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The influence of hypertension upon the normal cardiovascular responses to hemorrhagic hypotension and shock[1]

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Summary. The data suggest that rats genetically inbred to be hypertensive (SHR) are less able to compensate for hemorrhage and shock than their normotensive controls (WKY). Two reasons for this genetic dysfunction are: 1) SHRs seem to depend more on innervated alpha 1 than noninnervated alpha 2 adrenoreceptors for vasoconstriction; and 2) the vascular smooth muscle hypertrophy noted in SHRs may interfere with effective vasoconstriction.
Key words. Hypertension; hypotension; vascular compensation; vascular decompensation; hemorrhage; shock.

The determination of the pretrauma cardiovascular state of patients admitted into a trauma unit is of paramount importance before rational diagnosis and therapy can be instituted. For example, a chronically hypertensive patient may well be in a state of cardiovascular shock even though his arterial pressure is within the normal range for a normotensive individual. If the physician is not aware of the preexisting hypertension, he may not know if his patient's normal cardiovascular compensatory response to trauma is compromised. This situation would be made even more complex if the patient were undergoing antihypertensive drug therapy which would also interfere with the normal mechanisms used by the body to return blood pressure to normal. The purpose of this study, therefore, was to examine the hypothesis that unmedicated spontaneously hypertensive rats (SHR), which have been shown to be an appropriate model for human hypertension[2], are less able to compensate for severe hemorrhage than genetically matched normotensive rats (WKY).

Methods. 10 rat pairs were evaluated in these studies. One of the rats in each pair was genetically inbred to be spontaneously hypertensive (SHR), while its control was a genetically matched normotensive Wistar Kyoto rat (WKY). The weight of the WKYs averaged 263 ± 14 g compared to 298 ± 14 g for the SHRs. All rats were acquired from the Charles River Breeding Laboratories Inc. of Wilmington, Massachusetts. Following anesthetic induction by i.p. administration of 35 mg/kg sodium pentobarbital[3], the right femoral vein and right carotid arteries were cannulated with PE 10 and PE 60 tubing respectively. Supplemental anesthesia and heparin sodium (10 mg/kg) were administered through the venous cannulae, while mean arterial pressure (MAP) and controlled hemorrhage were accomplished through the arterial cannulae. To insure an adequate airway a short (2 cm) PE 240 cannula was inserted into the trachea.

The following is a brief description of the hemorrhage protocol used in these studies. For a more detailed description including a diagram of the experimental apparatus, the reader is directed to a prior publication[4]. The arterial cannula of each rat in a pair (SHR and WKY) was connected to a T-tube. One arm of the T-tube was attached to a P23DB Statham pressure transducer, while the 2nd arm was afixed to the bottom of a calibrated 25-ml buret. The two burets, one for each rat in the pair, were connected by tubing and another T-tube in such a manner that a well controlled back pressure could be applied equally to the fluid surfaces in both burets. The back pressure exerted upon the fluid could be precisely controlled using a syringe and pressure regulating clamp[4]. The pressure on the surface of the fluid in the buret in contact with the SHR was then set at the inherent pressure of the WKY control, and the stopcock between the buret and SHR opened allowing blood to move into the buret (see fig.). Normally 3–5 min were required for the pressure in the SHR to fall to the WKY pressure. Once the MAPs were equalized, both buret pressures were set at 60 mm Hg for 15 min, then 30 mm Hg where they were maintained by carefully adjusting the syringe and pressure regulating clamp. The movement of blood from the rats into the burets represented a total body cardiovascular compensatory adjustment to the desired level of hypotension. The maximum blood volume removed divided by b.wt was consid-

	WKYs	SHRs
MAP (mm Hg)	123 ± 4	187 ± 3**
Comp. vol. (ml/kg)	32.1 ± 6	24.7 ± 1.4**
Comp. vol./p (ml·mm Hg/kg)	0.349 ± 0.02	0.154 ± 0.007**
Comp. time (min)	98 ± 6	77 ± 9*

MAP = mean arterial pressure. Comp. vol. = maximum shed blood volume at 30 mm Hg MAP. Comp. vol./p = maximum shed blood volume at 30 mm Hg at MAP normalized for pressure drop. Comp. time = time from onset of hemorrhage to maximum blood loss at 30 mm Hg MAP. Values are expressed as mean ± 1 SEM. * indicates statistical difference (WKYs vs SHRs) at $p < 0.05$; and ** indicates statistical difference at $p < 0.01$.

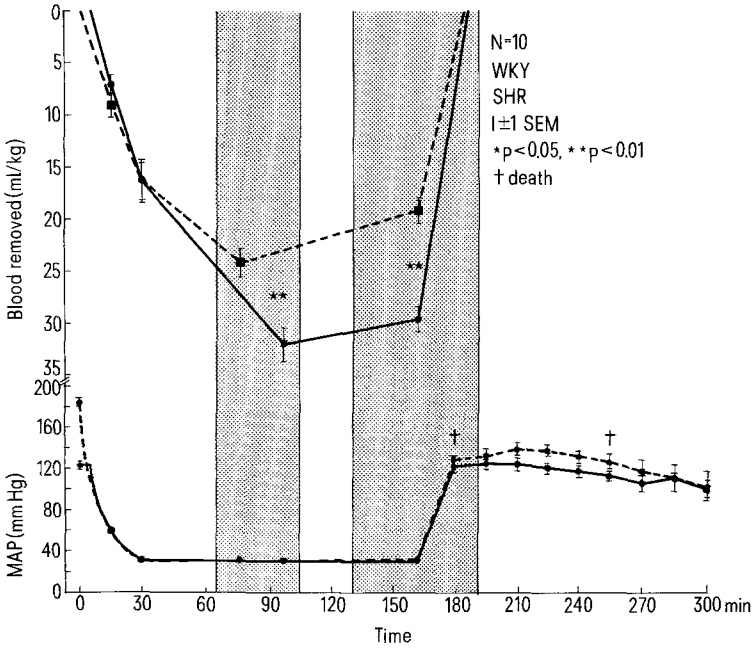
ered to be a measure of the ability of the rat to compensate for the stress. Within a variable period of time the animals showed signs of cardiovascular decompensation by requiring blood uptake from the burets in order to maintain the 30 mm Hg MAP. After one of the rats, usually the SHR, reached the transfusion point of 20%, the blood remaining in both burets was simultaneously reinfused by applying additional pressure to the fluid surfaces. The rats were then followed for an additional 2 h. All data were evaluated statistically using Student's t-test for paired data and presented as the mean + 1 SEM.

Results. The data presented in the figure and the table show that the prehemorrhage control MAPs for WKYs and SHRs were 124 ± 3 and 185 ± 4 mm Hg respectively. The maximum blood removed (i.e. compensatory effort) was 32.2 ± 1.6 ml/kg for WKYs and 24.2 ± 1.4 ml/kg for SHRs. This difference was made even greater when the maximum blood removed was normalized for the greater fall in MAP noted in the SHRs compared to the WKYs (i.e. 185 − 30 = 155 mm Hg pressure drop for SHRs compared to 124 − 30 = 94 mm Hg for WKYs). This normalization which is presented in the table shows that the WKYs pushed out 0.349 ± 0.02 ml·mm Hg/kg compared to only 0.154 ± 0.007 ml·mm Hg/kg for the SHRs. Also note that the compensation time was longer for WKYs (100 ± 8 min) than for SHRs (81 ± 10 min). Following reinfusion at 180 min (fig.) the MAPs of the WKYs returned to prehemorrhage levels. The SHRs MAPs also increased to approximately 125 mm Hg, but that value was still far below their prehemorrhage value which averaged 185 mm Hg. During the 2-h post-reinfusion observation period two SHRs died while all WKYs survived.

Discussion. The data presented strongly suggest that SHRs are far less able to respond positively to a hypotensive episode than their normotensive controls. The reasons for this reduced compensatory capability may be both physiological and morphological in nature. Andresen et al.[5] have shown that the baroreceptors of SHRs are reset in such a way that receptor strain sensitivity was increased, thus, making the baroreceptor reflex in the SHRs more responsive to hypotension than the WKYs. This observation is consistent with a report by Lundin and Thoren[6] who documented that not only was the baseline sympathetic activity to the kidney greater in SHRs than WKYs, but during stress the increase was even more pronounced in SHRs. In addition, Lee et al.[7] have shown that the nerve density in mesenteric arteries was greater in SHRs than WKYs. Even though the sympathetic nervous activity and the density of these fibers was increased in the SHRs, Strecker et al.[8] reported that the NE affinity at the adreneregic receptor sites was not different from normotensive animals.

Microcirculatory evidence provided by Hutchins and Darnell[9] indicated that the small arteries of SHRs are larger in diameter but fewer in number than normotensives. These small resistance vessels have been shown to develop significant smooth muscle hypertrophy; however, when normalized to muscle mass even though the contractile and metabolic properties of the smooth muscle cells were not different than those found in normotensive animals[10–12].

In conclusion, the reduced compensatory capability of the SHRs to respond to hypotension and shock was probably related, at least in part, to the physical constraints of the hypertrophied smooth muscle found in the resistance vessels to constrict. However, recent studies from our laboratory[4] may suggest another possible explanation. This report suggested that animals relying upon innervated alpha 1 adrenoreceptor activation through the baroreceptor reflex for compensation to hypotension vasoconstrict less effectively than those responding primarily through noninnervated extrasynaptic alpha 2 receptors. The suggested mechanisms for this reduced compensatory ability were related to the alpha 1 response which contains two endogenous negative feedback loops that serve to inhibit vascular tone by reducing the presynaptic release of NE. These mechanisms which occur during times of high sympathetic activity are: 1) the interaction of elevated catecholamines with the presynaptic alpha 2 receptors; and 2) the presumed release of prostaglandins of the E series[13] from the vascular endothelium during high vascular tone. Since SHRs have an increased baroreceptor sensitivity to hypotension[5], and increased peripheral sympathetic tone[6] and an increased peripheral nerve density compared to WKYs, one might expect the SHRs to rely more heavily upon the alpha 1 mechanism for compensation than the non-innervated alpha-2s. Thus, based upon previous reports[4–13] it was not surprising that the SHR compensated less effectively than the WKYs when subjected to hemorrhagic hypotension and shock.



This time/function graph compares the hemorrhage response of normotensive (WKY) with spontaneously hypertensive rats (SHR). Each of the 10 studies utilized one rat from each group. All data are plotted as the mean ± 1 SEM. The first shaded bar between 65 and 105 min represents the SEM range over which the maximum blood removed occurred. The 2nd shaded area represents the mean ± 1 SEM for 20% uptake by the SHRs.

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A potential difference across mouse ovarian follicle

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Summary. A small potential difference (antrum positive) has been measured with fine-tipped glass microelectrodes across the epithelial cell layers of the mouse ovarian follicle wall. As ovulation approached the potential in the antrum became more positive compared to the outside. Metabolic inhibitors and locally active hormones also altered the potential difference. The ionic basis and the significance of the potential difference are unknown.
Key words. Mouse; ovarian follicle; potential difference; ovulation.

A potential difference (PD) and a flow of fluid commonly develop across epithelia as a result of active ion transport by epithelial cells. The cell layers of the wall of the mammalian ovarian follicle form a compound epithelium. I have found in mouse, that a very small PD exists across a follicle wall. This PD becomes more positive inside as ovulation approaches, becomes more positive with inhibition of active ion transport processes and shifts in a negative direction after exposure to prostaglandins of the E and F series.

Methods. Naturally cycling and hormonally primed mice of the Porton strain were used. Vaginal smears were taken daily to determine the stage of the natural cycle. Hormonally primed animals were injected i.p. with 5 IU pregnant mare's serum gonadotrophin (PMSG) and 48 h later with 5 IU human chorionic gonadotrophin (HCG), to induce superovulation 11–13 h later[2]. Most experiments were made using an in vitro preparation. (The same results were obtained from experiments in vivo.) Mice were anesthetized with an i.p. injection of urethane. An ovary and adjacent fat was excised and pinned to the Sylgard base of a perspex bath through which pre-warmed (33 °C) physiological salt solution flowed. The composition of the superfusate was, mM/liter: Na Cl, 145; K Cl, 4.7; Mg SO_4, 1.2; K H_2PO_4, 1.2; Ca Cl_2, 2.5; glucose, 10. It was buffered with tris maleate and the pH adjusted to 7.4. Microelectrodes filled with 3 M KCl with tip potentials < 10 mV and resistances of 10–40 MΩ were used for voltage recording. The probing microelectrode mounted on a step-motor was advanced in 1 μm steps (2/sec) through the follicle wall and into the fluid-filled antrum. Potential measurements were amplified and displayed in the convential manner. Results are expressed as mean ± SE of the mean (SEM).

Results. A microelectrode traversing the follicle wall records several negative-going intracellular potentials as it pushes through the cells in its path. A steady potential arises when the fluid-filled antrum is entered and this is maintained as the electrode continues to move through the follicular fluid (fig. 1a). This latter is the trans-follicle wall potential difference (FWPD). To account for variations in follicular anatomy, I define this as any stable potential value recorded with the tip of the electrode more than 60 μm deep to the follicular surface and maintained during continued forward travel of the microelectrode for at least 50 μm.

The FWPD values showed considerable scatter in naturally cycling animals (fig. 1b) and had a mean of $+1.2 \pm 0.3$ mV ($n = 194$). This is significantly different from zero ($p < 0.001$: modified Student's t-test; Bailey, 1981)[3]. No difference was obvious between different stages of the cycle. In hormonally primed animals, 12 h post-HCG, that is around the time of ovulation, FWPD had increased to $+3.8 \pm 0.8$ mV ($n = 19$; fig. 1c). This is significantly different from the mean of the naturally cycling group ($p < 0.01$: modified Student's t-test; Bailey, p. 48, 1981)[2].

Pharmacological experiments. 1) *Sodium pentobarbitone* (10 mM/liter, substituted for equimolar sodium chloride). Six follicles from two animals both hormonally treated and immediately pre-ovulatory were tested. Sodium pentobarbitone in the perfusate caused a small positive going shift in FWPD, ranging from +1 mV to +4 mV, in all follicles (fig. 2a). The mean response was $+2.4 \pm 0.6$ mV. The new potential remained steady until the drug was washed out, as much as 10 min later, and the original value was re-established.

2) *Sodium cyanide* (5 mM/liter, replacing equimolar sodium chloride). Eight follicles were tested from two animals in the estrus stage of the natural cycle. In all cases sodium cyanide caused a small positive shift in FWPD. Responses ranged from 0.5 mV to 1.5 mV with a mean ± SEM of $+1.0$ mV ± 0.1 mV (fig. 2b). The effect was reversible.

3) *Oubain* (10^{-3} M/liter). Eight follicles from four animals were examined. Three of the animals had been primed with hormones and were immediately pre-ovulatory, the other animal was in estrus. Seven of the eight follicles showed positive shifts in FWPD shortly after exposure to oubain. Responses ranged from +1 mV to +7 mV, with a mean value of $+2.0 \pm 0.6$ mV. The potential shift was reversed on wash-out.

4) *Prostaglandin* E_1. A concentration of 1×10^{-5} M/liter was ineffective. 7×10^{-5} *M/liter.* Sixteen follicles from six naturally cycling mice were tested. Three animals were in metestrus, one in diestrus, one in pro-estrus and one in estrus. In fourteen of the sixteen follicles, a small negative shift in FWPD was seen. This ranged from -1 mV to -10 mV, with a