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Effect of Sulfonylurea Treatment and Fasting on the Levels of Plasma Amino Acids in the Rat*

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Summary

Rats chronically treated with two daily doses of tolbutamide, glibenclamide or glipentide were compared with animals treated with placebo. Plasma individual amino acids were determined at 0, 3, 7, 10, 12, 14, 17, 24, 27 and 29 days of treatment 16 hours after the administration of the drug. Rats were fasted for 48 h periods at days 10 to 12 and 27 to 29 of the experiment. Sulfonylurea treated animals show minor changes in the plasma aminogram, although glipentide and glibenclamide produced greater effects than tolbutamide. At the 3rd day after the onset of the treatment, plasma levels of glutamate + glutamine, arginine and histidine appeared significantly reduced in glipentide and glibenclamide treated animals. When plasma samples were collected 3 h after the drug administration at the 24th day of treatment, the only observed change was a decrease in the levels of arginine in the glipentide treated animals. Fasting produced decreases in plasma levels of alanine, proline, cysteine, tyrosine, methionine +ornithine and tryptophan, there were no changes in serine, aspartate + asparagine, threonine citruline, arginine and lysine; and glycine, glutamate + glutamine and leucine + isoleucine show increases. These changes were rapidly compensated with refeeding, appearing a "rebound effect" in certain amino acids. Both fasting and refeeding affect very little the effect of sultonylureas on plasma amino acid levels, although for some individual amino acid they reduce or enhance the effect of the fasting. These small effect of sulfonylureas on plasma amino acid levels could be the result of the juxtaposition of different factors, including the effects of these drugs on circulating insulin levels, on protein biosynthesis and amino acids transamination and hepatic gluconeogenesis.

Key-Words: *Sulfonylureas – Amino Acids – Fasting*

Introduction

The plasma concentration of amino acids changes with the administration of insulinotropic factors. Actually, as early as 1922 was shown that following carbohydrate ingestion, the plasma level of amino acid nitrogen is lowered (*Foling and Berglund* 1922) and this observation has been confirmed repeatedly (*Harris and Harris* 1974; *Munro and Thomson* 1953; *Crofford, Felts and Lacy* 1964; *Zinneman, Nuttall and Goetz* 1966), being attributed either to effects due to changes in circulating insulin itself (*Luck, Morrison and Wilbur* 1928) or to the concomitant effect of enhanced metabolization of glucose (*Adibi, Morse and Amin* 1975). Other factors known to alter the pancreatic insulin secretion rate, such as starvation and sulfonylurea treatment had also been shown to produce alterations in the amino acid plasma concentrations (*Metzger, Hare and Freinkel* 1971; *Adibi, Morse and Amin* 1975). Most of the studies carried out with sulfonylurea drugs "in vivo" have been done after acute drug administration, and it is not known how they affect the plasma amino acid concentrations after chronic treatment, a condition that is more similar to the use of these drugs as hypoglycaemic agents in humans. The problem is particularly suggesting, as it has been shown that the insulinotropic and hypoglycaemic effects of these drugs are minimized after prolonged treatment (*Sodoyez, Sodoyez-Goffaux, Dunbar and Foà* 1970; *Codina, Lasunción and Herrera* 1977). Starvation has been shown to decrease the insulinotropic effect of sulfonylureas (*Feldman and Lebovitz* 1970; *Feldman and Lebovitz* 1973) although this has not always being observed (*Ramahandridona, DiCampo-Rougerie and Vague* 1975), and thus it could also influence the effect of these drugs on the plasma amino acid levels. In the present paper it has been studied the effect of tolbutamide and two other high hypoglycaemic sulfonylureas of the so called second generation: glipentide (*Morell* 1974; *García-Rafanell, Lasunción, Morell and Herrera* 1977; *Alric* 1973) and glibenclamide (*Loubatières, Mariani, Ribes and Alric* 1973) after different times of treatment, and the effect of two subsequent periods of 48 hours of starvation on the plasma individual amino acid levels in the rat.

Material and Methods

Female virgin Wistar rats, weighing initially 140-150 g were used. Rats were housed in a temperature controlled animal room (23°C) with light cycle 12-on 12-off. Animals were fed rat chow and tap water *ad libitum*. Rats were kept in individual metabolic cages and sulfonylureas were administered to them twice daily, at one half and eight hours after the beginning of the light cycle respectively. The drugs were given by stomach tube, without anesthesia, suspended in 0.5% CM-cellulose containing 0.3% Tween 80, at the following concentrations: 100 mg/kg of body wt. for tolbutamide, and 5 mg/kg of body wt. for both glibenclamide and glipentide. These concentrations were reputed as equihypoglycaemic (*García-Rafanell* et al. 1977; *Codina, Lasunción and Herrera* 1977) when administered in this form. Blood samples were collected dropwise from the cut tip of the tail into heparinized china plates just prior to the morning administration of the drugs in the indicated days, except in day 24, in which the rats were bled 3 hours after the drug administration.

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Table 1. Effect of the stress on the plasma individual amino acid concentrations of rats kept in isolation cages (IC), treated twice daily with dissolvent by means of gastric sonda (GS), during one week in both cases, as compared to controls (C) kept in collective cages without further hindrance.

Amino acids	C	IC	GS
Ala	734 ±65 (1)	752 ± 12	695 ± 52
Glu + Gln	952 ± 44	894 ± 85	869 ± 40
Ser	387 ± 25	318 ± 19	396 ± 21
Gly	272 ± 24	256 ± 30	261 ± 12
Asp + Asn	157 ± 13	126 ± 15	122 ± 12
Thr	173 ± 9	168 ± 13	187 ± 10
"Gluconeogenic"	2160 ± 89	2513 ±140	2529 ±102
Pro	383 ± 34	356 ± 58	328 ± 25
Hyp	54 ± 4	50 ± 2	34 ± 3**
Amino Acids	437 ± 36	405 ± 55	363 ± 28
Leu + Ile	312 ± 18	345 ± 38	346 ± 19
Val	180 ± 14	175 ± 13	195 ± 35
Branched Chain	492 ± 23	520 ± 31	501 ± 19
Lys	397 ± 36	446 ± 50	402 ± 25
Arg	176 ± 36	168 ± 10	182 ± 13
His	87 ± 14	75 ± 14	81 ± 6
Cit	62 ± 7	50 ± 13	44 ± 11
Basic	777 ± 27	739 ± 81	690 ± 44
Trp	256 ± 18	275 ± 25	290 ± 25
Tyr	62 ± 7	90 ± 22	85 ± 7
Phe	64 ± 7	66 ± 7	97 ± 19
Aromatic	403 ± 29	431 ± 49	472 ± 29
Taurine	361 ± 39	246 ± 30	313 ± 28
Cysteine'	107 ± 6	101 ± 67	112 ± 13
Met + Orn	176 ± 37	174 ± 42	153 ± 4
Sulphur	615 ± 32	520 ± 65	600 ± 58
Total	5334 ± 94	5129 ± 344	5156 ± 150

Plasma was obtained by centrifugation of the blood samples and deproteinized with cold acetone as described previously (*Arola, Herrera and Alemany* 1977a). Deproteinized acetone supernatants were used for individualized amino acid determinations using a radiochemical method based on the dansyl chloride reaction with the amino acids (*Arola, Palou, Herrera and Alemany* 1976) and were processed statistically using a Compucorp 445 "Statistician" desk computer.

Amino acids were distributed in several groups according to their structure and main physiological functions. The group of aromatic amino acids included tyrosine, tryptophan and phenylalanine, basic amino acids included lysine, histidine, arginine and citrulline; sulphur amino acids included taurine, methionine (that also incorporated the data on ornithine, due to the inability of the method to discriminate between both) and "cysteine", composite value for cysteine, cysteate and half-cystine, given as a whole for better physiological interpretation; branched chain amino acids included valine and leucine + isoleucine (also not discriminated); imino acids included proline and hydroxyproline; "gluconeogenic" amino acids included alanine, glycine, serine, glutamate + glutamine and aspartate + asparagine (these values are given together due to partial glutamine – and asparagine – deamidation during storage) all these amino acids are gluconeogenic but there are other gluconeogenic amino acids not included into this group (proline, phenylalanine, etc.) because of better adscription to other groups.

In order to check the possible effect of stress on the plasma concentration of individual amino acids, three groups of five animals each were studied under different conditions of stress; the group used as control was kept in collective cages during one week, and no treatment was applied to them; the animals of the second group were housed in individual (metabolic) cages for one week and no manipulation was inflicted on them; the third group was housed in collective cages, but each rat received twice daily a solution of CM-cellulose and Tween-80 via gastric sonda for one week.

Results

Effects of the Stress

The results of mild stress on plasma amino acid profiles can be seen in Table 1. With the exception of a significant decrease in the plasma levels of hydroxyproline in the rats of the third group, neither the plasma concentration of total amino acids nor the individual figures differed significantly between the second and third groups and the controls.

Effect of Chronic Sulfonylurea Treatment

As shown in Figure 1, with the exception of a significant decrease in the total plasma amino acid concentration observed after three days of treatment with glipentide, the mean total plasma amino acid concentrations of rats treated twice daily with either tolbutamide, glibenclamide or glipentide, differ very little from those of controls that received no drugs but only the CM-cellulose excipient. After 48 hours of fasting, in the 12th day of treatment, there is a slight decrease in the total amino acid concentrations of both controls and glipentide treated rats, not observed in the animals treated with either tolbutamide or glibenclamide, being this difference statistically significant. After the fasting period there is a "rebound effect" in the levels of total plasma amino acids, most marked in tolbutamide treated animals and the controls. The decrease in plasma total amino acid concentrations is repeated after a second 48 hour fasting on day 29th of treatment. Both periods of fasting produce a no significant decrease in the plasma proline and hydroxyproline concentrations (Fig. 1). The refeeding after the first period of starvation produces also a "rebound effect" that overshoots the values of proline and hydroxyproline previous to the fasting. The treatment with the sulfonylurea drugs did not affect significantly the plasma concentrations of proline and hydroxyproline in the fed animals, but considerably postponed the "rebound effect" of these imino acids with refeeding; this effect is specially marked in the hydroxyproline concentrations of rats treated with either glipentide or glibenclamide.

The plasma concentrations of the "gluconeogenic" amino acids are shown in Figure 2. In the case of glutamate + glutamine and alanine it can be seen that both glipentide and glibenclamide produce a decrease in their levels at the third day of treatment, which is not observed in the controls nor in the rats treated with tolbutamide. Tolbutamide produces an increase in some "gluconeogenic" amino acids, significant for

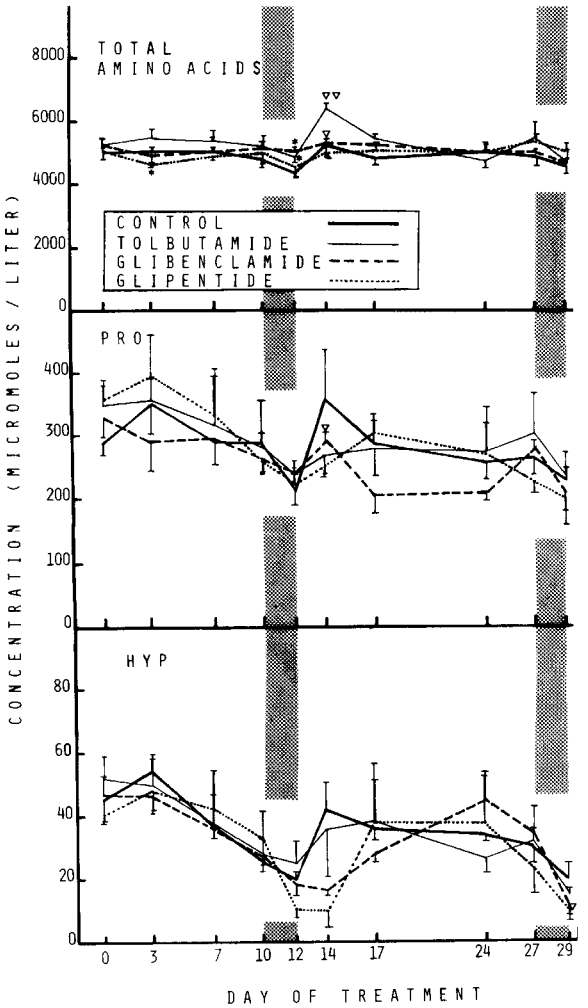

Fig. 1. Effect of chronic treatment with sulfonylureas and 48 h of fasting on the plasma concentration of total amino acids, proline and hydroxyproline in the rat. The drugs were administered twice daily by stomach tube at the concentrations of 100 mg/kg of body weight of tolbutamide and 5 mg/kg of body weight for both glibenclamide and glipentide. The fasting periods are indicated by the shadowed areas, corresponding to the days 10th to 12th and 27th to 29th of treatment. Triangles correspond to the P values for each group versus the amino acid levels found on the previous determination: $\Delta = P < 0.05$; $\Delta\Delta = P < 0.01$; $\Delta\Delta\Delta = P < 0.001$ Asterisks correspond to the P values versus the controls that did not received the drugs: * = $P < 0.05$; ** = $P < 0.01$.

glutamate + glutamine at days 10 and 12 of treatment and for serine and aspartate + asparagine at day 14. Fasting produced different effect on the "gluconeogenic" amino acids concentrations. While neither glutamate + glutamine, serine, threonine nor aspartate + asparagine levels show significant variations with fasting in the four groups of rats studied, alanine concentration is significantly reduced in both periods of fasting studied, whereas glycine is significantly increased. Refeeding after the first 48 hours of fasting produces a "rebound effect", most marked in the case of alanine regardless of the drug treatment applied to the rats.

In Figure 3 are summarized the plasma concentrations of individual branched chain amino acids, which, in general, show very slight variations with either fasting, refeeding or sulfonylureas treatment. Glibenclamide and glipentide potentiate, in some way, the fasting-induced rise or leucine + isoleucine, and, together with tolbutamide, they produce a greater increase in the valine levels with refeeding than those observed in the controls. In Figure 3 are also shown the plasma concentration values of basic amino acids. Both arginine and histidine levels are significantly reduced at the third day of treatment, either with glipentide or glibenclamide, while there are no significant changes at this time in tolbutamide treated animals nor in the controls. The plasmatic levels of aromatic amino acids can be seen in Figure 4. All of them follow a similar pattern, showing a decrease during the two fasting periods studied, recovering the pre-fasting levels with refeeding, although this recuperation seems to be slower than the one observed in "gluconeogenic" amino acids and imino acids. The treatment with sulfonylureas practically did not affect the plasma concentrations of aromatic amino acids throughout all the experiment. The concentrations of plasma sulphur amino acids can also be seen in Figure 4. It can be observed that fasting produces decreases in the concentrations of "cysteine", taurine and methionine + ornithine although the changes in taurine seem to be delayed and maintained up to the second day of refeeding that follows the 48 hours fast. In the rats treated with glibenclamide or glipentide, the changes in sulphur amino acids are similar to those observed in controls, whereas in the rats treated with tolbutamide the changes in taurine during the fasting-refeeding period reverse totally the control pattern.

All the previous data are referred to blood samples collected 16 hours after the daily drug administration to the rats. In order to check whether shorter periods between sulfonylureas administration and blood samples extraction could in any way alter the observed plasma amino acids concentrations, on the day 24th of treatment, blood was collected three hours after the first drug administration of the day. The plasma amino acids concentrations found are shown in the Table 2. With the only exception of a significant reduction in the arginine levels in the rats treated with glipentide, the individual (and also the composite group) levels of amino acids are not statistically different neither with the values in the controls not with those of the samples obtained 15-hours after drug administration.

Discussion

In general agreement with other authors (*Metzger* et al. 1971; *Adibi* 1971), in this work it is shown that fasting produces decreases in the plasmatic levels of several amino acids, being alanine the best example.

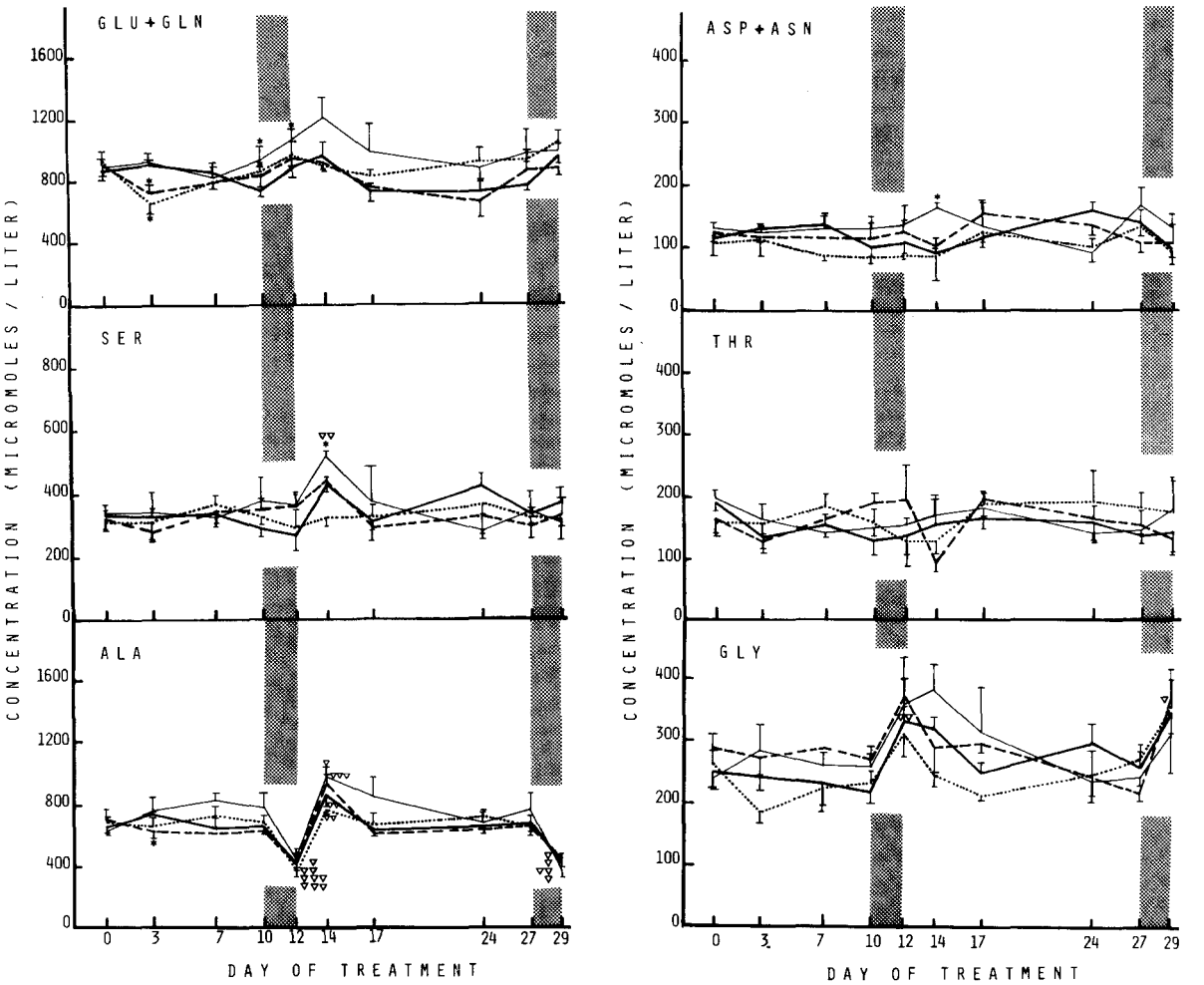

Fig. 2. Effect of chronic treatment with sulfonylureas and 48 h of fasting on the plasma concentration of 'gluconeogenic' amino acids in the rat. The treatment and symbols used are the same as for Figure 1.

This is not a generalized trend; the concentrations of other amino acids remain unaltered with fasting (serine, aspartate + asparagine, threonine, citrulline, arginine and lysine); other amino acids show an increase, being glycine the amino acid with more marked increase. Fasting is associated with an increased release of gluconeogenic substrates from the non-splanchnic tissues (*Blackshear, Holloway and Alberti* 1974; *Felig* et al. 1970; *Pozefsky* et al. 1969); the concentrations of gluconeogenic amino acids in plasma would, therefore, be the result of the balance between their release and utilization their by the splanchnic bed organs for gluconeogenesis (*Aikawa* et al. 1973; *Felig* 1973). In accordance with the well known role of alanine as gluconeogenic substrate (*Ishikawa, Aikawa and Matsutaka* 1972; *Felig* et al. 1970), the present results are in support of the notion that endogenic utilization of this amino acid for gluconeogenesis exceeded its release from peripheral organs, mainly skeletal muscle. A similar argument could be used to explain the fall of the concentrations of several other amino acids, such as proline, hydroxyproline, cysteine etc., that, although more indirectly than alanine, can also be used as gluconeogenic substrates. The net increase in the plasma glycine concentration found with fasting agree with the data observed by *Metzger, Hare and Freinkel*(1971). Using the same reasoning, this increase of glycine could be the result of a slower utilization of this amino acid as compared with the rate of production (and hence of its appearance in the plasma) of this and other gluconeogenic amino acids.

All these changes in plasmatic amino acid concentrations become rapidly compensated with refeeding, but we have observed that this compensation is preceded by a "rebound effect" in most of the amino acids, suggesting the existence of a temporal imbalance between the increase of release into the bloodstream from the gastrointestinal tract, the decrease of their utilization for energetic purposes and its enhanced uptake by extra-splanchnic organs and tissues.

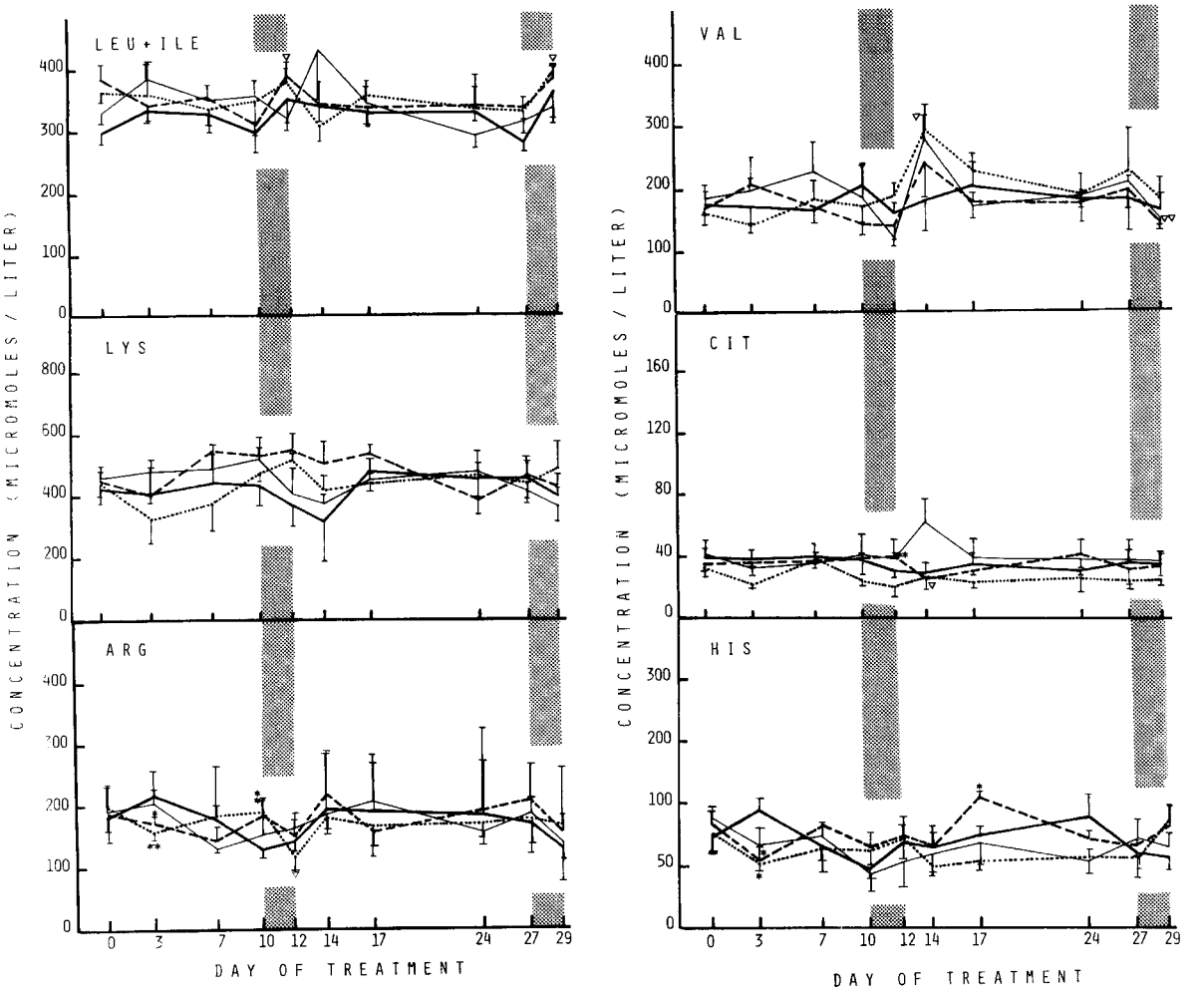

Figure 3. Effect of chronic treatment with sulfonylureas and 48 h of fasting on the plasma concentration of branched chain and basic amino acids in the rat. The treatment and symbols used are the same as for Figure 1.

Further studies will be required to determine the comparative quantitative contribution of these factors upon the homeostatic balance of amino acids concentration in the plasma. Prolonged treatment with sulfonylurea drugs produces small changes in the plasma amino acid levels; the most consistent effect is found in a significant decrease in the concentrations of glutamate + glutamine, alanine, arginine and histidine produced at day 3th of treatment with glibenclamide or glipentide. These two sulfonylureas are more powerful hypoglycaemic drugs than tolbutamide, and as shown recently (*Codina* et al. 1977), their effects on glycaemia fade out after a prolonged treatment. Thus, it is not surprising that their hypoaminoacidemic effects will also fade out during a more prolonged treatment. When blood samples were obtained shortly afther the administration of the drugs, the effects of glipentide decreasing the levels of arginine are still evident, in spite of the fact that the animals were already on their 24th day of treatment. Here again, the observed effect parallels in some way those found with regard to the glycaemia, at this time of treatment the hypoglycemia can be observed only shortly after the daily drug administrations (*Codina* et al. 1977).

The effects detected upon amino acids concentrations on the plasma of rats treated with sulfonylureas can be precisely attributed to the administration of these drugs and not to artifact induced by the handling of the animals, as ascertained by the lack of change of most of the individual amino acid levels when studied the effects of mild stress, induced by the isolation of the animals or the gastric intubation. The only significant differences found versus controls are those related with hydroxyproline, which levels drop significantly after one week of gastric sonda treatment stress. This behaviour of hydroxyproline concentrations is clearly apparent along the chronic treatment, being probably the considerable variation found in Figure 1 due mainly to the stress. No other amino acid showed any significant changes with both isolation and gastric tube treatment.

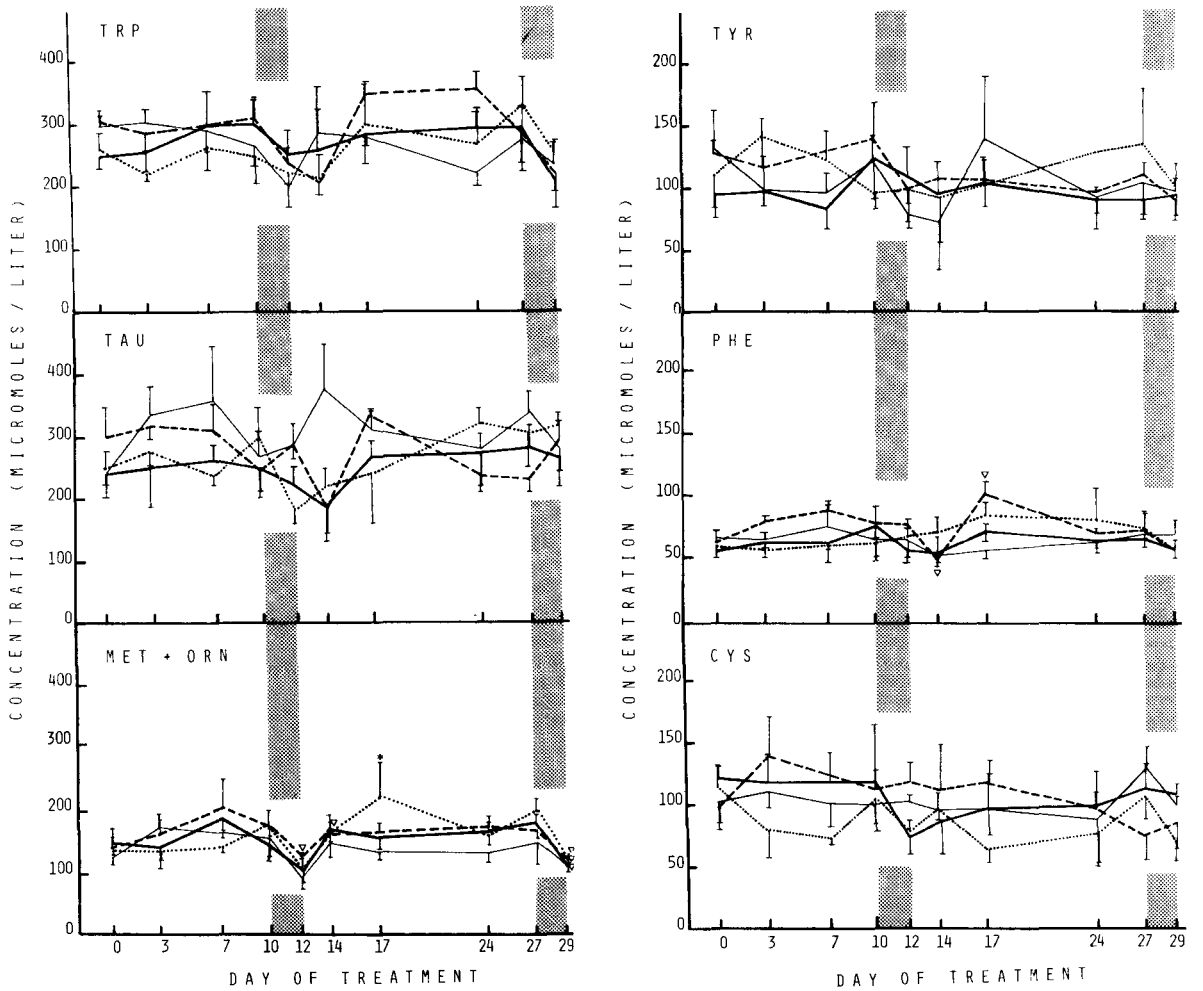

Fig. 4. Effect of chronic treatment with sulfonylureas and 48 h of fasting on the plasma concentration of aromatic and sulphur amino acids in the rat. The treatment and symbols used are the same as for Figure 1.

Although the effect of these drugs on the plasma individual levels of amino acids can be influenced by their insulinotropic action (*Madsen* 1967), as insulin itself is known to affect the circulating concentration of amino acids (*Luck, Morrison and Wilbur* 1928; *Adibi, Morse and Amin* 1975), their action is more complex than that, as it has been shown that these drugs have a direct effect both on protein biosynthesis (*DeChatelet and McDonald* 1968; *Fulks, Li and Boldbug* 1975) and amino acid transamination (*Bendit* 1957). On the other hand, sulfonylureas have been shown to inhibit hepatic gluconeogenesis (*Dehmel* et al. 1971), fact that can also influence the imbalance between amino acids release and uptake and metabolization. It is possible that this imbalance will be specially enhanced with food withdrawal, when gluconeogenesis must be proceeding at its maximum capability, a fact that could be put foward to explain the small decrease in plasma total amino acids concentration in the fasting rat treated with sulfonylureas.

Table 2. Effect of the immediacy of drug administration (3 h) on the plasma individual amino acid concentrations of rats tre treated chronically with sulfonylureas (at day 24 of treatment).

Amino acid	Control	Tolbutamide	Glibenclamide	Glipentide
Ala	701 ± 68 (1)	674 ± 29	713 ± 45	672 ± 31
Glu + Gln	959 ± 75	959 ± 50	861 ± 91	871 ± 43
Ser	380 ± 10	379 ± 34	362 ± 40	357 ± 30
Gly	235 ± 8	269 ± 21	250 ± 31	227 ± 9
Asp + Asn	138 ± 19	146 ± 16	161 ± 8	119 ± 15
Thr	124 ± 11	157 ± 15	168 ± 17	137 ± 11
"Gluconeogenic"	2553 ± 135	2555 ± 129	2696 ± 138	2372 ± 60
Pro	272 ± 32	287 ± 41	231 ± 25	251 ± 32
Hyp	33 ± 1	44 ± 12	33 ± 5	32 ± 6
Imino acids	300 ± 32	331 ± 47	264 ± 24	282 ± 38
Leu + Ile	338 ± 15	320 ± 24	336 ± 20	319 ± 27
Val	209 ± 36	170 ± 13	188 ± 18	190 ± 9
Branched Chain	547 ± 48	490 ± 20	505 ± 18	509 ± 30
Lys	536 ± 130	461 ± 29	510 ± 52	510 ± 26
Arg	174 ± 3	195 ± 17	190 ± 17	146 ± 5**oo
His	67 ± 19	49 ± 10	66 ± 6	67 ± 13
Cit	44 ± 10	50 ± 1	38 ± 2	35 ± 5
Basic	820 ± 84	739 ± 39	806 ± 45	758 ± 22
Trp	299 ± 20	242 ± 36	291 ± 17	282 ± 63
Tyr	118 ± 8	106 ± 16	105 ± 16	107 ± 14
Phe	69 ± 9	63 ± 7	56 ± 2	57 ± 9
Aromatic	559 ± 62	597 ± 30	590 ± 28	484 ± 51
Taurine	282 ± 36	314 ± 14	298 ± 37	295 ± 24
'Cysteine'	122 ± 22	124 ± 35	119 ± 20	97 ± 15
Met + Orn	146 ± 53	162 ± 12	173 ± 18	154 ± 11
Sulphur	486 ± 22	411 ± 54	452 ± 29	432 ± 63
Total	5265 ± 313	5113 ± 169	5324 ± 218	4838 ± 173

(1) Values given are μmoles/liter; mean ± S.E.M. of 5 determinations. Significance versus controls: ** = $p < 0.01$. Significance versus values obtained (day 24th of treatment) 18 hours after drug administration: oo = $p < 0.001$.

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Comparison of Endocrine Secretion by Monolayer Cultures Derived by Different Procedures from Neonatal Hamster and Rat Pancreas*

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Summary

Monolayer cultures derived from neonatal hamster or rat pancreas by two different epithelioid cell-enriching gravity sedimentation procedures varied in ability to maintain uniform levels of insulin secretion with increased culture age. Rat pancreatic cultures were superior in this respect to identically derived hamster preparations, depending on the preparative procedure employed. Quantitative differences in the temporal pattern of insulin secretion by different rat pancreatic culture preparations were ascribed to plating cell density and consequent terminal cell density as a function of preparative procedure such that reduced densities favored sustained secretory levels. These findings suggest the importance of tissue species and preparative procedure in deriving pancreatic monolayer cultures capable of sustained levels of insulin secretion with age.

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Introduction

The successful application of cell culture methods to the endocrine pancreas is documented by a number of reports concerned with the morphological and functional characterization of pancreatic monolayer cultures derived from the hamster (*Macchi and Blaustein* 1969; *Macchi, Beyer, Gapp, Blaustein and Beaser* 1973; *Scheid and Macchi* 1974), guinea pig (*Hilwig, Schuster, Heptner and Wasielewski* 1968), human (*Hilwig* et al. 1968; *Hilwig and Schuster* 1970; *Leach, Ashworth, Barson and Milner* 1973), rabbit (*Leach* et al. 1973), pig (*Hilwig and Vrbanec* 1970; *Hilwig* 1972a, b), mouse (*Leiter, Coleman and Waymouth* 1974), and rat (*Hilwig* et al. 1968; *Hilwig and Vrbanec* 1970; *Lambert, Blondel, Kanazawa, Orci and Renold* 1972; *Chick* 1973; *Chick, Lauris, Flewelling, Andrews and Woodruff* 1973). Sustained rates of insulin release with increased culture age have been reported for monolayer cultures derived from fetal

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