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2-MERCAPTO-1-(β-4-PYRIDETHYL) BENZIMIDAZOLE INHIBITION OF BASAL AND ALDOSTERONE-STIMULATED SODIUM TRANSPORT BUT PROLONGATION OF THE TRANSIENT THEOPHYLLINE-INDUCED STIMULATION IN THE TOAD BLADDER

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Abstract—2-Mercapto-1-(β-4-pyridethyl) benzimidazole (MPB) was originally introduced as a reversible inhibitor of RNA synthesis, but subsequent findings made this suggestion doubtful. We examined the effect of MPB on active sodium transport, measured as short-circuit current (scc), across the isolated urinary bladder of the toad (*Bufo marinus*). The drug caused a rapid, dose-dependent inhibition of baseline scc; 25 μg/ml MPB reduced it by 70%. Sensitivity to MPB was the same in the presence and absence of metabolizable substrate. The transport stimulation by aldosterone (7×10^{-8} M) was abolished entirely when MPB was introduced 30 min before the hormone. In bladders incubated with MPB with or without aldosterone, removal of both agents resulted in a rise in scc, which was more rapid in the aldosterone-pretreated hemibladders; a significant difference was observed after 30 min. This suggests that MPB inhibited transport at a site distal to messenger RNA accumulation. The effect of 3 hr of pretreatment with MPB on the response of the bladders to antidiuretic hormone (ADH, 20 mU) and cyclic AMP (cAMP, 10 mM) was then examined. The absolute increment in scc due to these agents was the same as in the absence of MPB, though the baseline was much reduced by the drug. After challenging MPB-pretreated bladders with theophylline (22.5 mM), sodium transport rose continuously for 90 min, in contrast to the small, short-lived rise in the absence of MPB. It is proposed that, in the toad bladder, MPB may: (1) inhibit cAMP-dependent protein kinase, as found by us in other tissues; and (2) counteract the accumulation of a transport inhibitor, possibly calcium or cyclic GMP, in tissues treated with endogenous or exogenous cAMP.

The urinary bladder of the toad, *Bufo marinus*, an organ shaped like a thin, bilobal sack, performs active transport of sodium ions from the mucosal (urinary, apical) side to the serosal (basolateral) side of the bladder; aldosterone and antidiuretic hormone (ADH)† are among the hormones that stimulate this process *in vitro* as well as *in vivo* [1–3]. The aldosterone-induced increase in sodium transport occurs after a latent period of about 75 min, and it is well established that synthesis of RNA and protein is essential in the action of this hormone [1, 3–5]. In bladders depleted of oxidizable substrate, the effect of the induced protein is not expressed [6]. ADH, an adenylate cyclase-stimulating hormone [1, 2], elicits an immediate response, as do theophylline and exogenous cyclic AMP (cAMP) [1, 2]. The effect is transient, in spite of the continuous presence of the stimulating agents, and only after an incubation period in the absence of stimulants does this refractoriness disappear [7].

The present study describes the effects of 2-mercapto-1-(β-4-pyridethyl) benzimidazole (MPB) on sodium transport in the toad urinary bladder. MPB was first introduced as a reversible inhibitor of RNA and DNA synthesis, since it had been found to block the incorporation of radioactive nucleosides and deoxynucleosides into the respective macromolecules [8]. However, it was shown later that MPB inhibited the uptake and/or phosphorylation of nucleosides [9, 10] and, unlike actinomycin D, did not interfere with the RNA polymerase reaction in isolated nuclei [11]. MPB was eventually found to inhibit a wide variety of cell functions (for references see [12]).

The experiments described were performed several years ago. The original purpose was to use MPB to reversibly inhibit RNA synthesis; however, the results were unexpected and incompatible with the alleged mechanism of action of the drug. More recently, we showed that MPB inhibited the cAMP-dependent protein kinase activity in the cytosol of a testicular tumor cell line [12] and in homogenates of uteri and ovaries of immature rats (M. Lahav, unpublished results). We believe that inhibition by MPB of the cAMP-dependent protein kinase in the toad bladder may be a possible explanation for some of the findings described here.

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† Abbreviations: ADH, antidiuretic hormone; MPB, 2-mercapto-1-(β-4-pyridethyl) benzimidazole; scc, short-circuit current; cAMP, cyclic AMP; cGMP, cyclic GMP; and DMSO, dimethyl sulfoxide.

MATERIALS AND METHODS

MPB was a gift of the Midlands Yorkshire Tar Distillers, Ltd., Birmingham, England. *d*-Aldosterone was purchased from CalBiochem. ADH (pitressin) was obtained from Parke, Davis & Co., and cyclic AMP and theophylline were products of Sigma.

All experiments were done in winter (January–March). Toads (*B. marinus*) were purchased from Tarpon Zoo, Tarpon Springs, FL, and stored without food. Active sodium transport was measured by the short-circuit current (scc) method of Ussing and Zerahn [13] as described previously [14]. (The scc has been shown to be equivalent to the rate of active transepithelial sodium transport under a wide variety of physiological conditions [1].) The toads were double pithed, and the hemibladders were removed and immersed in frog Ringer's solution (Na^+, 113.4 mM; K^+, 3.4 mM; Ca^{2+}, 2.7 mM; Cl^-, 117.1 mM; HCO_3^-, 2.4 mM; pH 8.1 in air and total solute 225 mOsmoles). Each hemibladder was mounted as a diaphragm between glass or plastic half-chambers and supported from the serosal side by a nylon mesh stretched over the opening of the half-chamber. After one rinse with frog Ringer's solution, the chambers were filled with fresh frog Ringer containing 700 units/ml penicillin G, and 0.8 mg/ml streptomycin sulfate, and, except when indicated, also 12 mM glucose. After an overnight incubation, the media were replaced with fresh solutions of the same composition, except for the glucose concentration, which was only 6 mM. One hour later ("time zero"), the experiment was started. In some experiments [Figs. 3 (right panel), 4 and 5] there was no overnight incubation; the mounted bladders were rinsed once, the chambers were filled with glucose-supplied Ringer's solution, and 3–4 hr later the experiment was started. A solution of MPB in dimethyl sulfoxide (DMSO) was prepared fresh daily (a 1000-fold of the final concentration) [11] and added to the chambers serosally and mucosally. The control tissues received an equal volume of DMSO. Aldosterone was added mucosally and serosally to give a final concentration of 7×10^{-8}M. ADH (final concentration 20 mU/ml) was added serosally. Theophylline was dissolved in medium on the day of the experiment to give 25 mM theophylline, and then water was added (1 ml/10 ml solution); thus, the final theophylline concentration was 22.5 mM. Freshly prepared cAMP solution in water (0.3 M) was brought to pH 8.0 and added serosally (final concentration, 10 mM).

In each experiment there were two treatment groups; of the two hemibladders obtained from each toad, one was included in each treatment group. The scc of each hemibladder was expressed as the normalized value, obtained by dividing each of the readings at time "t" by that recorded for the same hemibladder at time zero.

RESULTS

MPB and the response to aldosterone. Bearing in mind that RNA synthesis is an essential step in aldosterone action [1, 3–5], MPB, allegedly an inhibitor of RNA synthesis, was tested for its ability to interfere with the aldosterone-induced transport stimulation. MPB, dissolved in DMSO, was given to one hemibladder of each pair in a final concentration of 2.5, 10 or 25 μg/ml; the other member of the pair received DMSO only. Thirty minutes later aldosterone was added to the bathing media of all tissues. At a concentration of 2.5 μg/ml, MPB had no effect on the response to aldosterone; however, a slight (10–15%) depression of scc appeared to occur during the latent period. Addition of 10 μg/ml MPB caused an immediate fall in the rate of sodium transport, which at the end of the latent period tended to stabilize at a value of 55% of the control. The response to aldosterone eventually evolved was inhibited to the same extent (data not shown). When the concentration of MPB was increased to 25 μg/ml, the aldosterone-induced rise was abolished entirely; the transport rate at the end of the experiment was 30% of that found at time zero (Fig. 1).

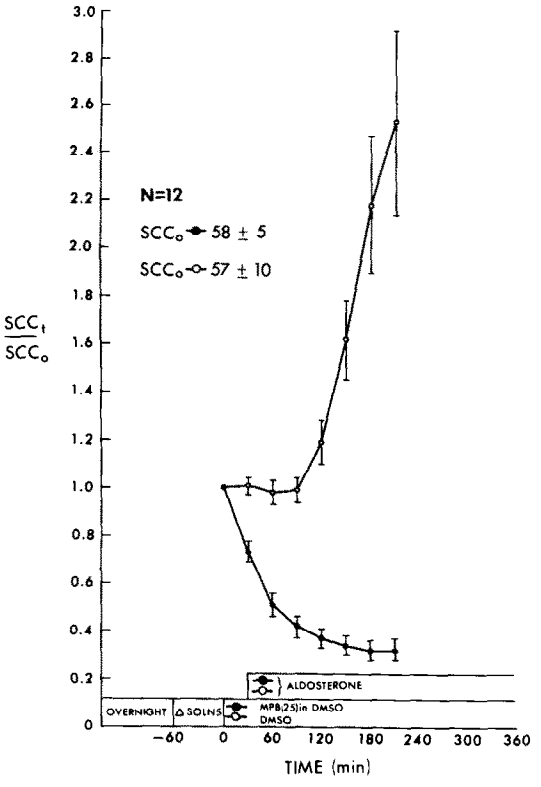

Fig. 1. The effect of MPB on the response to aldosterone (7×10^{-8}M). Key: (—●—) MPB (25 μg/ml) + DMSO (0.1%), and (—○—) DMSO (0.1%). Values are means ± S.E.M.

The marked inhibition of basal scc by MPB was very different from the effects of actinomycin D or cycloheximide, which affected basal transport very slightly, or not at all [4, 5].

We then thought to define more precisely the step at which MPB blocked the action of aldosterone. In the experiment described in Fig. 2, all hemibladders were given 25 μg/ml MPB, and 30 min later aldosterone was added to one member of each pair. After 2 more hr, both MPB and aldosterone were removed

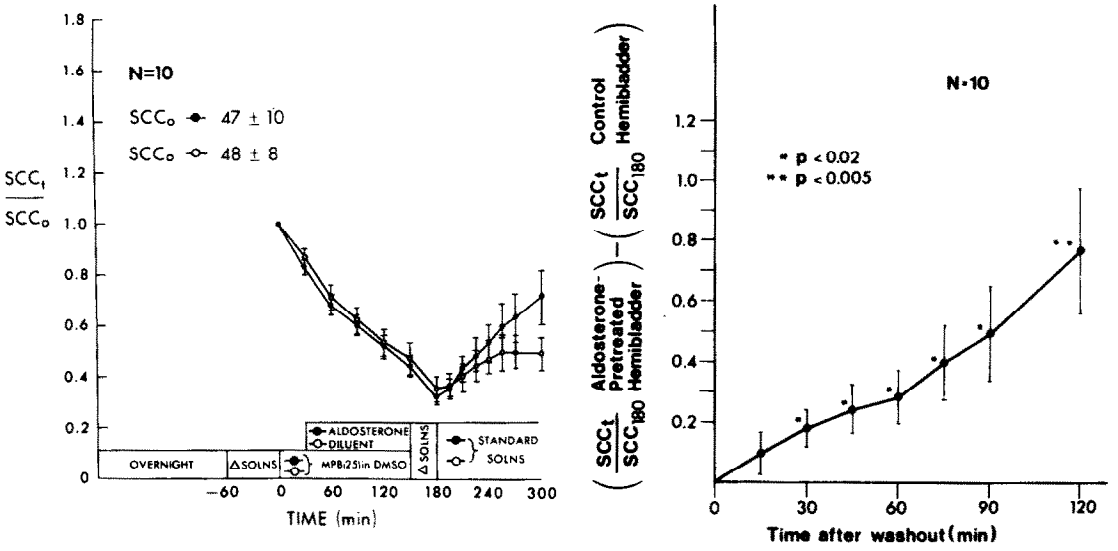

Fig. 2. Left panel: Effect of exposure to aldosterone (7×10^{-8}M) in the presence of MPB (25 μg/ml) on scc recovery after removal of both MPB and aldosterone. Key: (—●—) aldosterone, and (—○—) diluent. Right panel: Difference between the recovery of scc in tissues pretreated with MPB and aldosterone and those pretreated with MPB alone. The data are the same as in the left panel. The scc values of each hemibladder were normalized to the scc of the same tissue as the end of the washout (t = 180 on the left panel). The difference between the aldosterone-pretreated and control hemibladder within each pair was calculated. Key: (—●—) aldosterone, and (—○—) diluent.

by changing the bathing solutions three times. In the aldosterone-pretreated tissues the scc rose continuously and more steeply than in the controls. Because of the large standard errors, the difference between the two groups became statistically significant only at the end of the experiment (Fig. 2, left panel). However, within each pair of hemibladders, a larger slope was consistently observed in the aldosterone-pretreated tissue. Therefore, the data were recalculated as follows: (a) the scc readings of each hemibladder were normalized to the scc recorded for the same tissue at t = 180 min (i.e. immediately after the washout) rather than to that recorded at t = 0; (b) within each pair of hemibladders, and for each time point, the difference between the transport rate of the aldosterone-treated and the control tissue was calculated. Using the paired *t*-test, it was found that the difference between the two groups became statistically significant as early as 30 min after the end of the washout (Fig. 2, right panel). The latent period of aldosterone action is known to be more than 75 min [4, 6, 14]. Thus, it seems that MPB blocked the response to aldosterone at a late step.

MPB and substrate-stimulated transport. If bladders of *B. marinus* are incubated overnight in glucose-free frog Ringer's solution, a subsequent addition of an oxidizable substrate results in an increase in the rate of sodium transport; of the compounds tested, oxaloacetate was the most effective [6]. The sensitivities of bladders to MPB in the absence and presence of substrate were then compared. Bladders were depleted of endogenous stores of oxidizable metabolites by incubating them for 15 hr in the absence of glucose. The bathing media were then changed to fresh substrate-free solutions, and 60 min later 25 μg/ml MPB was added to one hemibladder of each pair. After another 120 min, when the treated tissues showed a transport rate of approximately 50% of the controls, oxaloacetate (5 mM) was added to all. The scc in both groups of hemibladders doubled, and the ratio between the transport rates in the MPB-treated and control group was the same as before the addition of oxaloacetate.

MPB and the response to ADH and cyclic AMP. In the experiment presented in Fig. 3 (left panel), 25 μg/ml MPB was given to one hemibladder of each pair, and 120 min later, when the scc was inhibited by approximately 60%, more MPB was added to the pretreated tissues, to give a final concentration of 62.5 μg/ml (it is possible that some of the MPB has precipitated out [8]). This brought the scc down to 28% of the DMSO-treated controls within the next 60 min. ADH (20 mU/ml) was then added to all hemibladders. Surprisingly, the absolute increment in scc was identical in the presence of MPB and, since the baseline was severely suppressed in the MPB-treated bladders, the relative response to ADH in those tissues was much higher (316%) than in the controls (86%).

The effect of exogenous cAMP (10 mM) in tissues pretreated with MPB (25 μg/ml, 3 hr) was then examined (Fig. 3, right panel). As with ADH, the magnitude of the cAMP-induced peak was approximately the same in both groups, though at the point of cAMP addition the scc in the MPB-treated tissues was only 35% of that of the controls.

MPB and the response to theophylline. The effect of MPB (25 μg/ml) on the stimulation of transport by theophylline (22.5 mM) was tested in the next experiment. This inhibitor of phosphodiesterase has been shown to increase intracellular cAMP, as well as scc, in the toad bladder [2, 15]. Figure 4 (left panel) shows that the theophylline-induced transport stimulation was affected rather dramatically by the

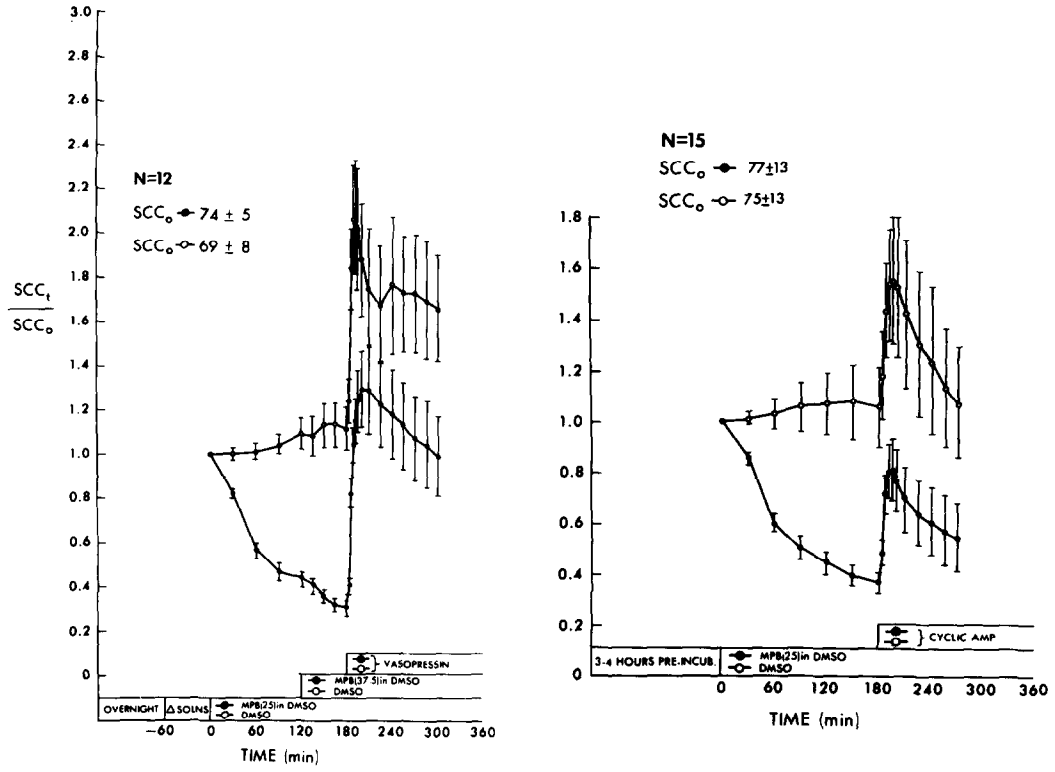

Fig. 3. Left panel: Effect of high concentration of MPB on basal scc and the response to ADH (20 mU/ml). Key: (—●—) MPB (62.5 μg/ml) + DMSO (0.1%), and (—○—) DMSO (0.1%). Right panel: Effect of MPB on the scc stimulation by cAMP (10 mM). Key: (—●—) MPB (25 μg/ml) + DMSO (0.1%), and (—○—) DMSO (0.1%).

presence of MPB. At about 15 min, the absolute increment in scc was the same in both groups. At this point the transport rate in the control tissues began to fall, returning to the baseline 30 min later. In contrast, the scc in the MPB-treated bladders rose continuously during the 90 min of exposure to theophylline, and during the last 45 min it actually exceeded the scc of the controls. Thus, in the pres-

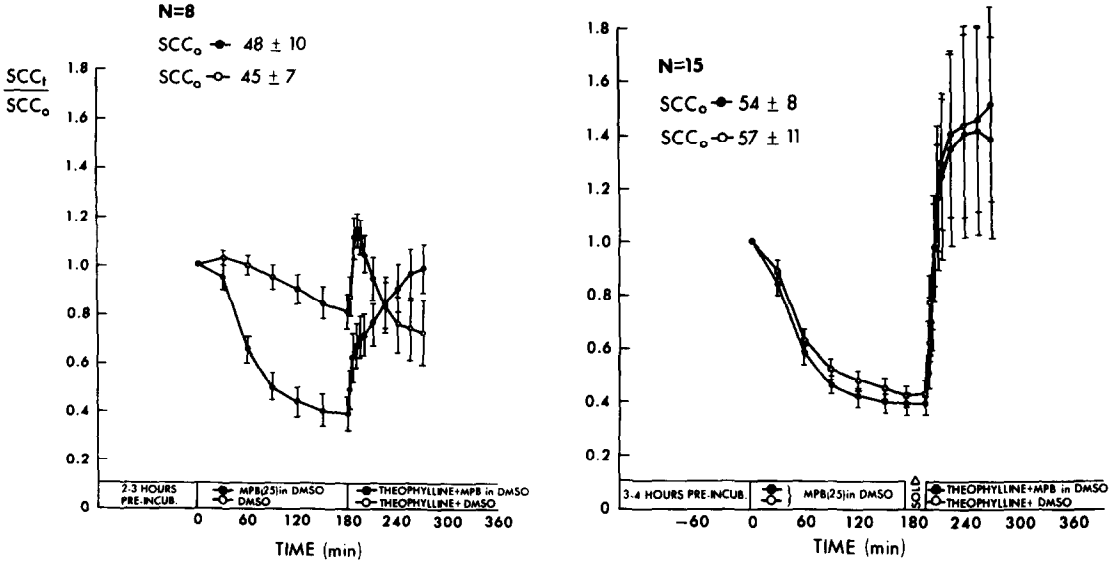

Fig. 4. Left panel: Effect of MPB on the scc stimulation by theophylline (22.5 mm). Key: (—●—) MPB (25 μg/ml) + DMSO (0.1%), and (—○—) DMSO (0.1%). Right panel: Effect of theophylline in bladders pretreated for 3 hr with MPB, with and without readdition of MPB simultaneously with theophylline (22.5 mM). Key: (—○—) MPB (25 μg/ml) + DMSO (0.1%) simultaneously with theophylline, and (—○—) DMSO (0.1%) simultaneously with theophylline.

ence of MPB the absolute response to theophylline was doubled, and at the same time its transiency was lost.

In this experiment, as in those of Fig. 3, the stimulant was added after a preincubation of 180 min with MPB, and with the continued presence of the drug; in the next experiment the need for this continuous presence was examined (Fig. 4, right panel). All hemibladders were first treated with 25 μg/ml MPB for 180 min; after three sequential changes of the bathing media the scc was determined; the media were then changed to a theophylline-containing standard solution, which for one hemibladder of each pair contained also MPB (25 μg/ml). In both treatment groups the response to theophylline was similar; it was very marked and did not decline during the 90 min of exposure to theophylline. Thus, MPB readded together with theophylline seems to have no effect; still, in interpreting these results one should keep in mind that both the onset of the effect of MPB and the recovery from it seem to be slow and gradual compared to the time-course of the theophylline response (see the various figures in this study).

The augmentation of the response to theophylline by MPB was apparent within each of the eight pairs of hemibladders of the previous experiment (left panel of Fig. 4), and in all but one of the MPB-treated tissues the rise in scc was stable. On the other hand, the response in the controls, exposed to theophylline alone, was more variable: in two hemibladders the rise was stable, in two others the scc returned to the basal level, and in the remaining four the scc proceeded to decline to values well below the original baseline. The lower-than-baseline value could not be explained by spontaneous baseline decline (a decrease of 30–80% in the 90 min of exposure to theophylline, compared to a 2–17% during the preceding 90 min in the same tissues).

These observations, masked in the averaged results of Fig. 4, became apparent when the same data were plotted in a different way (Fig. 5). In this figure, each of the sixteen hemibladders is represented by a circle, open (control) or closed (MPB-treated). For each circle, the abscissa value is the relative maximal response ($scc_{max}/scc_{180\,min}$), and the ordinate is the residual response at the end of the incubation ($scc_{270\,min}/scc_{180\,min}$) of the corresponding tissue. The straight solid line (in which the abscissa and ordinate values are identical at each point) is the theoretical curve for a non-transient response, in which the transport rate persists at the maximal value achieved. It is obvious from Fig. 5 that the sharpest deviations from this theoretical curve were observed in tissues with poor initial response, whereas the large responses (with at least doubling of the baseline) were stable.

DISCUSSION

The present study describes the complex interference of MPB with the regulation of sodium transport in the toad bladder. Since RNA synthesis is an essential step in the action of aldosterone [1, 3–5], the effect of MPB, allegedly an inhibitor of RNA synthesis [8], was first examined on the aldosterone-induced stimulation. We found that MPB at 25 μg/ml (a concentration in the range needed in other systems [8–12]) abolished the effect of aldosterone; however, unexpectedly, MPB also caused a rapid fall in the basal transport rate.

It is possible that MPB eliminated the short-lived messenger RNA of short-lived protein(s) essential for basal sodium transport; however, available data argue against this suggestion. First, cycloheximide (0.5 μg/ml) prevented 90% of leucine incorporation into epithelial proteins, but did not affect basal transport at all [4, 5]. Second, in the presence of actinomycin D, the scc declined continuously, with a $T_{1/2}$ of more than 6 hr [4, 5], whereas MPB caused an immediate and sharp fall ($T_{1/2}$ of 45 min) down to a new transport rate; the new rate was stable during an incubation of 270 min with MPB (M. Lahav, unpublished data). Third, MPB eliminated the aldosterone-induced stimulation by acting distally to the hormone-induced RNA synthesis. In bladders pretreated with MPB, with or without aldosterone, the subsequent rise following removal of drugs was sharper in the former case; the difference became significant after 30 min (Fig. 2). It is very unlikely that "*de novo*" response to residual aldosterone started after removal of MPB. Aldosterone-stimulated transport is first observable 90 min after addition of the hormone [4, 6], and most of this lag is due to synthesis and processing of RNA, prior to the onset of polypeptide chain synthesis [5]. In addition, the washout procedure used here had been shown to effectively remove aldosterone from the medium [5]. During the last few years, effects of MPB unexplainable by inhibition of RNA synthesis were reported for other systems (see [12] for references). Since the extent of inhibition of basal transport by MPB was the same in the presence and absence of oxaloacetate, it is unlikely that MPB

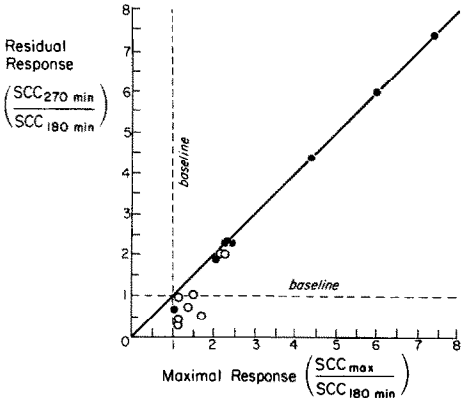

Fig. 5. Stimulatory response of theophylline (22.5 mM) in the presence and absence of MPB (25 μg/ml) in individual bladders (same tissues as in Fig. 4, left panel). Key: (●) MPB-pretreated, and (○) controls. The abscissa of each point is the maximal response (relative to the baseline) at the time of addition of theophylline, and the ordinate is the relative residual response at the end of the incubation. The straight line is the theoretical curve for a stable response (abscissa = ordinate).

acted by preventing the utilization of exogenous substrate for the energy-requiring transport process. The results obtained with agents that act via cAMP (Figs. 3, 4 and 5) suggest that direct inactivation of the sodium transport machinery itself is less likely than interference with a regulatory mechanism thereof.

Recently, we showed that MPB inhibited the cAMP-dependent, but not the cAMP-independent, protein kinase activity in three cell types (see beginning of paper) [12]; the percent inhibition became smaller as the concentration of cAMP increased, but it could not be reversed entirely. MPB bears structural resemblance to purine nucleotides, and this could be the basis for its interference with protein kinase activation.

Inhibition by MPB of cAMP-dependent protein kinase may thus underlie also the suppression of scc by this drug; it is well established that, in the toad bladder, protein·kinase activation mediates the stimulatory effect of cAMP on sodium transport [2]. Several years ago, Greengard and his colleagues found that the labeling by radioactive phosphate of a 49,000 dalton protein is reduced by treatment of the bladder with ADH or dibutyryl cAMP; the available data strongly indicate that this protein is the regulatory subunit of protein kinase, which is subjected to autophosphorylation when inactive [2, 16–18]. Interestingly, aldosterone reduced the phosphorylation of the same protein, both in the presence and absence of cAMP, and this effect was abolished by spirolactone, an aldosterone antagonist at the receptor level, and by inhibitors of RNA and protein synthesis [2]. Thus, if protein kinase is essential in the aldosterone-induced transport stimulation, inhibition of this enzyme by MPB can explain also the abolishment of the aldosterone response by MPB (Figs. 1 and 2). Since the same MPB dose allowed for 30% of the basal transport (Fig. 1), a cAMP-independent component in baseline scc is implied.

In view of the inhibitory effects of MPB described above, it was very surprising to find that the transport stimulation due to exogenous and endogenous cAMP was unimpaired, or even augmented, in the presence of MPB (Figs. 3–5). Differential effects on basal and stimulated scc of various experimental manipulations have been reported previously. Ekblad *et al.* [19] found that 10 μM diazodiiodo sulfanilic acid did not affect the baseline while it enhanced the response to cAMP plus theophylline. Taylor *et al.* [20] reported that quinidine, calcium ionophores, and reduction of serosal sodium inhibited basal scc, but did not impair, or even augment, the response to ADH.

It may be argued that, since in the presence of high cAMP concentrations MPB was less effective in inhibiting protein kinase [12], the elevated intracellular cAMP levels brought about by the addition of ADH, theophylline, or exogenous cAMP may partly reverse the inhibition of protein kinase by MPB.

However, other explanations for our data can be offered. MPB may counteract an inhibitory mechanism which is responsible for the transient nature of the response to cAMP.

It has been observed by several groups [1, 7, 18, 20] as well as by us (Figs. 3 and 4) that the stimulation of sodium transport by ADH, cAMP or theophylline does not persist at its maximal value despite the continuous presence of the stimulant. Indirect data suggest that, in the response to ADH, protein kinase activity may decline in parallel to the decline in scc. As mentioned above, addition of ADH resulted in dephosphorylation of a protein identified as the regulatory subunit of protein kinase, and this was assumed to reflect enzyme activation [2, 16, 17]. Intriguingly, rephosphorylation resumed at the same time that sodium transport began to decline [18]. A decrease in protein kinase activity could result from a fall in cAMP concentration. Such a fall was not observed in bladders treated with ADH and theophylline for 30–75 min [7, 15, 21]. However, since there may be more than one pool of cAMP in epithelial cells [2], these results are inconclusive.

The transiency of the response to cAMP could not be attributed simply to the depletion of a factor required for maintaining elevated transport rate. First, in some theophylline-treated tissues (Fig. 5), or substrate-depleted, ADH-treated hemibladders (unpublished results), the scc eventually declined to values which were far below the baseline, even after taking into account the spontaneous decrease in basal scc; moreover, this decline was most remarkable in tissues that showed small initial stimulation. Also, to restore the sensitivity to the stimulant, interim incubation in the absence of the agent was required; with the continued presence of the stimulant, the tissue remained refractory although by that time the transport rate could have been as low as the original baseline [7].

It seems to us that these findings are best explained by postulating that the initial rise in cAMP concentration triggers the accumulation of an inhibitor of cAMP action or, alternatively, the inactivation of a site essential for sodium transport. Taylor *et al.* have speculated before that the transiency of the response to ADH is due to calcium accumulation [20], and this suggestion seems attractive to us. Many experimental manipulations that elevate cytosolic calcium were reported to inhibit sodium transport (e.g. quinidine [20], reduction of serosal sodium and elimination of serosal potassium [20], cholinergic agents [22, 23], hypertonic serosal solution [24], and treatment with calcium ionophores [20, 23, 25]). As pointed out [22, 23], the inhibitor could be also cyclic GMP (cGMP), which was shown in many tissues, including the toad bladder [22, 23], to accumulate as a consequence of elevated calcium. And indeed, a permeable structural analog of cGMP, 8-Cl-S-cGMP, was found to inhibit the oxytocin-induced stimulation of scc in frog skin [26]. Moreover, isobutyl-methylxanthine which, in the toad bladder elevated cGMP as well as cAMP, caused no change in scc in the same tissues [23].

Interestingly, an increase in calcium efflux was reported for bladders exposed to ADH and to theophylline, though the mechanisms involved could be different for the two effectors [27].

Figure 6 summarizes the proposed mechanisms of action of the various agents studied here. Heavy arrows indicate an increase in concentration or rate, and dashed-line arrows indicate inhibition. Both

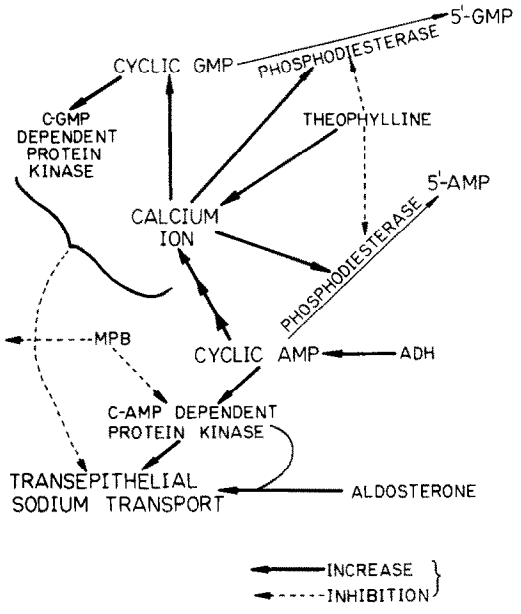

Fig. 6. Schema summarizing the suggested mechanisms of action of various transport stimulants and of MPB.

aldosterone and ADH are thought to act on the same cell population [1]; there is evidence that both hormones increase the number of active sodium channels in the apical membrane, but it is not clear whether both stimulants recruit the same pool of channels [28, 29]. Aldosterone also acts to increase energy metabolism, and substrate is required for channel recruitment by this hormone [1, 3, 6, 29]. ADH stimulates cAMP production, and cAMP, via protein kinase, stimulates transport [1, 2]. Protein kinase probably participates also in the action of aldosterone [2, 17]. Increased cAMP concentration induces a rise in cytosolic calcium, which, in turn, increases cGMP level [22, 23]. Cyclic GMP (via cGMP-dependent protein kinase), or calcium, or both, inhibit sodium transport [20, 22–26]. Calcium may also stimulate phosphodiesterase [30]. After addition of ADH or exogenous cAMP, phosphodiesterase participates in determining the new steady-state cAMP concentration, which may be lower than the initial elevated value; phosphodiesterase may thus contribute to the biphasic kinetics of the response to these agents. Theophylline inhibits phosphodiesterase, and thus brings about cAMP accumulation with its consequences. Theophylline may directly trigger calcium release from intracellular stores (e.g. [31]), and presumably it inhibits cGMP degradation, thus contributing to the inhibitory process in additional ways.

MPB inhibits cAMP-dependent protein kinase; high cAMP levels may partly reverse this inhibition [12]. MPB also blocks the inhibition by calcium and/or cGMP. Thus, in the presence of both MPB and theophylline—when cAMP degradation, as well as the inhibition of cAMP action, are blocked—the scc stimulation is stable. This scheme, though attractive to us, is by no means the only possible one. For instance, inhibitors of phospholipase A_2 and/or cyclo-oxygenase were shown to suppress basal scc [32] and the response to aldosterone [33], while prostaglandins were shown to inhibit ADH-induced rise in scc, though not the cAMP-induced stimulation [34]. Thus, some of the effects of MPB may be due to inhibition of prostaglandin production. In conclusion, while the use of MPB helped to expose some characteristics of the regulatory mechanisms in the bladder, the actual site(s) of action of MPB in this tissue, as well as in the many other systems affected by it, is not yet elucidated.

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