CHLORPROPAMIDE AND TOLBUTAMIDE INHIBITION OF ADENOSINE 3'5' CYCLIC MONOPHOSPHATE PHOSPHODIESTERASE*

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SUMMARY

These sulfonylurea agents inhibit the cyclic AMP phosphodiesterase, and thereby could increase the steady state level of cyclic AMP in various tissues, depending upon the tissue concentrations achieved after oral or parental administration.

This report presents results which indicate that tolbutamide and chlorpropamide are inhibitors of cyclic nucleotide phosphodiesterase, the enzyme that inactivates cyclic AMP by converting it to 5' AMP.

These two sulfonylurea compounds have been widely used in the treatment of diabetes mellitus (1). They have been shown to cause release of endogenous insulin from pancreatic beta cells (2). In addition, oral chlorpropamide (3) and intravenous tolbutamide (4) have been shown to have antidiuretic vasopressin-like effects in patients with diabetes insipidus.

The mechanism of the above effects has as yet not been elucidated. It may be pertinent to this problem that cyclic AMP has been implicated as the mediator of insulin release from the pancreas and in the action of vasopressin on the kidney. Cyclic AMP has been postulated as the mediator of these physiological functions because direct administration of cyclic AMP to the perfused pancreas caused insulin release (5-6) and its application

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to the isolated toad bladder and isolated perfused rabbit kidney tubule mimicked the effect of vasopressin (7-8). The methylxanthines, which are phosphodiesterase inhibitors (9), when applied to the toad bladder mimicked the effects of vasopressin and increased the tissue concentration of cyclic AMP. Vasopressin action in the toad bladder is associated with elevation of the tissue level of cyclic AMP. In the tissue cultured rat pancreas, caffeine (another methylxanthine) increased insulin release into the culture media (10-11). We have recently shown (12) that both chlorpropamide and vasopressin therapy are associated with a rise in the urinary excretion of cyclic AMP in patients with diabetes insipidus. Chlorpropamide also potentiated the effects of vasopressin. Takahashi et al., have also shown that vasopressin increases the urinary excretion of cyclic AMP in patients with diabetes insipidus (13). Beck et al., have also shown that 10^{-2} M chlorpropamide and vasopressin (10^{-3} units/ml) caused a three fold increase above control levels of cyclic AMP in slices of rat renal medulla (14). In the toad bladder, chlorpropamide (3.3mM) significantly increased the osomotic water flow (15). In view of the above facts it appeared to be important to investigate whether these agents act by inhibiting the cyclic AMP phosphodiesterase.

METHODS

Rat kidney was homogenized in 10 volumes of water per gram of tissue in a teflon pestle homogenizer for 2 minutes at $0^{o}C$. The homogenate was centrifuged (30,000 x g) for 30 minutes at $0^{o}C$. The supernatant was used to assay phosphodiesterase. Cyclic AMP phosphodiesterase was measured by monitoring the conversion of H^3 cyclic AMP to H^3 5' AMP. The H^3 5' AMP formed by the phosphodiesterase was converted to H^3 adenosine by King Cobra venom nucleotidase, separated and counted by liquid scintillation as described by Brooker et al., for the assay of cyclic AMP (16). The reaction was conducted in a volume of 100µl for 10 minutes at $30^{o}C$. The solution contained 1×10^{-6} M cyclic AMP and

0.1μCi of H^3 cyclic AMP (sp. act = 16.3 Ci/mMole), 10mM $MgCl_2$, 100mM tris HCl pH 8.0, sufficient phosphodiesterase for about 40% reaction, 1μg King Cobra venom and either Chlorpropamide, sodium tolbutamide or caffeine. The reaction was stopped by 0.8ml of a 50% slurry of Dowex 1 resin (chloride form, minus 400 mesh), scintillation solvent was added, and the samples were counted. Tolbutamide, chlorpropamide, or caffeine (10mM) did not inhibit the conversion of H^3 5' AMP to adenosine.

RESULTS AND DISCUSSION

Table I shows that both chlorpropamide and tolbutamide are inhibitors of phosphodiesterase from kidney, although they are not as potent as caffeine. Similar results were obtained for phosphodiesterase prepared from rat pancreas. Because the homogenate cyclic AMP phosphodiesterase does not obey simple Michaelis-Menten Kinetics (16) the Ki for these inhibitors was not calculated.

These concentrations of tolbutamide and chlorpropamide which effectively inhibit the cyclic nucleotide phosphodiesterase are slightly higher than serum levels obtained with these agents during therapy. Serum levels during therapy are close to 10^{-3} M (17-18) for both these drugs even though the daily dose is quite different. This indicates a considerable difference in their pharmacokinetics and in their probably distribution in body compartments and tissues. In addition, caffeine, another agent which also inhibits cyclic AMP phosphodiesterase, has neither hypoglycemic nor antidiuretic effects, although it has recently been shown to markedly increase the serum level of insulin in humans (19). In fact, methylxanthines have been used as mild diuretics and in some cases can potentiate epinephrine-induced hyperglycemia (20). When directly administered to the pancreas, moreover, caffeine induces insulin release and when applied to toad bladders it mimics the effects of the antidiuretic hormone, vasopressin. It is possible that differences in the tissue distribution between

TABLE I

Addition	N	% Inhibition (+ S.E.M.)
None	8	0.0 ± 2.97
Chlorpropamide		
5×10^{-4}M	4	6.0 ± 2.34
5×10^{-3}M	3	25.7 ± 2.60
1×10^{-2}M	4	37.3 ± 2.29
Tolbutamide		
5×10^{-4}M	3	4.0 ± 0.58
5×10^{-3}M	3	23.0 ± 1.53
1×10^{-2}M	4	41.0 ± 0.85
Caffeine		
5×10^{-4}M	4	40.3 ± 2.56
5×10^{-3}M	4	87.3 ± 2.96
1×10^{-2}M	4	90.0 ± 1.08

the sulfonylurea agents and methylxanthines in vivo might explain why they act similarly in vitro, but differently in vivo.

These sulfonylurea compounds which are phosphodiesterase inhibitors could reduce the rate of cyclic AMP degradation and lead to higher steady state levels of the nucleotide in target organs. This might partially explain how chlorpropamide and tolbutamide potentiate insulin release in patients with diabetes mellitus, and create antidiuresis in patients with diabetes insipidus.

It also seems possible that cyclic AMP might be involved in atherogenesis possibly as an inducer of the enzymes necessary for collagen synthesis. Many physiological conditions related to a high risk of atheroscler-

osis seem also related to hormonal conditions which would predispose elevated tissue cyclic AMP levels. It seems possible that inhibition of cyclic AMP phosphodiesterase by tolbutamide could be considered part of the reason that long term cardiovascular side effects (21) occur when diabetes mellitus is treated with this drug.

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