Sequential Metabolic Characteristics following Portacaval Shunt in Rats

J.E.G. de Boer, R.J. Oostenbroek, J.J. van Dongen, M.A. Janssen, P.B. Soeters

Departments of *Biochemistry and bSurgery, University of Limburg, Maastricht, The Netherlands

Abstract. A portacaval shunt (PCS) model is frequently employed to study phenomena inherent in portal-systemic shunting of splanchnic blood. In many species, a PCS induces hepatic insufficiency, accompanied by encephalopathy. Rats operated on with a 'nonsuture' technique tolerate a PCS better and exhibit no or only slight encephalopathy. Age and environment seem to have a large impact on the ability to tolerate a PCS. This explains the discrepancies between the results of different investigators and the varying time periods reported between the PCS operation and the optimal time for experiments. To characterize the PCS model (button technique) in rats with respect to metabolic parameters in our field of interest, we studied three groups of male Sprague-Dawley rats - non-operated (n = 12); sham-operated (n = 12) and PCS (n = 13) - for 4 weeks following surgery. Body weight in the PCS group decreased for 1 week after surgery and then increased at about the same rate as in the control groups. Plasma immunoreactive insulin, plasma immunoreactive glucagon (IRG) and aromatic amino acid concentrations were highest 1 week after surgery and tended to normalize in the next weeks. Plasma branched-chain amino acid (BCAA) concentrations were decreased in the 1st, 2nd and 3rd week after surgery, after which normalization occurred. These data demonstrate that after 3–4 weeks, male Sprague-Dawley rats start to recover from the metabolic disturbances caused by PCS with regard to the parameters measured. Therefore, experiments in this area, especially those relating to BCAA metabolism, should be carried out 2–3 weeks after the shunt operation (button technique).

Introduction

Animals with end-to-side portacaval shunt (PCS) have been used extensively to study the metabolic consequences of the portal-systemic shunting of blood around the liver. In most species, a portal-systemic shunt eventually leads to hepatic failure, encephalopathy and death. During the last 10 years, alternative theories have been proposed, linking hepatic encephalopathy to a disturbed neutral amino acid pattern [Fischer
and Baldessarini, 1971; Hoyumpa et al., 1974; James et al., 1979; Munro et al., 1975; Soeters et al., 1977]. This pattern has been reported in man and in several animal species and consists of decreased plasma levels of branched-chain amino acids (BCAA) and increased levels of aromatic amino acids (AAA). To obtain a rat model exhibiting these abnormalities we initially used rats 5-6 weeks after a PCS operation [Child, 1963; James et al., 1976; Lee et al., 1974; Pector et al., 1980] and did not find consistent differences in plasma immunoreactive insulin (IRI) and in plasma amino acids compared with controls. Apparently, after 5-6 weeks, PCS rats had, at least partly, adapted to the PCS status. Therefore, it was necessary to redefine our PCS rat model. A series of relevant parameters, namely body weight, plasma amino acids, plasma IRI and plasma immunoreactive glucagon (IRG), were measured before operation and 1, 2, 3 and 4 weeks after operation in PCS rats, sham-operated rats and nonoperated controls. In addition, a separate group of rats was subjected to splenoportography at 2.5 and 4.5 weeks after a PCS operation and compared with control rats.

Materials and Methods

Male Sprague-Dawley rats weighing 190-200 g were used. They were housed under standard laboratory conditions. Thirty-seven rats were divided into three groups: unoperated controls (n = 12), sham-operated rats (n = 12) and PCS rats (n = 13). PCS operations were performed under ether anesthesia according to the technique described by Lee and Fisher [1961], and modified by employing a 'nonsuture' or 'button' technique [Funovics et al., 1975]. The size of the Teflon button was adapted to the diameter of the portal vein. The PCS operation takes about 25 min. The sham-operated group underwent laparotomy and had the portal vein occluded for 10 min while the inferior caval vein was partially occluded for 5 min. The control group was not treated at all. Three milliliters of blood were drawn from rats in the fasted state by ocular puncture before surgery and 1, 2, 3 and 4 weeks after surgery. The blood was collected in heparinized tubes and immediately cooled on ice. Protease inhibitor aprotinin (Trasylol®) was added to the cooled test tube used for the determination of IRG. Plasma was frozen immediately at −70 °C until analysis.

Plasma IRI was determined with a commercially available radioimmunoassay kit (Wellcome, Beckenham, UK) with human standards and antihuman antisera. The normal range for fasted rats was 18.2 ± 8.1 μU/ml (mean ± SD).
Plasma IRG was measured with a commercial radioimmunoassay kit (Biolab, Brussels, Belgium) with human standards and antihuman antisera, 30,000 daltons. The normal range for fasted rats was 225 ± 79 pg/ml (mean ± SD).
Plasma amino acids were determined on an LKB 4400 amino acid analyzer [Dilley and Rocek, 1979]. The plasma was deproteinized with sulfosalicylic acid (5% w/v) and filtered through 0.22-μm pores (Millipore filters). Normal values for rats were determined in 75 untreated male Sprague-Dawley rats of about 200-300 g (table 1). In this study, overnight-fasted values are given for IRI, IRG and amino acids in order to eliminate interindividual variations due to differences in food intake.

For statistical calculations the Wilcoxon signed-rank test for interweekly differences within a group and the Wilcoxon rank-sum test for differences between two groups [Lehmann and d'Arrera, 1975] were used.

Results

Body Weight

Figure 1 shows the body weight curves in PCS rats, sham-operated rats and nonoperated controls. Characteristic in the curve of PCS rats is a weight loss of 5-15% during the 1st week after surgery, a minimum body weight 1 week postoperatively and an increase thereafter at a rate comparable to
Fig. 1. Postoperative body weight curves (mean ± SD) of control (n = 12), sham-operated (n = 12) and PCS (n = 13; shunted according to the button technique) male Sprague-Dawley rats.

Fig. 2. Plasma IRI (a) and IRG (b) in sham-operated and PCS male Sprague-Dawley rats (mean ± SEM). Open symbols indicate statistical significance compared with the preoperative value. Asterisks above the X-axis indicate statistically significant differences between PCS and sham-operated rats. Unoperated controls fell within the normal range (see Materials and Methods).

Table 1. Mean (± SD) pre- and postoperative plasma amino acid levels (μmol/l) in PCS, sham-operated and unoperated (controls; institutional laboratory control values) male Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Plasma amino acids</th>
<th>Unoperated controls (n = 75)</th>
<th>Sham-operated (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 1</td>
</tr>
<tr>
<td>Valine</td>
<td>167 ± 34</td>
<td>178 ± 27</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>85 ± 18</td>
<td>88 ± 13</td>
</tr>
<tr>
<td>Leucine</td>
<td>125 ± 25</td>
<td>135 ± 27</td>
</tr>
<tr>
<td>Threonine</td>
<td>268 ± 50</td>
<td>268 ± 50</td>
</tr>
<tr>
<td>Serine</td>
<td>287 ± 49</td>
<td>304 ± 43</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>67 ± 15</td>
<td>69 ± 9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>45 ± 13</td>
<td>37 ± 9</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>47 ± 27</td>
<td>47 ± 10</td>
</tr>
</tbody>
</table>

All rats were fasted for at least 14 h.
sham-operated and nonoperated control rats. The preoperative weight of PCS rats was reached 2 weeks after the operation.

**Plasma IRI and IRG**

Figure 2 demonstrates that postabsorptive plasma levels of IRI in PCS rats were 60% higher than the preoperative values (p < 0.04) and that the levels of IRG were about 300% higher (p < 0.02) 1 week after surgery, after which they tended to normalize. Sham-operated animals showed the same pattern but to a lesser extent, namely about 10% rise in IRI (p < 0.05) and about 100% in IRG (p < 0.02) 1 week after surgery. Three weeks after the sham-operation, they were completely normalized. Compared to the sham-operated rats, the PCS animals had significantly higher plasma IRI levels up to 3 weeks after surgery (44, 51 and 38% 1, 2 and 3 weeks after surgery, respectively) and significantly higher plasma IRG levels up to at least 4 weeks after surgery (69, 100, 113 and 78% at week 1, 2, 3 and 4 after surgery, respectively).

**Aromatic Amino Acids**

The plasma levels of the AAA, tyrosine and phenylalanine, in the PCS group were significantly increased (p < 0.03) 1 week after the shunt operation; in the sham group, the plasma phenylalanine level was also significantly elevated (p < 0.05) 1 week after surgery but less than in the PCS group (p < 0.02; fig. 3, table 1). Compared with sham-operated rats, the PCS rats had significantly higher plasma tyrosine and phenylalanine levels (p < 0.03) 1 week after surgery. No significant difference was recorded 2 and 3 weeks after surgery between the PCS group and the sham-operated group, while at week 4 after surgery, the difference just reached significance (p < 0.05). Tryptophan was not significantly altered.

**Branched-Chain Amino Acids**

Plasma levels of the BCAA, valine, leucine and isoleucine, are shown in figure 4. In the PCS rats, plasma levels of all three BCAA decreased, stabilized at a low level 2–3 weeks after surgery and tended to normalize thereafter. Compared to preoperative values, levels of leucine and isoleucine in the PCS group 2 and 3 weeks after surgery were significantly lower (leucine p < 0.02 and isoleucine p < 0.03). The decrease in valine concentration did not reach statistical significance, due to greater individual variation; in 4 rats, the plasma valine concentration was completely unaffected.

**Splenoportography**

A separate group of rats shunted according to the button technique was subjected to
Fig. 3. Plasma AAA (mean ± SEM) in sham-operated and PCS male Sprague-Dawley rats. Unoperated controls fell within the normal range (see table 1, first column). Symbols are used as indicated in the legend of figure 2.

Fig. 4. Plasma BCAA (mean ± SEM) in sham-operated and PCS male Sprague-Dawley rats. Unoperated controls fell within the normal range (see table 1, first column). Symbols are used as described in the legend of figure 2.

Fig. 2. Plasma AAA and BCAA in sham-operated and PCS male Sprague-Dawley rats. Unoperated controls fell within the normal range (see table 1, first column). Symbols are used as indicated in the legend of figure 2.

splenoportography to evaluate the patency of the shunt at different postoperative time periods. Each rat had only one splenoportography. Consequently, results from different postoperative time periods are derived from different rats and may only give a rough indication of PCS rats with similar body weight curves. PCS rats that gained weight like the rats used in the study described above (n = 7) had 2.5 weeks postoperatively, open shunts...
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Fig. 5. a Splenoportography of control rat. Opacification of the spleen, splenic vein and portal vein, subsequent filling of the whole liver and, at the right side of the picture, some superposition of liver and heart. b, c Splenoportography of button-shunted rat 2.5 weeks after surgery. b Opacification of spleen, splenic vein and direct filling of inferior caval vein (some narrowing due to the button). No contrast in the liver, opacification in the heart. c Opacification of part of the spleen with subsequent filling of obstructed portal vein. Collaterals are visualized. No contrast in the liver. d Splenoportography of button-shunted rat 4.5 weeks after surgery. Open PCS. Opacification of the whole inferior and superior caval vein and extensive collaterals resulting in the visualization of the liver.

and no clear collaterals, while the liver was not visualized. In contrast, in rats that did not gain weight and/or were still below their initial body weight (n = 4), a clear obstruction of the shunt was visualized 2.5 weeks postoperatively as well as collaterals to the stomach and esophagus but not to the liver, evidenced by the absence of contrast in the liver. All rats subjected to splenoportography 4.5 weeks after surgery (n = 5) gained weight, including rats that did not gain weight at 2.5 weeks after surgery. All these rats had large collaterals and contrast in the liver, independent of an obstruction of the shunt. For representative pictures, see figure 5.

Rats that underwent PCS according to the 'suture' technique (n = 10) lost more weight (fig. 6) than button-shunted rats. Additionally, splenoportography was done in 5 of these rats after 2.5 weeks. Hardly any visualizable collaterals were seen and 2 of the 5 rats had an obstructed shunt. At 4.5 weeks after surgery, all rats (n = 5) had an obstructed shunt. Three of these rats had developed collaterals, 2 of which had contrast in the liver.
Discussion

In earlier experiments, we could not confirm, 5–6 weeks postoperatively, the metabolic characteristics described in the literature, using the nonsuture technique. Besides the condition of the rats and the time of shunting, other factors may have been responsible for this, including the rat species employed, the technical skill with which the operations were carried out and several environmental factors. A nonsuture technique was chosen because it permits standardization of the operation time, occlusion time of the portal vein, and within certain limits, standardization of the diameter of the anastomosis. The significance of these factors, however, cannot easily be quantified.

In order to eliminate fluctuations in plasma amino acids, plasma IRI and plasma IRG levels due to interindividual differences in food intake, blood samples, in this study, were taken in the overnight-fasted state. Pair-feeding might circumvent problems of differences in food intake, but causes an abnormal eating-behavior: consumption of the food that is granted to the control rats within a relatively short time and starving thereafter.

Body Weight

Reproducible body weight curves were recorded in rats of about 200 g at the time of the PCS operation. Rats with a body weight below 180 g had a higher mortality rate and greater variations in body weight and metabolic parameters after operation. Before undergoing the PCS operation, rats should be in optimal condition. Infections, stress due to transport, abnormal noise, etc., in the week before the PCS operation and possibly the use of other anesthetics than ether, inhibit growth. Most investigators report weight losses of up to 30% or more [Child, 1963; Pector et al., 1980; Rossouw et al., 1978] and the lowest body weights are generally recorded 4–6 weeks after surgery. Preoperative weights are not reached within 6 weeks or even 5 months. Sham-operated rats, on the other hand, start gaining weight 1 week after the operation or later, which also contrasts...
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with our results. Initial declines in body weight in the PCS group are most probably caused by a low food intake. The favorable body weight curves in PCS rats after 2-3 weeks warrant the conclusion that the metabolic changes observed after 2-3 weeks are specific for PCS and are not partly due to undernutrition.

Table II. Mean (± SD) plasma neutral amino acids (µmol/l) in PCS (button technique) and control rats 5.5 weeks after surgery

<table>
<thead>
<tr>
<th>Plasma amino acids</th>
<th>Control (n = 10)</th>
<th>PCS (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>165 ± 23</td>
<td>176 ± 28</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>90 ± 12</td>
<td>91 ± 15</td>
</tr>
<tr>
<td>Leucine</td>
<td>132 ± 14</td>
<td>147 ± 22</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>61 ± 12</td>
<td>74 ± 9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>49 ± 14</td>
<td>56 ± 9</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>46 ± 14</td>
<td>52 ± 14</td>
</tr>
</tbody>
</table>

Plasma Insulin and Glucagon

Decreases in plasma BCAA levels have been linked to hyperinsulinism [Munro et al., 1975]. A mild hyperinsulinemia is observed in all PCS rats 2 weeks after surgery (fig. 2), but the levels of insulin in some rats have normalized within 3 weeks. PCS rats exhibited a 3-fold increase in plasma glucagon levels. High glucagon levels persisted in PCS rats for at least 4 weeks after surgery. In the PCS group, peak values of insulin and glucagon were observed 1 week after surgery due to PCS and to operation trauma (see sham-operated rats). This is earlier compared with suggestions in the literature that glucagon levels increase gradually in the first weeks until a stabilized high level is achieved. No reference value for 1 week after surgery could be found in the literature.

Using the suture technique, Pector et al. [1980] reported normal plasma insulin levels in PCS Wistar rats and in pair-fed sham-operated controls 3 weeks after surgery, together with significant hyperglucagonemia in PCS rats compared to the controls. On the other hand, Rossouw et al. [1978], also using a suture technique, found lower insulin levels in their PCS group compared to the nonoperated control group, but their data are apparently derived from fed rats. As their PCS rats were virtually semistarved, insulin levels might have been influenced by the low food intake. Basal insulin levels in a lean 'maras-
cantly elevated plasma AAA levels that we recorded 1 week after surgery in PCS rats. Plasma BCAA levels in PCS rats were consistently depressed 2 and 3 weeks after operation, whereas these levels were almost normalized at 4 weeks and had completely normalized at 5.5 weeks after surgery (determined in another group of rats; table II). This is in contrast with reports of decreased BCAA levels 5 or more weeks after surgery [Lee et al., 1974]. The elevated plasma BCAA levels in sham-operated animals and the normal to slightly decreased levels in PCS rats at 1 week after surgery might be explained by operation trauma. Injury alters the plasma amino acid pattern. This pattern differs from that observed in other catabolic states and includes elevated AAA and BCAA levels [Askanazi et al., 1978]. That the BCAA levels in PCS rats are normal, or slightly increased 1 week after surgery indicates that the mechanism lowering the BCAA is already effective.

In an analogous experiment in fed rats, plasma neutral amino acid changes in the PCS group versus controls were comparable to those of fasted PCS rats. Phenylalanine, however, was increased 2–3 weeks after surgery.

**BCAA:AAA Ratio**

The development of hepatic encephalopathy is claimed to be indicated by a decreased BCAA:AAA ratio [Fischer et al., 1975]. For humans and dogs, the normal ratio (3:4) decreases to 1 in coma [Soeters et al., 1977]. Assuming that these normal values are also valid for rats, the sham-operated group in our experiment is within the normal range while the PCS group is significantly decreased 1–3 weeks after surgery compared to sham-operated rats and to the preoperative value (fig. 7). Normalization occurs at 4 weeks. No values below 2 were recorded and the rats exhibited no convincing signs of encephalopathy as reported in the literature [Grün et al., 1978]. However, we did not try to quantitate a possible encephalopathy.

Although ammonia may be one of the factors in the pathogenesis of hepatic encephalopathy, it was not determined in this study due to the limited amount of blood that could be drawn in vivo rat experiments. In this way, we diminished the risk of rats developing anemia, which would have influenced the results. From other experiments, it is clear that plasma ammonia levels are moderately elevated (1.5- to 3-fold) 2.5 weeks after PCS (controls 47 ± 7 and PCS 77 ± 12 μmol/l; mean ± SD).

This study was designed to characterize the PCS rat model operated on with a button technique, but it was felt necessary to add
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some findings of PCS rats shunted in our laboratory with the suture technique. There are, in general, important differences between both PCS rat models, when data from the same time periods after the operation are compared. We found differences in growth, encephalopathy, collaterals from the portal area to the liver, liver weight, amino acids and glucagon. Plasma enzymes (ALT, AST, GLDH and AP) are increased after the PCS operation in both nonsuture and suture-shunted rats, but tend to normalize, only in the button-shunted group, after 3 (GLDH) or 4 weeks. According to the literature [Vearman et al., 1981], serum IgA levels are also increased in some PCS rats, most pronounced in the nonsuture-shunted group, indicating a diminished excretory function [Birbeck et al., 1979; Orlans et al., 1978]. The PCS rats selected according to our criteria are relatively healthy rats; their condition is only influenced by the shunting of blood around the liver. Rats shunted with the nonsuture technique, that did not fulfill our criteria, and were less healthy, more closely resembled the suture-shunted rats. Suture-shunted rats are, in general, depleted animals at the time they are used in experiments, compared with button PCS rats fulfilling our criteria. In our opinion, it is likely that the parameters measured in suture-shunted rats are also influenced by other factors than the shunt itself, e.g. prolonged starvation and malnutrition. The process of normalization of metabolic disturbances of the PCS operation 4 weeks in our button-shunted rats may be due to the ability of rats to adapt when no extreme catabolic state has developed. The formation of collateral blood vessels may be crucial for the normalization, probably favored by an optimal condition of the rats. The presence of collaterals suggests that some degree of portal hypertension must be present, despite the fact that the shunts proved to stay open and care was taken to employ buttons with an inner diameter that allowed the passage of a normal-sized portal vein of the rats and despite the fact that twisting of the portal vein was prevented as much as possible. Close examination of the site of the button at sacrifice showed adhesions which might cause a slight stenosis. No signs of infection or positive cultures were found. Despite extreme standardization, some environmental factors will have an influence on the animal model. Therefore, every investigator, before starting experiments, should carefully define his/her own rat model in terms of metabolic characteristics specific for the model.

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Peter B. Soeters, MD,
University of Limburg,
Hospital St. Annaal,
Department of Surgery,
PO Box 1918
6201 BX Maastricht (The Netherlands)