Glomerulopressin Production by Isolated Rat Liver after Amino Acid Infusion (43299)

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Abstract. **The infusion of certain amino acids, such as serine, alanine, and proline (SAP), has been shown to increase the glomerular filtration rate, whereas branched chain amino acids (BCAA) leucine, isoleucine, and valine fail to modify the glomerular filtration rate. It has been suggested that this effect of amino acids on the glomerular filtration rate is mediated by the action of the hormone glomerulopressin. The purpose of this work was to study the action of SAP and BCAA on glomerulopressin production. Livers isolated from rats were perfused with (i) Krebs-Ringer-Bicarbonate, (ii) SAP, or (iii) BCAA. Results indicate that glomerulopressin production is stimulated by SAP, but inhibited by BCAA.** [P.S.E.B.M. 1991, Vol 198]

Liver vein blood ultrafiltrate from toads (1), rabbits (2), and dogs (3) contains a hormone-factor called glomerulopressin, which is also produced by isolated, perfused rat livers (4, 5). Glomerulopressin increases the glomerular filtration rate (GFR) in rats (2) and dogs (6).

Furthermore, this hormone-factor is found in pancreatectomized dogs (3), in dogs intraportally infused with glucagon (7), in normal humans treated with glucagon, in newly diagnosed Type I insulin-dependent diabetic patients (8), and in normal humans infused with amino acids (9).

Glomerulopressin is thermostable and has a molecular mass lower than 500 Da (1). Incubation with pronase does not destroy its activity (1), and extraction of peptides with glacial acetic acid (10) does not decrease the activity of glomerulopressin on the glomerular pressure of toads (11). Incubation of the ultrafiltrates with β-glucuronidase destroyed glomerulopressin activity. When β-glucuronidase was inhibited by boiled saccharate, a specific inhibitor of β-glucuronidase (12), glomerulopressin activity remained unaltered (3). These data suggest that glomerulopressin is not a peptide and that it may be a glucuronide. It has been obtained in a purified form by extraction with *n*-butanol and use of high performance liquid chromatography with different elution systems (13).

Intraportal or intravenous serine, alanine, and proline (SAP) infusion increases GFR in dogs, although not when it is infused intrarenally. In contrast, intraportal infusion of branched chain amino acids (BCAA) fails to alter renal hemodynamics (14).

The purpose of this experiment was to study the effect of SAP and BCAA on glomerulopressin production by isolated rat liver.

Materials and Methods

Technique for Isolation of Ultrafiltrates Containing Glomerulopressin. Sprague-Dawley male rats, weighing 200–300 g and fasted for 24 hr, were anesthetized with thiopental (25 mg/kg). The portal vein was cannulated *in situ* and infused with the different solutions described in Treatments at 5 ml/min flow rate and 22 mm Hg pressure. The solutions were continuously aerated with 95% O_2 and 5% CO_2 and maintained at 38°C. The liver was isolated by clamping the hepatic artery and inferior vena cava; then, the hepatic vein was cannulated for perfusate collection. Perfusate obtained during the first 5 min was discarded, then the perfusate was collected during the next 20 min. The liver was kept at 38°C during the time of perfusion. The criterion used in determining the success of the perfusion was the presence of a pale, uniform color in all the hepatic lobes. Perfusates were ultrafiltrated through YC-05 Diaflo membranes, which allow the passage of substances with a molecular mass lower than 500 Da. Ultrafiltrates were kept at −20°C until used, and the activity of each was assessed by measurement of the

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tension developed by stomach muscle from the isolated stomach fundus of the rat.

Technique for Assessment of the Tonic Tension Contraction Developed by Isolated Smooth Muscle from the Stomach Fundus of Rats. Sprague-Dawley female rats weighing 130–140 g were sacrificed by a blow to the head. The stomach fundi were removed and placed in petri dishes containing Krebs-Ringer-bicarbonate solution (KRB) at pH 7.4. Longitudinal (0.4 × 1.5 cm) strips were cut. One end of the strip was attached to a glass holder and immersed in tissue chambers filled with 1200 μl of KRB solution. This medium was continuously bubbled with a O_2:CO_2 gas mixture and maintained at 37°C. The other end of the tissue was connected to a force transducer (Gould Statham UC 3 with a UL 5 Micro scale) and registered by a Grass 7D polygraph. After a resting tension of 500 mg was applied to the preparations by means of a micrometric device, the tissues contracted in isometric conditions. Their mechanical activity was then determined in terms of tonic tension contraction, i.e., the sustained levels of contractile force, measured in milligrams, developed above the resting or basal tension.

Among the several substances that are known to increase the tonic tension contraction of the rat stomach fundus, prostaglandins are the most similar to glomerulopressin, because glomerulopressin acts through liberation of prostaglandins (15); therefore prostaglandin E_2 was used as a standard. The tension contraction produced by the glomerulopressin on the stomach fundus was converted into units by comparison with several doses of prostaglandin E_2 (0.625, 1.25, 2.50, and 5.00 ng/ml final bath concentration) using a 4-point assay (16).

Treatments. Animals were divided into three groups of seven each, and isolated livers were perfused with: (i) KRB; (ii) serine (1.11 m*M*), alanine (1.80 m*M*), and proline (0.72 m*M*); and (iii) leucine (0.68 m*M*), isoleucine (0.40 m*M*), and valine (0.80 m*M*).

In this work, SAP and BCAA concentrations in the perfusion fluid of isolated livers were four times higher than their concentration in normal rat plasma (17), but the total amino acid concentration in perfusion fluid (3.63 m*M*) was lower than that in rat plasma (4.16 m*M*) (17).

All amino acids were purchased from the Sigma Chemical Co, St. Louis, MO.

Statistical Analysis. Bartlett's test was employed to verify variance homogeneity. All data were processed by one-way analysis of variance. The significance of differences among group means was evaluated by Duncan's method, regarding a $P < 0.05$ value as statistically significant. Results are expressed as mean ± SE.

Results

Analysis of variance indicated a marked difference among the three groups, with $P < 0.005$.

On perfusing livers with KRB, glomerulopressin activity in the ultrafiltrate was 12.5 ± 2.34 units/ml.

The addition of SAP to the perfusion fluid led to considerable stimulation of glomerulopressin activity in the ultrafiltrate, reaching 20.6 ± 3.02 units/ml, whereas BCAA inhibited glomerulopressin production, which decreased to 6.4 ± 1.88 units/ml. (Fig. 1).

Discussion

Several authors have shown that either protein intake (18–22) or amino acid infusion (23–28) increases GFR, an effect attributed both to enhancement of glucagon production (29) and to stimulation of prostaglandin synthesis (18, 25, 28, 30, 31).

In order to explain the mechanism of amino acid activity, it has been demonstrated that intraportal or intravenous, but not intrarenal, infusion of serine, alanine, and proline increases GFR. In contrast, intraportal infusion of postprandial doses of the branched chain amino acids, leucine, isoleucine, and valine, fails to modify GFR (14).

In the isolated perfused kidney, amino acid-induced increase in GFR is much lower than *in vivo* (32) or is even absent (33). It may be concluded that the effect of amino acids on GFR is most likely mediated by a set-off signal by the amino acids in the whole organism.

As advanced by Brenner *et al.* (34), the mediator may be a blood-borne factor. Alvestrand and Bergström (35) postulated that glomerular hyperfiltration following protein intake, and during amino acid or glucagon infusion, was induced by release of glomerulopressin, and, furthermore, that the common factor was the increased amino acid uptake by the liver. Indeed, several authors have supported this hypothesis (18–28).

Glomerulopressin increases GFR in dogs (6) and rats (2, 36). Likewise, it has been observed that in humans, besides increasing GFR, peripheral amino acid

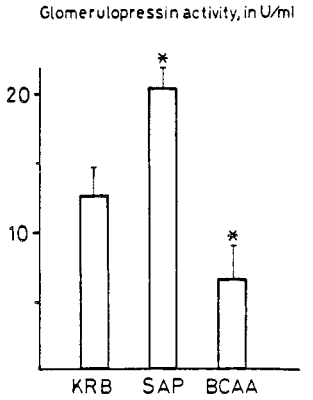

Figure 1. Activity of liver ultrafiltrates perfused with KRB, SAP (3.63 m*M*), or BCAA (1.88 m*M*). Values are expressed as means ± SE, with $n = 7$ in each group and $P < 0.05$, in comparison to the KRB-perfused group.

infusion also enhances hepatic production of glomerulopressin (9).

Both *in vivo* (7) and *in vitro* (4) perfusion with glucagon stimulate glomerulopressin production by the liver. In isolated livers, glucagon induces a prompt increase in cAMP concentration (37). Perfusion of isolated rat liver with dibutyryl cAMP also stimulates glomerulopressin production. There is a good log-dose relationship between infused dibutyryl cAMP concentration and glomerulopressin production, which suggests a direct action of cAMP on glomerulopressin production by isolated livers (5).

In the whole organism, amino acid infusion stimulates glucagon production (38), which in turn stimulates hepatic production of cAMP. The subsequent increase in cAMP stimulates amino acid transport into the hepatic cell (38). This could represent the mechanism for production of glomerulopressin when amino acids are infused *in vivo*, but not when isolated livers are perfused with glucagon (4). Since the perfusion solution contains no amino acids, the stimulating effect of glucagon on glomerulopressin production by the isolated liver seems to be mediated through cAMP.

In this work, fasted rats were used to increase uptake of amino acids by the liver (39). In this condition, SAP enhances and BCAA inhibits glomerulopressin production. Both amino acid groups act directly on the liver to modify glomerulopressin production.

It has been shown *in vivo* that GFR is increased due to the action of glucagon and of SAP. Both of these compounds directly increase hepatic glomerulopressin production. In turn, BCAA, which fails to increase GFR, inhibits glomerulopressin production. This suggests that amino acid-induced increases in GFR correlate with greater glomerulopressin production.

Research on rats (17, 30) and humans (25, 28, 31) demonstrates that an increase in GFR following either protein intake (30, 31) or amino acid infusion (25, 28) may be mediated by prostaglandin synthesis.

In previous work, it has been shown that the effect of glomerulopressin is blocked by cyclooxygenase inhibitors in various systems, including toad lymphatic heart (40), isolated rat stomach fundi, duodenum, and bladder (15), dog mesenteric artery (41) and ovarian flow (42), several arteries and veins of diverse species (43), and GFR of rat kidneys (36), suggesting that glomerulopressin effect is mediated by prostaglandin synthesis. It has also been demonstrated that superfusion of isolated glomeruli with glomerulopressin stimulates the production of prostacyclin (44).

The effect of amino acids, protein ingestion, glucagon infusion, and glomerulopressin on renal synthesis of prostaglandin indicates a common pathway for the increase in GFR.

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