The effect of sodium based hypo-osmolality on arterial smooth muscle reactivity in vitro

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Abstract. The study tested the hypothesis that the reduced $[Na^+]_e$ and hypo-osmolality of normal pregnancy are causally linked to the attenuation of vascular smooth muscle reactivity in vitro. Aortic rings from nonpregnant female rats were incubated in physiological medium containing 114 mM NaCl/l and the contractile responses to phenylephrine, KCl and CaCl$_2$ as well as the relaxations to acetylcholine and KCl were compared with those of rings incubated in normal medium containing 119 mM NaCl/l. There was no solute substituted for the lowered $[Na^+]$. Experiments with phenylephrine were repeated using de-endothelialized rings and intact rings pretreated with indomethacin. Contractile responses of intact rings ($n=11$) in hypo-osmolar solution to phenylephrine were significantly ($P<0.001$) lower than of those in normal medium ($n=11$). Responses were partially restored by endothelial denudation but not in the presence of indomethacin. Relaxations to acetylcholine ($n=7$ for hypomolar; $n=6$ for normal solution) and KCl ($n=7$ for each of hypo- and normal osmolar) were significantly enhanced ($P<0.05$) in rings incubated in hypomolar solution. There was no significant difference between the responses of the rings to KCl, and CaCl$_2$ in either solution. These effects are similar to some of those previously described for vascular smooth muscle in normal pregnancy suggesting that the reduced $[Na^+]_e$ and hypo-osmolarity of normal pregnancy may be contributing to the diminished vascular reactivity.

Key words: Hypo-osmolality – Reduced $[Na^+]_e$ – Vascular reactivity

Introduction

During normal human pregnancy, plasma osmolality decreases by about 8–10 mOsm/KgH$_2$O below values obtained in the nonpregnant state [1]. Most of the reduction is due to lowered serum concentration of sodium and
its attendant anions [1]. The pregnant woman defends the new level of osmolality by appropriate responses to water deprivation and water loading [2], suggesting that the altered serum osmolality may be of some physiological significance to the pregnancy. Normal human pregnancy is also characterized by attenuation of vascular reactivity to pressor agents both in vivo and in vitro [3–5]. The mechanism of this altered vascular reactivity remains unclear despite intense investigations. Vascular smooth muscle reactivity is sensitive to changes in extracellular sodium ion concentration, [Na’]e [6–8] as well as tonicity [9–11]. By investigating the effect of sodium-derived hypotonicity on the reactivity of aortic rings from nonpregnant rats in vitro, this study tests the hypothesis that the reduced [Na’]e and hypotonicity of normal pregnancy are causally related to diminished vascular reactivity.

Materials and methods

The protocol was approved by the Research and Ethics Committee of the Faculty of Medicine and Health Sciences, UAE University. Adult female virgin (200–250 g) Wistar rats obtained from the University Animal House were killed by stunning. The descending thoracic aorta was quickly dissected, freed of adhering connective tissues, and placed in normal physiological salt solution of the following composition: (mM/l) NaCl, 119; KCl, 4.7; CaCl2, 1.6; MgSO4.7H2O, 1.2; KH2PO4, 1.2; NaHCO3, 24.9 and glucose, 11.5. The medium was continuously bubbled with a gas mixture containing 95% O2 and 5% CO2 and maintained at a temperature of 37°C and pH of 7.4. The rings were carefully mounted between two L-shaped stainless steel holders in 5 ml organs baths containing the normal solution. The upper holders were connected to isometric transducers (Dynamometer UFI) which were coupled to a Lectromed Multitrace 2 recorder for displaying responses. The lower holders were hooked to a fixed base. A resting tension of 1 g, which was the optimal passive tension at which the rings generated the greatest contractile response to 10–7 M phenylephrine was applied to each ring. After a 30-min equilibration period, the tissue was stimulated with 10–7 M phenylephrine to confirm viability and was then relaxed to 10–7 M acetylcholine. Only those rings that demonstrated at least 42% relaxation to acetylcholine were deemed to have functional endothelium [12] and used for experiments requiring intact endothelium. Where indicated by the protocol, the vascular endothelium was removed mechanically by gently rubbing the intimal surface with a roughened glass. The effectiveness of this procedure was confirmed by absence of significant relaxation to 10–7 M acetylcholine [13]. Thereafter, the medium was changed to either hypotonic (see later) or normal osmolar solution, and a further 90-min equilibration time was allowed before the commencement of experiments.

Solutions

Hypo-osmolar solution contained omit 114 mM/l NaCl, while the normal osmolar solution contained 119 mM/l NaCl. The osmolality of the solution was determined using a vapour pressure osmometer (Viescor, Wescor, Utah) and the mean values were for hypo-osmolar solution, 270.9±2.6 mosm/kg H2O) and for normal osmolar solutions, 280.4±3.0 mosm/kg H2O.

Potassium-free solution was prepared by substitution of KCl with NaCl, and KH2PO4 with NaH2PO4 and calcium-free solution was prepared by omitting CaCl2. High-KCl, calcium-free solution was similarly prepared but with equimolar substitution of KCl with NaCl and addition of 10–6 M phenolamine to block the effects of norepinephrine released from adrenergic nerve endings in the blood vessel wall [14].