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CHANGES IN PLASMA AMINO ACIDS LEVELS AND "IN VIVO" GLUCONEOGENESIS FROM ALANINE IN RATS CHRONICALLY TREATED WITH SULFONYLUREAS (*)

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SUMMARY :

Female rats were treated with two daily equihypoglycemic doses (as observed in acute treatment) of tolbutamide, glibenclamide or glipentide by stomach tube, and were compared with control animals treated with the suspending medium alone. On day 29 the rats were subjected to a 48 h fast and then were injected intraperitoneally with 100 μMoles of C^{14} -alanine. Blood samples were collected before and 5, 15 and 30 minutes after the alanine injection, at which time the animals were killed.

Blood glucose levels increased after the injection of alanine in all groups, but at the different times studied, both the glibenclamide and glipentide treated animals showed hypoglycemia versus controls. The radioactivity found in blood glucose and liver glycogen and glyceride-glycerol decreased in the glibenclamide treated animals compared with controls while in the other groups it was similar. The increase in liver glycogen after the injection of alanine was also diminished in the glibenclamide treated animals. Alanine produced an increase in the plasma levels of gluconeogenic, basic, aromatic and sulphur-containing amino acids in the controls, while in the animals treated with glipentide the alanine effect was less pronounced.

The results show an impairment of gluconeogenic function in glibenclamide treated animals. The effects of both tolbutamide and glipentide were less dramatic. Nevertheless, the findings hinted at an effect of both drugs upon glycogen metabolism in liver.

Key Words : Sulfonylureas. Hypoglycemia. Glycogen. Amino acids. Gluconeogenesis.

RÉSUMÉ :

Variations du taux des amino-acides du plasma et de la gluconéogénèse « in vivo » à partir de l'alanine chez les rats sous traitement prolongé par sulfonylurées.

Des rates ont été traitées avec deux prises quotidiennes, administrées par sonde gastrique, de tolbutamide, de glibenclamide ou de glipentide à dose équivalente par leur effet hypoglycémiant (ainsi que cela résultait de leur effet à dose unique), et ont été comparées aux animaux de contrôle traités seulement par l'excipient. Au 29e jour, les rates étaient soumises à un jeûne de 48 heures après lequel elles recevaient une injection intrapéritonéale de 100 μMoles de C^{14} -alanine ; le sang était prélevé avant, puis 5, 15 et 30 minutes après cette injection et les animaux étaient aussitôt sacrifiés.

Après l'injection d'alanine le taux de la glycémie s'élevait dans tous les groupes d'animaux, une hypoglycémie par rapport aux témoins étant observée chez les rates traitées par glibenclamide ou par glipentide. La radioactivité du glucose sanguin et du glycogène et du glycéride-glycérol du foie s'abaissait chez les animaux traités par glibenclamide tandis qu'elle évoluait comme celle des témoins chez les animaux des deux autres groupes ; l'élévation du glycogène hépatique était également diminuée chez les rates traitées par glibenclamide. L'alanine produisait une augmentation du taux plasmatique des amino-acides gluconéogéniques, basiques, aromatiques et sulfurés chez les animaux de contrôle, tandis que cet effet était moins prononcé chez ceux traités par glipentide.

Ces résultats montrent une anomalie de la fonction gluconéogénique chez les animaux traités par glibenclamide. Les effets du tolbutamide et du glipentide étaient moins marqués. Néanmoins ces constatations sont en faveur d'un effet de ces deux dernières drogues dans le métabolisme hépatique du glycogène.

Mots Clés : Sulfonylurées. Hypoglycémie. Glycogène. Amino-acides. Gluconéogénèse.

The sulfonylurea drugs have been shown to decrease the activities of gluconeogenic enzymes when studied « in vitro » (1, 2) and the splanchnic output of glucose after short periods of « in vivo » administration (3, 4), but their effect after chronic administration simulating their clinical use, is not known.

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Amino acids are the main endogenous substrates for gluconeogenesis during fasting (5). Intravenous administration of alanine to humans provokes significant alterations in the plasma levels of other amino acids (6). Stimulation of insulin secretion with tolbutamide has been shown to lower the concentrations of free amino acids in plasma (7).

We have studied the effect of intraperitoneal radioactive alanine administration in the fasted rat, together with the effect on the response to three sulfonylureas: tolbutamide, glibenclamide and glipentide, administered chronically to the animals upon « in vivo » gluconeogenic capabilities, relating glucose and glycogen metabolism to that of amino acids.

MATERIALS AND METHODS

Female virgin Wistar rats, weighing initially 140 - 150 g were used. Animals were housed in a temperature controlled (23 °C) animal room with 12 hours-on 12 hours-off light cycle; they were fed standard rat chow and water *ad libitum*. Rats were kept in individual metabolic cages and sulfonylureas were administered twice daily, for 29 days, at one half and eight hours after the beginning of the light cycle. Drugs were administered by stomach tube without anaesthesia, suspended in 0.5 % CM-cellulose and 0.15 % Tween — 80 at the following concentrations: 100 mg/Kg of body weight for tolbutamide, and 5 mg/Kg for glibenclamide and glipentide, concentrations considered to be of equihypoglycemic strength (8, 9) when acutely administered in this form. Tolbutamide and glipentide were generously provided by Laboratorios Uriach, Barcelona, Spain, and glibenclamide by Hoechst Iberica, S.A., Barcelona, Spain.

On day 27 the animals were deprived of food, and 48h later (on day 29) at the beginning of the light cycle, 100 μMoles of C^{14}-alanine (UL) (0.1 μCi/μMol) /rat were injected intraperitoneally without anaesthesia. During the fasting period the drugs treatment was continued as indicated. Blood samples were collected from the tail before injection and 5 and 15 minutes later into heparinized receptacles. At 30 minutes after the injection, the animals were killed by decapitation, the liver rapidly dissected out and pieces frozen in liquid nitrogen. Blood samples were collected from the neck into heparinized beakers.

Aliquots of whole blood were deproteinized with perchloric acid and the supernatant neutralized with $KHCO_3$ for glucose estimation by the glucose oxidase method (10) and for chromatographic separation of labelled glucose and alanine. Other plasma samples were separated for amino acid analysis by a radiochemical micromethod (11) after deproteinization with acetone (12). Amino acids were distributed into six groups according to their structure and physiological functions (13). The aromatic amino acids included phenylalanine, tyrosine and tryptophan; the sulfur amino acids were taurine, methionine plus ornithine (because of inadecuate chromatographic separation the combined value is given) and « cysteine », the latter value representing cysteine, cysteate and half-cystine; the basic amino acids included arginine, histidine, lysine and citrulline; the branched chain amino acids were valine and leucine plus isoleucine (given also as a composite value); the imino acids included proline and hydroxyproline; the « gluconeogenic » amino acids, were so grouped by their physiological role, accepting that others may fulfill this metabolic function in addition.

Glycogen in the liver was extracted with ethanol after alkaline digestion (14) and determined, after acid hydrolysis, with glucose oxidase (10). Liver total lipids were obtained after extraction and purification with chloroform: methanol (2:1 by volume) (15); they were saponified and fractionated for assay of radioactivity in glyceride-glycerol (16).

Separation of labelled glucose and alanine in deproteinized blood samples was carried out by thin layer chromatography on activated plastic backed Silicagel G F254 (Merck, Darmstadt) plates, containing fluorescent indicator. Spots were eluted with 25 % ammonia: 96 % ethanol (1:1 by volume). The Rf values for glucose and alanine in this system were 0.42 and 0.69 respectively. Visualization was achieved running double standard strips on each 20 × 20 cm plates with glucose and alanine, and staining one of them with ninhidrin (0.2 % ninhidrin in acetone and gentle heat) for alanine spot visualization, and with 0.5 % KIO_4 in 0.05 M H_2SO_4 for detection of the glucose spot under 254 nm UV light after gentle heating. Strips of the developed chromatograms were counted for radioactivity in a PPO/POPOP toluene/triton X-100 based scintillation cocktail (17) in a scintillation counter. In the glucose band 98,9 % of radioactive glucose was recovered, containing only 0.32 % of alanine. The alanine fraction contained 99.23 % of alanine radioactivity and only 0.88 % of glucose. The other two fractions combined (including the origin) were virtually free of glucose and alanine radioactivity.

RESULTS

Table I shows the concentrations of blood glucose attained in the 48 hour fasted rats chronically treated with sulfonylureas, and in the controls, just before the intraperitoneal injection of 100 μMoles of radioactive alanine (time 0) and at different times after it. Both glibenclamide and glipentide treated animals show lower values than the controls, maintained for up to 30 minutes after the injection, while the values in the tolbutamide treated rats did not differ from the controls. In all groups there was a marked and significant increase in blood glucose after the alanine injection. The radioactivity incorporated into circulating glucose at different times after injection of labelled alanine is time-dependent in all groups, but the process is significantly slower in the glibenclamide-treated rats than in the other groups. The radioactivity remaining in the alanine fraction of the blood is shown in *Table II*. There is an increase of radioactivity in this amino acidic fraction with time after the administration of the tracer, which is similar for all the groups.

The liver glycogen content *(Table III)* did not differ significantly with treatment. The administration of alanine produced a significant increase in the liver glycogen content of the tolbutamide, glipentide and control groups, without significant changes in the glibenclamide-treated animals. Animals treated with glibenclamide incorporated a significantly smaller portion of alanine radioactivity into glycogen than controls, while values in glipentide and tolbutamide treated animals did not differ significantly. The radioactivity incorporated into glyceride-glycerol lipids *(Table III)* of the whole liver was significantly less in the glibenclamide-treated animals, but unchanged in glipentide and tolbutamide treated rats compared with controls. Little radioactivity was recovered in

TABLE I. — *Blood glucose concentration (G) (in mM/liter), and blood glucose radioactivity (R) (in x10³ dpm/liter) in rats treated chronically (29 days) with sulfonylureas at differeni times after the i.p. injection of labelled alanine.*

Mean ± S.E.M. of 5 determinations,
Significances versus 0 minutes : * = $p < 0.05$, ** = $p < 0.01$,
Significances versus controls: •• = $p < 0.05$, ••• = $p < 0.001$,
Tolbutamide: 2 × 100 mg/kg body weight/day,
Glibenclamide: 2 × 5 mg/kg body weight/day,
Glipentide: 2 × 5 mg/kg body weight/day,

TIME (minutes)		CONTROL	TOLBUTAMIDE	GLIBENCLAMIDE	GLIPENTIDE
0	G	4.06 ± 0.17	3.54 ± 0.18	2.42 ± 0.15•••	2.86 ± 0.17••
5	G	4.60 ± 0.32	3.79 ± 0.22	2.92 ± 0.20•••	2.96 ± 0.17••
	R	2.11 ± 0.33	1.68 ± 0.38	1.61 ± 0.33	2.05 ± 0.57
15	G	4.94 ± 0.27*	4.20 ± 0.16*	3.14 ± 0.20*•••	3.21 ± 0.22*•
	R	5.11 ± 0.80	4.10 ± 0.58	3.48 ± 0.52	5.98 ± 0.44
30	G	5.60 ± 0.32**	5.14 ± 0.30**	3.63 ± 0.34*•••	4.11 ± 0.26**••
	R	11.00 ± 0.44	12.46 ± 1.79	6.18 ± 0.55•••	11.46 ± 1.49

TABLE II. — *Radioactivity present in the blood (in x10³ dpm/ml) as alanine in rats treated chronically (29 days) with sulfonylureas at different times after the i.p. injection of labelled alanine.*

(Values are mean ± S.E.M. of 5 determinations).
Tolbutamide, glibenclamide, glipentide: see Table I.

TIME (minutes)	CONTROL	TOLBUTAMIDE	GLIBENCLAMIDE	GLIPENTIDE
5	9.86 ± 0.72	9.24 ± 2.14	6.03 ± 1.27	8.41 ± 1.90
15	15.69 ± 2.58	10.63 ± 2.32	12.66 ± 3.02	10.33 ± 1.12
30	18.20 ± 1.61	16.35 ± 1.60	18.32 ± 2.44	12.60 ± 1.78

TABLE III. — *Glycogen and lipid radioactivity in the liver of 48 hours fasted rats treated chronically (29 days) with sulfonylureas 30 minutes after the i. p. injection of labelled alanine.*

All values are mean ± S.E.M. of 5 determinations.

Significance versus controls: • = $p < 0.05$, •• = $p < 0.01$,
Significance versus no alanine administration: * = $p < 0.05$, ** = $p < 0.01$,

Tolbutamide, glibenclamide, glipentide: see Table I.

	CONTROL	TOLBUTAMIDE	GLIBENCLAMIDE	GLIPENTIDE
Glycogen content (mg/whole liver) of liver of animals not treated with alanine	2.07 ± 0.28	1.41 ± 0.19	1.97 ± 0.43	2.25 ± 0.29
Glycogen content (mg/whole liver) of liver of animals injected with alanine	6.61 ± 1.88*	2.92 ± 0.34*•	2.78 ± 0.73	12.17 ± 2.20**
Radioactivity incorporated in liver glycogen (x10³ dpm/whole liver)	28.63 ± 4.62	15.82 ± 2.45	6.33 ± 1.55••	28.83 ± 5.81
Radioactivity incorporated in liver glyceride-glycerol (x10³ dpm/whole liver)	25.76 ± 1.78	26.95 ± 4.00	14.66 ± 2.66••	25.50 ± 2.74

TABLE IV. — *Concentrations (in μmoles/liter) of plasma free amino acids in rats chronically treated (29 days) with sulfonylureas before (0) and after 30 minutes of an intraperitoneal injection of 100 μmoles of labelled alanine.*

Significances versus 0 time: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.
Significances versus controls: • = $p < 0.05$,
Tolbutamide, glibenclamide, glipentides: see Table I.

amino acids	CONTROLS		TOLBUTAMIDE		GLIBENCLAMIDE		GLIPENTIDE	
	before injection	after injection	before injection	after injection	before injection	after injection	before injection	after injection
Ala	414 ± 28	883 ± 161*	395 ± 59	840 ±179*	457 ± 38	996 ± 90**	450 ± 15	710 ± 31***
Glu + Gln	943 ± 49	950 ± 55	997 ± 35	1064 ±236	876 ± 43	916 ±105	1060 ± 66	854 ± 41•
Ser	373 ± 42	380 ± 54	310 ± 42	364 ± 46	334 ± 55	379 ± 47	327 ± 24	353 ± 28
Gly	347 ± 34	322 ± 28	321 ± 63	299 ± 41	366 ± 43	308 ± 29	348 ± 39	281 ± 28
Thr	145 ± 36	151 ± 39	178 ± 50	169 ± 17	139 ± 22	142 ± 41	176 ± 48	114 ± 20
Asp + Asn	100 ± 12	162 ± 23	131 ± 20	135 ± 20	115 ± 27	171 ± 12*	98 ± 20	101 ± 24
'Gluconeogenic' total	2316 ±123	2896 ± 231*	2368 ±244	2908 ±462	2274 ±185	3031 ±191*	2504 ±180	2426 ± 23
Pro	232 ± 17	238 ± 17	246 ± 36	251 ± 82	218 ± 32	350 ± 29*	213 ± 41	255 ± 31
Hip	20 ± 5	29 ± 7	16 ± 1	30 ± 11	12 ± 3	20 ± 4	10 ± 2	19 ± 2
Imino acids total	251 ± 19	267 ± 16	281 ± 53	281 ± 91	229 ± 34	370 ± 33*	221 ± 42	265 ± 31
Leu + Ile	365 ± 55	364 ± 48	342 ± 15	321 ± 35	380 ± 23	351 ± 45	398 ± 10	248 ± 39
Val	165 ± 20	239 ± 33	155 ± 12	222 ± 40	150 ± 12	177 ± 41	196 ± 31	168 ± 22
Branched chain total	548 ± 37	603 ± 51	507 ± 5	544 ± 46	529 ± 22	528 ± 45	593 ± 21	515 ± 51
Lys	436 ± 20	493 ± 30	382 ± 46	505 ± 77	458 ± 31	474 ± 64	507 ± 90	523 ± 71
Arg	131 ± 25	129 ± 22	132 ± 13	180 ± 43	155 ± 10	188 ± 52	162 ± 45	161 ± 19
His	70 ± 11	136 ± 16**	69 ± 10	93 ± 14	81 ± 16	144 ± 14*	86 ± 18	80 ± 12
Cit	42 ± 6	52 ± 4	45 ± 8	55 ± 20	40 ± 7	60 ± 11	29 ± 2	30 ± 9
Basic total	675 ± 15	810 ± 30**	651 ± 56	814 ±116	735 ± 22	866 ± 75	784 ±107	762 ± 66
Trp	212 ± 14	273 ± 32*	221 ± 38	285 ± 28*	216 ± 50	311 ± 18	256 ± 19	245 ± 10
Tyr	95 ± 15	132 ± 36	96 ± 22	115 ± 28	91 ± 16	146 ± 47	99 ± 1	132 ± 42
Phe	56 ± 1	71 ± 2**	66 ± 11	76 ± 21	57 ± 6	69 ± 6	56 ± 4	71 ± 10
Aromatic total	363 ± 39	476 ± 61**	372 ± 64	514 ±106	365 ± 57	526 ± 61	410 ± 21	478 ± 58
Taurine	264 ± 44	633 ± 74**	274 ± 32	625 ±141*	290 ± 28	575 ± 90*	314 ± 17	624 ±152*
'Cysteine'	113 ± 8	131 ± 22	103 ± 7	114 ± 13	87 ± 17	132 ± 25	73 ± 16	144 ± 10*
Met + Orn	102 ± 13	105 ± 20	103 ± 13	167 ± 47	117 ± 8	135 ± 24	111 ± 6	146 ± 32
Sulphur total	495 ± 44	869 ± 84**	492 ± 43	905 ±186*	493 ± 35	841 ± 76**	507 ± 34	914 ±158*
Total	4585 ±228	5872 ±395***	4673 ±403	5984 ±841	4626 ±197	6163 ±237**	5017 ±240	5395 ±147•

unsaponifiable lipids nor in the fatty acids fraction. The injection of alanine *(Table IV)* in control rats provoked a significant increase in alanine levels, composite « gluconeogenic » amino acids, histidine, composite basic amino acids, tryptophan, phenylalanine and composite aromatic amino acids, taurine, composite sulphur amino acids and in total amino acids. In tolbutamide treated animals the pattern of response was similar, although significant only in alanine, tryptophan, taurine and composite sulphur amino acids. In the glibenclamide group, the increase in alanine was more pronounced; there were also increases in aspartate - asparagine, composite « gluconeogenic » amino acids and total imino acids, proline, (also both significantly higher than controls), histidine, taurine, composite sulphur amino acids and total amino acids. In glipentide-treated animals, there were increases in alanine, taurine, « cysteine » and composite sulphur amino acids. Total amino acids and glutamate + glutamine were significantly lower in glipentide-treated animals than in controls.

DISCUSSION

After 48 hours fasting, rats treated chronically twice daily with oral glipentide or glibenclamide had lower blood glucose levels, while rats treated with tolbutamide had practically the same glucose levels as untreated controls. The doses of glipentide and tolbutamide had previously been found equihypoglycemic in acute administration (8), and those of glipentide and glibenclamide were also those found equihypoglycemic after short periods of chronic treatment (9). The lack of blood glucose changes in the tolbutamide treated animals is not surprising, because it has been recently shown that the hypoglycemic effect of this drug dissappears (and may even be reversed) after long term treatment (9).

Alanine injection raised blood glucose levels in all groups, but the relative hypoglycemia of the glibenclamide and glipentide treated rats was maintained. This blood glucose rise may be due to the conversion of alanine to glucose rather than the stress of hand-

ling, as the rats were fasted and so were liver glycogen depleted.

The amino acids pattern before and after alanine administration varied little between treatment groups and controls. The changes in the sulfonylurea-treated rats were generally not significant, but the controls showed increases in histidine, tryptophan, phenylalanine and taurine, in adition to alanine. Gilbert et al. (21) found considerable urinary taurine excretion after alanine injection in mice. The nature of the relationship between alanine and taurine remains to be established, as do the effects upon several essencial aminoacids such as tryptophan, histidine and phenylalanine; and the mechanism by which these sulfonylureas prevented the rise of certain amino acids after alanine administration.

The effects of tolbutamide, seen as a whole, indicate no significant alterations in the alanine to glucose conversion pathway with respect to controls, in accordance with published data (22). When the ratios of related metabolites are calculated *(Table V)* a clearer picture of the metabolic situation emerges. The ratio of plasma alanine to blood glucose concentrations does not differ between tolbutamide treated rats and controls, while the ratio of blood glucose to liver glycogen is significantly augmented in the former group. These results together with the low liver glycogen content of tolbutamide treated rats after alanine administration point to a restriction of glucose incorporation into glycogen, data which agree with the lowered glucose release from the liver reported by Recant and Fischer (4).

Glibenclamide treatment reduced the production of labelled glucose from radioactive alanine and increased the blood radioactive alanine to radioactive glucose ratio *(Table V)*, in the presence of low glucose levels. There was also reduced incorporation of label into liver glycogen and glyceride-glycerol; the reverse is true wherever liver gluconeogenesis is enhanced (18, 19, 20). This decreased conversion of alanine to glucose with glibenclamide is probably a consequence of impaired transamination observed in the presence of sulfonylureas by other authors (23, 24) and the same may be true of other changes in amino acids concentrations that follow the alanine pattern.

Glipentide behaves in a different way, as the concentrations of labelled and cold alanine were considerably lower than in controls 30 minutes after the alanine injection. The glucose concentration in blood was also lower, with significantly lower radioactivity ratios of alanine to glucose *(Table V)*, indicating an active process of alanine conversion into glucose, but with alanine to glucose ratio equal to that of controls, findings perhaps explained by relative enhancement of gluconeogenesis from alanine together with a continued release of glucose, increasing the blood specific activity. The ratio of radioactivity in liver glyceride-glycerol to blood glucose resembles that in controls suggesting little effect of glipentide on the conversion pathway. Similarly the ratio of blood glucose to liver glycogen radioactivity with glipentide is the same as in controls, indicating no changes in glycogen synthesis from glucose. However, the ratio of blood glucose to liver glycogen concentrations is significantly reduced *(Table V)* which, together with the increase of liver glycogen concentration after the treatment with alanine, seems

TABLE V. — *Relationships between metabolic parameters at 30 minutes after the i.p. injection of 100 µmoles of labelled alanine in rats chronically treated (29 days) with sulfonylureas.*
Significances versus controls: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.
Tolbutamide, glibenclamide, glipentides: see Table I.

QUOTIENT	UNITS	CONTROL	TOLBUTAMIDE	GLIBENCLAMIDE	GLIPENTIDE
Liver glyceride-glycerol rad. / Blood glucose radioactivity	10^3dpm/whole liver / 10^3dpm/ml	2.15 ± 0.26	2.38 ± 0.26	2.99 ± 0.38	2.85 ± 0.63
Blood glucose radioactivity / Blood glucosa concentration	10^3dpm/ml / µmoles/ml	2.00 ± 1.97	2.49 ± 0.48	2.09 ± 0.45	2.52 ± 0.40
Liver glycogen radioactivity / Liver glycogen cencentration	10^3dpm/whole liver / mg/whole liver	4.33 ± 0.74	5.42 ± 0.87 *	2.28 ± 0.32 *	2.37 ± 0.28 *
Blood alanine radioactivity / Plasma alanine concentration	10^3dpm/ml / µmoles/ml	17.46 ± 2.28	17.54 ± 3.41	14.64 ± 2.20	13.87 ± 2.30
Blood alanine radioactivity / Blood glucose radioactivity	10^3dpm/ml / 10^3dpm/ml	1.55 ± 0.18	1.02 ± 0.10 *	3.27 ± 0.48**	0.95 ± 0.08*
Plasma alanine concentration / Blood glucose concentration	µmoles/ml / µmoles/ml	0.18 ± 0.01	0.14 ± 0.02	0.31 ± 0.02***	0.17 ± 0.01
Blood glucose concentration / Livwe glycogen concentration	µmoles/ml / mg/whole liver	0.85 ± 0.17	1.76 ± 0.23**	1.31 ± 0.28	0.34 ± 0.02*
Blood glucose radioactivity / Liver glycogen radioactivity	10^3dpm/ml / 10^3dpm/whole liver	0.36 ± 0.03	0.62 ± 0.07**	1.41 ± 0.17***	0.32 ± 0.05

to indicate that glucose release via glycogen breakdown is impaired in glipentide treated animals.

In previous studies it was shown that the hypoglycemic effect of sulfonylureas decreased progressively with the duration of treatment (9), suggesting a reduction in their insulinotropic effects. It was shown previously (1, 2) that these drugs may directly affect the activities of gluconeogenic enzymes and also (24) the activities of alanine transaminases. Thus, the impaired gluconeogenesis observed after prolonged treatment with sulfonylureas could be the result of the combined effects of a direct action of the altered circulating insulin levels and indirect influences of these drugs on the gluconeogenetic pathway.

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