NOTE
Regulation of Muscle Fructose 2, 6-Bisphosphate Levels by Sulfonylureas

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Abstract
We examined the effect of sulfonylureas on the level of fructose 2, 6-bisphosphate (F-2, 6-P₂) in muscle using a mouse hindlimb flow-through perfusion system. The F-2, 6-P₂ level in muscle was increased by adding glibenclamide or tolbutamide in a dose-dependent manner. The stimulatory potency of each drug on F-2, 6-P₂ formation was parallel to its hypoglycemic potency. Sulfonylurea stimulation of muscle F-2, 6-P₂ formation is thought to be an important extrapancreatic action improving the deranged carbohydrate metabolism in diabetics.

It is well known that the hypoglycemic effect of sulfonylurea is attributed in part to its extrapancreatic action. The effect of sulfonylurea on carbohydrate metabolism in the liver and peripheral tissues is the most important one among various extrapancreatic actions of the drug. Sulfonylureas have been reported to stimulate glycogenesis and inhibit gluconeogenesis in the liver by itself or by enhancing insulin action (Fleig et al., 1984; Rinninger et al., 1984; Monge et al., 1986). In addition, the drug potentiates insulin-induced recruitment of glucose transport carrier in adipocytes (Jacobs and Jung, 1985) and enhances insulin action on glucose uptake in skeletal muscle (Feldman and Lebovitz, 1969; Daniels and Lewis, 1982).

Recently, we have reported that sulfonylurea stimulated liver fructose 2, 6-bisphosphate (F-2, 6-P₂) formation and the stimulatory effect of the drug is in proportion to its hypoglycemic potency (Matsutani et al., 1984; Hatao et al., 1985). F-2, 6-P₂ was found to be a potential activator of phosphofructokinase, a key enzyme of glycolysis (Pilkis et al., 1981), and the level of F-2, 6-P₂ is known to be regulated by pancreatic hormones, glucagon and insulin (Richards and Uyeda, 1980; Richards and Uyeda, 1982). Glucagon suppression of liver F-2, 6-P₂ formation is released by tolbutamide (Matsutani et al., 1984). These effects of the drug on the F-2, 6-P₂ level have not been demonstrated in peripheral tissues.

In the present study, we investigated the effect of sulfonylureas on the level of muscle F-2, 6-P₂ in a mouse hindlimb perfusion system.

Materials and Methods
Male ddY mice, weighing 25–30 gm, were
faeted overnight. Following anaesthesia with ether, the abdomen was opened and a poly-
ethylene catheter was inserted into the descending aorta. Perfusion was started with Krebs-Ringer bicarbonate buffer gassed with 95% O₂-5% CO₂ (pH 7.4, 37°C) containing 5 mM glucose. The vena cava was cut and the reproductive organs were ligated. A peristaltic pump delivered perfusate at a constant flow rate of 3 ml per minutes. Minimum dead space was obtained by utilizing a double peristaltic pump and three way plugs. After preperfusion for 10 minutes, perfusate containing sulfonylurea was applied. About 50 mg of biceps femoris was biopsied after the perfusion, and the sample was rapidly frozen in acetone-dry ice and kept at -80°C until assay of F-2, 6-P₂.

The frozen sample of liver was placed in 600 µl of 0.2 M iced cold Tris-HCl buffer (pH 7.2) containing 0.5 mM EGTA and 5 mM MgCl₂, and 200 µl of 0.5 N NaOH were added. The sample was homogenized at first with a glass homoge-
nizer and then with a sonifier. The homogenate was then heated at 80°C for 20 minutes and centrifuged at 50,000 × g for 30 minutes, and the supernatant was used for F-2, 6-P₂ determination.

The F-2, 6-P₂ was assayed by the method of Furuya and Uyeda (1980). The reaction mixture in a final volume of 1 ml consisted of the fol-
lowing: 100 mM Tris-HCl buffer (pH 7.5) con-
taining 2 mM Mg²⁺, 0.25 mM fructose-6-phos-
phate, 10 mM ATP, 2.5 mM dithiothreitol, 0.2 mM EDTA, 0.16 mM NADH, 0.01 U phospho-
fructokinase, 0.4 U aldolase, 2.4 U triose-
phosphate isomerase, and 0.4 U glyceral-3-
phosphate dehydrogenase. The sample (20 µl) was added to 0.98 ml of the reaction mixture in the assay cuvette, and the reaction velocity was determined spectrophotometrically at 37°C com-
pared with a known amount of standard F-2, 6-P₂ concentration was reflected by the difference in activity before and after the acid treatment.

Student’s t-test were used in the statistical evaluation of the results. A probability of p<0.05 was considered to be statistically significant.

Results

As shown in Fig. 1, tolbutamide (1 mM) and glibenclamide (1 µM) elevated the F-2, 6-P₂ level in the perfused muscle. The level of F-2, 6-P₂ reached equilibrium by 10 minutes after the start of perfusion with the effector. Accordingly, the perfusion with sulfonylurea was done for 10 minutes in all experiments. In this experiment the mean basal level of F-2, 6-P₂ of the mouse muscle was 3.5±0.5 nmol/g wet wt.

The effects of various concentrations of glibenclamide and tolbutamide on the F-2, 6-P₂ level during 10 min perfusion are shown in Fig. 2 and Fig. 3. glibenclamide

![Fig. 1. Time course of F-2, 6-
P₂ level in the absence of sulfonylureas (●), and in the presence of 1 µM gliben-
clamide (○) and 1 mM tol-
butamide (▲) in perfused mouse muscle. Hindlimbs of overnight fasted normal ddy male mice were perfused with KRB buffer (pH 7.4, 37°C) by a flow through technique. Each value is the mean for two separate experiments.](image-url)
Fig. 2. Effect of glibenclamide on F-2, 6-P$_2$ level in perfused mouse muscle. Hindlimbs of overnight fasted normal male mice were perfused with KRB buffer (pH 7.4, 37°C) containing 1 μM glibenclamide for 10 minutes by a flow through technique. Each value represents the mean ± SEM for six experiments. *: p<0.05 compared with the control level.

Fig. 3. Effect of tolbutamide on F-2, 6-P$_2$ level in perfused mouse muscle. Hindlimbs of overnight fasted normal male mice were perfused with KRB buffer (pH 7.4, 37°C) containing 1 mM tolbutamide for 10 minutes by a flow through technique. Each value represents the mean ± SEM for six experiments. *: p<0.05 compared with the control level.
at the concentration of $10^{-7}$ M stimulated F-2, 6-P$_2$ formation significantly and the maximum effect of the drug was obtained at $10^{-6}$ M. But a higher concentration of the drug ($10^{-5}$ M) did not exhibit any effect on the activator formation (Fig. 2). The maximum effect of tolbutamide was observed at a concentration of $10^{-8}$ M (Fig. 3). There was no effect of tolbutamide at a higher concentration ($10^{-2}$ M) as in the case of glibenclamide (data not shown).

**Discussion**

The present study demonstrated that the F-2, 6-P$_2$ level in mouse muscle was increased by adding sulfonylureas using a mouse hindlimb flow-through perfusion system. The concentrations of glibenclamide (1 μM) and tolbutamide (1 mM) which produced the maximum effect on F-2, 6-P$_2$ formation corresponded with their therapeutical levels at oral administration (Zecca et al., 1985).

As shown in Fig. 2, higher concentration of sulfonylurea than that producing the maximal biologic effect exhibited smaller stimulatory effects on F-2, 6-P$_2$ formation compared with those in the lower concentrations. In muscle as well as in liver (Hatao et al., 1985), sulfonylurea within a limited range of concentration stimulates F-2, 6-P$_2$ production in a dose-dependent manner. Although it is often observed that a high concentration of the drug has an opposite effect to a low concentration, the mechanism and significance of the decreased effect of sulfonylurea at a higher concentration on F-2, 6-P$_2$ formation remains to be clarified.

On the other hand, the physiological role of F-2, 6-P$_2$ in skeletal muscle has not been clearly understood since the muscle F-2, 6-P$_2$ level is significantly lower than the level in the liver. Dall’aglio et al. (1986) reported that a decrease in F-2, 6-P$_2$ associated with insulin deficiency was observed in the liver but not in skeletal muscle. However, the F-2, 6-P$_2$ level in muscle reported by Dall’aglio et al. (1986) was much lower than that obtained in our study or in previous studies (Kuwajima and Uyeda, 1982; Thomas and Uyeda, 1986). Our results clearly show that sulfonylurea raises the muscle F-2, 6-P$_2$ level by twice the basal level or more. Daniels and Lewis (1982) reported that tolbutamide potentiated the glucose uptake in perfused rat hindlimbs, and our observations appear to support their results with respect to the mechanism of drug action. In addition, Uyeda et al. (1981) demonstrated that F-2, 6-P$_2$ counteracts ATP or citrate inhibition of phosphofructokinase and decreases the Km value of the enzyme for fructose-6-phosphate in muscle.

These facts strongly suggest that the stimulatory effect of sulfonylurea on muscle F-2, 6-P$_2$ formation plays an important role in improving the deranged carbohydrate metabolism in the muscle of diabetics by stimulating glycolysis.

**References**


