Altered Vascular Reactivity in Isolated Aortic Rings from Potassium-adapted Wistar Rats

R.I. Ozolua, E.E.I. Omogbai and A.B. Ebeigbe
Departments of Pharmacology and Toxicology and Physiology, University of Benin, PMB 1154, Benin City, Edo State, Nigeria

Abstract: The observation that chronic oral potassium supplementation could cause a decrease in blood pressure in normotensive rats led to the design of the present study aimed at understanding the nature of vascular reactivity to some drugs. Responses of aortic ring preparations were obtained from two groups of Wistar rats: One given normal feeds and tap water (control) and the other given normal feeds and 0.75% KCl solution for 5 weeks (potassium-adapted). Isometric contractions were measured in rings exposed to noradrenaline (NA), 5-HT, KCl and CaCl₂, while relaxations were measured in the presence of acetylcholine (ACh), sodium nitroprusside (SNP) and Levcromakalim. Results show significant decreases (p<0.05) in both pD₂ and E₅₀ values for NA and 5-HT in rings from K-adapted rats. In the case of KCl, although the pD₂ values were significantly different, the E₅₀ were the same. Maximum responses to CaCl₂ were not significantly altered but threshold concentrations were significantly raised in rings from K⁺-adapted rats. Following NA pre-contraction, responses to ACh in endothelium-intact vessels did not change but relaxation was significantly enhanced in endothelium-denuded vessels from K⁺-adapted rats. Responses to SNP and Levcromakalim were significantly enhanced and were only partially reversed by tetraethylammonium (TEA). The results suggest the non-involvement of endothelial nitric oxide but suggest the possible roles of potassium-channels in the altered vascular reactivity in aortic rings from normotensive K⁺-adapted rats.

Key words: Potassium adaptation, potassium supplementation, potassium channels, rat aorta, vascular reactivity

INTRODUCTION

Hypertension has remained a leading cause of mortality and morbidity in affluent and poor societies alike (Kearney et al., 2005; Fuentes et al., 1998). Current opinions favour the use of non-pharmacologic methods, such as potassium-rich diets for reducing the incidence of the disease, regardless of the etiology (Gu et al., 2001). In addition, chronic oral potassium intake higher than what is ordinarily available in foods has been reported by several authors to cause a decrease in blood pressure in several animal models (Suzuki et al., 1981; Tolvanen et al., 1998) and human forms (Cappuccio and MacGregor, 1991) of hypertension, although there has been a contradictory report as well (Sacks et al., 1995). While the beneficial effects do not depend on the salt of potassium (He et al., 2005), the wide variety of protocols that have been used to effect potassium supplementation may account for the differences in observations among workers. Potassium supplementation has been found to be more effective in hypertensive than in normotensive human subjects (Cappuccio and MacGregor, 1991) and in addition, salt sensitivity and other variables may be important (Mu et al., 2005). In the rat, chronic ingestion of potassium results in adaptation involving various mechanisms, which help the animal to cope with the extra load of potassium (Obika 1993; McCabe et al., 1993).

Several vascular and non-vascular mechanisms have been suggested to be involved in the blood pressure-lowering effect of chronic potassium intake. Among the possible mechanisms are: enhanced endothelium-dependent relaxations (Tolvanen et al., 1998; Rajj et al., 1988), increase in the activity of vascular Na⁺, K⁺-ATPase and superoxide dismutase (SOD) enzymes (Dolson et al., 1995; McCabe et al., 1994) and reduced vascular reactivity to pressor agents (Campbell and Schmitz, 1978). We have in a previous report showed that the activities of these two enzymes also increase in aortic strips from potassium-adapted normotensive rats (Ozolua et al., 2003). It is possible that the influence of these enzymes may alter the responsiveness of vessels from potassium-adapted rats through mechanisms...
that involve nitric oxide and potassium channels (Ozolua et al., 2003).

Although most studies on the blood pressure-lowering effects of potassium have been on hypertensive animals or humans, the effect of potassium adaptation on normotensive blood pressure and the nature of vascular responses under this condition have not been fully studied. Our protocol of potassium adaptation decreases mean arterial blood pressure in normotensive rats (Omobgbai et al., 2005) and other workers (Naismith and Braschi, 2003) have reported similar observations in normotensive humans. These observations led us to the present investigation, which was to study the possible vascular mechanisms that may explain this phenomenon using aortic rings obtained from normotensive Wistar rats given chronic oral potassium solution.

MATERIALS AND METHODS

Animals: Age-matched adult Wistar rats weighing between 200 and 300 g were obtained from the animal house colony of Ambrose Alli University, Ekpoma, Nigeria. After 2 weeks of acclimatization they were divided into two groups. One group served as control and was fed normal growers’ mash (Bendel Feeds and Flour Mill Plc., Nigeria) and given tap water ad libitum. The other group was fed with normal growers’ mash and in addition given 0.75% KCl solution ad libitum for a duration of 5 weeks. This latter group was considered K⁺-adapted (Obika, 1993; Ozolua et al., 2003; Omobgbai et al., 2005). Rats were housed five per cage with a 12 h light-dark cycle for the 5 weeks of treatment and handled according to standard protocols for the use of laboratory animals as approved by the Faculty of Pharmacy, University of Benin, Nigeria, Committee for Use of Animals for Experiments. The experiments comply with the laws of the Federal Republic of Nigeria.

Drugs: Noradrenaline (NA) was obtained from Stirling Drug Inc. (USA), 5-hydroxytryptamine (5-HT), acetylcholine (ACh), sodium nitroprusside (SNP), ethylene glycol tetra-acetic acid (EGTA), tetraethylammonium (TEA) and pentobarbitone sodium were all obtained from Sigma (UK) while levomakalamin was obtained from SmithKline Beecham (UK). The drug solutions were prepared fresh in distilled water. Levomakalamin was dissolved first in 70% ethanol with further dilutions carried out in 10% ethanol-water mixture. High K⁺ PSS was prepared by equimolar substitution of NaCl with KCl. Ca-free PSS contained no added CaCl₂ with or without 2.0 mM EGTA. All chemicals were of analytical reagent grade.

Concentration-Response Experiments: At the end of 5 weeks, the animals were anaesthetized by injecting excessive doses of pentobarbitone sodium (ip) and exsanguinated. The thoracic aortae were quickly dissected out and placed in physiological salt solution (PSS), cleaned of adherent connective tissues as much as possible and cut into rings of 2 mM length. The rings were suspended in L-shaped wire loops in 10 mL organ baths containing PSS. The upper loop was attached to a Grass Model FT03 force transducer connected to a Grass Model 7P polygraph (Grass Instruments Co., Quincy, MA, USA). The PSS was made of the following composition (mM/L): NaCl 119, KCl 4.7, NaHCO₃ 24.9, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 1.6, glucose 11.5. The PSS was bubbled throughout with 95% O₂-5% CO₂ gas mixture (BOC Gases, Nig. Plc) with the pH and temperature maintained at 7.4 and 37°C, respectively. The rings were given a resting force of 1 g (Ebeigbe and Aloamaka, 1987, Mian and Martin, 1995). An equilibration period of 60 min was allowed after which rings were stimulated twice at 15 min intervals with 80 mM K⁺ (Engler et al., 2000). The average of these contractions represented the reference (100%) as internal standards for comparing subsequent contractile responses (Oppgaard et al., 2000). Aortic tissue viability was closely monitored within 6 h period of experiments. This was ascertained by the observation that force produced by 80 mM K⁺ for each ring was reproducible within this period.

Experimental protocol

Concentration-response tests to NA, 5-HT, KCl and CaCl₂: Rings from both the control and K⁺-adapted groups were exposed to cumulative concentrations of NA or 5-HT to evaluate whether there were changes in vasoreactivity to these contractile agents. Contractions induced by these agents were expressed as a percentage of the reference 80 mM K⁺ contraction. The contractile responses to high K⁺ were studied by exposing the rings from both groups to cumulative concentrations of KCl (1.0×10⁻² to 7.0×10⁻² M).

In calcium concentration-response experiments, normal PSS was replaced by a Ca²⁺-free PSS containing 2.0 mM EGTA for 20 min. Rings were stimulated repeatedly, during the Ca²⁺-free, EGTA exposure, with 80 mM K⁺, to deplete intracellular Ca²⁺ pools (Ebeigbe and Aloamaka, 1987). At the end of this exposure, the tissues were rinsed four times with Ca²⁺-free + EGTA PSS and subsequently, with Ca²⁺-free, 80 mM K⁺, 0 mM EGTA PSS; followed by cumulative additions of Ca²⁺ to the bath.

Concentration-response tests to ACh, SNP and levomakalamin: Vasorelaxation studies were carried out by pre-contracting the rings with NA at a concentration
producing 75% of maximal NA contraction: 10^{-7} M for controls and 10^{-6} M for K-adapted group (concentrations were obtained from concentration-response experiments). This was followed by the addition of the relaxant, after steady contraction had been established (time-dependent control experiments showed that the pre-contraction induced by NA remained stable for the duration of the concentration-response tests). The relaxation responses to acetylcholine (ACh), sodium nitroprusside (SNP) and levromakalim were examined in order to assess the possible roles of endothelium-derived nitric oxide (NO), exogenously generated NO and potassium channel opening, respectively. In ACh experiments, endothelium denudation was effected by gently rubbing the intimal surface of the rings with a wire.

Following the responses of aortic rings from K'-adapted rats to levromakalim and SNP, fresh rings from both groups were exposed to 10 mM tetraethylammonium (TEA), 10 min prior to pre-contraction with NA. Subsequently, increasing concentrations of levromakalim or SNP were added (TEA remained in the bath throughout this protocol). The responses plotted as percentage relaxation were compared to the effects of these drugs in the absence of TEA.

**Serum and Aortic K' analyses:** K'-adapted and control rats were anaesthetized with pentobarbital sodium (40 mg kg^{-1}, ip). About 4 mL of blood samples were withdrawn from the carotid artery into bottles by use of a cannula. The blood was allowed to clot and then centrifuged at 2000 g for 5 min in order to obtain the serum.

Pieces of the thoracic aorta from both groups were cleaned of adherent fat and connective tissues as much as possible and transferred to a filter paper in which they were dabbed before weighing. Each aortic tissue was homogenized in 5 mL of deionised water and centrifuged at 2000 g for 5 min to obtain 2 mL supernatant. Samples of sera and supernatants were analysed for K' using a Corning (UK) clinical flame emission photometry.

**Statistics:** Values are presented as Means ± Standard Error of the Mean (SEM) and n represents the number of rats from which aortic tissues or sera were obtained. EC_{50} (molar concentration producing 50% of maximum response) were computed for NA, 5-HT, Ca^{2+} and K' using a programme for logit transformation of concentration-response curves and presented as the negative logarithm (pD_{20}). In the case of ACh, SNP and Leveromakalim, values are presented as IC_{50} (Molar concentrations producing 50% relaxation after pre-contraction with NA). The maximal response (E_{max}) was determined graphically in all cases. Comparisons of EC_{50}, IC_{50} and E_{max} values were made where appropriate, by using the Student’s t-test (GraphPad software, UK). p<0.05 was taken to denote statistical significance in all cases.

**RESULTS**

Table 1 shows the E_{max} obtained from the respective concentration-response experiments to K', Ca^{2+}, NA and 5-HT. While responses to maximum concentrations of K' and Ca^{2+} are not significantly different between the control and potassium-adapted groups, there are significant differences (p<0.05) in responses to maximum concentrations of NA and 5-HT.

Concentration-response curves for NA and 5-HT are shown in Fig. 1. In NA experiments, the pD_{20} values were significantly (p<0.05) higher in control rats (7.79±0.10) than in K'-adapted rats (7.06±0.13). Responses to 5-HT were similar to those for NA: the respective pD_{20} values were 6.10±0.02 vs. 5.88±0.10 (p<0.05).

Figure 2 shows KCl-induced contraction in which although E_{max} remains unchanged for both groups, pD_{20} values were significantly different (p<0.05): 1.83±0.04 vs. 1.63±0.03 for control and K'-adapted rats, respectively.

**Dose-response to CaCl_{2}:** Cumulative additions of Ca^{2+} following Ca^{2+}-depletion and stimulation with 80 mM K' (Fig. 3) resulted in graded contractile responses in aortic rings from both groups of rats. Although the pD_{20} values were not significantly different, the threshold concentration of Ca^{2+} in rings from K'-adapted rats was higher than in controls. Responses to 5·10^{-7} M were 23.41±5.8% (Control) and 0.00% (K'-adapted).

**Effects of ACh, SNP and levromakalim:** The magnitudes of the pre-contraction induced by 10^{-7} M NA (control rings) and 10^{-4} M NA (K'-adapted rings) were:

<table>
<thead>
<tr>
<th>Table 1: The maximal contractile responses (E_{max}) by aortic rings from control and K'-adapted rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>K' (70 mM)</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>K'-adapted</td>
</tr>
<tr>
<td><em>Significant difference (p&lt;0.05) compared to corresponding value in the column, n = 5.</em> Ca^{2+}-loading was preceded by stimulation with 80 mM K' and NA and 5-HT responses were related to maximal responses produced by 80 mM K'</td>
</tr>
</tbody>
</table>

**Effects of ACh, SNP and levromakalim:** The magnitudes of the pre-contraction induced by 10^{-7} M NA (control rings) and 10^{-4} M NA (K'-adapted rings) were:

<table>
<thead>
<tr>
<th>Table 2: Serum and aortic tissue K' content in control and K'-adapted rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>K' content</td>
</tr>
<tr>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>K'-adapted</td>
</tr>
</tbody>
</table>

Aortic tissue K' content is significantly higher in the K'-adapted group (p<0.05), n = 5 per group.
Fig. 1: The responses of aortic rings from K⁺-adapted and control rats to noradrenaline (a) and 5-HT (b). The responses to the contractile agents are attenuated in K⁺-adapted rats; both the pD₂ and Eₘₐₓ values are significantly lower (p<0.05) in this group but threshold concentrations are comparable with controls. n = 8 for each of the groups.

Fig. 2: Concentration-dependent contractile responses to potassium chloride. Although the pD₂ values and threshold concentrations are significantly different (p<0.05), the Eₘₐₓ values are the same but are attained at higher concentrations by the rings from the adapted rats. n = 6 for each group.

Fig. 3: Concentration-dependent contractile responses to Ca²⁺ in Ca²⁺-depleted 80 mM K⁺-stimulated aortic rings from control and K⁺-adapted rats. Rings from K⁺-adapted rats show less sensitivity at lower concentrations although Eₘₐₓ is not altered. For each group, n = 6, *p<0.05.

Fig. 4: Relaxant effects of acetylcholine (ACh) in endothelium-intact (EI) and endothelium-denuded (ED) aortic rings from control and K⁺-adapted rats. Rings were pre-contracted with 10⁻² or 10⁻⁴ M noradrenaline for control and K⁺-adapted rats, respectively. Responses were comparable in endothelium-intact vessels but Eₘₐₓ was significantly higher in endothelium-denuded rings from K⁺-adapted rats* (p<0.05), n = 6-7 per group.

542.5±18.3 mg and 550.0±24.2 mg, respectively. In aortic rings with intact endothelium, the relaxation responses to ACh from both groups were not significantly different but after endothelium denudation, there was significant decrease (p<0.05) in the Eₘₐₓ values in the adapted (42.10±4.2%) compared to the controls (28.5±4.7%) (Fig. 4). Responses induced by levromakalim and SNP were significantly enhanced in rings from K⁺-adapted rats: IC₅₀ values for levromakalim (Fig. 5a) were (6.46±0.81)×10⁻⁷ M and (3.80±0.69)×10⁻⁷ M, for control and K⁺-adapted rats, respectively (p<0.01). In the presence of 10 mM TEA, the enhanced vasorelaxant
Fig. 5: The relaxant effects of levcromakalim (a) and SNP (b) on aortic rings from control and K⁺-adapted rats. Rings were pre-contracted with 10⁻⁷ or 10⁻⁶ M noradrenaline for control and K⁺-adapted rats, respectively. Effects of levcromakalim and SNP are significantly enhanced by K⁺-adaptation but partially reversed by 10 mM TEA. Comparing IC₅₀ values: p<0.001 and p<0.05 (levcromakalim) and p<0.01 and p<0.05 (SNP), in the presence or absence of 10 mM TEA, respectively. n = 6-7

action of levcromakalim was significantly reduced thereby bringing the IC₅₀ value (2.95±0.74)×10⁻⁵ M closer to that of the control but still significantly different from it (p<0.05). The presence of TEA reduced the Eₘₑₓ of the rings from K⁺-adapted rats from 100.0 to 94.8±1.2%. Similarly, for SNP (Fig. 5b), the respective IC₅₀ values for control and K⁺-adapted rats were (7.08±0.74)×10⁻⁶ M and (1.29±0.81)×10⁻⁶ M, p<0.01. The presence of 10 mM TEA did not reduce the effect of SNP in rings from K⁺-adapted rats to levels obtained in rings from the control. The IC₅₀ value for TEA + SNP in rings from K⁺-adapted rats is (3.39±0.83)×10⁻⁶ M (compared to (7.08±0.74)×10⁻⁶ M in rings from control rats in the absence of TEA p<0.05). The Eₘₑₓ value obtained by rings from K⁺-adapted rats in the presence of TEA was reduced from 100.0±0.0 to 93.2±2.0%.

In rings from control rats, TEA significantly (p<0.5) increased the IC₅₀ and decreased Eₘₑₓ values for both levcromakalim and SNP. The IC₅₀ values are: (6.46±0.81)×10⁻⁵ M vs (3.50±0.48)×10⁻⁷ M for levcromakalim and (7.08±0.74)×10⁻⁶ M vs (3.30±0.27)×10⁻⁷ M for SNP. The Eₘₑₓ values are: 83.7±2.1% vs 91.1±0.4% for levcromakalim and 85.2±1.7%±58.2±0.0% for SNP.

Table 2 shows that while aortic tissue K⁺ level was significantly higher (p<0.05) the adapted than the control, serum K⁺ levels were comparable in the two animal groups.

**DISCUSSION**

We have observed that compared with controls, contractile responses to NA and 5-HT in aortic rings from K⁺-adapted rats were significantly attenuated (Fig. 1). This observation is comparable to other reports (Obika, 1993; Omogbai et al., 2005) in normotensive K⁺-adapted rats showing reduced vascular responses to bolus intravenous injections of NA. Although we have reported that our protocol of potassium supplementation reduces blood pressure in normotensive as well as hypertensive animals (Omogbai et al., 2005), most studies by other workers on the effect of potassium supplementation have been on hypertensive animals (Gu et al., 2001; Suzuki et al., 1981; Tolvanen et al., 1998).

Blunted responses to vasoconstrictors could be due to factors such as reduced intracellular Ca²⁺ concentration and/or delivery to the contractile apparatus (Gurney, 1994), increased production and/or protection of relaxant second messengers such as NO, endothelium-derived hyperpolarizing factor (EDHF) and cGMP (de Wit et al., 2000; Feletou and Vanhoutte, 2000) and reduced sensitivity of the contractile machinery (Pfitter, 2001). The reason for the lower maximal responses by rings from the K⁺-adapted rats to NA and 5-HT is not clear but a probable explanation is reduced sensitivity of the contractile machinery to Ca²⁺. This tenet is supported by a report that reduced sensitivity of the contractile machinery to Ca²⁺ is associated with increased intracellular level of cGMP (Pfitter, 2001), which accompanies NO production. Increased SOD activity which suggests enhanced NO availability has been shown in K⁺-adapted rats (Ozolua et al., 2003). It is known that both SOD and Na⁺K⁺-ATPase facilitate vascular smooth muscle relaxation through different mechanisms. While SOD is known to scavenge superoxide anion thereby increasing
basal NO (Mian and Martin, 1995). Na⁺, K⁺-ATPase is believed to cause hyperpolarization and resetting of the resting membrane potentials of vascular smooth muscle cells (Hermsmeyer, 1982). NO itself causes the opening of membrane Kᵦ channels through the cyclic GMP-protein kinase C pathway (Brayden, 1996).

Results in the present study show that maximal responses did not change in KCl and CaCl₂ concentration-response experiments (Fig. 2 and 3) following potassium supplementation. Increased threshold to K⁺ and Ca²⁺ may indicate increased hyperpolarization in vascular smooth muscle cells (VSMC) since K⁺ is often used to reverse hyperpolarization (Adeagbo and Triggle, 1993) and there is reduced Ca²⁺ influx in hyperpolarized vascular smooth muscle cells (VSMCs) (Brayden, 1996). In separate experiments (not presented), we have observed that the phasic contractile response to NA in Ca²⁺-free, BQTA-containing medium (presumed to be due to mobilization of intracellular Ca²⁺ pools (Ebeigbe and Alomanaka, 1987)) was not significantly altered by potassium adaptation. Thus it appears that while potassium adaptation may delay Ca²⁺ influx through voltage-operated channels, it does not alter the NA-mobilizable Ca²⁺ pool. The unchanged Eₘ₉ to KCl in both groups is suggestive of complete reversal of hyperpolarization with higher K⁺ concentrations (Adeagbo and Triggle, 1993).

These same mechanisms may explain at least in part, the enhanced relaxant effects of SNP (an exogenous NO donor) and levromakalim (a K⁺ channel opener) in aortae from K⁺-adapted rats (Fig. 5). That the effects of these drugs were only partially reversed by 10 mM TEA may indicate a higher open-state of K⁺ channels as a result of potassium adaptation (Brayden, 1993; Nelson and Quayle, 1995). Potassium channels such as Kᵦ, are thought to regulate membrane potentials through hyperpolarization (Brayden, 1996) and have been implicated in determining the resting membrane potentials and myogenic tone regulation (Nelson and Quayle, 1995).

The present finding of unchanged ACh-induced relaxation in endothelium-intact rings from K⁺-adapted rats is at variance with those of other workers (Tolvanen et al., 1998; Rajj et al., 1988) in hypertensive rats. It is not clear if the pathological state could be a modifier to the response but our present data suggest an unimportant role for ACh-induced endothelial NO release as a mechanism for the altered vascular reactivity in K⁺-adapted normotensive rats. The presence of significant relaxation to ACh in endothelium-denuded rings indicates a role for intracellular mechanisms that may involve inducible NO synthase.

Our protocol of manipulating dietary potassium for the achievement of potassium adaptation has not produced any significant difference in serum potassium concentrations compared to those of control rats. We have however observed that aortic tissue potassium content was significantly higher in the K⁺-adapted than in control rats (Table 1). Although the site of storage of the potassium in the VSMC is not known, it may well be part of the adaptive processes that involve increased non-renal cell potassium content (Giebisch, 1986). It is noteworthy that reports on potassium levels in plasma (or serum) and tissues after chronic ingestions of potassium have been contradictory; while some workers have reported elevated plasma levels with no change in skeletal muscle potassium levels in spontaneously hypertensive rats receiving oral potassium supplements (Workman and Palier, 1985) others have reported no change in both serum and rectus abdominis muscle potassium content (Rajj et al., 1988).

In conclusion, our protocol of potassium adaptation causes attenuation of maximal contractile responses induced by NA and 5-HT while enhancing relaxation induced by sodium nitroprusside and levromakalim. The mechanisms responsible for the altered reactivity may involve cellular potassium channel activation.

ACKNOWLEDGEMENTS

This work was supported by the University of Benin Research Grant to RIO. The authors are grateful to BOC Gases Nigeria Plc for their generous donation of 95% O₂-5% CO₂ gas mixture.

REFERENCES


