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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 amino acid, 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, citrulline, 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 sulfonylureas 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 acid changes with the administration of insulinotrophic factors. Actually, as early as 1922 was shown that following carbohydrate ingestion, the plasma level of amino acid nitrogen is lowered (Folin and Berghlund 1922) and this observation has been confirmed repeatedly (Harris and Harris 1974; Munro and Thomson 1953; Crofford, Felts and Lacy 1964; Zinnmann, Nutall 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 metabolism 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 hypoglycemic agents in humans. The problem is particularly suggesting, as it has been shown that the insulinotropic and hypoglycemic effects of these drugs are minimized after prolonged treatment (Sodoyez, Sodoyez-Goffaux, Dunbar and Foué 1970; Codina, Lascuion 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 (Ramahandridina, DiCamp_Wordrie 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 hypoglycemic sulfonylureas of the so called second generation: glipentide (Morell 1974; Garcia-Rafanell, Lasuncion, Morell and Herrera 1977; Arib 1973) and glibenclamide (Loubabières, Marijani, Ribes and Arib 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 equihypoglycemic (Garcia-Rafanell et al. 1977; Codina, Lasucion 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.
Plasma was obtained by centrifugation of the blood samples and deproteinized with cold acetone as described previously (Arola, Herrera and Alemamy 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 Alemamy 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 – deamination 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 adsorption 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 glibenclamide, the mean total plasma amino acid concentrations of rats treated twice daily with either tolbutamide, glibenclamide or glibenclamide, 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 glibenclamide 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 glibenclamide 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 glibenclamide 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: ∆ = P < 0.05; ∆∆ = P < 0.01; ∆∆∆ = P < 0.001 Asterisks correspond to the P values versus the controls that did not received the drugs: * = P < 0.05; ** = P < 0.01.

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 of 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 Table 2. With the only exception of a significant reduction in the arginine levels in the rats treated with glibenclamide, the individual (and also the composite group) levels of amino acids are not statistically different neither with the values in the controls nor 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.
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 nonsplanchnic 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 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.
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 after 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 hypoglycaemia 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.
Although the effect of these drugs on the plasma individual levels of amino acids can be influenced by their insulinoergic 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 forward 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 treated chronically with sulfonylureas (at day 24 of treatment).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Control</th>
<th>Tolbutamide</th>
<th>Glibenclamide</th>
<th>Gliquidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>701 ± 68 (1)</td>
<td>674 ± 29</td>
<td>713 ± 45</td>
<td>672 ± 31</td>
</tr>
<tr>
<td>Glu + Gln</td>
<td>959 ± 75</td>
<td>959 ± 50</td>
<td>861 ± 91</td>
<td>871 ± 43</td>
</tr>
<tr>
<td>Ser</td>
<td>380 ± 10</td>
<td>379 ± 34</td>
<td>362 ± 40</td>
<td>357 ± 30</td>
</tr>
<tr>
<td>Gly</td>
<td>235 ± 8</td>
<td>269 ± 21</td>
<td>250 ± 31</td>
<td>227 ± 9</td>
</tr>
<tr>
<td>Asp + Asn</td>
<td>138 ± 19</td>
<td>146 ± 16</td>
<td>161 ± 8</td>
<td>119 ± 15</td>
</tr>
<tr>
<td>Thr</td>
<td>134 ± 11</td>
<td>157 ± 15</td>
<td>168 ± 17</td>
<td>137 ± 11</td>
</tr>
<tr>
<td>“Glucogenic”</td>
<td>2553 ± 135</td>
<td>2555 ± 129</td>
<td>2695 ± 138</td>
<td>2372 ± 60</td>
</tr>
<tr>
<td>Pro</td>
<td>272 ± 32</td>
<td>287 ± 41</td>
<td>231 ± 25</td>
<td>251 ± 32</td>
</tr>
<tr>
<td>Hyp</td>
<td>33 ± 1</td>
<td>44 ± 12</td>
<td>33 ± 5</td>
<td>32 ± 6</td>
</tr>
<tr>
<td>Imino acids</td>
<td>300 ± 32</td>
<td>331 ± 47</td>
<td>264 ± 24</td>
<td>282 ± 38</td>
</tr>
<tr>
<td>Leu + Ile</td>
<td>338 ± 15</td>
<td>320 ± 24</td>
<td>336 ± 20</td>
<td>319 ± 27</td>
</tr>
<tr>
<td>Val</td>
<td>209 ± 36</td>
<td>170 ± 13</td>
<td>188 ± 18</td>
<td>190 ± 9</td>
</tr>
<tr>
<td>Branched Chain</td>
<td>547 ± 48</td>
<td>490 ± 20</td>
<td>505 ± 18</td>
<td>509 ± 30</td>
</tr>
<tr>
<td>Lys</td>
<td>536 ± 130</td>
<td>461 ± 29</td>
<td>510 ± 52</td>
<td>510 ± 26</td>
</tr>
<tr>
<td>Arg</td>
<td>174 ± 3</td>
<td>195 ± 17</td>
<td>190 ± 17</td>
<td>146 ± 5**oo</td>
</tr>
<tr>
<td>His</td>
<td>67 ± 19</td>
<td>49 ± 10</td>
<td>66 ± 6</td>
<td>67 ± 13</td>
</tr>
<tr>
<td>Cit</td>
<td>44 ± 10</td>
<td>50 ± 1</td>
<td>38 ± 2</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>Basic</td>
<td>820 ± 84</td>
<td>739 ± 39</td>
<td>806 ± 45</td>
<td>758 ± 22</td>
</tr>
<tr>
<td>Trp</td>
<td>299 ± 20</td>
<td>242 ± 36</td>
<td>291 ± 17</td>
<td>282 ± 63</td>
</tr>
<tr>
<td>Tyr</td>
<td>118 ± 8</td>
<td>106 ± 16</td>
<td>105 ± 16</td>
<td>107 ± 14</td>
</tr>
<tr>
<td>Phe</td>
<td>69 ± 9</td>
<td>63 ± 7</td>
<td>56 ± 2</td>
<td>57 ± 9</td>
</tr>
<tr>
<td>Aromatic</td>
<td>559 ± 62</td>
<td>597 ± 30</td>
<td>590 ± 28</td>
<td>484 ± 51</td>
</tr>
<tr>
<td>Taurine</td>
<td>282 ± 36</td>
<td>314 ± 14</td>
<td>298 ± 37</td>
<td>295 ± 24</td>
</tr>
<tr>
<td>‘Cysteine’</td>
<td>122 ± 22</td>
<td>124 ± 35</td>
<td>119 ± 20</td>
<td>97 ± 15</td>
</tr>
<tr>
<td>Met + Orn</td>
<td>146 ± 53</td>
<td>162 ± 12</td>
<td>173 ± 18</td>
<td>154 ± 11</td>
</tr>
<tr>
<td>Sulphur</td>
<td>486 ± 22</td>
<td>411 ± 54</td>
<td>452 ± 29</td>
<td>432 ± 63</td>
</tr>
<tr>
<td>Total</td>
<td>5265 ± 313</td>
<td>5113 ± 169</td>
<td>5324 ± 218</td>
<td>4838 ± 173</td>
</tr>
</tbody>
</table>

(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 Pancreatic Monolayer Cultures


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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 ascribable 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.

Key-Words: Pancreatic Monolayer Cultures, Hamster, Rat – Secretion, Insulin, Glucagon – Culture Cell Density-Dependent Inhibition

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 Beasser 1973; Scheid and Macchi 1974), guinea pig (Hilwig, Schuster, Heptner and Wastiewski 1968), human (Hilwig et al. 1968; Hilwig and Schuster 1970; Leach, Ashworth, Barson and Milner 1973), rabbit (Leach et al. 1973), pig (Hilwig and Vrbane 1970; Hilwig 1972a, b), mouse (Leiter, Coleman and Waymouth 1974), and rat (Hilwig et al. 1968; Hilwig and Vrbane 1970; Lambert, Blondel, Kanazawa, Orci and Renold 1972; Chick 1973; Chick, Lauris, Fletwell, Andrews and Woodruff 1973). Sustained rates of insulin release with increased culture age have been reported for monolayer cultures derived from fetal

*Supported in part by a research grant from the Upjohn Company, Kalamazoo, Michigan, U.S.A. Part of a dissertation submitted by David A. Gapp to Boston University Graduate School in partial fulfillment of the requirements for the Doctor of Philosophy degree.

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Received: 29 Aug. 1977 Accepted: 1 Nov. 1977

0018-5043/78 1132-0489 $ 05.00 © 1978 Georg Thieme Publishers