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EFFECT OF THEOPHYLLINE AND OTHER DRUGS ON RABBIT RENAL CYCLIC NUCLEOTIDE PHOSPHODIESTERASE, 5′-NUCLEOTIDASE AND ADENOSINE DEAMINASE

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Abstract—The effect of theophylline and several other drugs on cyclic AMP and cyclic GMP phosphodiesterase, 5′-nucleotidase and adenosine deaminase was tested in homogenates of rabbit renal cortex and medulla.

Theophylline was a competitive inhibitor of cyclic nucleotide phosphodiesterase, a non-competitive inhibitor of 5′-nucleotidase and alkaline phosphatase but did not influence adenosine deaminase. Cyclic AMP and cyclic GMP hydrolysis were inhibited to an equal extent by theophylline, furosemide, caffeine, 3-isobutyl-methylxanthine, and SQ 20006. Dipyridamol, ZK 62.711, Ro 20-1724 and ICI 63.197 inhibited cyclic AMP hydrolysis in at least ten times lower concentrations than cyclic GMP hydrolysis. Conversely, M & B 22948 and dilazep were more potent as inhibitors of cyclic GMP than of cyclic AMP hydrolysis.

All the diuretic agents tested (furosemide, ethacrynic acid, chlorthalidone, cyclopentiazide and theophylline) inhibited 5′-nucleotidase at 1 mM concentration or lower.

Of the drugs tested at 1 mM concentration only dipyridamol and chlorthalidone produced significant (30 per cent) inhibition of adenosine deaminase activity.

Methylxanthines, including theophylline, have a diuretic action in several species including man [1]. There is evidence that the effect is due both to an increase in renal blood flow leading to an increased glomerular filtration rate and to direct tubular actions [1]. In addition theophylline abolishes autoregulation of renal blood flow both following intravenous [2, 3] and intratubular application [4]. Theophylline also increases renin release from the kidney [5–8], an effect that may be independent of the action on renal blood flow [7].

The mechanisms behind these renal effects of theophylline are not known. Following the finding by Butcher and Sutherland [9] that theophylline is an inhibitor of cyclic nucleotide phosphodiesterase (EC 3.1.4.17c) it has been assumed that many, if not all, actions of the methylxanthine are secondary to elevated cyclic AMP levels. However, as regards the renal actions of theophylline the validity of this assumption has been questioned [4, 7, 8]. Another possible explanation for the renal effects of theophylline was offered by the finding of Osswald [3] that the drug is a potent competitive antagonist of the renal actions of adenosine. Adenosine reduces urine volume, sodium excretion and glomerular filtration rate [3] and renin secretion [10]. Furthermore, it enhances the vascular effects of noradrenaline and sympathetic nerve stimulation, while the release of transmitter is reduced [11].

As a part of a study of the mechanisms of theophylline in the rabbit kidney we have therefore studied the effect of some methylxanthines and other compounds on the hydrolysis of cyclic AMP and cyclic GMP by rabbit renal homogenates. Furthermore, in view of the possible involvement of adenosine in the actions of theophylline, its effect on 5′-nucleotidase and adenosine deaminase have been studied. For comparison the effects of other methylxanthines as well as other structurally unrelated phosphodiesterase inhibitors and diuretic agents were also studied.

MATERIALS AND METHODS

[^{32}P] Guanosine 3′,5′-cyclic monophosphate, triethylammonium salt (^{32}P-cGMP, 19-31 Ci/m-mole) and [^{32}P] adenosine 3′,5′-cyclic monophosphate, triethylammonium salt (^{32}P-cAMP 20-33 Ci/m-mole) were supplied by New England Nuclear. 8-[^{3}H] adenosine 3′,5′-cyclic monophosphate, ammonium salt (^{3}H-cAMP, 26 Ci/m-mole), 8-[^{14}C] adenosine 5′-monophosphate, ammonium salt, (^{14}C-AMP, 60 mCi/m-mole), 2-[^{3}H] adenosine (20 Ci/m-mole) were obtained from the Radiochemical Centre, Amersham. α,β-methylene adenosine diphosphate (AOPCP) was obtained from PL Biochemicals. 5′-nucleotidase was obtained from Sigma. Adenosine deaminase, adenosine, inosine, 5′-AMP, cAMP and cGMP were obtained from Boehringer, Mannheim. 3-isobutyl-1-methylxanthine (IBMX) was obtained from Aldrich Chemicals. Dilazep was a gift from Dr. N. Brock, Astra werke, Brackwede; indomethacin from MSD; dipyridamol from Boehringer, Mannheim. Theophylline and caffeine were obtained from ACO,

Sweden. 4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidin (ZK 62.711) was a gift from Dr. Kehr of Scheering AB, 2-*O*-propoxyphenyl-8-azapurin-6- one (MB 22.948) of Dr. Davis of May and Baker Ltd, and erythro-9-(2-hydroxy-3-nonyl) adenine hydrochloride (ENHA) of Wellcome, U.S.A. Ro 20-1724 and ICI 63.197 were provided by Dr. Kjell Fuxe and SQ 20006 by Dr. Tamas Bartfai. SP-sephadex was obtained from Pharmacia and AG 1 × 2 (-Cl) from Bio Rad. All other chemicals were reagent grade or best commercially obtainable.

Female white New Zealand rabbits (2.5–3 kg) were anaesthetized with sodium pentobarbital. The renal artery was cannulated and the kidneys flushed with ice-cold Tyrode's solution. Thereafter, kidneys were excised, freed from adherent fat and capsule, sliced, the cortex and the medulla separated and homogenized.

Cyclic nucleotide phosphodiesterase (EC 3.1.4.17c) was determined either by the method of Thompson *et al.* [12] using ^{3}H cyclic AMP as substrate or according to Selstam and Rosberg [13] using ^{32}P-cyclic AMP or ^{32}P-cyclic GMP as substrate and separation of products and substrate by inorganic salt co-precipitation with $ZnSO_4$–Ba $(OH)_2$ or $ZnSO_4$–Na_2CO_3 [14]. The tissue was homogenized in 20–40 vol of 40 mM Tris–acetate pH 7.8 containing 7 mM 2-mercaptoethanol using a Teflon-glass homogenizer operating at 200 rpm for 30–90 sec. 10–40 μg of kidney protein was incubated for 5–30 min at 30°, depending on the source of the enzyme. Conditions were chosen so that the assay was linearly dependent on amount of protein and on time of incubation.

5′-nucleotidase (EC 3.1.3.5) was determined by a radioenzymatic procedure, essentially as described by Gentry and Olsson [15], using ^{14}C-AMP (or ^{3}H-AMP) as substrate. The assay was conducted in 1 ml 50 mM Tris-acetate pH 7.4 containing 0.1 mM Mg Ac_2 using approximately 50 μg kidney protein as source of the enzyme. The incubation was proceeded for 30 min at 37°, and was stopped by the addition of 0.2 ml each of 0.15 M $ZnSO_4$ and $Ba(OH)_2$. After centrifugation an aliquot of the supernatant, containing the product, was counted in a liquid scintillation counter. The activity was determined in the absence and in the presence of 25 μM α,β-methyleneadenosine diphosphate (AOPCP) a specific inhibitor of 5′-nucleotidase [16]. The $ZnSO_4$–Ba $(OH)_2$ precipitation removed more than 98.5 per cent of the substrate and less than 14 per cent of the product. The assay was conducted under conditions where it was linear with amount of protein and with time. Conditions were chosen so that less than 20 per cent of the substrate was consumed.

Adenosine deaminase (EC 3.4.5.4) was determined essentially as described by Gustin and Kemp [17]. The tissue was homogenized in 50 mM Tris–acetate pH 7.4 containing 1 mM EDTA and 7 mM 2-mercaptoethanol. 50–75 μg protein was incubated in a total volume of 0.5 ml of the same buffer without 2-mercaptoethanol [^{3}H]adenosine (0.1 μCi/nmole) was used as substrate. Incubation was for 10 min at 37°. The reaction was stopped by the addition of 0.05 ml 5 M formic acid. The entire sample was added on a 2.5 ml SP-sephadex column. Inosine was eluted with 0.1 M formic acid and adenosine by sodium formate pH 5.5.

Protein was determined according to Lowry *et al.* [18] using bovine serum albumin as standard. ^{32}P-radioactivity was determined in a liquid scintillation counter without scintillator using the Čerenkov-effect. ^{3}H- and ^{14}C-radioactivity in the water containing samples was determined in a liquid scintillation counter using 4 g scintillator (98% PPO and 2% *bis*-MSB) per liter of toulene/triton X-100 (2:1, w/v).

RESULTS

Cyclic nucleotide phosphodiesterase(s). Both in renal cortex and renal medulla the specific activity of cyclic AMP PDE was higher in the 10,000 *g* supernatant than in the corresponding pellet (Table 1). Of the total activity approximately one third was present in the pellet. Assays over a range of substrate concentrations (0.05 to 50 μM) revealed an apparent K_m in the μmolar range in all fractions of the kidney extract (1.2–12 μM). In the supernatant fraction from rabbit kidney cortex the presence of a high K_m enzyme was also indicated (Fig. 1) [19, 20]. The present findings in the rabbit kidney are in essential agreement with studies in the rat [19, 20]. A double reciprocal plot of the data of which some are presented in Table 1 suggested that theophylline inhibited cAMP PDE in both cortex and medulla in an apparently competitive manner. The data suggest that the cortical

Table 1. Effect of theophylline on rabbit kidney cAMP/PDE activity

Enzyme source	cAMP μM	Activity (nmol·min^{-1}·mg^{-1})	Theophylline 0.2 mM	(% inhibition) 2.0 mM
Cortex sup.	0.1	0.019	46 ± 2	71 ± 4
	1.0	0.145	34 ± 3	60 ± 1
	10	1.01	27 ± 10	51 ± 3
Cortex pellet	0.1	0.009	39 ± 4	70 ± 4
	1.0	0.046	35 ± 6	66 ± 6
Medulla sup.	0.1	0.021	19 ± 4	56 ± 3
	1.0	0.142	20 ± 5	41 ± 3
Medulla pellet	0.1	0.012	13 ± 7	29 ± 11
	1.0	0.069	4 ± 4	22 ± 2

Rabbit kidney tissue was separated into cortex and medulla. Each part was homogenized in 0.25 M sucrose containing 40 mM Tris–acetate pH 7.8 containing 7 mM 2-mercaptoethanol. Each fraction was separated into a particulate and soluble fraction by centrifugation at 10,000 *g* for 30 min. Mean ± S.E.M. of triplicate determination.

Table 2. Inhibition of cyclic AMP and cyclic GMP phosphodiesterase from rabbit renal cortex. A 10.000 *g* supernatant of rabbit renal cortex was prepared. 0.2 μM ^{32}P-cyclic AMP or ^{32}P-cyclic GMP was used as substrate. Incubation for 5 min at 30°

Drug	Cyclic AMP		Cyclic GMP		IC_{25} cAMP/ IC_{25} cGMP
	IC_{25}	IC_{50}	IC_{25}	IC_{50}	
SQ 20006	$5 \cdot 10^{-6}$	$1.3 \cdot 10^{-5}$	$1.5 \cdot 10^{-5}$	$8.7 \cdot 10^{-5}$	0.3
IBMX	$8.7 \cdot 10^{-6}$	$5.4 \cdot 10^{-5}$	$1.4 \cdot 10^{-5}$	$4.7 \cdot 10^{-5}$	0.6
Dipyridamol	$1 \cdot 10^{-5}$	$6.2 \cdot 10^{-5}$	$3 \cdot 10^{-4}$	—	0.03
M & B 22948	$2.9 \cdot 10^{-5}$	$1 \cdot 10^{-4}$	$1.1 \cdot 10^{-5}$	$5.6 \cdot 10^{-5}$	2.6
Dilazep	$3.9 \cdot 10^{-5}$	$1.3 \cdot 10^{-4}$	$8.5 \cdot 10^{-6}$	$3.8 \cdot 10^{-5}$	4.6
ZK 62711	$6.6 \cdot 10^{-5}$	$4.9 \cdot 10^{-4}$	$1.4 \cdot 10^{-3}$	—	0.05
RO 20-1724	$1 \cdot 10^{-4}$	$6 \cdot 10^{-4}$	$2 \cdot 10^{-3}$	—	0.05
ICI 63197	$1.1 \cdot 10^{-4}$	$3 \cdot 10^{-4}$	$> 10^{-3}$*	—	<0.1
Theophylline	$1.2 \cdot 10^{-4}$	$6 \cdot 10^{-4}$	$1.5 \cdot 10^{-4}$	$6.3 \cdot 10^{-4}$	0.8
Furosemide	$1.3 \cdot 10^{-4}$	$5.2 \cdot 10^{-4}$	$1.8 \cdot 10^{-4}$	$5.4 \cdot 10^{-4}$	0.7
Caffeine	$3.4 \cdot 10^{-4}$	$1.4 \cdot 10^{-4}$	$5.1 \cdot 10^{-4}$	$1.5 \cdot 10^{-3}$	0.7
Adenosine	$>10^{-3}$†	—	$>10^{-3}$†	—	—

* Less than 20% inhibition at 1 mM.
† Less than 5% inhibition at 1 mM.

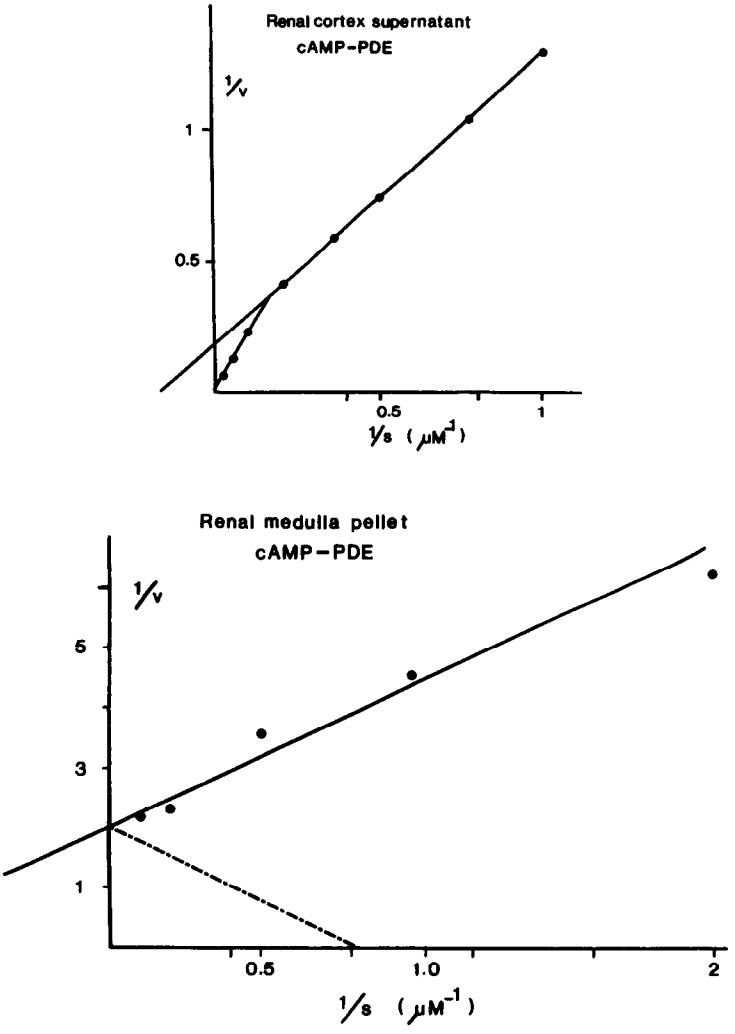

Fig. 1. Hydrolysis of cyclic AMP by a supernatant of renal cortex (a) and by the 10,000 *g* pellet from renal medulla (b) (for details see Table 3). Mean of duplicate determinations. The estimated maximal rates of hydrolysis (in the low substrate range) were 1.1 and 0.15 nmol·min^{-1}·mg^{-1}, respectively.

Table 3. Kinetic constants of 5'-nucleotidase from rabbit kidney

	K_m app.	V_{max}
	(μM)	(nmole · min^{-1} · mg^{-1})
Whole kidney	20	14
Cortex	14	11
Medulla	37	15

Whole kidney, kidney cortex and kidney medulla was homogenized in 40 mM Tris–acetate pH 7.8 containing 7 mM 2-mercaptoethanol. Assay in 0.5 ml 40 mM Tris–acetate pH 7.8 containing 1 mM $MgAC_2$. AMP concentration ranged from 0.1 to 100 μM. Determinations in triplicate from one experiment.

Table 4. The effect of drugs on rabbit kidney cortex 5'-nucleotidase (and alkaline phosphatase). Mean ± S.E.M. of duplicate determination from each of two experiments

Drug	Conc. (mM)	% of control 5'-nucleotidase	% of control Alkaline phosphatase
Theophylline	0.1	44 ± 2	86 ± 3
	1.0	31 ± 2	57 ± 4
Furosemide	0.1	84 ± 12	96 ± 1
	1.0	61 ± 7	86 ± 1
Ethacrynic acid	0.1	40 ± 2	96 ± 1
	1.0	34 ± 12	78 ± 2
Chlorthalidone	0.1	84 ± 4	97 ± 1
	1.0*	74	82
Cyclopentiazide	1.0*	54	85
Papaverine	1.0	92 ± 2	107 ± 3
Dilazep	1.0	102 ± 5	93 ± 0

The assay was conducted at 0.8 μM AMP concentration for 30 min at 37°. 52 μg protein/ml. The activity was 0.21 nmole · min^{-1} · mg^{-1} (5'-nucleotidase) and 0.86 nmole · min^{-1} · mg^{-1} (alkaline phosphatase). * One experiment only.

enzyme is inhibited to a larger extent than the medullary (Table 1).

In another set of experiments the effect of a number of drugs on cyclic AMP and cyclic GMP hydrolysis in the rabbit renal cortex was examined. These results are summarized in Table 2. In agreement with the results of Kakiuchi *et al.* [19] in rat kidney, we found that the rate of cyclic GMP hydrolysis was somewhat higher than the rate of cyclic AMP hydrolysis at equal substrate concentrations (72 vs 55 pmole.min^{-1}.mg^{-1} at 0.2 μM substrate). It is further seen from Table 2 that theophylline inhibits both cyclic AMP and cyclic GMP hydrolysis at this low substrate concentration by 50% at a concentration of approximately 0.6 mM. SQ 20006, IBMX, Dipyridamol, M & B 22.948, ZK 62.711, Ro 20-1724 and ICI 63.197 were, in that order, more potent than theophylline as inhibitors of cyclic AMP hydrolysis, while furosemide and caffeine were less potent and adenosine apparently ineffective. Dilazep was the most potent inhibitor of cyclic GMP hydrolysis followed by, in that order, M & B 22.948, IBMX, SQ 20006, theophylline, furosemide, caffeine, dipyridamol, ZK 62.711, Ro 20-1724, ICI 63.197. It may be seen that there were large differences between the phosphodiesterase inhibitors with regard to their relative activity on cyclic AMP and cyclic GMP hydrolysis.

5'-nucleotidase. The 5'-nucleotidase in rabbit kidney had an apparent K_m for AMP in the 10 μM range, both in the cortical and medullary part (Table 3). This is in agreement with results obtained in other tissues such as pig small intestine [16] and rat liver [21]. There were no clearcut differences in activity between cortex and medulla.

Both the 5'-nucleotidase and alkaline phosphatase catalyzed degradation of 5'AMP was inhibited by theophylline (Fig. 2 and Table 4). Our finding thus confirms the observation of Tsuzuki and Newburgh [22] in rat brain homogenates. The inhibition is of a noncompetitive type (Fig. 2). It may be seen that several

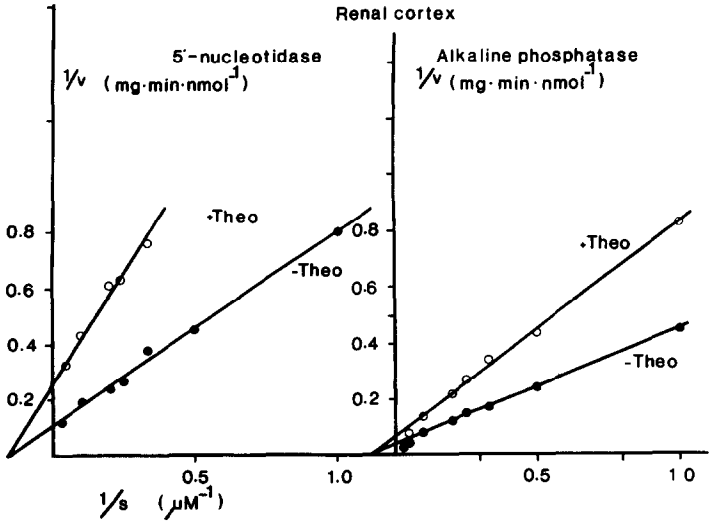

Fig. 2. The effect of theophylline (1 mM) on the hydrolysis of 5'-AMP by a rabbit renal cortex homogenate. 5'-nucleotidase measured as the difference between hydrolysis in the presence and in the absence of 25 μM AOPCP, alkaline phosphatase as hydrolysis in the presence of 25 μM AOPCP (see ref. 15). 51 μg protein ml. Incubation 10 min at 37°.

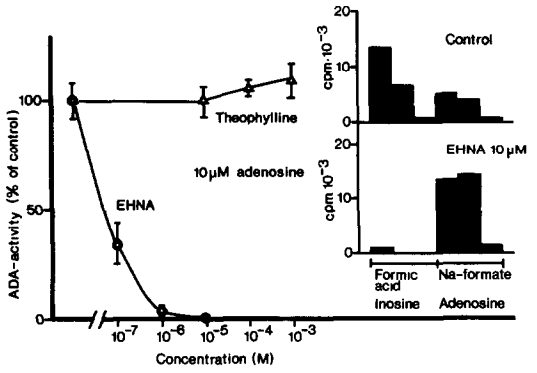

Fig. 3. Adenosine deaminase in rabbit kidney cortex. Inhibition by EHNA and lack of effect of theophylline. Insert shows the separation of inosine and adenosine on SP-sephadex from incubation in the presence and in the absence of 10 μM EHNA.

Table 5. Effect of drugs on rabbit kidney adenosine deaminase

Drug 1	Conc.	% of control activity (±S.D.) (n = 3 – 6)
EHNA	0.1 μM	34.5 ± 9.7
EHNA	1.0	4.2 ± 2.4
EHNA	10.0	0.1 ± 0.2
Theophylline	0.1 mM	106.7 ± 3.0
Theophylline	1.0	109.3 ± 8.1
Papaverine	1 mM	87.9 ± 0.3
Dilazep	1 mM	88.9 ± 1.9
Indomethacin	1 mM	105.9 ± 0.4
Dipyridamol	1 mM	67.3 ± 2.6
Furosemide	1 mM	100.2 ± 3.5
Cyclopentiazide	1 mM	93.6 ± 1.0
Ethacrynic acid	1 mM	87.6 ± 0.9
Chlorthalidone	1 mM	70.9 ± 0.6

Incubation for 10 min at 37°. 1 ml 50 mM Tris–acetate pH 7.4 with 1 mM EDTA. Started with 73 μg protein (2,000 g supernatant). 10 μM adenosine as substrate. Basal activity 6.9 nmole · min^{-1} · mg^{-1}. Results with Indomethacin, cyclopentiazide, ethacrynic acid compared to control with EtOH (20% inhib.) and chlorthalidone with DMSO (12% inhib.)

other diuretic agents, including ethacrynic acid, furosemide, chlorthalidone and cyclopentiazide also inhibited 5′-nucleotidase. The effect on alkaline phosphatase was less pronounced. By contrast, papaverine and dilazep, two phosphodiesterase inhibitors which are also inhibitors of adenosine transport, were ineffective.

Adenosine deaminase. The typical elution pattern from the SP-sephadex column is shown in the insert in Fig. 3. The figure also shows the inhibitory effect of EHNA on adenosine deaminase from kidney (IC_{50} < 0.1 μM). Theophylline had no significant effect on adenosine deaminase (Fig. 3, Table 5). Of the drugs tested at 1 mM concentration only dipyridamol and chlorthalidone gave a significant (30 per cent) inhibition of ADA-activity.

DISCUSSION

Theophylline has pronounced renal effects already in low concentrations (1–10 μM; 3,7,23). However, even in renal cortex, where the cyclic nucleotide phosphodiesterase(s) appeared to be most sensitive to inhibition by theophylline, 25 per cent inhibition required more than 10^{-4} M theophylline although low concentrations of the substrate (0.1 μM) were used in the assay. It has been found that the renal actions of theophylline are not potentiated by cyclic AMP [7]. Furthermore, there is no evidence for increased renal secretion of cyclic AMP following administration of theophylline in doses that cause marked blood flow changes as well as increased renin production [8]. Thus, the actions of theophylline may not be due to inhibition of cyclic nucleotide phosphodiesterase.

It could, however, be argued that theophylline specifically inhibits one type of cyclic AMP phosphodiesterase, that specifically regulates a small, but important, pool of cyclic AMP in the kidney. It is clear that the kidney phosphodiesterase system is very complex [19, 20]. The molecular forms and the kinetic behaviour of the enzyme complex is modified by a number of factors including a calcium-plus-magnesium-ion-dependent protein activator [19, 20]. Thus, although neither the literature nor the present studies provide any evidence for a particular form of cyclic nucleotide phosphodiesterase that is potently inhibited by theophylline, the possibility that it exists cannot be ruled out.

It has been suggested that other structurally unrelated diuretics are also active via an action on cyclic nucleotides [24], since they inhibit renal adenylate cyclase activity [24, 25]. When one of these diuretics, furosemide, was given *in vivo* the renal cyclic AMP levels were increased rather than decreased, however [26]. This *in vivo* action could possibly be explained by inhibition of cyclic AMP hydrolysis [27 and present results]. The finding that cyclic GMP levels in the kidney are also increased by furosemide [26] may have a similar explanation (Table 2). However, the concentration of furosemide necessary to produce inhibition of cyclic nucleotide breakdown was high and the significance of that finding as an explanation for the diuretic effect of furosemide is in doubt. Also for other reasons the role of cyclic nucleotides in the diuretic action of this drug has been questioned [25, 26].

The present results confirm previous findings in other tissues that different forms of phosphodiesterases may be affected to an unequal degree by inhibitors [e.g. 28–33]. Thus, in agreement with results from other tissues, ZK 62.711 was more potent as an inhibitor of cAMP hydrolysis [32]. The same is true for Ro 20-1724, ICI 63.197 [31] and dipyridamol [33]. By contrast M & B 22.948 appeared to preferentially inhibit cyclic GMP hydrolysis, in agreement with results obtained in the lung [30, 31]. Dilazep was found to show a very marked preferential inhibition of cyclic GMP hydrolysis. The methylxanthines, theophylline, caffeine and IBMX, as well as SQ 20006 and furosemide did not exhibit any selectivity.

The present data confirm the finding that theophylline is an inhibitor of 5′-nucleotidase [22], and suggest that nonspecific 5′-AMP hydrolysing activity, presumably alkaline phosphatase, is also inhibited by theophylline. The inhibition of both types of activity appeared to be noncompetitive rather than competitive. It is unclear if this finding has any significance for

our understanding of the mechanisms by which theophylline exerts its diverse biological effects. However, it serves to emphasize that theophylline is not a specific cyclic nucleotide phosphodiesterase inhibitor. Since theophylline is known to antagonize the effects of adenosine in a variety of cell types, including the kidney, the finding that the drug may also inhibit adenosine formation is interesting. Newman and McIlwain [34] recently reported that drug-induced increases in adenosine content of brain slices was significantly reduced by theophylline, which might be explained by inhibition of 5'-nucleotidase.

Inhibition by theophylline of 5'-nucleotidase might also possibly influence those assays of phosphodiesterase that employ 5'-nucleotidase to convert the formed 5'-mononucleotides to nucleosides. For that reason we have determined the phosphodiesterase inhibitory effect of theophylline by two different methods. There was a slight difference in results (for example, 0.2 μM theophylline inhibited hydrolysis of 0.2 μM cyclic AMP by a renal cortical supernatant by 42 per cent using the 5'-nucleotidase dependent assay and by 31 per cent using the ^{32}P-cyclic AMP assay). However, the assays were carried out on different occasions and with different batches of enzyme and it cannot be concluded that the difference is due to inhibition of 5'-nucleotidase by theophylline.

It was also found that several other diuretic drugs, particularly ethacrynic acid, inhibited 5'-nucleotidase. This enzyme is located in cell membranes [16], just as Na^+, K^+-ATPase and adenylate cyclase. Since all three enzymes are inhibited by ethacrynic acid, and also, to a lesser extent, by other diuretic drugs [cf. 35] it is possible that the inhibition is due to an interaction with some structurally important membrane component rather than to a direct inhibitory effect at the enzyme(s). Such a possibility directly reflects upon the many studies concerning the biochemical basis of diuretic action [35].

While theophylline was inhibitory on phosphodiesterase and the phosphatases there was no effect on adenosine deaminase. In fact none of the drugs tested at 1 mM concentration produced but a minor inhibition, with the exception of the well known adenosine deaminase inhibitor EHNA. In particular dilazep and dipyridamol, which influence adenosine metabolism *in vivo* had a minor effect on adenosine deaminase *in vitro*. One explanation of their *in vivo* inhibitory effect is that they inhibit adenosine transport thereby limiting the access of the substrate to the enzyme.

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