Venous hydrostatic indifference point as a marker of postnatal adaptation to orthostasis in swine

PHILLIP S. BUCKNER, ANTHONY W. QUAIL, DAVID B. F. COTTEE, AND SAXON W. WHITE
Discipline of Human Physiology and Neuroscience Group, Faculty of Medicine and Health Sciences, University of Newcastle, Callaghan, New South Wales 2308, Australia

Buckner, Phillip S., Anthony W. Quail, David B. F. Cottee, and Saxon W. White. Venous hydrostatic indifference point as a marker of postnatal adaptation to orthostasis in swine. J. Appl. Physiol. 87(3): 882-888, 1999.—The postulate that venous adaptation assists postural baroreflex regulation by shifting the hydrostatic indifference point (HIP) toward the heart was investigated in eight midazolam-sedated newborn piglets. Whole body head-up (+15, +30, and +45°) and head-down (−15 and −30°) tilt provided a physiological range of orthostatic strain. HIP for all positive tilts shifted toward the heart (P < 0.05), +45° HIP shifted most (6.7 ± 0.3, 5.9 ± 0.5, and 3.6 ± 0.3 (SE) cm caudal to right atrium on days 1, 3, and 6, respectively). HIP for negative tilts (3.0 ± 0.2 cm caudal to right atrium) did not shift with postnatal age. Euthanasia on day 6 caused 2.1 ± 0.3-cm caudal displacement of HIP for positive and negative tilts (P < 0.05). HIP proximity to right atrium was not altered by α-, β-adrenoceptor and cholinoceptor blockade on day 5. It is concluded that early HIP migration reflects enhancement of venous pressure control to head-up orthostatic strain. The effect is independent of baroreflex-mediated adrenoceptor and cholinoceptor mechanisms.

postural baroreflex; tilt; newborn; swine; autonomic blockade

EXAGGERATED ARTERIAL pressure fluctuations are associated with morbidity, especially for extremely premature human neonates (15). Attempts to examine the development of baroreflex regulation have focused on heart rate and arterial pressure responses in term and preterm newborns to whole body tilting. Data are inconsistent, suggesting variation in levels of baroreflex control (12, 22, 25). Cardiac output is highly sensitive to changes in central venous filling pressure (11, 20), but the contribution of the developing venous system to these observations on the postural baroreflex has not been investigated.

Deviation in central venous pressure with body reorientation were recognized in the 19th century to relate directly to distance from the hydrostatic indifference point (HIP) (24). The HIP is a unique axial reference within the column of venous blood where pressure is not altered by a given postural reorientation. HIP location is determined by the distribution of venous mural compliance, which, in turn, has many potential controlling mechanisms (6). The venous HIP of adult humans resides just below the cupola of the diaphragm (6), and in the mature dog it is within the right ventricle (8). This proximity of the HIP to the heart ensures relative stability of cardiac filling and stroke volume despite changes in body posture.

Organisms have evolved a degree of postural baroreflex control commensurate with the requirements for survival in their natural habitats. For example, head-up tilt is poorly tolerated in aquatic reptiles taken out of water compared with closely related terrestrial and especially arboreal species (19). In humans, postural reflexes need to be maintained by regular conditioning, as demonstrated by the tendency to orthostatic hypotension after prolonged bed rest or spaceflight (2, 5). The veins of the late-gestation human fetus are buoyed by pressure transmitted from the amniotic fluid and so are not exposed to orthostatic strain (14). We postulated that, should an active venous pressure control system exist in mammals, at birth it will be poorly conditioned to the extrauterine environment. Native maturation of such a system would be evidenced by improved stability of cardiac filling pressure during body tilt, marked by postnatal shifting of the venous HIP toward the heart.

The piglet is a well-established model for the investigation of human neonatal cardiovascular control (7). In this study, changes in the HIP were traced in the piglet by using both head-up and head-down tilt during the first 6 days of life and after lethal injection. As there are no previous data describing HIP location in the living neonate, a range of tilts was employed to determine the effect of tilt magnitude and direction on HIP location. A separate group of piglets was studied on postnatal day 5, before and after autonomic blockade, to establish the influence of adrenoceptor and cholinoceptor mechanisms on venous HIP location.

METHODS

Eight domestic piglets, opposite-sex pairs from four litters, underwent surgery within 12 h of birth. They were studied after recovery and also on postnatal days 3 and 6. Four other piglets, two of each sex, were studied on day 5 before and after drug-induced total autonomic blockade (TAB). The experimental procedures and care of the piglets were in accord with the Code of Practice adopted by the Animal Care and Ethics Committee of the University of Newcastle.

Tilt-table design. The rigid cradle that supported the piglets in these experiments was fashioned from a polyvinyl chloride pipe (75 mm diameter, 500 mm length) cut longitudinally to produce a U-shaped trough. Four oval-shaped holes were located to accommodate the legs, and multiple 8-mm holes were drilled at 10-mm centers to provide skin traction and allow visualization of a cross marking, on the lateral thorax, the center of the piglet’s right atrium. The entire cradle was heat molded and padded so as to provide snug...
support; urinary balloon catheters were incorporated into the cradle front and back so that, when the catheters were inflated with warm water, either the buttocks or shoulders were supported during tilting. These measures ensured that the piglet shifted <5 mm relative to the plastic cradle over the entire range of head-up and head-down tilt.

On either side of the cradle, 10-mm-wide Perspex strips were attached longitudinally with measuring tape glued to their outer surfaces. These strips acted as rigid rails for two shuttles made of polyvinyl chloride to which the venous and arterial pressure transducers were attached. Each shuttle could be precisely anchored anywhere along its rail by a thumbscrew.

The plastic cradle was supported on a stainless steel (16-mm² section tubing) rectangular frame with a central axle, allowing rotation on a base made of the same stainless steel tubing. The axle was geared to a 12-V motor, and the motor and drive chain were adapted from an automobile window winding mechanism. Another gear meshed with a rotary potentiometer so that the tilt-table inclination could be continuously recorded. At one end of the axle was also attached a semicircular sheet metal protractor with holes drilled at 15° intervals around its perimeter. The holes allowed triggering of an optical sensing device that cut power to the drive motor, provided an override button on the remote control box was not activated. Thus the tilt table could be driven precisely to any of the preset inclinations by using a direction switch and the optical sensor override button on a box controlling a transformed power source. The voltage source was adjusted so that the tilt table rotated quietly and smoothly at 5°/s.

Surgery. Piglets were anesthetized with 1% isoflurane in oxygen via a dose-fitting mask. An umbilical artery was catheterized by using a 3.5-Fr polyvinyl chloride umbilical vessel catheter (Argyle, St. Louis, MO) inserted 1 cm further than the distance between the umbilicus and shoulder tip. This procedure is the same as that widely performed on sick human neonates, and ultrasound confirmed that it also results in the catheter tip lodging within the midthoracic aorta in the newborn piglet. The right external jugular vein was dissected through a 1-cm incision in the root of the neck and another 3.5-Fr catheter was inserted 4–5 cm. A double-lumen catheter (4-Fr, Arrow Pediatric, Reading, PA) was used for the venous catheterization in the four piglets who underwent the autonomic blockade experiments to allow simultaneous drug infusion and central venous pressure monitoring. The venous catheter was tunneled subcutaneously to emerge between the scapulae. Both catheters were primed and flushed daily with saline containing heparin (2 U/ml), Penicillin (60 mg/kg) and gentamicin (2.5 mg/kg) were given preoperatively, and the surgery was completed within 30 min. Piglets recovered in the neutral thermal environment of a Humidicrib. They were fed milk formula every 4 h (Dil-Vetalact, Sharpe Laboratories) via a bottle and nipple.

Determination of location of the right atrium. Immediately after the surgery, the center of the right atrium, as a point of reference, was determined by two-dimensional ultrason, and its location was marked on the lateral chest in two planes by using an indelible marker pen. The locations of the venous and arterial catheters were checked by ultrason to confirm their tips were in the right atrium and midthoracic aorta, respectively.

Protocol. Before each experiment, piglets were given intravenous (iv) midazolam (Roche; 0.1 mg/kg body wt); this rendered them less excitable but still able to walk and drink. They were loaded into the polyvinyl chloride cradle, which was then attached to the tilt-table frame by locating bolts. The arterial and venous pressure transducers (DT-NN, Ohmeda, Madison, WI) were calibrated before and after each experiment by using mercury and saline manometers, respectively. These lightweight transducers were chosen because their small domes allowed them to be accurately centered at a desired point relative to the piglet. They have a stable and linear response over the pressure ranges of interest (±20 cmH₂O for venous pressure and 0–100 mmHg for arterial pressure).

Catheters and transducers were flushed carefully with heparinized normal saline to exclude visible air bubbles, and the distal transducer port was then occluded by closing a rigid three-way tap. The pressure transducers were firmly fixed to the shuttle devices on either side of the cradle such that, with the tilt table in the horizontal position, their domes were at the same height as the right atrial reference point marked on the lateral chest of the piglet. Each shuttle was then fixed along its rail such that the pressure domes were at one of four standard locations: aligned with the right atrium, 5 or 10 cm more caudal to it, or 5 cm more rostral to it. Transducers were enclosed in aluminum foil to exclude radiant heat exposure. After epochs of quiet sleep, evidenced by shallow regular respiration, no rapid-eye movements or ear flicks, and steady heart rate, piglets were then tilted from horizontal to each of the following inclinations: 15, 30, and 45° head up and 15 and 30° head down, maintaining each new position for at least 60 s. The 45° head-down position was abandoned, as this frequently caused arousal and movement in the earliest piglets studied.

Room temperature was stable at 22°C, and the piglets were warmed or cooled by adjusting the intensity of an infrared lamp as indicated by restlessness, sweating, piloerection, and perfusion of extremities. In this environment there was no variation in temperature monitored by a rectal probe. Animals were fed before each experiment, and plentiful urine output was noted.

Autonomic blockade. In four additional piglets, a- and b-adrenoceptor and cholinceptor blockade were employed on day 5 to investigate the role of autonomic control on the HIP. The blocking procedures were those used in the newborn lamb by Jones et al. (13). Restated briefly, b-adrenoceptor blockade was achieved with iv propranolol (2 mg, Inderal, ICI) followed by an infusion at 0.17 mg/min through the side port of the double-lumen central venous catheter; the block was confirmed by observing ablation of the tachycardic response to boluses of iv isoproterenol (1 µg, Isuprel, Winthrop). a-Adrenoceptor blockade was achieved with iv phentolamine (Regitine, Ciba) by 2-mg bolus injection and 0.32 mg/min infusion; the block ablated the pressor response to iv phentylphrine (125 µg). An iv bolus of the cholinceptor-antagonist atropine (1 mg, Apex Laboratories) was followed by an infusion at 0.2 mg/min. The drop in arterial pressure due to a test bolus of iv acetylcholine (50 µg, Sigma Chemical, St. Louis, MO) was ablated by the atropine dose. The study protocol was conducted during combined infusion of the three blocking agents; after completion of the tilts, the blockade was again checked by serial bolus injections of the three agonists.

HIP in the deceased piglet. While still in the tilt table on postnatal day 6, piglets were given iv heparin (5,000 U) and killed by rapid iv injection of 1 ml/kg of pentobarbital sodium (Nembutal, Abbott). The HIP was determined by repeating the tilt protocol, commencing immediately after asystole. During this period of ~20 min, the venous pressure deviations with tilts remained stable and the HIP estimates were highly reproducible.
distance from the right atrium, and \( \theta \) is the tilt angle (see Eq. A2 in the Appendix). Tilts were repeated with the transducer in the four locations relative to the right atrium to test reproducibility and also to ensure measurements were obtained with the transducer far enough from the true HIP so that the pressure changes recorded due to tilt were large relative to the background venous pressure fluctuations. These HIP estimates were averaged for each piglet on each day according to tilt angle.

Data analysis. Data were not included in the analysis if, because of piglet arousal and movement, the venous pressure did not quickly settle to a stable level after tilt. Overall, some 15% of tilt events were excluded from the analysis, but this varied among piglets and on different days; in particular, older piglets were more likely to arouse. Because the HIP can be calculated from the change in venous pressure recorded at a single transducer location, the HIP for each tilt angle on each day is represented in the analysis even though data from some transducer positions are missing. Data are expressed as means \( \pm \) SE.

Venous pressure and HIP data (Table 1) were not included for analysis for a pair of day 1 piglets (because of signal drift from a faulty pressure transducer) and from one of these piglets on day 6 (because the venous line occluded). During all other experiments the venous pressure signal was stable, with \(<1 \text{ cmH}_2\text{O}\) difference for the two-point calibrations (0 and 20 cmH\(_2\)O) measured before and after completion of the protocol. Thus data from six piglets were available for within-piglet analysis comparing day 1 with day 3, from seven piglets for day 3 vs. day 6, and from six piglets for day 1 vs. day 6. The matrix, including missing values, was handled by Sigmaplot software, applying ANOVA for repeated measures. A \( P < 0.05 \) was assumed to be significant. The HIP estimates were compared among the three different ages and between presence or absence of autonomic blockade.

RESULTS

Piglets weighed 1.62 \( \pm \) 0.04 (SE) kg, with body length (snout to rump) 36.1 \( \pm \) 1.8 cm on day 1; on day 6, the corresponding measurements were 1.72 \( \pm \) 0.07 kg and 37.1 \( \pm \) 0.9 cm, respectively. On day 1, the ratio of the distances from the snout to the center of the right atrium and right atrium to rump was 0.47 \( \pm \) 0.02, whereas in the transverse plane across the thorax, the ratio of the distance from dorsal thoracic spinous process to right atrium and right atrium to sternum was 0.73 \( \pm \) 0.03.

Table 1. CVP, resting HR, and MAP in all piglets during quiet sleep while the tilt table was in the horizontal position

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 1 (n = 6)</th>
<th>Day 3 (n = 8)</th>
<th>Alive (n = 7)</th>
<th>Dead (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVP, cmH(_2)O</td>
<td>1.3 ( \pm ) 0.3*</td>
<td>2.9 ( \pm ) 0.4</td>
<td>2.9 ( \pm ) 0.3</td>
<td>9.6 ( \pm ) 0.7</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>169 ( \pm ) 4</td>
<td>152 ( \pm ) 13</td>
<td>178 ( \pm ) 12</td>
<td>0</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>67 ( \pm ) 2</td>
<td>68 ( \pm ) 2</td>
<td>79 ( \pm ) 3</td>
<td>7.4 ( \pm ) 0.5</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE. n. No. of piglets; CVP, central venous pressure; HR, heart rate; MAP, mean arterial pressure. Note different units for MAP and CVP. *Significantly different from postnatal days 3 and 6, \( P < 0.05 \). †Significantly different from postnatal days 1 and 6, \( P < 0.05 \).
A 30-s epoch of baseline data was collected while piglets were in quiet sleep before each tilt event; these data segments were averaged on each day to provide resting data (Table 1). Within-piglet comparison revealed a rise in the central venous pressure between days 1 and 3 but no further rise between days 3 and 6. The rising trend in resting arterial pressure evident after day 3 failed to achieve statistical significance ($P = 0.07$). The resting heart rate was lower on day 3 than on either of the other days.

Analysis of the $+45^\circ$ tilt data from all piglets revealed a cephalad shifting of the HIP toward the heart (Fig. 2). In addition, statistically significant but smaller shifts were evident for the other ($+30$ and $+15^\circ$) positive tilts (Fig. 3). In all but one piglet, this shifting of the HIP for head-up tilt (HIP$_{up}$) occurred after day 3. With head-down tilt (HIP$_{down}$, $-30$ and $-15^\circ$), HIP was also caudal to the heart but was not influenced by postnatal age (Fig. 3).

On all days there were differences in the measured HIP, depending on grade and direction of tilt and the extremes of tilt tested; i.e., $+45$ and $-30$ had the most disparate HIP values (Fig. 3). These differences due to the nature of the tilt became smaller with increasing age, reflecting improving HIP stability over the range of tilt stimuli. For instance, the separation of the HIP values for $+45^\circ$ (HIP$_{up}$) and $-30^\circ$ (HIP$_{down}$) tilt was $3.9 \pm 0.3$, $3.6 \pm 0.7$, and $1.3 \pm 0.3$ cm on days 1, 3, and 6, respectively.

In the deceased 6-day-old piglet, the HIP was the same regardless of the direction or grade of tilt and was more caudal to the heart than on the same day while the piglet was alive by $2.1 \pm 0.3$ cm (Fig. 3).

Resting data from the separate group of four piglets who underwent TAB with phentolamine, propranolol, and atropine on day 5 are shown (Table 2). TAB caused no significant change in resting heart rate, arterial pressure, or central venous pressure. HIP location was
5-day-old piglets before and after TAB

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>TAB</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVP, cmH2O</td>
<td>5.7 ± 0.9</td>
<td>5.2 ± 1.3</td>
<td>0.70</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>147 ± 5</td>
<td>158 ± 19</td>
<td>0.62</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>62 ± 1</td>
<td>77 ± 7</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Values are means ± SE. TAB, total autonomic blockade.

similar to that seen in the other 6-day-old piglets and was not affected by TAB (Fig. 4).

DISCUSSION

Using an appropriate tilt table, we have shown that HIP location can be tracked in a small neonatal animal model without the need for deep anesthesia, which has the potential to ablate cardiovascular responses of interest. HIP up migrated cranially, converging on both the heart and HIP down. This appears to be a useful postnatal adaptation as a HIP close to the heart implies minimal changes in cardiac filling during alterations in posture and hence improved cardiac output homeostasis.

Midazolam has been reported to cause a small reduction in arterial pressure and vascular resistance (9) but has no significant effect on central venous pressure (16), nor does the rate of midazolam metabolism appear to be influenced by early postnatal age (3). There was no trend in HIP up during experiments (which took ~1.5–2 h to complete), and so any age-related change in stroke volume, thus maintaining the observed HIP in proximity to the heart. In the application of this postulate to the piglet, it might be argued that the increased separation of the piglet’s heart and HIP up in the first 3 days of life might be due to the relative noncompliance of the ventricles at this age (12). Reduced right ventricular compliance would imply that stroke volume adjustments are relatively insensitive to changes in filling pressure, resulting in a caudal HIP up and HIP down. HIP down is not more caudally located in the first 3 days, and so our data would support the proposition that the venous HIP is determined by characteristics of the veins rather than the heart.

Clark et al. (4) described a passive model for venous column hydrostatics. In any inert, fluid-filled container, the HIP marks the arithmetic center of the compliances distributed in the walls of that container. For instance, in the simplest model, that of a fluid-filled rigid tube sealed at either end by compliant caps, the HIP will be closest to the end with the most compliant cap and is the same no matter how the tube is reoriented relative to gravity (4). The deceased 6-day-old piglet is analogous to such an inert system in that the same HIP was recorded regardless of tilt direction or grade. The situation in the living animal is more complicated in that the distribution of compliances among the veins may change in response to body tilt and also the pressurization of the veins may be altered by mechanisms that adjust total venous capacity. Notably, in the living animal it cannot be assumed that the measured HIP will be independent of the direction and magnitude of tilt.

In life, constriction of the hindlimb, pelvic, and splanchnic veins during head-up tilt would effect a cephalad shift in the HIP for two reasons. First, it would significantly reduce total venous capacity and so enhance average venous pressure in the head-up position. Second, the caudal location of these veins would mean that the reduction in their compliance that accompanies their constriction would result in a cranial shift in the HIP analogous to the effect of stiffening one of the caps in the fluid-filled-cylinder model (4). Thus the caudal veins may well be important in HIP control because they have a large capacity and they are relatively distant from the HIP.

Our observations of the piglet, confirming those previously reported of the dog, demonstrate that the venous HIP is not materially shifted by autonomic blockade (8). Thus unlike the arterial HIP (21), the venous HIP is not maintained by centrally integrated baroreflexes but by the local properties of the veins. For
instance, changes in pelvic and splanchnic venous tone might be due to their myogenicity, that is, their ability to respond to the changing transmural hydrostatic pressures that accompany whole body tilt. A significant myogenic response has recently been documented in human and canine saphenous veins to physiological changes in distending pressure (1). Vascular myogenic responses are controlled by voltage-operated calcium channels, which are in turn modulated by many factors including nitric oxide released from endothelium, as well as prostaglandins and epinephrine. Alternatively, venous constriction during head-up tilt might be due to the release of a nonadrenergic transmitter through a barosensitive axonal reflex analogous to the dermal venules in the reticulospinal reflex activated during orthostatic stress (10, 23). The local stretch set point and pressure sensitivity about which these myogenic or axonal responses operate would need to relate to distance from the HIP.

Whatever the mechanism that controls intrathoracic venous pressure during postural changes, in the newborn the process appears to require conditioning or resetting. From the moment of birth, the veins have to adapt to the loss of the supra-atmospheric pressure and buoyancy provided by the amniotic fluid. The rise in resting central venous pressure observed during the first 3 days of life is perhaps a manifestation of this process; the shift in the HIP up occurs after postnatal day 3 and is therefore not simply a result of this correction in resting venous pressure. The extremely premature human neonate has limited cerebral auto-regulation in the face of fluctuations in arterial pressure and is prone to intraventricular hemorrhage (15). The time course of the maturation in human HIP control and the influence of premature birth on this process may have important clinical relevance. In addition, adaptation in venous HIP regulation may occur in adults undergoing prolonged bed rest, repeated acceleration, or zero-gravity spaceflight (2, 5).

On days 1 and 3, there are significant differences in the measured HIP, depending on tilt direction and grade. The HIP down but not the HIP up values at these ages are different from those in the deceased piglet, suggesting that there is active control of venous pressure at birth but only in the direction that can diminish venous pressure and so produce an appropriate response to head-down tilt. If the data on the 6-day-old piglets were studied in isolation, the juxtaposition of heart, HIP up and HIP down, might be interpreted simply that the heart resides at the passive center of venous compliance. The day 6 HIP up and HIP down values, however, are both different from those measured in the deceased animal and thus appear to require active regulation.

In summary, this study details a convenient method for characterizing the neonatal venous system by determining its HIP. In the mature animal, HIP control is precise and not susceptible to chemical autonomic blockade and thus is difficult to unravel by conventional means. In this context, a newborn model is useful because the control system during the first days of life is not as effective as in the adult. These data support the hypothesis that there is an active mechanism that maintains the HIP close to the heart and therefore attenuates changes in cardiac filling pressure during alterations in posture. In the newborn, this control is achieved for head-down tilt before it is achieved for head-up tilt. The implications for the development of human cardiovascular control, especially in the preterm infant, need further study.

**APPENDIX**

Derivation of the Formula Used to Calculate the HIP From a Single Tilt Event

The venous system is considered to be a horizontal, elongated, fluid-filled cylinder connected via a catheter filled with fluid of the same density to a pressure transducer fixed to the Earth’s surface. The rise in pressure at point x centimeters along the column from RA (ΔP0) because of the tilt will be

\[ \Delta P_x = P_x^d - P_0 = [P_x^d + (d - x) \sin \theta] - P_0 \]

By definition, the HIP will be located along the column where \( \Delta P_x = 0 \), i.e., at

\[ x = (P_x^d - P_0) \sin \theta + d \text{ cm from the RA} \]

i.e., HIP (cm) = \( \Delta P / \sin \theta \) + d (A2)

where \( \Delta P \) is the change in pressure during tilt recorded by a transducer in centimeters along a fluid column from a reference location.

The authors gratefully acknowledge Dr. Yamei Cui for devoted laboratory assistance, Dr. Gary Warner for performing the thoracic ultrasounds, Trevor Oldham for building the tilt table, Geoffrden Davis for the loan of the AMLAB system, and Timothy Wylie for writing computer software. These studies were funded by a grant from the Charitable Trust Fund of John Hunter Hospital, Newcastle, Australia.

Address for reprint requests and other correspondence: A. W. Quail, Discipline of Human Physiology, Univ. of Newcastle, Callaghan, NSW 2308, Australia (E-mail: hpaog@mail.newcastle.edu.au).

Received 10 November 1998; accepted in final form 3 May 1999.

**REFERENCES**


