ADENOSINE 3′, 5′-MONOPHOSPHATE-DEPENDENT PROTEIN KINASE IN ADIPOSE TISSUE: INHIBITION BY TOLBUTAMIDE

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Summary

Incubation of rat epididymal fat pads with 4 mM sodium tolbutamide for two hours produced half-maximal inhibition of adenosine 3′, 5′-monophosphate-dependent protein kinase. Related compounds (sodium paratoluenesulfonate, butylurea and sodium sulfadiazine) had minimal effects at similar concentrations. The antilipolytic effect of tolbutamide may be explained by its inhibition of protein kinase.

Introduction

The biochemical mechanism of hormonally-stimulated lipolysis has recently been established. The activation of adipose tissue hormone-sensitive lipase occurs with its phosphorylation by adenosine 3′, 5′-monophosphate (cyclic AMP)-dependent protein kinase (1-3). Lipolytic hormones increase the activity of protein kinase by stimulation of adenyl cyclase and elevating intracellular cyclic AMP (4-5). Insulin and prostaglandin E_1 inhibit lipolysis by interfering with the accumulation of intracellular cyclic AMP (4); whereas, tolbutamide blocks the effect of lipolytic hormones without diminishing the increase in intracellular cyclic AMP (6-7). This communication demonstrates that tolbutamide inhibits cyclic AMP-dependent protein kinase in rat epididymal fat pads.

Methods

Diced epididymal fat pads from fed Wistar rats (175-225 gm) were obtained after decapitation and incubated at 37° C for two hours in Krebs-bicarbonate buffer containing 1.27 mM $CaCl_2$. When added, tolbutamide and related compounds

were present only during the incubation. After incubation fat pads were rinsed and sonicated in cold Krebs-bicarbonate buffer. The aqueous supernatants from centrifugation at 50,000 X g for 30 min. at 4° C contained 0.75 to 1.25 mg protein per ml (8) and were assayed for cyclic AMP-stimulated protein kinase activity (9). The assay was performed in 0.2 ml with these additions, 10 μmoles sodium glycerophosphate pH 7.0, 2 μmoles sodium fluoride, 0.4 μmoles theophylline, 0.1 μmoles ethylene glycol bis (β-aminoethyl ether)-N, N´-tetraacetic acid, 3 μmoles magnesium chloride, 0.3 mg mixed histone, 2 nmoles (γ-^{32}P) ATP, 1 nmoles cyclic AMP when indicated, and 0.05 ml of supernatant.

Results and Discussion

Sodium tolbutamide inhibited both the basal and the cyclic AMP-stimulated protein kinase activities (Fig. 1A) and half maximally inhibited the cyclic

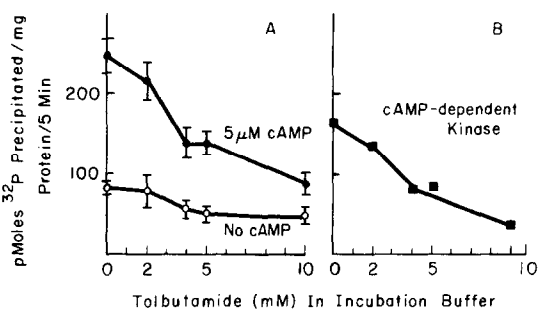

Figure 1. Effect of various concentrations of sodium tolbutamide on cyclic AMP-stimulated protein kinase. In A enzyme activities with and without cyclic AMP in assay tubes are shown with the bars representing S.E.M. In B cyclic AMP dependent enzyme activities were obtained from A by substracting the basal values from the cyclic AMP-stimulated values.

AMP-dependent kinase activity at 4 mM (Fig. 1B). Similar tolbutamide concentrations are required for half maximal inhibition of in vitro lipolysis induced by hormones (norepinephrine and ACTH) or by dibutyryl cyclic AMP plus theophylline (10-11). The components of the tolbutamide molecule, sodium para-toluenesulfonate and butylurea, had no effect alone or in combination

at 4 mM and had minimal effects at 10 mM (Table 1). Sodium sulfadiazine, a sulfonamide without antilipolytic properties (12), also had minimal effect of protein kinase activity at 10 mM (Table 1). When tested against a partially purified protein kinase from bovine adipose tissue, tolbutamide was a potent inhibitor; whereas, the other compounds in Table 1 had no effect

Table 1

Effect of Tolbutamide and Related Compounds

	% Inhibition of Protein Kinase
Na Tolbutamide 4 mM	50
Na p-toluenesulfonate 10 mM	5
Butylurea 10 mM	20
Butylurea 10 mM and Na p-toluenesulfonate 10 mM	20
Na Sulfadiazine 10 mM	10

The percent inhibition was obtained for each compound in experiments similar to those in Figure 1.

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at 10 mM (15). Tolbutamide also inhibits both soluble and membrane-bound protein kinase from canine heart (13). The tolbutamide inhibition of adipose tissue cyclic AMP-dependent protein kinase is one possible explanation for the antilipolytic effects of this drug.

This is the first report of drug action on cyclic AMP-dependent protein kinase in a tissue where the drug effect can be explained by its inhibition of protein kinase. In addition to its well-documented role in the cyclic AMP effects on lipolysis and glycogenolysis (14-15), protein kinase has been postulated as the common mediator of the widely varied responses believed to be controlled by cyclic AMP (16) and is a likely site for the action for

other drugs which antagonize cyclic AMP-mediated processes without alterations in intracellular levels of cyclic AMP.

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