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EFFECTS OF SULFONYLUREAS (TOLBUTAMIDE, GLIPENTIDE AND GLIBENCLAMIDE) ON *IN VITRO* GLYCEROL METABOLISM IN ADIPOSE TISSUE FROM RATS.[1]

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SUMMARY

The effects of tolbutamide, glipentide and glibenclamide on the utilization of glycerol in adipose tissue from fed rats was studied. Glipentide and glibenclamide reduced the production of glycerol, inhibited the uptake of 1-^{14}C glycerol and inhibited the conversion of 1-^{14}C glycerol to $^{14}CO_2$ and ^{14}C-glyceride-glycerol. Tolbutamide had less of an effect on the parameters studied;only at a high concentration (1mg/ml) did it reduce the formation of ^{14}C-glyceride-glycerol. The differences between the effects of sulfonylurea compounds versus modulators of intracellular cyclic-AMP levels (insulin, epinephrine, theophylline) on lipid metabolism is pointed out. The data support the possibility that sulfonylureas might act as uncoupling agents in adipose tissue.

Most of the pharmacological effects of sulfonylurea compounds are believed to be due to the stimulant action of these drugs on the release of insulin from the pancreas (1-5).However, there are also some effects of sulfonylureas that are probably not mediated by pancreatic insulin secretion.One of the clearest examples of an extrapancreatic action is the inhibition of adipose tissue lipolysis (6-9). While the mechanism responsible for

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the effects of sulfonylureas on lipolysis is unknown, it has been suggested that they might act as uncoupling agents of oxidative phosphorylation in adipose tissue (10).

Since adipose tissue metabolizes large amounts of glycerol via a pathway which requires the ATP dependent phosphorylation of glycerol (11-16), support for the notion that sulfonylureas act as uncoupling agents would be obtained if they were found to inhibit the utilization of glycerol in adipose tissue. This point was studied in the present investigation in which the effect of tolbutamide and two other hypoglycemic sulfonylureas, glipentide (UR-661) (N-4-{β(O-anisamide)-ethyl}-benzenesulfonyl-N'-cyclopentylcarbamide) and glibenclamide (N-4-{2-(5-chloro-2-methoxybenzamide)-ethyl}-phenyl-sulfonyl-N'-cyclohexil-urea) on the *in vitro* metabolism of glycerol in adipose tissue was determined.

MATERIALS AND METHODS

Pieces of lumbar fat pads (18 ± 3 mg/vial) from fed female Wistar rats weighing 159 ± 8 g were incubated in 1 ml of Krebs Ringer bicarbonate buffer, pH 7.4 (17) containing 0.5 μCi of 1-^{14}C-glycerol (32.5 mCi/mmole) and 10 mg of bovine albumin purified by the method of Chen (18).The concentrations of tolbutamide, glipentide and glibenclamide used were 1,10,50,100 or 1.000 μg/ml. Dilution of the drugs was carried out in chloroform. The proper amount of the drug was added to each empty vial in a volume of 100 μl. Chloroform alone (100 μl) was added to control vials. The vials were blown to dryness under N_2 at 37° and shaken intensely with the incubation medium for 90 min before placing the tissues in them. Incubation was carried out for 180 min at 37°C under O_2/CO_2 (95:5), with shaking (100 cycles/min). The samples were processed as previously described (13,14) for the development and analysis of $^{14}CO_2$, the enzymatic determination of glycerol in the media (19) and the purification (20) and fractionation of lipids in the tissues (14).

RESULTS

The effects of tolbutamide, glipentide and glibenclamide on parameters of lipid metabolism in adipose tissue *in vitro* is presented in Table 1. In accordance with previous reports (21),

Table 1. The effects of sulfonylurea compounds on *in vitro* glycerol metabolism in adipose tissue from rats.

Drug conc (µg/ml)	Production of glycerol (µmoles/100mg)	Uptake of (1-^{14}C) glycerol†	Formation of $^{14}CO_2$†	Formation of ^{14}C-glyceride-glycerol†
No Drug Control	0.256±0.027	5.87±0.17	2.16±0.43	3.71±0.32
Tolbutamide				
1	0.314±0.034	7.03±0.92	2.75±0.77	4.27±0.68
10	0.362±0.129	5.79±1.30	2.49±0.61	3.29±0.84
50	0.313±0.062	6.04±0.76	2.72±0.66	3.32±0.46
100	0.280±0.034	6.09±0.94	2.18±0.71	3.92±1.20
1000	0.211±0.055	4.57±1.01	2.29±0.55	2.28±0.46*
Glipentide				
1	0.213±0.023	6.26±0.97	2.38±1.05	3.89±0.55
10	0.226±0.025	6.71±1.41	2.92±1.50	3.79±0.32
50	0.180±0.015*	6.38±1.34	2.42±0.78	3.97±0.92
100	0.149±0.025*	5.17±1.25	1.85±0.45	3.33±0.97
1000	0.079±0.037**	3.06±0.73**	1.09±0.59*	1.05±0.24***
Glibenclamide				
1	0.250±0.066	5.80±1.21	2.10±0.68	3.70±0.73
10	0.298±0.071	5.31±1.11	1.19±0.59	3.03±0.63
50	0.260±0.038	5.18±1.12	2.29±0.68	3.15±0.43
100	0.169±0.060*	4.85±1.15	2.15±0.45	1.61±0.43***
1000	0.049±0.018***	1.51±0.37***	0.83±0.22***	1.07±0.05***

Values are means ± S.E.M. for 5 rats/group
† = expressed as % of initial radioactivity/100 mg
* = statistically significant compared to Control values
(* = $p<.05$, ** = $p<.01$, *** = $p<.001$).

tolbutamide in concentrations up to 1 mg/ml in the incubation medium failed to significantly influence the production of glycerol. In contrast, both glipentide and glibenclamide had very intense effects on basal lipolysis, significantly reducing the production of glycerol at concentrations of 50 µg/ml and 100 mg/ml, respectively. Tolbutamide in concentrations up to 1 mg/ml

failed to significantly alter the uptake of 1-^{14}C glycerol while glibenclamide and glipentide both significantly reduced this parameter. The effect of glipentide on the uptake of 1-^{14}C glycerol was greatest and at a concentration of 1 mg/ml the value was 26% of the control level.

The control values for the formation of $^{14}CO_2$ and ^{14}C-glyceride-glycerol show that practically all the labelled glycerol taken up by the tissue was transformed into these two metabolites. This agrees with previous experiments in which the incubation of glycerol was carried out in the absence of glucose (13-16) as in the present study. Tolbutamide in the concentrations employed did not exert a statistically significant effect on the formation of $^{14}CO_2$ while both glipentide and glibenclamide at high concentrations decreased it significantly.The formation of ^{14}C-glyceride-glycerol from 1-^{14}C-glycerol was reduced by all three sulfonylureas, but higher concentrations of tolbutamide and glipentide were required compared to glibenclamide in order to produce a statistically significant reduction in the formation of this metabolite.

DISCUSSION

The present findings show that the well-known antilipolytic effect of sulfonylurea compounds in adipose tissue (10) is accompanied by an intense inhibition of glycerol uptake and metabolism. The so-called second generation sulfonylureas (22), glipentide and glibenclamide, were more potent than tolbutamide, a first generation compound, in their effects on glycerol metabolism in adipose tissue. The comparative potencies of these drugs on adipose tissue *in vitro* can be associated to their hypoglycemic effects *in vivo* as it is well known that both glipentide and glibenclamide are more potent hypoglycemic agents than tolbutamide (22,23). It is to be noted that the inhibitory action of the sulfonylureas on glycerol uptake might have been underestimated in the present investigation. The dilution of isotopic glycerol by cold glycerol produced by the tissue during incubation could affect the calculation of glycerol uptake. Since the production of glycerol was reduced by the sulfonylurea agents, the specific activity of labelled glycerol was greater in the vials incubated with the drugs than in the control samples. As a result, the ac-

tual inhibitory effect of the drugs on the uptake of glycerol by the tissue was probably greater than the level calculated from the data obtained. It is reasonably certain that the effects of sulfonylurea compounds on glycerol metabolism in adipose tissue *in vitro* is not mediated by insulin since this hormone typically enhances the uptake and oxidation of glycerol in adipose tissue (24) while the sulfonylureas inhibited these parameters. It is also unlikely that the effect of sulfonylureas on lipolysis and glycerol metabolism in adipose tissue can be accounted for on the basis of their enhancing effect on cyclic AMP levels (9) since epinephrine and theophylline also enhance the level of cyclic AMP in adipose tissue (10,25,26) but they have opposite effects to sulfonylureas on lipolysis in adipose tissue and do not affect the production of CO_2 from glycerol (24,27).

The action of tolbutamide as an uncoupling agent in brown fat cells (28) raises the possibility that oxidative phosphorylation might be uncoupled by sulfonylureas in white adipose tissue as well. Thus, one possible explanation for the effects of sulfonylurea compounds on glycerol metabolism is that they might reduce the availability of ATP which is required to activate the hormone-sensitive lipase (29) and to phosphorylate glycerol (11, 12) for metabolism in the tissue (14). Further studies are required to test this hypothesis, but whatever the mechanism for the influence of sulfonylureas on glycerol metabolism turns out to be, it is evident from the present study that these intense effects must be taken into account in order to understand the action of these compounds on adipose tissue.

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