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SHORT COMMUNICATIONS

Effects of chlorpropamide and tolbutamide on adenylate cyclase activity in rat heart and liver*†

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The mechanisms of sulfonylurea action are still not understood completely. Previous data have implicated adenylate cyclase as a possible target for sulfonylurea action, but the observations on the effects of sulfonylureas on this enzyme have been inconsistent. Lasseter *et al.* reported a stimulation of myocardial adenylate cyclase activity by sulfonylureas *in vitro* [1], whereas we have noted an inhibition of renal adenylate cyclase activity [2]. Experiments on direct or indirect consequences of altered adenylate cyclase activities, caused by sulfonylureas, do not clearly support a stimulation of this enzyme activity by these drugs [3–6]. Therefore, we have carried out studies on the effects of chlorpropamide and tolbutamide on myocardial and hepatic adenylate cyclase activity *in vitro*.

Fresh heart or liver, obtained from female Sprague–Dawley rats, 200–250 g, was used in each experiment. After removal, the tissues were placed immediately in iced sucrose (0.25 M). They were next minced with surgical scissors and homogenized in 8 vol. of 25 mM Tris–HCl (pH 7.5) with Ten Broeck glass or stainless steel homogenizers. The homogenate was filtered once over gauze and centrifuged, as described by Marx and Aurbach [7], to isolate partially purified plasma membranes in a 2000 *g* pellet.

Adenylate cyclase activity was assayed, as described by us previously [8, 9], with 1.6 mM adenosine triphosphate (ATP) and an ATP-regenerating system [10]. Cyclic AMP was separated from ATP by elution over alumina, as described by Ramachandran [11]. Neither chlorpropamide nor tolbutamide (10^{-4} or 10^{-2} M) altered the elution of cAMP from alumina by 10 mM Tris–HCl (pH 7.4), as judged by the elution of [^{3}H]cAMP. The protein content of each assay tube was measured by the method of Lowry *et al.* [12]. Values are expressed as means ± S.E.M. The significance of the difference between means was judged by the nonpaired *t*-test with a TI-59 calculator, using programs supplied by the manufacturer (Texas Instruments, Houston, TX).

Chlorpropamide powder was a gift of Pfizer, Inc. (New York, NY); tolbutamide powder was donated by Upjohn, Inc. (Kalamazoo, MI). Both substances were dissolved in ethanol because of their insolubility in water. Therefore, ethanol alone was added to all controls. Epinephrine bitartrate, sodium fluoride, sodium lauryl sulfate, adenosine triphosphate, creatine phosphokinase, creatine phosphate, theophylline, cyclin 3′, 5′-adenosine monophosphate, and Tris–HCl were all purchased from the Sigma Chemical Co. (St. Louis, MO). Neutral alumina was obtained from ICN Pharmaceuticals (Eschwege, West Germany). Disposable plastic chromatography columns with Whatman No. 1 filter paper in the bed support were obtained from the Kontes Glass Co. (Vineland, NJ). [α^{32}P]-ATP and [^{3}H]cAMP, used as recovery standards, and Aquafluor II were purchased from the New England Nuclear Corp. (Boston, MA).

Concentrations of chlorpropamide, equivalent to average serum levels achieved after standard doses of this drug [13], were added alone or with epinphrine (5×10^{-4} M) to partially purified plasma membranes from rat heart or liver (Table 1). Chlorpropamide caused significant inhibition of basal and epinephrine-stimulated enzyme activity. Similar results were obtained with the same concentrations of tolbutamide. Basal and epinephrine-stimulated adenylate cyclase activities in rat liver (Table 2) and heart (Table 3) were decreased by tolbutamide. Similarly, tolbutamide diminished the stimulation of adenylate cyclase activity by glucagon in both tissues (Tables 2 and 3).

To exclude the possibility that these results were caused by the ethanol diluent, we added ethanol alone to all controls. In addition, the effect of the ethanol diluent

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Table 1. Effect of chlorpropamide on adenylate cyclase activity in partially purified plasma membranes from rat heart and liver

Addition	Adenylate cyclase activity* [pmoles cAMP formed·(mg protein)$^{-1}$·min^{-1}] Heart	P	Liver	P
Ethanol†	17.86 ± 1.12		7.42 ± 0.19	
Chlorpropamide, 10^{-4} M	14.72 ± 0.95	< 0.05‡	6.94 ± 0.09	< 0.05‡
Chlorpropamide, 10^{-2} M	5.64 ± 0.49	< 0.001‡	5.58 ± 0.37	< 0.01‡
Epinephrine, 5×10^{-4} M	28.76 ± 0.41§		10.93 ± 0.29§	
Epinephrine, 5×10^{-4} M, + chlorpropamide, 10^{-4} M	15.11 ± 1.46	< 0.005‖	8.98 ± 0.41	< 0.01‖
Epinephrine, 5×10^{-4} M, + chlorpropamide, 10^{-2} M	7.21 ± 0.70	< 0.002‖	4.84 ± 0.30	< 0.0001‖
NaF, 2×10^{-3} M	28.78 ± 2.46§		17.10 ± 0.77§	

* Each value is the mean ± S.E.M. of six determinations.
† Diluent of chlorpropamide, added in appropriate concentrations.
‡ Significance of the difference versus ethanol alone.
§ $P < 0.001$ for difference between this value and value with ethanol alone.
‖ Significance of the difference versus epinephrine alone.

Table 2. Effect of tolbutamide on adenylate cyclase activity in partially purified plasma membranes from rat liver

Addition	Number of observations	Adenylate cyclase activity* [pmoles cAMP formed·(mg protein)$^{-1}$·min^{-1}]	P
Ethanol†	16	7.72 ± 0.22	
Tolbutamide, 10^{-4} M	16	6.74 ± 0.25	< 0.01‡
Tolbutamide, 10^{-3} M	16	5.63 ± 0.27	< 0.001‡
Epinephrine, 5×10^{-4} M	4	15.48 ± 0.99§	
Epinephrine, 5×10^{-4} M, + tolbutamide, 10^{-4} M	4	10.97 ± 0.52	< 0.01‖
Epinephrine, 5×10^{-4} M, + tolbutamide, 10^{-3} M	4	10.47 ± 0.95	< 0.01‖
Glucagon, 10^{-9} M	4	12.01 ± 0.62§	
Glucagon, 10^{-9} M, + tolbutamide, 10^{-4} M	4	8.79 ± 0.92	< 0.01¶
Glucagon, 10^{-9} M, + tolbutamide, 10^{-3} M	4	6.06 ± 0.98	< 0.001¶
Glucagon, 10^{-7} M	4	17.22 ± 0.63§	
Glucagon, 10^{-7} M, + tolbutamide, 10^{-4} M	4	15.22 ± 0.79	< 0.03**
Glucagon, 10^{-7} M, + tolbutamide, 10^{-3} M	4	13.44 ± 0.47	< 0.01**

* Values are means ± S.E.M
† Diluent of tolbutamide, added at appropriate concentrations.
‡ Significance of difference versus ethanol alone.
§ $P < 0.001$ for difference between this value and the value with ethanol alone.
‖ Significance of difference versus epinephrine alone.
¶ Significance of difference versus glucagon (10^{-9} M), alone.
** Significance of difference versus glucagon (10^{-7} M) alone.

Table 3. Effect of tolbutamide on adenylate cyclase activity in partially purified plasma membranes from rat heart

Addition	Number of observations	Adenylate cyclase activity* [pmoles cAMP formed·(mg protein)$^{-1}$·min^{-1}]	P
Ethanol†	8	17.32 ± 0.54	
Tolbutamide, 10^{-4} M	8	15.32 ± 0.58	< 0.01‡
Tolbutamide, 10^{-3} M	8	15.09 ± 0.51	< 0.01‡
Epinephrine, 5×10^{-4} M	4	22.61 ± 1.28§	
Epinephrine, 5×10^{-4} M + tolbutamide, 10^{-4} M	4	19.18 ± 0.88	< 0.02‖
Epinephrine, 5×10^{-4} M, + tolbutamide, 10^{-3} M	4	15.90 ± 1.64	< 0.001‖
Glucagon, 10^{-9} M	4	22.64 ± 0.39§	
Glucagon, 10^{-9} M, + tolbutamide, 10^{-4} M	4	20.56 ± 0.88	< 0.02¶
Glucagon, 10^{-9} M, + tolbutamide, 10^{-3} M	4	16.64 ± 0.77	< 0.001¶

* Values are means ± S.E.M.
† Diluent of tolbutamide added at appropriate concentrations.
‡ Significance of difference versus ethanol alone.
§ $P < 0.001$ for difference between this value and value with ethanol alone.
‖ Significance of difference versus epinephrine alone.
¶ Significance of difference versus glucagon alone.

on adenylate cyclase activity in partially purified plasma membranes from rat liver was studied. Basal adenylate cyclase activity [8.22 ± 0.76 pmoles cAMP formed·(mg protein)$^{-1}$·min^{-1}] was unchanged by the addition of the same concentration of absolute ethanol, 10 μl in 145 μl assay volume, as was added in all other experiments. Ethanol did not modify the stimulation of adenylate cyclase caused by 5×10^{-4} M epinephrine.

Sulfonylureas have complex effects on cyclic AMP metabolism. Not only do they alter adenylate cyclase activity, but they also inhibit cAMP-dependent phosphodiesterase activity [14–16]. The changes induced in cyclic AMP accumulation may be variable, depending upon the tissue studied and the experimental system used [2–4, 16–20). Differences in effect on cyclic AMP metabolism may be determined by the magnitude of phosphodiesterase inhibition, and by any alterations of adenylate cyclase activity [2]. In some studies, reported effects of sulfonylureas on certain tissues are inconsistent with the observed changes in cyclic AMP accumulation [3, 17, 18, 21]. In contrast, the enhancement of vasopressin action on renal medulla [22, 23], or reductions in effects of epinephrine on lipolysis, glucagon on liver, or parathyroid hormone on renal cortex may be explained by parallel changes in tissue concentrations of cyclic AMP [2, 4, 18, 19, 24].

Since sulfonylureas inhibit, with non-uniform potency, cAMP-dependent phosphodiesterase activity in every tissue studied thus far [2, 14–16, 25], variable reductions in adenylate cyclase activity in the same tissues by these agents may explain their differential effects on cyclic AMP accumulation in different tissues. Sulfonylurea-induced stimulation of adenylate cyclase would not account for these overall effects on cyclic AMP. We previously noted that chlorpropamide decreased basal and hormonally stimulated adenylate cyclase activities in renal cortex and medulla [2]. The present study indicates similar effects by both chlorpropamide and tolbutamide on adenylate cyclase activity in rat liver and heart. Therefore, we propose that sulfonylureas decrease adenylate cyclase activity in target tissues as one of their actions.

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