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THE EFFECTS OF CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE AND GUANOSINE 3′,5′-MONOPHOSPHATE AND THEOPHYLLINE ON RENIN SECRETION IN THE ISOLATED PERFUSED KIDNEY OF THE RAT

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1 The influence of cyclic adenosine 3′,5′-monophosphate (cyclic AMP), cyclic guanosine 3′,5′-monophosphate (cyclic GMP) and theophylline on renin secretion was examined in the isolated kidney of the rat perfused with Krebs dextran solution.

2 Neither cyclic AMP (10^{-6} to 10^{-4} M) nor dibutyryl cyclic AMP (10^{-5} M) produced an increase in renin secretion.

3 Cyclic GMP and 8 Br-cyclic GMP caused a small rise in renin secretion in some experiments but this effect was independent of the dose and its physiological significance is uncertain.

4 Theophylline (10^{-6} to 10^{-4} M) caused a significant elevation in renin secretion which was not blocked by (+)-propranolol. Theophylline with cyclic AMP or cyclic GMP did not produce an amplified effect.

5 Despite previous suggestions that cyclic AMP stimulated renin secretion, this could not be confirmed in the present preparation. Since there is no evidence that cyclic AMP or cyclic GMP (or their derivatives, dibutyryl cyclic AMP and 8 Br-cyclic GMP) enter the cells, it will be necessary to study their activity in isolated juxtaglomerular cells to define a possible rôle.

Introduction

There is now much evidence that renin secretion is markedly influenced by the sympathetic nervous system and catecholamines acting on renal β-adrenoceptors (Vander, 1965; Wathen, Kingsbury, Stouder, Schneider & Rostorfer, 1965; Assaykeen, Clayton, Goldfien & Ganong, 1970; Ueda, Yasuda, Takabatake, Iizuka, Iizuka, Ihori & Sakamoto, 1970; Johnson, Davis & Witty, 1971; Winer, Chokshi & Walkenhorst, 1971; Ganong, 1972; Tanigawa, Allison & Assaykeen, 1972; Davis, 1973; Vandongen, Peart & Boyd, 1973). It is believed that the effect of many hormones on their target cells is mediated by stimulation of adenyl-cyclase leading to increase in 3′,5′ cyclic adenosine monophosphoric acid (cyclic AMP), regarded as the 'second messenger' (Robison, Butcher & Sutherland, 1971; Butcher, Robison & Sutherland, 1972). The original observation was of course that adrenaline worked in this way (Sutherland & Robison, 1966). Isoproterenol, a β-receptor stimulator, is a very effective stimulus to renin release in the isolated perfused kidney (Vandongen *et al.*, 1973) and also produces a considerable elevation of the cyclic AMP content of the kidney cortex in experimental animals (Beck, Reed & Murdaugh, 1972). Glucagon has been shown to stimulate the adenyl-cyclase/cyclic AMP system in different organs (Murad & Vaughan, 1969) and also increases renin secretion from the isolated perfused kidney (Vandongen *et al.*, 1973). This effect was not prevented by the β-adrenoceptor blocking agent, propranolol. It has been postulated that theophylline, which in isolated systems inhibits the phosphodiesterase enzyme that removes cyclic AMP, may exert its effect on whole cells by allowing accumulation of cyclic AMP (Butcher & Sutherland, 1962). This might also apply to cyclic guanosine 3′,5′-monophosphate (cyclic GMP) (Ishikawa, Ishikawa, Davis & Sutherland, 1969). Plasma renin activity was increased by theophylline in animals and in man (Winer, Chokshi, Yoon & Freedman, 1969; Reid, Stockigt, Goldfien & Ganong, 1972; Johns & Singer, 1973). It has previously been claimed that renin secretion was increased by cyclic AMP *in*

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vitro and *in vivo* (Michelakis, Caudle & Liddle, 1969; Winer *et al.*, 1971; Yamamoto, Okahara, Abe, Ueda, Kishimoto & Morimoto, 1973), but this was not confirmed (Tagawa & Vander, 1970). Cyclic GMP has been viewed for a long time as a compound in search of a function (Ashman, Lipton, Melicow & Price, 1963). The kidney is rich in this substance and its physiological role is unknown (Ishikawa *et al.*, 1969) although in some secretory cells it has been suggested that there may be a common pathway, guanosine cyclase/cyclic GMP, which opposes the action of the adenyl-cyclase/cyclic AMP pathway (Goldberg, Haddox, Hartle & Hadden, 1973; Kolata, 1973). It therefore seemed important to determine whether cyclic AMP, cyclic GMP and theophylline had an effect on renin secretion and might indicate a final common pathway for stimulation of the juxta-glomerular cells.

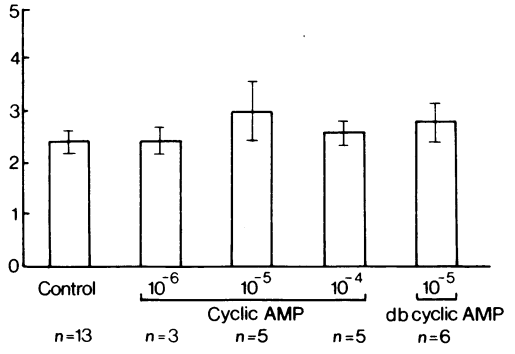

Figure 1 The effect of cyclic AMP and dibutyryl cyclic AMP (db cyclic AMP) on renin secretion in isolated perfused kidney of the rat. Molar concentrations of the drugs are expressed on the abscissae and the change of renin concentration is expressed on the ordinates as the ratio of the value at 24 min over that immediately before the start of drug administration (0 minute). Vertical bars show s.e. mean.

Methods

Kidney perfusion

Male rats (300 g to 350 g) maintained on a regular diet were anaesthetized with sodium pentobarbitone (0.1 mg/g) intraperitoneally and given 100 units heparin intravenously. The left kidney was isolated and perfused as described previously (Vandongen *et al.*, 1973). The perfusion fluid was Krebs-Ringer dextran saline equilibrated with 95% O_2 and 5% CO_2 at 37°C and was delivered as pulsatile flow at a constant rate (usually 8 ml/min) by roller pump. Perfusion pressure was measured by transducer and Devices M2 recorder. Experiments were begun when the perfusion pressure had stabilized at 55-80 mmHg, 6-8 min after the start. A control collection was then made during 1 min, when drug administration was started, and further similar samples were then collected every 4 minutes. The drugs were dissolved in perfusion fluid and infused separately into the arterial line through a needle in the tubing at 0.04 ml/minute. Total duration of perfusion did not exceed 30 minutes. The drug concentrations achieved in the perfusate were calculated from:

$$\frac{\text{Drug, mol/min}}{\text{Rate of flow, ml/min}}$$

Renin assay

One ml from each perfusate sample (8 ml) was dialysed for 24 h (Visking tubing) at 4°C against 0.16 M phosphate buffer (pH 4.5) containing disodium edetate, following the method for plasma renin activity (Skinner, 1967). Further dialysis against phosphate buffer (pH 7.5) for 24 h was carried out. Both buffers contained gentamycin (10 μg/ml). The samples were then incubated at 37°C with plasma from rats nephrectomized 24 h beforehand (1 ml perfusate with 0.4 ml plasma), which had been treated by similar dialysis procedures to destroy angiotensinase activity. The angiotensin I produced on incubation was measured by radioimmunoassay (Boyd, Adamson, Fitz & Peart, 1969) and the renin concentration was expressed as nanogram equivalent of 5-isoleucine-angiotensin I generated per ml of perfusate per hour of incubation. Since the peak renin concentration was reached in all experiments by 24 min from the start, results were expressed as the ratio of the renin concentration at 24 min to the renin concentration at zero time. Since flow rate was very nearly constant, concentration was used rather than secretion rate. All values given are means ± s.e. and significance was measured by Student's paired *t* test. Comparisons were always made between a control group of perfusions (n = 13) and the treated group.

Materials

Adenosine 3',5' cyclic monophosphoric acid (cyclic AMP), N_6O_2-dibutyryl-adenosine 3',5' cyclic monophosphoric acid Na salt, guanosine 3',5' cyclic monophosphoric acid Na salt, 8 Br-guanosine 3',5' cyclic monophosphoric acid Na salt, theophylline (1,dimethylxanthine) (Sigma Chemical Co.) and propranolol (ICI) were used.

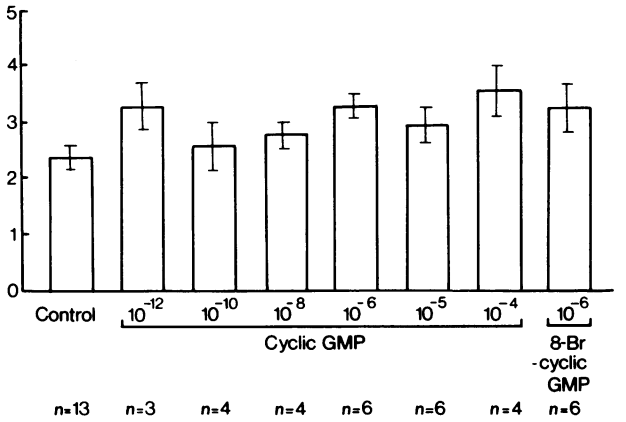

Figure 2 The effect of cyclic GMP and 8 Br-cyclic GMP on renin secretion in isolated perfused kidney of the rat. Molar concentrations of the drugs are expressed on the abscissae and the change of renin concentration is expressed on the ordinates as the ratio of the value at 24 min over that immediately before the start of drug administration (0 minute). Vertical bars show s.e. mean.

Results

Effect of cyclic AMP and dibutyryl cyclic AMP

Neither of these two substances produced an effect on renin secretion (Figure 1) and there was no alteration in perfusion pressure or flow.

Effect of cyclic GMP and 8 Br-cyclic GMP

An increase significant at $P < 0.05$ level was seen with cyclic GMP in concentrations of 10^{-4}, 10^{-6} and 10^{-12} M but with concentrations of 10^{-5}, 10^{-8} and 10^{-10} M, no significant effect was observed ($P > 0.1$) (Figure 2). Since some of the numbers of animals in the individual groups are small ($n = 3$), this might account for the variability. When 8 Br-cyclic GMP (10^{-6} M) was used, the effect was similar to that of cyclic GMP itself (10^{-6} M) (Figure 2). There was no change in perfusion pressure or flow in any experiment.

Effect of theophylline

While infusion of theophylline (10^{-7} M) did not produce a significant increase in renin secretion ($P > 0.01$), there was a significant increase of renin when the theophylline concentration was increased to 10^{-6} and 10^{-4} M ($P < 0.01$) (Figure 3). Propranolol (3.4×10^{-7} M), which was the concentration previously found to block renin release due to isoprenaline (Vandongen *et al.*, 1973), did not abolish the stimulating effect of theophylline (10^{-6} M), although the increase in renin secretion was smaller than with theophylline alone (difference not significant, $P > 0.05$). Propranolol alone was without effect (Figure 4). There was no effect on perfusion pressure or flow with theophylline or propranolol.

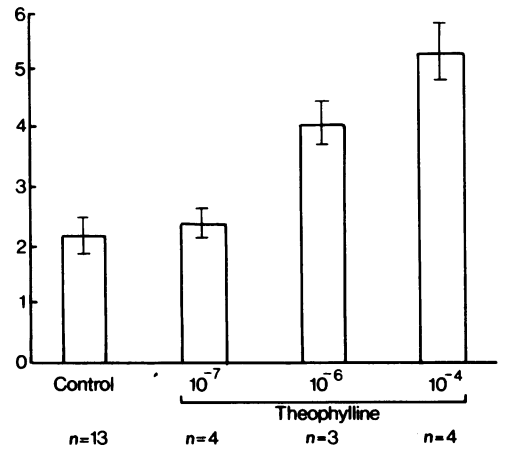

Figure 3 The effect of theophylline on renin secretion in the isolated perfused kidney of the rat. Molar concentrations of the drug are expressed on the abscissae and the change of renin concentration is expressed on the ordinates as the ratio of the value at 24 min over that immediately before the start of drug administration (0 minute). Vertical bars show s.e. mean.

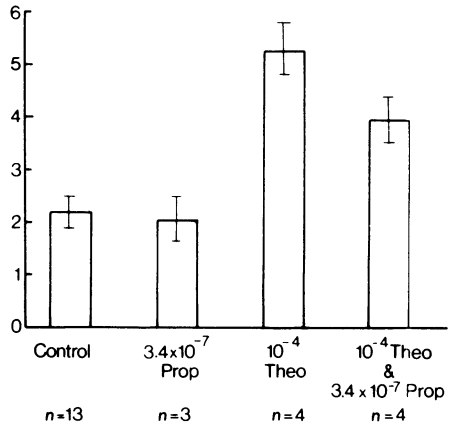

Figure 4 The effect of propranolol (Prop), theophylline (Theo) and theophylline plus propranolol on renin secretion in the isolated perfused kidney of the rat. Molar concentrations of the drugs are expressed on the abscissae and the change of renin concentration is expressed on the ordinates as the ratio of the value at 24 min over that immediately before the start of drug administration (0 minute). Vertical bars show s.e. mean.

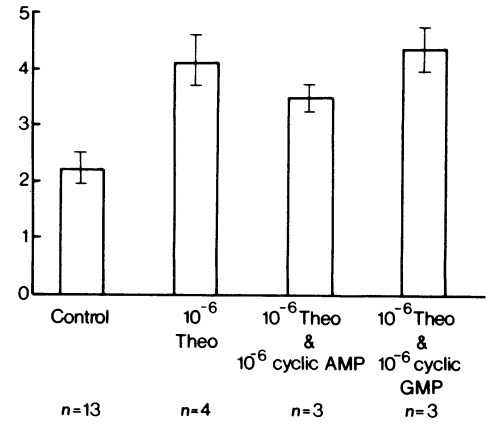

Figure 5 The effect of theophylline (Theo) plus cyclic AMP and theophylline plus cyclic GMP on renin secretion in the isolated perfused kidney of the rat. Molar concentrations of the drugs are expressed on the abscissae and the change of renin concentration is expressed on the ordinates as the ratio of the value at 24 min over that immediately before the start of drug administration (0 minute). Vertical bars show s.e. mean.

Effect of theophylline with cyclic AMP or cyclic GMP

The usual elevation of renin secretion was seen in both these studies but in neither case was the increase significantly different from that seen with theophylline alone ($P > 0.1$) (Figure 5).

Discussion

It has clearly been shown that neither cyclic AMP nor dibutyryl cyclic AMP had any effect on renin secretion. This might be because cyclic AMP could not enter the cell in sufficient quantity during the limited perfusion time (20 min) or that cyclic AMP was not on the final intracellular pathway for stimulation of renin. It has been suggested that the dibutyryl derivative passes the cell membrane more easily than cyclic AMP because it is more fat soluble and this could account for the bigger biochemical effect of this substance in some systems (Robison *et al.*, 1971; Sutherland, 1972). This idea was not confirmed in tissue culture studies (Ryan & Durich, 1972) and there is no definite evidence in its favour at the present time. It should be stated that even in experiments where cyclic AMP or dibutyryl cyclic AMP is known to stimulate biochemical processes, it is quite unknown whether this is a membrane effect or requires the entry of the substances into the cells. The present results are therefore opposed to those either in the whole animal (Winer *et al.*, 1971) or from renal cell suspensions (Michelakis *et al.*, 1969). It was of interest to study cyclic GMP not only because it occurs in the kidney but because it has been implicated in the function of some secretory cells (Eichhorn, Salzman & Silen, 1974). The very variable response at different concentrations does not carry much conviction about a real role in stimulation of renin since at best the response is not very great. The derivative 8 Br-cyclic GMP, which like dibutyryl cyclic AMP is more fat soluble, was no more active than cyclic GMP. Similar reservations apply to the evidence about its entry into cells (Estensen, Hili, Quie, Hogan & Goldberg, 1973). Theophylline has a direct effect on renin secretion without changes in perfusion pressure and flow. While it is a phosphodiesterase inhibitor, there is no evidence that this is how it releases renin and its action was not amplified by cyclic AMP or cyclic GMP. Theophylline can cause release of dopamine β-hydroxylase and noradrenaline from sympathetic nerve endings (Wooten, Thoa, Kopin & Axelrod, 1973), but there was no increase in perfusion pressure which would have been expected with sympathetic stimulation or noradrenaline release, and further this increase in renin secretion was not abolished by propranolol. This is

important since it is the β-receptors that are significant in renin release. Recently it has been found that caffeine, theophylline and theobromine can decrease calcium sequestration in the sarcoplasmic reticulum in cells and can increase the calcium influx in cardiac cells (Blinks, Olson, Jewel & Branevy, 1972). While a relation between calcium flux in juxtaglomerular cells and renin release has been suggested (Vandongen & Peart, 1974), it remains uncertain how theophylline stimulates renin release. A possible rôle for cyclic AMP or cyclic GMP will require study of changes in their intracellular concentration or activity in isolated juxtaglomerular cells.

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