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Hemodynamic studies in a parabiotic model of portal hypertension

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Summary. Splanchnic and systemic hemodynamic studies were performed in a rat model of parabiosis and portal hypertension. A portal hypertensive and a normal rat were surgically united side to side. A hyperdynamic circulation, characterized by increased cardiac index (413 ± 26 vs 318 ± 23 ml·min^{-1}·kg^{-1}; $p < 0.05$) and portal venous inflow (9.61 ± 1.29 vs 6.33 ± 0.36 ml·min^{-1}·100 g b.wt^{-1}; $p < 0.05$), was found in all the portal hypertensive rats but not in the normal parabiotic partners. These results do not support the existence of a transferable humoral factor mediating the hyperdynamic circulatory state of chronic portal hypertension.
Key words. Parabiosis; portal hypertension; hyperdynamic circulation.

Vasoactive humoral factors have recently been suggested as possible mediators of the hyperdynamic circulatory state[2,3] observed in portal hypertensive patients[4,5] and experimental models of portal hypertension[6,7]. Chronic plasma exchange between portal hypertensive and normal animals may induce this syndrome in the normal animal and will therefore confirm the existence of a humoral factor. To explore this possibility we performed splanchnic and systemic hemodynamic studies in a model of parabiosis in which a portal hypertensive and a normal rat were united. Parabiosis, the union of two living individuals through a capillary bed, has been a profitable tool in evaluating humoral transferable effects especially in endocrine studies[8]. Previous studies in this model have shown that plasma exchange between two united rats ranged from 0.47 to 1.04% of one animal's plasma volume per min[9].

Material and methods. Male, Sprague-Dawley litter mate rats were used. Portal hypertension was induced by partial ligation of the portal vein (PVL). The operative procedure for portal vein constriction has been described in detail[10]. In brief, the portal vein was isolated and the stenosis was created by a single ligature of 3-0 silk around the portal vein and a 20 gauge blunt-tipped needle. The needle was then removed and the portal vein was allowed to reexpand distal to the stenosis. 1 week later parabiosis was performed by uniting a portal hypertensive and a normal rat according to the surgical technique described by Bunster and Meyer[11]. The rats were anesthetized by ketamine 100 ml·kg^{-1}. After appropriate shaving, an incision was made from the base of the ear to the tail. Then each pair of rats was joined by sutures of surgical silk (No. 3-0) through scapula and musculature of abdomen and thigh. Matching skin edges were joined with metal autoclips. Each pair was housed in an individual plastic cage and allowed free access to rat chow and water. Weight gain was an indicator of healthy animals in a successful parabiotic union. 10 parabiotic pairs were prepared. 4 weeks after the parabiosis, hemodynamic studies were performed under ketamine anesthesia (100 mg·kg^{-1}). Four pairs were excluded earlier because of failure to thrive. One pair was excluded because of severe bleeding during the hemodynamic study. Five pairs were successfully studied. Cardiac output, organ blood flow and portal systemic shunting (PSS) were determined by a radioactive microsphere technique which was described in detail in previous communications from our laboratory[12]. Arterial pressure and portal pressure were also measured. Portal venous inflow (PVI) was the sum of arterial blood flow of the stomach, intestine, spleen, pancreas and mesentery. The venous outflow of each of these organs is into the portal venous system, PVI therefore, represents the total splanchnic arterial inflow entering into the portal system. In order to confirm the existence and the amount of the exchange of plasma, 0.5 ml of a 20% Evans blue solution was injected into the penile vein of the portal hypertensive rat. 15 min later arterial blood samples were taken from the two rats. The serum was separated and stored at −70 °C until dye concentration was measured. In each pair, plasma exchange was calculated from the formula:

$$r = \frac{Cn}{Cp} \times \frac{100}{15}$$

where r = rate of exchange in 1 min expressed as a percentage of one animal's plasma volume, Cn and Cp = Evans blue concentration in normal and portal hypertensive rats respectively. At the end of the experiment the rates were sacrificed by a bolus injection of KCl, separated and weighed individually.

Data is expressed as mean ± SEM. Statistical analysis was performed by the unpaired t-test.

Results and discussion. At the time of the study the weight of the PVL and the normal rats was similar 326 ± 31 vs 344 ± 16 g. The rate of plasma exchange in 1 min was 1.4 ± 0.3% with a range of 0.5 to 2.3%. The hemodynamic data are depicted in the table. Previous studies from our laboratory have shown that portal vein constriction in the rat provides a reproducible model of portal hypertension in which portal systemic shunting and a hyperdynamic systemic and splanchnic circulation develop in a

Splanchnic and systemic hemodynamic data in five parabiotic pairs

	PVL (mean ± SEM)	Normal
CI ml·min^{-1}·kg^{-1}	413 ± 26*	318 ± 23
MAP mm Hg	117 ± 10	140 ± 6
PP mm Hg	15.4 ± 1.2*	11.1 ± 1.1
PSS %	95.9 ± 4.1**	0.3 ± 0.2
PVI ml·min^{-1}·100 g b.wt^{-1}	9.61 ± 1.29*	6.33 ± 0.36

CI, cardiac index; MAP, mean arterial pressure; PP, portal pressure; PSS, portal systemic shunting; PVI, portal venous inflow. * $p < 0.05$; ** $p < 0.001$.

predictable period of time (less than 2 weeks)[3]. Similar results were obtained in all the portal vein constricted mates of the parabiotic pair. However, a hyperdynamic circulatory state was not found in the normal parabiotic partners. In all the parabiotic pairs, plasma exchange was evident and the rate of exchange suggests that within several hours complete mixing of the plasma between the two animals takes place.

The results of the study do not support the existence of a transferable humoral factor mediating the hyperdynamic syndrome. On the other hand the existence of such an agent cannot be completely excluded by our findings. Benoit et al.[2] found a decrease in the intestinal arteriolar resistance of an isolated intestinal preparation of a normal rat when the preparation was perfused with blood from a portal hypertensive rat. It is possible that in the parabiotic rats despite a significant plasma exchange, the plasma levels of vasoactive agents sufficient to produce a hyperdynamic state may not be achieved because of dilution of these factors or their rapid metabolization by the liver of the normal rat. In the portal hypertensive rats most of the portal blood is diverted from the liver by the extensive portal systemic shunting. Although there is some increase in the hepatic arterial flow, the total hepatic blood flow is severely reduced[3]. In the normal rats, since the hepatic blood flow is unaltered the hypothetical vasoactive agents could be rapidly metabolized. The hyperdynamic syndrome could also be mediated by a change in receptor response which may further explain the lack of hyperdynamic changes in the normal rats.

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The inner ear structures of the echidna – an SEM study

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Summary. Surface structures of the echidna cochlea were investigated using a scanning electron microscope technique. It was found that unlike typical mammalian cochleas, the echidna cochlea possesses four rows of inner hair cells and 6–9 rows of outer hair cells, and that the arrangements of the stereocilia of the outer hair cells were not uniform throughout the length of the basilar membrane.
Key words. Echidna; cochlea; inner hair cells; outer hair cells; basilar membrane; scanning electron microscopy.

Monotremes are unique animals in that they possess some reptilian and some mammalian anatomical characteristics. There are three extant species: the platypus, *Ornithorhyncus anatinus,* the echidna, *Tachyglossus aculeatus* and the long-beaked echidna, *Zaglossus bruijnii*[2]. Available data suggest that the overall structure of the inner ear of the echidna, *Tachyglossus,* is closest in structure to that of other mammals. There are some interesting dissimilarities, however, such as the numbers of rows of inner and outer hair cells present on the basilar membrane[3,4]. A scanning electron microscope (SEM), which has been used extensively in investigation of mammalian cochlear surface structures, was used to clarify further the surface structures of the inner ear of *Tachyglossus.* It is possible to conclude from the present results that although it is probably most similar to that of other mammals, the echidna inner ear has several features characteristic of birds and reptiles.

Examination of available literature indicates that the mammalian cochlea has the following main characteristics: a) It is generally in a spiral of varying turns and widths, depending on the species examined[5]. b) It has three main fluid-filled canals (the scala vestibuli, the scala media and the scala tympani) with well defined round and oval windows at the basal end[6]. c) There are two types of hair cells; one row of inner hair cells (IHCs) and usually three rows of outer hair cells (OHCs) separated by a tunnel rod[7,8]. Exceptions to this are often found in humans and monkeys which frequently have four or five rows of OHCs at the apical turn of the cochlea[7]. d) Mature hair cells lack a true kinocilium[8]. e) Stereocilia of the OHCs are usually arranged in a 'W' shape[9] or a 'V' shape[10].

The similarities and dissimilarities of the echidna's cochlear structures to those of other orders of mammals can be summarized as follows: The cochlear duct of the echidna forms a banana-shaped curve rather than a spiral as in other orders of mammals, with a lagena at the distal end[8,11]. The cochlea has well defined oval and round windows, although the oval window is, in fact, round as is the case in birds and many reptiles[11]. There are the usual three fluid-filled canals within the cochlea (see fig. 1). Tunnel rods separate the inner and outer hair cells[8] (see fig. 2). There is a slight disagreement as to the number of rows of OHCs the IHCs on the basilar membrane. Previous studies[3,4] have found there to be three rows of IHCs and 4–6 rows of OHCs. Our results show that the numbers of rows of hair cells are quite different from those in other orders of mammals, and it is better to view the echidna as having four rows of IHCs and six