND DISSECTED BLADDERS TO CYCLIC AMP

Addition	*n*	*Control hemibladder** (*I*)	*Dissected hemibladder** (*II*)	*II —I*	*% of control*
Cyclic AMP (5 mM)	8	0.76 ± 0.11	0.23 ± 0.03	0.51 ± 0.16	37.8 ± 12.0 ($P<0.025$)
Probenecid (1 mM) + cyclic AMP (5 mM)	8	2.61 ± 0.20	1.63 ± 0.15	0.98 ± 0.29	66.3 ± 8.2 ($P<0.01$)

* Mean increase in water net flux ($\mu l \cdot min^{-1} \cdot cm^{-2}$) at the peak of the response.

bladder in the group pretreated with probenecid. This compound thus reduced but did not abolish the difference between dissected and control hemibladder in their response to cyclic AMP (Table III).

A positive interaction was also observed between probenecid and theophylline: pretreatment by probenecid (1 mM) increased the response to theophylline (5 mM) from 0.33 ± 0.07 $\mu l \cdot cm^{-2} \cdot min^{-1}$ in control to 0.92 ± 0.19 ($n = 5$) in pretreated hemibladder, the difference being significant at the level of 0.025 when paired values were considered.

Influence on short-circuit current

A few experiments were done to investigate whether natriferic actions of oxytocin and cyclic AMP were modified in the same manner as their hydrosmotic actions.

A reduction of resting short-circuit current (mean decrease: 2.94 ± 1.13 $\mu A \cdot cm^{-2}$, $n = 14$) was observed under the influence of probenecid alone (1 mM).

The increase in short-circuit current produced by oxytocin (0.22 nM) was reduced (by 17.5, 20 and 9 $\mu A \cdot cm^{-2}$ in three experiments) under the influence of probenecid (0.5 mM), while on the contrary the natriferic response to cyclic AMP (5 mM) was enhanced (by 10.5, 4.5 and 7.5 $\mu A \cdot cm^{-2}$ in three experiments).

DISCUSSION

Probenecid produces important and opposite modifications in the bladder response to neurohypophyseal hormones on one hand, and to cyclic AMP and theophylline on the other hand.

As far as the inhibition of the response to oxytocin is concerned, our results are in agreement* with the similar inhibition observed in toad urinary bladder by DANTZLER *et al.*[14].

As mentioned earlier, however, the inhibitory effect of probenecid was also found for supramaximal concentrations of arginine–vasotocin, an observation at variance with the reversal of probenecid action by arginine–vasotocin described by these workers. Whether this apparent contradiction is due to a species difference or to the fact that arginine–vasotocin concentrations were not raised high enough in our experiments is not established. However, the observation that probenecid can increase the effect of compounds such as cyclic AMP and theophylline made it difficult to interpret its action in terms of interference with the first steps of hormone action only.

The observed enhancement of cyclic AMP action is indeed drastic and very reproducible. Enhancement of theophylline response, although statistically significant, was found in some series of experiments to vary largely from one animal to the other. Considerable variations in sensitivity to theophylline have already been reported, namely in relation to differences in physiological conditions of the animals[15], and this finding could perhaps explain the lack of significant theophylline enhancement reported by DANTZLER *et al.*[14].

If the same primary action of probenecid is involved in its interferences with the neuropeptides and with cyclic AMP and theophylline, this action should logically take place at a step subsequent to the production of cyclic AMP.

As far as the transfer of the nucleotide is concerned, the action of probenecid could take place either on entry into the cell (in the case of exogenous cyclic AMP) or on transfer between cells or subcellular compartments.

Probenecid could simply produce an increase in the passive permeability of cellular membrane. This would explain both the increased sensitivity to exogenous cyclic AMP and, due to the greater dilution of endogenous cyclic AMP, the reduction in oxytocin activity. A concomitant increased theophylline action would, however, not be expected if this was the case.

An inhibition of cyclic AMP active extrusion could also result in higher cellular levels. This would explain the increased response to exogenous cyclic AMP and theophylline, but not the observed decrease in the oxytocin action.

These opposite effects could be explained if it is assumed that this compound somehow modified the movements of cyclic AMP between two separate pools in the tissue: a localized one, the level of which is controlled by the hormone and a general one, the level of which is increased only during stimulations by cyclic AMP and theophylline. The transfer between them would result both in a dilution of cyclic AMP produced under control of the hormone and to an increased action of exogenous cyclic AMP and theophylline.

* Since the appearance of the preliminary report[6], similar results were reported by DANTZLER *et al.*[14].

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