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Uncoupling Action of Sulfonylureas on Brown Fat Cells

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SUMMARY

Tolbutamide markedly enhanced glucose oxidation to carbon dioxide and inhibited lactate formation by rat brown fat cells in the presence of insulin or cysteine. Tolbutamide alone increased oxygen uptake, and this stimulation was enhanced by insulin or cysteine. The effects of tolbutamide were similar to those of carbonyl cyanide *m*-chlorophenylhydrazone, an uncoupler of oxidative phosphorylation. The increase in glucose oxidation by tolbutamide or the uncoupling agent was not due to the stimulation of glucose metabolism via the pentose shunt pathway. The hypoglycemic sulfonylureas, tolazamide and glyburide, also stimulated glucose oxidation and respiration in the presence of insulin, but to a lesser extent than tolbutamide. Carboxytolbutamide, which has no hypoglycemic action, did not affect brown fat cell metabolism. Tolbutamide also stimulated respiration and glucose oxidation by rabbit brown fat cells in the presence of insulin. Tolbutamide did not affect the oxidation of glucose by white fat cells in either the presence or absence of insulin. Phenformin stimulated lactate formation by rabbit and rat brown fat cells and rat white fat cells. Phenformin inhibited glucose oxidation by rat white or brown fat cells in the presence of insulin. The results suggest that the effects of tolbutamide on brown fat cells are probably due to an uncoupling action, while those of phenformin are due to inhibition of respiration.

INTRODUCTION

Sulfonylureas are used in the treatment of mild maturity onset diabetes mellitus. However, their mechanisms of action are still obscure. Most believe that the hypoglycemic effect is due to the stimulation of insulin release in pancreatic β-cells (1, 2). Others favor the idea that sulfonylureas act on extrapancreatic sites, since the release of insulin alone cannot account for the long-term metabolic effects of tolbutamide (3). Many extrapancreatic sites of action have been investigated and reported. Among these are the inhibition of hepatic ketogenesis in rat liver slices (4), increased glucose uptake and glycogen synthesis in rat white adipose tissue (5), and potentiation of insulin action on glucose uptake by skeletal muscles (6). Some have suggested that sulfonylureas directly potentiate insulin action by other extrapancreatic mechanisms (7, 8). De Schepper and de Beer (9, 10) found that chlorpropamide and tolbutamide increased oxygen uptake by rat liver mitochondria and reduced ATP levels in liver slices.

Recent interest in the effects of sulfonylureas on adipose tissue is due to the work of Stone and his associates (11–13). They found that sulfonylureas exert an antilipolytic action in rat white adipose tissue and isolated rat white fat cells.

The present studies were designed to

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investigate the effects of tolbutamide and its derivatives on isolated brown fat cells of rats and rabbits in order to enhance our general understanding of the effects of sulfonylureas on metabolism. We also compared the effect of tolbutamide with that of phenformin on the metabolism of brown and white fat cells.

MATERIALS AND METHODS

Brown fat cells were isolated from the dorsal interscapular brown tissue of 130–160-g Sprague-Dawley female rats (Charles River CD strain) fed laboratory chow ad libitum. Cells were isolated by digestion of the minced brown fat pads with crude bacterial collagenase (Worthington) at a concentration of 1 mg/ml in phosphate buffer containing 4% albumin (14). The buffer was prepared fresh daily, and the pH was adjusted to 7.4 after addition of bovine fraction V albumin powder (lot 30,709) obtained from Armour Pharmaceutical Company. The phosphate buffer contained NaCl, 128 mM; $CaCl_2$, 1.4 mM; $MgSO_4$, 1.4 mM; KCl, 5.2 mM; and Na_2HPO_4, 10 mM (pH adjusted to 7.4 with HCl). In the studies using rabbits, brown fat cells were obtained similarly from the dorsal interscapular brown fat pads of young rabbits (New Zealand strain, weighing around 500 g). White fat cells were obtained by digestion of the parametrial adipose tissue with 0.5 mg/ml of collagenase (15).

Oxygen consumption was measured in a Gilson respirometer at 37°. The cells were added to siliconized Warburg side arm respirometer flasks containing 3 ml of medium and gassed with 100% oxygen. CO_2 was absorbed by the addition of 0.2 ml of 5% KOH to rolled strips of filter paper (5 × 80 mm) in the center well. Respiration was measured after an initial 30 min of incubation, which served as the equilibration period. In the experiments with labeled substrates, the filter papers were removed at the end of the incubation period and counted in Bray's solution (16).

In experiments when respiration was not measured, the cells were incubated in 17 × 100 mm polyethylene tubes containing 1.5 ml of medium. Brown fat cells were gassed with 100% oxygen, and the tubes were capped and incubated in a water bath at 37°. Carbon dioxide was collected on rolled filter papers in disposable plastic center wells (Kontes Glass Company) attached to the rubber caps. At the end of the incubation period, 0.2 ml of Hyamine hydroxide (Packard Instrument Company) was added to the filter paper, and 0.25 ml of 1 N H_2SO_4 to the medium. The filter papers and wells were removed after 30 min and counted in Omnifluor-toluene solution.

At the end of incubation period, aliquots of the medium were taken for lactate analysis (17). Total lipids were extracted from the remaining medium plus cells by a modification of the procedure of Dole and Meinertz (18) (hexane was substituted for heptane), and aliquots of the hexane layer were evaporated and then counted in 10 ml of Omnifluor-toluene scintillation solution (14). Total fatty acids were obtained by saponification of an aliquot of the hexane extract, followed by acidification and extraction with hexane (14). Glyceride-glycerol formation was calculated by subtraction of the radioactivity in total fatty acids from that in total lipids (14). The formation of carbon dioxide, total lipids, fatty acids, and lactate is expressed as micromoles of glucose converted to these products during the incubation period per millimole of triglyceride. Triglyceride content was determined from the total fatty acid content (14) and is used for convenience to express the amount of cells present in each experiment.

The sources of the chemicals were as follows. Tolazamide [Tolinase; 1-(hexahydro-1*H*-azepin-1-yl)-3-(*p*-tolylsulfonyl) urea, lot ZK 610], carboxytolbutamide [1-butyl-3-(*p*-carboxyphenylsulfonyl)urea, lot 7262-CHV-77], and glyburide [glibenclamide; HB-419; 1 - [*p* - [2 - (5 - chloro - *o* - anisamido)phenyl]sulfonyl]-3-cyclohexylurea, lot 9734-PEM-161] were gifts of Dr. William E. Dulin of the Upjohn Company. Crystalline porcine insulin was a gift of Eli Lilly and Company; it contained less than 0.0003% glucagon by weight. Glucose-U-^{14}C,[1] glucose-6-^{14}C, and glucose-1-^{14}C were

[1] The abbreviations used are: glucose-U-^{14}C, glucose uniformly labeled with ^{14}C; *m*-CCP, carbonyl cyanide *m*-chlorophenylhydrazone.

obtained from the New England Nuclear Corporation. Carbonyl cyanide *m*-chlorophenylhydrazone, valinomycin, phenformin (*N* - β - phenethylbiguanide hydrochloride), tolbutamide, and other chemicals were obtained commercially.

RESULTS

In isolated rat brown fat cells, tolbutamide alone increased, but not significantly, the conversion of glucose to CO_2 (Table 1). However, in the presence of insulin, glucose oxidation was markedly increased by tolbutamide (Table 1).

Cysteine is one of a number of thiol compounds which at relatively high concentrations stimulate glucose metabolism by white (19) and brown (20) fat cells. Cysteine, like insulin, increased glucose conversion to carbon dioxide in brown fat cells (Table 2). The stimulation by tolbutamide of glucose oxidation was almost 20 times greater in the presence than in the absence of cysteine (Table 2).

Tolbutamide at a concentration of 1 mg/ml tripled the rate of respiration in the presence of 2.8 mM (Table 2) or 30 mM glucose (Table 3). The stimulation of respiration by tolbutamide was doubled in the presence of cysteine (Tables 2 and 3). The effect of tolbutamide on respiration in either the presence or absence of cysteine was unaffected by increasing the concen-

TABLE 1

Effect of tolbutamide with and without insulin on glucose metabolism by brown fat cells

Brown adipose tissue of normal fed rats was digested with 1 mg/ml of collagenase for 1 hr. The cells were incubated (1.92 μmoles of triglyceride per tube) for 3 hr in 1.5 ml of medium containing 4% albumin and 2.8 mM glucose-U-^{14}C. The values without tolbutamide are the means of three experiments, and the increments due to added tolbutamide are the means ± standard errors of the paired differences.

Addition	Glucose conversion to CO_2			
	Without tolbutamide	Increment due to tolbutamide at		
		0.13 mg/ml	0.33 mg/ml	1 mg/ml
	μmoles/mmole triglyceride			
None	4.5 ± 1.0	0.3 ± 0.3	1.1 ± 0.8	5.0 ± 2.7
Insulin, 0.083 milliunit/ml	10.0 ± 4.0	2.3 ± 3.1	15.1 ± 2.2	34.7 ± 6.8
Insulin, 0.4 milliunit/ml	22.3 ± 4.0	3.7 ± 0.8	29.4 ± 8.5	46.0 ± 6.4

TABLE 2

Effects of tolbutamide and cysteine on brown fat cell metabolism in the presence of 2.8 mM glucose

Brown adipose tissue from normal fed rats was digested with 1 mg/ml of collagenase for 1 hr. The cells (4.85 μmoles of triglyceride per flask) were incubated for 2 hr in 3 ml of medium containing 4% albumin and 2.8 mM glucose-U-^{14}C. The values without tolbutamide are the means of three experiments, and the increments due to added tolbutamide are the means ± standard errors of the paired differences.

Addition	Oxygen consumption			Glucose conversion to CO_2		
	Without tolbutamide	Increment due to tolbutamide at		Without tolbutamide	Increment due to tolbutamide at	
		0.33 mg/ml	1 mg/ml		0.33 mg/ml	1 mg/ml
	μl/μmole triglyceride			*μmoles/mmole triglyceride*		
None	5.3 ± 0.4	1.9 ± 0.6	11.4 ± 3.4	4.3 ± 0.4	1.0 ± 0.2	3.2 ± 0.4
Cysteine, 1 mM	17.3 ± 2.8	7.1 ± 4.8	27.3 ± 11.0	18.2 ± 1.6	16.4 ± 2.6	57.1 ± 2.5

TABLE 3

Effects of tolbutamide with cysteine and cysteine + insulin on respiration in the presence of 30 mM glucose

Brown adipose tissue from normal fed rats was digested with 1 mg/ml of collagenase for 1 hr. The cells (3.92 μmoles of triglyceride per flask) were incubated for 2 hr in 3 ml of medium containing 4% albumin and 30 mM glucose. The values without tolbutamide are the means of three experiments, and the increments due to added tolbutamide are the means ± standard errors of the paired differences.

Addition	Without tolbutamide	Increment due to tolbutamide at	
		0.33 mg/ml	1 mg/ml
	μl O_2 consumed/μmole triglyceride		
None	5.7 ± 0.7	4.0 ± 3.1	13.9 ± 3.5
Cysteine, 1 mM	13.1 ± 5.8	8.0 ± 2.2	23.4 ± 6.7
Cysteine, 1 mM, + insulin, 0.4 milliunit/ml	13.3 ± 1.9		24.3 ± 9.6
Insulin, 0.4 milliunit/ml	5.2 ± 0.7		

TABLE 4

Effect of tolbutamide and phenformin on brown fat cell metabolism in the presence of 3 and 30 mM glucose

Brown adipose tissue from fed rats was digested with 1 mg/ml of collagenase for 1 hr. The cells (6 μmoles of triglyceride per flask) were incubated for 2 hr in 1.5 ml of medium containing 4% albumin and 3 or 30 mM glucose-U-^{14}C. The basal values are the means ± standard errors of three experiments, and the increments due to added insulin are the means ± standard errors of paired differences. The concentration of tolbutamide was 1 mg/ml, and that of phenformin was 240 μg/ml (1 mM).

Addition	Glucose concentration	Glucose-U-^{14}C conversion to CO_2		Glucose conversion to lactate	
		Without insulin	Increment due to insulin	Without insulin	Increment due to insulin
	mM	*μmoles/mmole triglyceride*		*μmoles/mmole triglyceride*	
None	3	1.9 ± 0.6	12 ± 1.5	5 ± 0.8	36 ± 6
	30	3.5 ± 1.5	15.8 ± 4.0	9 ± 6	48 ± 5
Tolbutamide	3	4.9 ± 2.5	28 ± 6.0	1 ± 0.4	24 ± 10
	30	11.3 ± 4.3	31.3 ± 3.0	6 ± 3	38 ± 7
Phenformin	3	1.9 ± 0.4	0.8 ± 0.4	18 ± 3	38 ± 5
	30	3.5 ± 0.3	2.4 ± 0.9	38 ± 7	63 ± 11

tration of glucose from 2.8 to 30 mM (Tables 2 and 3).

The effect of tolbutamide on glucose metabolism in 3 mM as compared with 30 mM glucose was examined in the experiments shown in Table 4. Glucose oxidation in both the presence and absence of tolbutamide was doubled by increasing the concentration of glucose 10-fold (Table 4). The increment in glucose oxidation due to insulin or to tolbutamide plus insulin was not significantly affected by the concentration of glucose in the medium (Table 4).

Phenformin (phenethylbiguanide) is one of a number of biguanide derivatives which are oral hypoglycemic agents used in the treatment of diabetes mellitus. Phenformin inhibits glucose oxidation by white adipose tissue and increases lactate formation (21–23). Phenformin at a concentration of 1 mM did not significantly inhibit basal glucose conversion to carbon dioxide but did significantly inhibit that due to insulin in rat brown fat cells (Table 4). Phenformin increased lactate formation but was without affect on the marked stimulation of lactate formation seen in the presence of insulin (Table 4).

We carried out experiments using glucose-1-^{14}C and glucose-6-^{14}C as substrates to see whether the increase in glucose

metabolism by tolbutamide was due to stimulation of the pentose shunt pathway. The effects of tolbutamide were also compared with those of the uncoupling agent *m*-CCP and the antibiotic valinomycin. The C-1:C-6 ratio of the basal values for CO_2 formation from glucose oxidation was 1.3, while that for the increase due to insulin was 1.8 (Fig. 1). The C-1:C-6 ratio of the increase due to tolbutamide at 0.33 mg/ml was 1.0 and rose to 1.3 in the presence of insulin (Fig. 1). At a higher concentration of tolbutamide (1 mg/ml), the C-1:C-6 ratio was 0.9 in the absence and 1.1 in the presence of insulin.

The C-1:C-6 ratio due to *m*-CCP (2.5×10^{-5} M) alone was 0.8 in the absence and 1.0 in the presence of insulin (Fig. 2). The C-1:C-6 ratio of the increase in glucose oxidation due to valinomycin was 1.1 in the absence and 1.0 in the presence of insulin. No significant potentiation of valinomycin action on glucose metabolism was seen in the presence of insulin (Fig. 2).

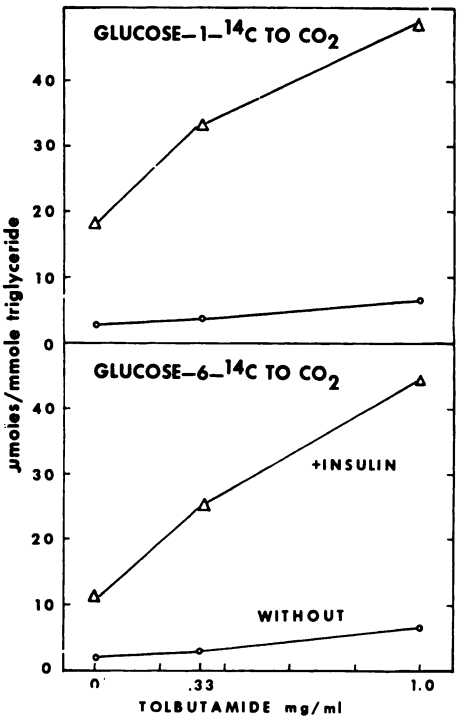

FIG. 1. *Stimulation of carbon dioxide formation from glucose-1-^{14}C or glucose-6-^{14}C by tolbutamide with and without insulin*

Brown fat cells (5.9 μmoles of triglyceride) were incubated for 2 hr in 1.5 ml of medium containing 4% albumin buffer and 2.8 mM glucose-1-^{14}C (upper half of the figure) or glucose-6-^{14}C (lower half). ○, values in the absence of insulin; △, those in the presence of 0.4 milliunit/ml of insulin. The values for lactate formation and glucose conversion to total lipid in these experiments are shown in Table 5.

The data in Table 5 indicate that while tolbutamide, *m*-CCP, and valinomycin increased glucose oxidation, these agents not only failed to stimulate glucose conversion to total lipid and lactate formation but in some cases actually decreased their formation. In the presence of insulin the results were even more striking; neither tolbutamide, *m*-CCP, nor valinomycin increased glucose conversion to total lipid or lactate (Table 5).

The effects of tolbutamide on glucose metabolism by white fat cells were quite different from our observations with brown fat cells (Table 6). Tolbutamide had no significant effect on glucose oxidation or conversion to fatty acids in either the presence or absence of insulin (Table 6). The C-1:C-6 ratio of the basal values was approximately 0.6 in white fat cells, while the ratio of the increments in glucose oxidation due to insulin was 5.2, which is considerably greater than was seen in brown fat cells (Fig. 2).

Phenformin stimulated lactate formation by white fat cells (Table 6). However, this drug inhibited the basal conversion of glucose to carbon dioxide and total lipid by white fat cells and almost completely blocked the stimulation by insulin of glucose oxidation and conversion to lactate and fatty acids (Table 6). All the studies with white fat cells were performed in the presence of 0.4 mM theophylline so that the antilipolytic action of tolbutamide and phenformin could be examined. In the studies shown in Table 6 with white fat cells there was a marked inhibition of glycerol release by phenformin and tolbutamide (not shown), and we have seen similar antilipolytic effects of both drugs on brown fat cells.[2]

Tolazamide and glyburide are sulfonyl-

[2] Unpublished observations.

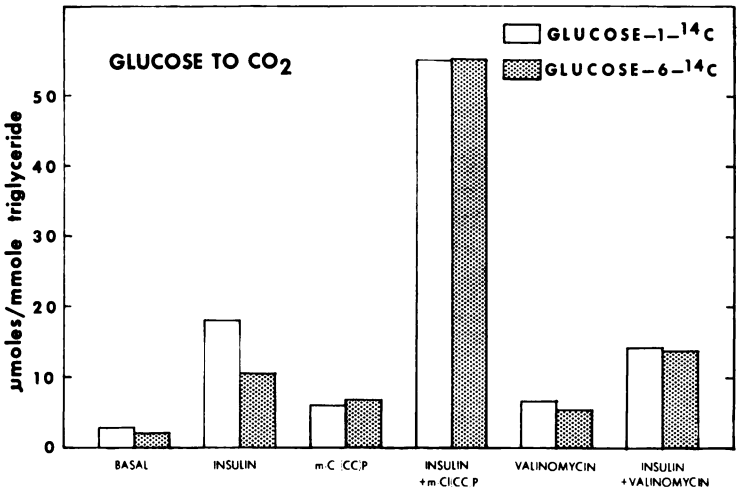

FIG. 2. *Effects of insulin on glucose-1-^{14}C or glucose-6-^{14}C conversion to CO_2 in brown fat cells incubated with carbonyl cyanide m-chlorophenylhydrazone [m-Cl(CC)P] or valinomycin*

Brown fat cells (5.9 μmoles of triglyceride per flask) were incubated for 2 hr in 1.5 ml of medium containing 4% albumin. The unshaded bars represent cells incubated with 2.8 mM glucose-1-^{14}C, and the stippled bars, those incubated with 2.8 mM glucose-6-^{14}C. The concentrations of the different reagents were as follows: insulin, 0.4 milliunit/ml; carbonyl cyanide *m*-chlorophenylhydrazone, 2.5 × 10^{-5} M; valinomycin, 2 μg/ml. The values for lactate formation and glucose conversion to total lipid in these experiments are shown in Table 5.

TABLE 5

Stimulation of lactate and total lipid formation by insulin in brown fat cells incubated with tolbutamide, m-CCP, or valinomycin

Brown adipose tissue from fed rats was digested with 1 mg/ml of collagenase for 1 hr. The cells (5.9 μmoles of triglyceride per flask) were incubated for 2 hr in 1.5 ml of medium containing 4% albumin and 2.8 mM glucose-1-^{14}C or glucose-6-^{14}C. The basal values are the means of three experiments, and the increments due to added insulin (0.4 milliunit/ml) are the means ± standard errors of the paired differences. Glucose conversion to carbon dioxide in the same experiments is shown in Figs. 1 and 2.

Additions	Glucose conversion to lactate		Glucose-1-^{14}C conversion to total lipid		Glucose-6-^{14}C conversion to total lipid	
	Basal	Increment due to insulin	Basal	Increment due to insulin	Basal	Increment due to insulin
	μmoles/mmole triglyceride		*μmoles/mmole triglyceride*		*μmoles/mmole triglyceride*	
None	−0.5 ± 0.8	21.2 ± 2.5	1.1 ± 0.2	10.2 ± 0.7	1.9 ± 0.2	10.4 ± 1.2
Tolbutamide, 0.33 mg/ml	−1.0 ± 0.2	14.9 ± 2.2	1.2 ± 0.2	11.4 ± 1.6	1.9 ± 0.5	12.3 ± 1.7
Tolbutamide, 1 mg/ml	−2.1 ± 1.9	14.0 ± 2.9	0.9 ± 0.4	8.2 ± 0.2	1.4 ± 0.4	6.7 ± 0.6
m-CCP, 2.5 × 10^{-5} M	−1.6 ± 1.6	10.3 ± 1.4	0.7 ± 0.2	9.5 ± 0.3	1.2 ± 0.4	8.9 ± 1.0
Valinomycin, 2 μg/ml	−1.1 ± 1.9	14.3 ± 0.6	0.6 ± 0.4	3.1 ± 1.4	0.4 ± 0.3	2.0 ± 0.2

ureas which are clinically effective at lower concentrations than tolbutamide (24, 25). Carboxytolbutamide is a tolbutamide metabolite found to have no hypoglycemic action (3). Tolbutamide at concentrations from 0.33 to 1 mg/ml increased glucose-U-^{14}C conversion to $^{14}CO_2$ and oxygen consumption by brown fat cells in the presence

TABLE 6

Effect of tolbutamide and phenformin on metabolism of white fat cells

White parametrial adipose tissue from fed rats was digested with 0.5 mg/ml of collagenase for 1 hr. The cells (40 μmoles of triglyceride per flask) were incubated for 4 hr in 1.5 ml of medium containing 4% albumin, 0.45 mM theophylline, and 2.8 mM glucose-1-^{14}C or glucose-6-^{14}C. The basal values are the means ± standard errors of three experiments, and the increments due to added insulin (80 μunits/ml) are the means ± standard errors of the paired differences. The concentration of tolbutamide was 1 mg/ml, and that of phenformin was 120 μg/ml (0.5 mM).

Addition	Glucose label	Glucose conversion to CO_2		Glucose conversion to fatty acids		Glucose conversion to lactate	
		Without insulin	Increment due to insulin	Without insulin	Increment due to insulin	Without insulin	Increment due to insulin
		μmoles/mmole triglyceride		*μmoles/mmole triglyceride*		*μmoles/mmole triglyceride*	
None	1-^{14}C	0.5 ± 0.1	10.0 ± 2.8	0	15.1 ± 4.0	0.3 ± 0.1	5.8 ± 0.1
	6-^{14}C	0.8 ± 0.3	1.9 ± 0.4	0.1 ± 0.1	27.6 ± 4.0		
Tolbutamide	1-^{14}C	0.7 ± 0.1	11.3 ± 2.2	0.1 ± 0.1	15.8 ± 3.5	0.7 ± 0.4	6.8 ± 0.8
	6-^{14}C	0.7 ± 0.2	0.6 ± 0.1	0.6 ± 0.2	26.6 ± 8.0		
Phenformin	1-^{14}C	0.3 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0	2.5 ± 0.5	1.8 ± 0.6
	6-^{14}C	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.3	0		

of 0.4 milliunit/ml of insulin (Fig. 3). Both tolazamide and glyburide at dosages between 0.37 and 0.7 mg/ml increased oxygen consumption and CO_2 formation, while at 1 mg/ml the effects did not increase with the dosage. In contrast to the above three hypoglycemic sulfonylureas, carboxytolbutamide actually inhibited both $^{14}CO_2$ formation and oxygen consumption (Fig. 3). Lactate formation and glucose conversion to glyceride-glycerol were unaffected by carboxytolbutamide in the same experiments, while the highest concentrations tested for the other sulfonylureas produced about a 50% decrease in their formation (not shown).

Experiments were carried out with different substrates in the absence of glucose. As shown in Table 7, tolbutamide stimulated oxygen consumption in brown fat cells almost 2-fold when no substrate was present. This stimulatory effect was similar to that seen when 2.8 mM glucose was added to the medium (Table 2). When substrates were added to the medium, there was a 2-fold increase in basal respiration. The increment due to insulin was the same in either the presence or absence of substrates. The effect of insulin plus tolbutamide was greater than that of either agent alone when oxalacetate or succinate was the substrate. Insulin and tolbutamide together did not give a greater effect than tolbutamide alone when α-glycerophosphate was the substrate. In one experiment 10 mM pyruvate was added, and respiration, both basal and that due to added agents, was similar to that observed with succinate or oxalacetate.

In rabbit brown fat cells, tolbutamide alone at 1 mg/ml had a significant stimulatory effect on oxygen consumption, which was not significantly enhanced by insulin (Fig. 4). In the presence of 0.4 milliunit/ml of insulin, 1 mg/ml of tolbutamide enhanced glucose conversion to CO_2 (Fig. 4). As shown in Fig. 4 and Table 8, neither oxygen uptake nor glucose metabolism was affected by 0.4 milliunit/ml of insulin alone. *m*-CCP at 2.5×10^{-5} M stimulated oxygen uptake, and this effect was enhanced in the presence of insulin. Phenformin increased lactate formation from glucose, particularly in the presence of insulin (Table 8).

DISCUSSION

In our experiments with rat brown fat cells, tolbutamide potentiated the action of insulin on glucose oxidation to carbon

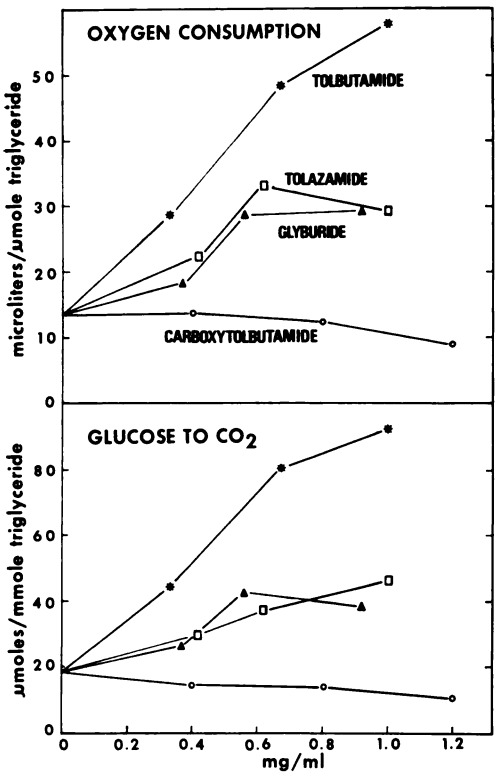

FIG. 3. *Effects of sulfonylurea derivatives on brown fat cell metabolism*

Brown fat cells (8.3 μmoles of triglyceride per flask) were incubated for 2 hr in 3 ml of 4% albumin buffer containing 2.8 mM glucose and 0.4 milliunit/ml of insulin. *, values for different doses of tolbutamide; □, those for tolazamide; ▲, those for glyburide; ○, those for carboxytolbutamide.

dioxide. High concentrations of cysteine or other thiols containing sulfhydryl groups partially mimic the action of insulin on white (19) and brown (20) fat cells. The present results also indicate that cysteine had the same effect as insulin on stimulation of glucose oxidation by tolbutamide. Possibly, in the presence of insulin or cysteine, transport is no longer the rate-limiting step for glucose metabolism and under these conditions an effect of tolbutamide on the oxidation of glucose can be seen.

Sulfonylureas have been reported to stimulate respiration in rat liver mitochondria (10) and in isolated pancreatic islet cells of mice (24). The increase was more marked at glucose concentrations below 5.5 mM. In rat brown fat cells, tolbutamide stimulated oxygen consumption in the absence of added substrate and in the presence of succinate, oxalacetate, α-glycerophosphate, or glucose.

Sulfonylureas have been found to decrease the ATP level in rat diaphragm and liver slices and mice β-cells, and to inhibit protein synthesis in rat diaphragm, liver slices, kidney, and adipose tissue (9, 10, 25). The decrease in ATP level and increase in oxygen uptake may have been due to the uncoupling effects of the sulfonylureas. *m*-CCP is a potent uncoupling agent of oxidative phosphorylation (26). The effects of tolbutamide and *m*-CCP on glucose oxidation by rat brown fat cells and oxygen consumption by rabbit brown

TABLE 7

Effects of tolbutamide and insulin with different substrates on metabolism of brown fat cells

Brown fat cells (7.5 μmoles of triglyceride per flask) were incubated in 3 ml of 4% albumin buffer for 2 hr. The basal values are the means of five experiments. The increments due to insulin (0.4 milliunit/ml), tolbutamide (1 mg/ml) or both (at the same concentrations) are the means ± standard errors of the paired differences. All cells were isolated and incubated in the absence of glucose.

Substrates	Basal	Increment due to insulin	Increment due to tolbutamide	Increment due to tolbutamide + insulin
		μl O_2 consumed/μmole triglyceride		
None	6.7 ± 1.9	7.3 ± 1.4	11.3 ± 2.5	16.4 ± 7.1
Oxalacetate, 10 mM	14.5 ± 6.0	6.2 ± 1.7	11.5 ± 1.6	24.8 ± 4.3
Succinate, 10 mM	15.2 ± 4.4	6.7 ± 0.4	13.8 ± 1.5	23.5 ± 3.4
α-Glycerophosphate, 10 mM	15.8 ± 3.7	5.9 ± 1.2	10.6 ± 3.8	10.8 ± 2.6

TABLE 8

Effects of phenformin and m-CCP on metabolism of rabbit brown fat cells

Brown adipose tissue from normal rabbits (2–3 weeks old) was digested with 1 mg/ml of collagenase for 1 hr. The cells (20.5 μmoles of triglyceride per flask) were incubated for 2 hr in 3 ml of medium containing 4% albumin, 0.1 mg/ml of pancreatic trypsin inhibitor, and 2.8 mM glucose-U-^{14}C. The values without addition of phenformin or *m*-CCP are the means of four experiments, and the changes due to phenformin or *m*-CCP are the means ± standard errors of the paired differences. The effects of tolbutamide in the same experiments are shown in Fig. 4.

Insulin	Oxygen consumption			Glucose conversion to lactate		
	No addition	Change due to phenformin (1 mM)	Change due to *m*-CCP (2.5×10^{-5} M)	No addition	Change due to phenformin (1 mM)	Change due to *m*-CCP (2.5×10^{-5} M)
milliunit/ml	*μl/μmole triglyceride*			*μmoles/mmole triglyceride*		
0	1.9 ± 0.8	−0.1 ± 0.4	+4.7 ± 0.4[a]	3.9 ± 0.7	+2.0 ± 0.4[a]	−0.1 ± 2.0
0.4	2.0 ± 0.7	+0.2 ± 0.4	+7.1 ± 2.0[a]	6.8 ± 2.0	+9.7 ± 3.2[a]	+4.0 ± 2.0

[a] Significant effect ($p < 0.05$ by paired comparisons).

fat cells were indistinguishable and suggest that the observed effects of tolbutamide may be secondary to its uncoupling action. Previous studies from this laboratory have also shown that *m*-CCP is a potent stimulator of respiration in rat brown fat cells (27).

The action of tolbutamide was somewhat different from that of valinomycin, whose uncoupling action is secondary to stimulation of K^+ flux (28, 29). Valinomycin also stimulates respiration in brown fat cells, but its action is dependent on the presence of K^+ in the medium (29).

The stimulation of respiration by tolbutamide in the absence of glucose or insulin indicates that this effect of the drug is not dependent on the availability of glucose. The stimulation by insulin of respiration in the absence of glucose is another effect of this hormone which is independent of any action on glucose transport.

The therapeutically effective concentrations of tolbutamide in man are generally considered to be in the range of 0.08–0.18 mg/ml (30). The minimally effective concentration of tolbutamide with respect to probable uncoupling effects on brown fat cell metabolism in our experiments was within this range, but demonstration of maximal effects required concentrations in the range of 0.5–1 mg/ml (Fig. 3). Significant effects of tolbutamide on insulin secretion by the perfused rat pancreas require concentrations in the range of 0.07–0.2 mg/ml of perfusate (31). However, it is clear that there is no relationship between the probable uncoupling actions of glyburide and tolazamide on brown fat cells and their effects on insulin release, since these agents were less effective than tolbutamide on brown fat cell metabolism but are much more potent than tolbutamide with respect to insulin release and dosage required for a hypoglycemic effect (32, 33). Possibly glyburide and tolazamide act solely as pancreatic stimulants whereas the hypoglycemic action of tolbutamide also involves potentiation of insulin action. We suggest that further experiments should be performed to test the hypothesis that the extrapancreatic actions of tolbutamide (30) may be due to an uncoupling action, and this should be considered as a possible explanation of the effect of sulfonylureas on the pancreas. At the very least, the probable uncoupling action of sulfonylureas might explain their antilipolytic action on white (11–13) and brown fat cells.[2]

Carboxytolbutamide is a metabolite of tolbutamide which is devoid of hypoglycemic action (3) and is ineffective on isolated pancreatic islet cells of mice (24). Our studies indicate that carboxytolbutamide is also inactive as a probable uncoupling agent on brown fat cells (Fig.

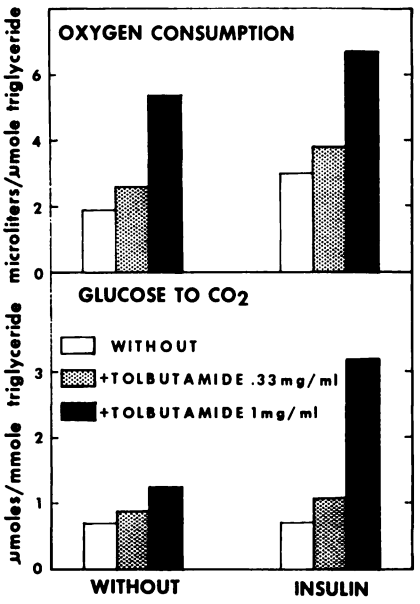

FIG. 4. *Effects of tolbutamide on rabbit brown fat cells*

Isolated rabbit brown fat cells (20.5 μmoles of triglyceride per flask) were incubated in 3 ml of medium containing 4% albumin buffer, 2.8 mM glucose-U-^{14}C, and 0.1 mg/ml of pancreatic trypsin inhibitor. The left half of each graph shows the values in the absence of insulin, while the right-hand values are those in the presence of 0.4 milliunit/ml of insulin. The unshaded bars represent the basal values without tolbutamide; the stippled bars, values with 0.33 mg/ml of tolbutamide; and the solid bars, values with 1 mg/ml of tolbutamide. The stimulation of respiration and glucose oxidation by 1 mg/ml of tolbutamide was statistically significant ($p < 0.05$ by paired comparisons) except in the case of glucose oxidation in the absence of insulin.

3). These data suggest that there is some specificity with regard to sulfonylurea effects on brown fat cell metabolism.

Rudman and DiGirolamo (34) found that the white adipose tissue of rabbits was relatively insensitive to insulin. Therefore, we included a small amount of pancreatic trypsin inhibitor in the medium used for incubation of rabbit brown fat cells because of the possibility that the fat cells might readily inactivate insulin. There was no significant stimulation of glucose oxidation by insulin alone in rabbit brown fat cells, in contrast to rat brown or white fat cells. Insulin did appear to increase glucose oxidation in the presence of tolbutamide.

The stimulation by phenformin of lactate formation by brown and white fat cells is similar to previous observations with oligomycin (35). Both phenformin and oligomycin (35) inhibited glucose oxidation by white fat cells, and oligomycin (35) stimulated glucose oxidation by brown fat cells, while phenformin was without affect on basal but did inhibit the increase due to insulin. The effects of oligomycin and phenformin appear to be quite similar and confirm previous reports with respect to the effects of phenformin on white adipose tissue (21–23).

Insulin preferentially stimulated the hexose monophosphate pathway of glucose metabolism in brown fat cells, although to a lesser extent than in white fat cells. Winegrad and Renold (36) originally reported the preferential oxidation of C-1 of glucose by white adipose tissue in the presence of insulin, and this was confirmed in the present experiments. Tolbutamide had effects opposite to those of insulin with regard to stimulation of the shunt pathway in brown fat cells. The effect of tolbutamide on the metabolism of glucose labeled at C-1 compared with C-6 was examined because of the report that tolbutamide preferentially stimulated the shunt pathway in white adipose tissue (4). There was a small, but not significant, stimulation of glucose-1-^{14}C oxidation by tolbutamide in white fat cells, but this was insignificant compared to the effect of insulin (Table 6). The difference between the effects of tolbutamide on brown as compared to white fat cell glucose oxidation is probably related to the much greater rate of oxidative metabolism in brown fat cells (14).

The conclusion from these studies is that the effects of tolbutamide on brown fat cell metabolism appear to be due to uncoupling of oxidative phosphorylation. The stimulation of glucose oxidation by tolbutamide is secondary to its uncoupling action and is dependent on the presence of insulin to increase the intracellular availability of glucose.

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