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EFFECTS OF TOLBUTAMIDE AND OF CHLORPROPAMIDE ON FATTY ACID SYNTHESIS

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Abstract—Livers of fasted or fed-rats, that received an intraperitoneal injection of 10 mg of tolbutamide, were perfused for 45 min with a medium containing or not ATP, and containing 20 μc of 2-^{3}H-acetate and 100 μc of U-^{14}C-glucose. Tolbutamide did not stimulate fatty acid synthesis from U-^{14}C-glucose in the livers of fasted rats, whether or not the perfusion medium contained glucose or ATP. There was actually an inhibition in the synthesis of fatty acids from 2-^{3}H-acetate when glucose was not present in the perfusion medium.

Groups of rats fasted for 24 hr, or fasted for 24 hr and then re-fed, were killed 30 min after the injection of 50 μc of U-^{14}C-glucose and of 50 μc of 2-^{3}H-acetate, and 60 min after intravenous administration of either tolbutamide or chlorpropamide.

In these experiments *in vivo*, both chlorpropamide and tolbutamide stimulated lipogenesis depressed by fasting, and increased fatty acid synthesis in re-fed animals. These effects were similar to, and appeared to be of the same degree as, those induced by insulin but were elicited only by large concentrations of the sulfonylureas.

SEVERAL studies have shown that insulin can restore lipogenesis *in vivo*[1,2] and *in vitro*[3] after inhibition due to fasting, provided the period of food deprivation lasts no more than 48 hr. Recently, however, it has been reported[4] that chlorpropamide, contrary to insulin, cannot restore hepatic lipogenesis and has very little effect on the adipose tissue and carcass of fasted animals, even though the drug is administered in doses capable of inducing a degree of hypoglycemia similar to that which follows an injection of insulin.

The latter observation is rather disconcerting since sulfonylureas are thought to exert their hypoglycemic action via a stimulation of insulin secretion.[5–8] Furthermore, this difference in the response to chlorpropamide and to insulin has been observed not only in fasted but also in fed animals, *in vivo*[9] as well as *in vitro*.[10] However, in the literature there are data which indicate that sulfonylureas may act independently of the pancreas or even in its absence. Indeed, these drugs appear to be able to reduce ketogenesis even in alloxanized-fasted animals, and to control the activity of many of the enzymes of glycolysis and gluconeogenesis.[10–13] On the other hand, chlorpropamide and tolbutamide are known to reduce the concentration of ATP in liver slices[14,15] and in brown adipose tissue,[16] presumably by uncoupling oxidative phosphorylations. They are also supposed to promote glucose uptake by white adipose tissue, glucose conversion into glycogen[17] and to have an anti-lipolytic action.[18–20]

The present study was undertaken in an attempt to clarify the action of sulfonylureas on the synthesis of fatty acids. For this purpose, we have studied the effects of tolbutamide with isolated, perfused rat livers in the presence or absence of ATP in the perfusion medium. Concomitantly, we have tried to establish whether the lack of restoration of lipogenesis *in vivo*, observed with chlorpropamide in fasted animals, is shared by other hypoglycemic agents, specifically tolbutamide, when acting at high concentrations such as those attained after intravenous administration. This part of our studies was performed in fasted and in fasted-refed animals, finally, we have compared directly the action of chlorpropamide and of tolbutamide on the synthesis of fatty acids *in vivo*.

EXPERIMENTAL AND METHODS

Experiments with perfused livers. Male Wistar rats, weighing about 250 g were used. They were divided into 3 major groups, two of which were fasted for 12 hr before the experiment ("fasted groups"), while the rats of the third group were fed a synthetic rat diet (Nafag cubes) until the time of the experiment ("fed group"). Two hours before the beginning of liver perfusion, the animals were anesthetized with an intraperitoneal injection of Numal Roche 0·1 ml/100 g of body wt. One hour later, tolbutamide (10 mg/100 g of body wt) was injected intraperitoneally into the rats of one of the fasting groups. Heparin (100 I.U.) was injected into all animals just before laparatomy.

Liver perfusion was performed with the technique and medium described by Shimassek[21] as modified by Hems *et al.*,[22] 150 ml of medium were used to perfuse each rat liver. In some experiments, 10 mg of glucose (fasted-group livers), or 140 mg (fed-group livers) were added to each 100 ml of perfusion medium. The first 5 ml of outflow perfusate were discarded in view of their high content of hepatic blood. Perfusion was allowed to stabilize for the next 15 min, then 2-^{3}H-acetate (20 μc) or U-^{14}C-glucose (100 μc) were injected into the pre-hepatic cannula. In certain cases, 40 μM of ATP were added to the perfusion medium every 15 min. The perfusion period was 45 min, after which the liver was taken and immersed into boiling alcoholic KOH for extraction of fatty acids.

Experiments in vivo. Wistar rats weighing about 200 g were divided into 2 major groups. The animals of one group were fasted for 24 hr before the experiment, while those of the other group were fasted for 24 hr but then re-fed (Nafag cubes) for either 6 or 12 hr before the experiment. Tolbutamide or chlorpropamide (10 mg/100 g of body wt) were administered intravenously in the tail vein) 60 min before the animals were killed. U-^{14}C-glucose (50 μc) or 2-^{3}H-acetate (50 μc) were injected also intravenously, 30 min after the administration of tolbutamide or chlorpropamide. The liver and epididymal fat pads were removed and immersed in boiling alcoholic KOH for extraction of fatty acids.

Fatty acid extraction and radioactivity measurement. Fatty acids were extracted with petroleum ether after a 4 hr saponification, elimination of the unsaponifiable and a washing with 50% ethanol in water containing unlabeled precursors. The solvents were then evaporated under nitrogen, and aliquots of the fatty acids were dissolved in 15 ml of toluene containing, per 1000 ml, 5 g of 2,5-diphenyloxazole (PPO) and 300 mg of 1,4-bis [2-(4-methyl-5-phenyloxazolyl)] benzene (POPOP). Radioactivities were measured in a 3 channel liquid scintillation spectrometer

(Tri-Carb, Packard). Quenching was determined by the channel-ratio method described by Hendler.[23]

RESULTS

Effects of tolbutamide, glucose and ATP on the incorporation of 2-^{3}H-acetate and U-^{14}C-glucose into fatty acids of perfused livers from fasted rats. The results presented in Table 1 show that:

(1) Tolbutamide inhibits the incorporation of 2-^{3}H-acetate into liver fatty acids when the perfusion medium contains no glucose, but not if glucose is present.

(2) The incorporation of U-^{14}C-glucose into liver fatty acids is not significantly changed by tolbutamide, irrespective of whether or not glucose is added to the perfusion medium.

(3) ATP added to the perfusion medium does not change the effects of tolbutamide on the incorporation of the two labeled precursors.

(4) The radioactivities attained by the fatty acids of perfused livers from fasted rats—whether or not the latter received tolbutamide, or glucose is present in the medium—never reach the levels observed with livers from fed animals.

Effect of tolbutamide on the incorporation in vivo *of 2-^{3}H-acetate and U-^{14}C-glucose into liver and adipose tissue fatty acids of fasted and of fasted-refed rats.* The results presented in Table 2 show that:

(1) Tolbutamide strongly stimulates fatty acid synthesis from both U-^{14}C-glucose and 2-^{3}H-acetate in the liver of fasted-refed rats. The stimulation, however, appears to be greater when glucose is the precursor since the average ^{3}H to ^{14}C ratio is 3·43 in control animals and 2·16 in tolbutamide-treated rats.

(2) The stimulatory effect of tolbutamide is more pronounced in the epididymal adipose tissue than in the liver of the fasted-refed rats. Indeed, radioactivities incorporated from glucose or acetate were 10- and 3-times, respectively, as high in tolbutamide-treated rats than in controls.

(3) In fasted rats, tolbutamide has a stimulatory effect on fatty acid synthesis quite similar to that observed in fasted-refed animals. In all cases, however, fasting caused an increase of the ^{3}H to ^{14}C ratio indicating that fasting depresses the incorporation of glucose more severely than that of acetate.

Comparison between the effect of chlorpropamide and of tolbutamide on fatty acid synthesis in liver and adipose tissue of fasted-refed rats. The results presented in Table 3 show that:

(1) As in the case of tolbutamide, chlorpropamide administered intravenously stimulates efficiently lipogenesis in both liver and adipose tissue.

(2) In liver, tolbutamide stimulates fatty acid synthesis from glucose about 4 times and from acetate about 3 times. The stimulation due to chlorpropamide seems to be less marked but is only apparently so, since there is a greater variability of results and there is no statistically significant difference between the means for the chlorpropamide- or tolbutamide-treated animals.

(3) In adipose tissue, the two sulfonylureas cause similar increases in the incorporation of U-^{14}C-glucose, but no significant stimulation of fatty acid synthesis when the precursor is 2-^{3}H-acetate. Thus, the fatty acid ^{3}H to ^{14}C ratio is reduced, especially in rats treated with chlorpropamide.

TABLE 1. EFFECTS OF TOLBUTAMIDE, GLUCOSE AND ATP ON THE SYNTHESIS OF FATTY ACIDS BY PERFUSED RAT LIVERS

	No. of rats	2-^{3}H-acetate		U-^{14}C-glucose		^{3}H/^{14}C	
I. Perfusion medium containing no glucose							
Fasted (12 hr)	6	24,742		5478		4·70	
		↑ P < 0·001 (±780)		↑ N.S. (±2650)		↑ P < 0·01 (±1·24)	
Fasted (12 hr) + tolbutamide	6	↓ 8346	P < 0·001 (±820)	↓ 9150	N.S. (±1550)	↓ 0·75	P < 0·01 (±1·02)
		↑ N.S. (±790)		↑ N.S. (±2310)		↑ N.S. (±0·46)	
Fasted (12 hr) + tolbutamide + ATP	6	↓ 8896		↓ 7020		↓ 1·30	
II. Perfusion medium containing glucose							
Fasted (12 hr)	6	32,357		10,818		3·07	
		↑ N.S. (±4750)		↑ N.S. (±1550)		↑ N.S. (±0·45)	
Fasted (12 hr) + tolbutamide	6	↓ 29,939	P < 0·001 (±41,100)	↓ 8,247	P < 0·01 (±6080)	↓ 3·6	P < 0·01 (±1·45)
		↑ P < 0·001 (±43,300)		↑ P < 0·01 (±6480)		↑ P < 0·01 (±1·28)	
Fed	6	↓ 238,374		↓ 30,346		↓ 7·97	

Each value represents the mean, in dpm, of the total radioactivity in liver fatty acids (S.D. in brackets). Tolbutamide (10 mg/100 g of body wt) was injected intraperitoneally into three of the five groups of fasted rats, 60 min before the beginning of the perfusion. In all cases, 20 μc of 2-^{3}H-acetate and 100 μc of U-^{14}C-glucose were introduced into the prehepatic cannula, 45 min before the perfusion was terminated. ATP, 45 μM every 15 min, was also added to the medium used to perfuse the livers of one of the groups of fasted rats. Glucose was added before the beginning of perfusion, 70 mg/100 ml or 140 mg/100 ml of medium in the case of fasted or fed rats, respectively.

TABLE 2. EFFECT OF TOLBUTAMIDE ON THE INCORPORATION *in vivo* OF 2-^{3}H-ACETATE AND U-^{14}C-GLUCOSE INTO LIVER AND ADIPOSE TISSUE FATTY ACIDS OF RATS FASTED FOR 24 hr OR FASTED FOR 24 hr AND THEN REFED FOR 12 hr

	No. of rats	Liver			Adipose tissue		
		^{14}C	^{3}H	^{3}H/^{14}C	^{14}C	^{3}H	^{3}H/^{14}C
Fasted re-fed	6	29,520	90,280	3·43	11,880	10,020	1·05
		↑ P < 0·001 (±26,600)	↑ P < 0·001 (±48,600)	↑ P < 0·01 (±0·36)	↑ P < 0·001 (±24,380)	↑ P < 0·001 (±4200)	↑ P < 0·001 (±0·15)
Fasted-refed + tolbutamide	6	156,830	323,000	2·16	137,200	30,680	0·24
Fasted (24 hr)	6	1257	13,768	11·05	1122	1865	1·95
		↑ P < 0·001 (±1215)	↑ P < 0·01 (±9500)	↑ P < 0·01 (±1·53)	↑ P < 0·001 (±1430)	↑ P < 0·001 (±528)	↑ P < 0·01 (±0·407)
Fasted (24 hr) + tolbutamide	6	7302	44,536	6·06	8135	4512	0·59

Each value represents the mean, in dpm (S.D. in brackets), of the total radioactivity in fatty acids of the liver or of the epididymal fat tissue. The animals were killed 30 min after the simultaneous, intravenous injection of 50 μc each of 2-^{3}H-acetate and U-^{14}C-glucose. Tolbutamide, at a dose of 10 mg/100 g of body wt., was injected also intravenously 30 min before the labeled precursors.

TABLE 3. COMPARISON BETWEEN THE EFFECTS OF CHLORPROPAMIDE AND OF TOLBUTAMIDE ON FATTY ACID SYNTHESIS *in vivo* IN THE LIVER AND ADIPOSE TISSUE OF RATS FASTED FOR 24 hr AND THEN REFED FOR 6 hr

		Liver			Adipose tissue		
	No. of rats	^{14}C	^{3}H	$^{3}H/^{14}C$	^{14}C	^{3}H	$^{3}H/^{14}C$
Controls	6	20,800	80,000	3·85	6,900	7,720	1·12
		P < 0·05 (±25,400)	P < 0·05 (±44,400)	P < 0·01 (±0·47)	P < 0·001 (±1,600)	N.S. (±4,530)	P < 0·01 (±0·16)
		P < 0·01 (±9,670)	N.S. (±33,000)	P < 0·01 (±0·49)	P < 0·01 (±3,420)	N.S. (±2,530)	P < 0·01 (±0·17)
Tolbutamide	6	85,400	190,500	2·23	15,170	14,067	0·58
		N.S. (±22,500)	N.S. (±62,200)	N.S. (±0·51)	N.S. (±3,350)	N.S. (±4,050)	P < 0·05 (±0·04)
Chlorpropamide	6	51,600	112,500	2·18	18,640	8,910	0·46

Each value represents the mean, in dpm (S.D. in brackets). of the total radioactivity in fatty acids of the liver or of the epididymal adipose tissue. The animals were killed 30 min after the simultaneous, intravenous injection of 50 μc each of 2-^{3}H-acetate and U-^{14}C-glucose. Tolbutamide and chlorpropamide, both at a dose of 10 mg/100 g of body wt. were injected also intravenously 30 min before the labeled precursors.

DISCUSSION

The chronological study of the events which lead to an inhibition of fatty acid synthesis in the liver of fasted rats has shown that a deficiency of ATP should be considered as the primary factor, while enzymatic disturbances would play a secondary role.[2]

Tolbutamide, administered *in vivo*, does not restore lipogenesis in perfused livers of fasted rats, contrary to what should be expected. It actually induces a further inhibition of fatty acid synthesis when acetate is the precursor. Uncoupling of oxidative phosphorylations by tolbutamide[16] would appear not to be responsible for these effects of the sulfonylurea, since ATP added to the perfusion medium does not restore lipogenesis. It can be objected, however, that ATP probably does not pass through cell membranes, or may be catabolized immediately after its uptake. Indeed, the absence of a further inhibition of acetate incorporation, when the medium is rich in glucose, tends to support the hypothesis of a tolbutamide-induced deficiency of ATP. It is in fact known that glucose does not stimulate directly the enzymes involved in fatty acid synthesis, and that insulin needs at least 3 hr to restore to normal the enzymatic activities impaired by a 24 hr fasting period. Furthermore, results obtained by other authors,[10,16] show that a stimulation of fatty acid synthesis does not occur after incubation of adipose-tissue pieces in the presence of tolbutamide. This is true whether or not glucose is present in the incubation medium (unpublished results). Thus, also in this case, the primary effect of tolbutamide appears to be an uncoupling of oxidative phosphorylations.

For these reasons, it is highly surprising to find that tolbutamide stimulated the synthesis *in vivo* of fatty acids, in the liver of both fasted and fasted-refed animals, an effect which is similar to that induced by insulin. Even after stimulation, however, the degree of fatty acid synthesis in fasting animals is always smaller than that seen in fasted-refed controls (Table 2). This finding may indicate that the delay (30 min) between the administration of tolbutamide and that of the labeled precursors is not sufficient to correct the enzymatic deficiencies resulting from a fasting period of 24 hr.

Tolbutamide restores fatty acid synthesis *in vivo* in the adipose tissue of both fasted and fasted-refed rats, as in the liver. In fasted animals, however, tolbutamide seems to be more efficient since the values observed in the treated animals are closer to those obtained in the fasted-refed controls (Table 2). This result is undoubtedly due to the later impairment of the enzymatic activities of this tissue which occurs with fasting. Also, refeeding the animals for 12 hr instead of 6 favors the stimulatory action of sulfonylureas, corroborating the importance of insulin in the action of these substances.

It is interesting to note, finally, that tolbutamide has an effect on fatty acid synthesis comparable to that of chlorpropamide in both fasted and fasted-refed animals.

The above considerations suggest therefore that:

(1) Sulfonylureas, like insulin, are able to stimulate lipogenesis depressed by fasting, and in fed animals as well. This stimulatory, insulin-like effect occurs only *in vivo* and with high concentrations such as those which result from intravenous administration of the drugs (it is indeed not seen with perfused-liver preparations after intraperitoneal administration to the animals). In the latter case, as well as *in vitro*, sulfonylureas cause an uncoupling of oxidative phosphorylations, which obliterates their stimulatory effect, and results in an inhibition of fatty acid synthesis even though insulin may be present in sufficient amounts.

(2) Chlorpropamide and tolbutamide have the same stimulation properties on lipogenesis.

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