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European Journal of Pharmacology 474 (2003) 241–247
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High salt diet modulates cAMP- and nitric oxide-mediated relaxation responses to isoproterenol in the rat aorta

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Received 21 November 2002; received in revised form 16 June 2003; accepted 24 June 2003

Abstract

This study tested the hypothesis that nitric oxide (NO) production contributes to relaxation induced by 3′,5′-cyclic adenylate monophosphate (cAMP)-elevating agents and that high salt diet impairs this mechanism of relaxation. Relaxation response to isoproterenol but not sodium nitroprusside, a NO donor, was reduced in the thoracic aorta from rats that were placed on a high salt diet (8% NaCl; 60 ± 4%, $P < 0.001$). 1*H*-[1,2,4]oxadiazolol [4,3,-α]quinoxalin-1-one (ODQ, 10 μM), a soluble guanylate cyclase inhibitor, but not N^{ω}-nitro-L-arginine methyl ester (L-NAME, 100 μM), an inhibitor of NO synthase (NOS), attenuated the relaxation to isoproterenol (59 ± 16%, $P < 0.01$). High salt diet also impaired the relaxation responses to forskolin, an activator of adenylate cyclase, or 8-Bromo-cAMP (8-Br-cAMP). (*N*-[2-((*p*-bromocinnamyl)aminoethyl]-5-isoquinolinesulfonamide hydrochloride (H-89) (8 μM), an inhibitor of cAMP-dependent protein kinase, did not affect the relaxation produced by isoproterenol. These data suggest that high salt diet impairs relaxation response to isoproterenol by a dual mechanism involving diminished NO/NOS pathway linked to cGMP pathway and diminished cAMP pathway that is independent of protein kinase A.

Keywords: Salt; Aorta; Isoproterenol; Nitric oxide (NO); cAMP; cGMP

1. Introduction

High salt diet can result in elevated blood pressure in such experimental animal models as the spontaneously hypertensive rat (Wu et al., 1998) or Dahl salt-sensitive rat (Nishida et al., 1998) as well as in normal Sprague–Dawley rats (Miyajima and Bunag, 1985; Sofola et al., 1993; Giardina et al., 2001) and Wistar rats (Kagota et al., 2001). High salt diet has also been implicated in the pathogenesis of human hypertension, particularly in salt-sensitive individuals as high salt diet was correlated with the level of blood pressure (Intersalt, 1988; Stamler et al., 1991). Conversely, salt restriction resulted in a small but significant reduction in blood pressure (Sacks et al., 2000). In contrast, in salt-resistant individuals, high salt diet is only associated with modest increases in blood pressure while major endothelial cell dysfunction or alterations in vascular reactivity are usually not seen (Bragulat et al., 2001). Studies in salt-sensitive experimental animals such as the Dahl salt-sensitive rat have shown that elevated blood pressure resulting from a high salt diet is associated with endothelial cell dysfunction as well as enhanced vascular reactivity to vasoconstrictor stimuli (Wu et al., 1998; Adegunloye and Sofola, 1997; Obiefuna et al., 1991a), which may contribute, at least in part, to the high blood pressure (Nishida et al., 1998; Hayakawa et al., 1999).

From the results of many studies, there is no consensus regarding the effect of high salt diet on the relaxation to many agonists including but not limited to acetylcholine. For example, attenuated relaxation response to acetylcholine was observed in spontaneously hypertensive rat (Kagota et al., 2001) or Dahl salt-sensitive rat rats (Nishida et al., 1998) but not in Sprague–Dawley rats (Adegunloye and Sofola, 1997; Obiefuna et al., 1991b; Giardina et al., 2001). However, others have observed that high salt diet caused reduction of acetylcholine-induced relaxation (Lenda et al., 2000). The involvement of different signalling mechanisms and the downstream pathways in salt-

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doi:10.1016/S0014-2999(03)02011-9

induced changes in vascular responses is the subject of many recent studies but available information produce conflicting data. For example, Kagota et al. (2001) observed that high salt diet caused a reduction in acetylcholine-mediated release of cGMP in the spontaneously hypertensive rat without affecting the initial step involving nitric oxide (NO) production. However, high salt diet was reported to impair acetylcholine-induced dilation in Dahl salt-sensitive rats by a mechanism involving reduced release of NO from the endothelium (Nava and Luscher, 1995). The lack of a consensus on the effects of salt loading on acetylcholine-mediated relaxation, therefore, necessitates an evaluation of other relaxant mechanisms during salt loading. The involvement of other relaxant mechanisms in salt-induced vascular dysfunction is supported by recent studies in which evidence was provided that high salt diet affects the cAMP system (Frisbee et al., 2001). However, as with the NO/cGMP system, available studies produced conflicting data as Frisbee et al. (2001) reported that high salt did not alter vascular cAMP production whereas an earlier report demonstrated a rise in cAMP level in thoracic aorta of desoxycorticosterone acetate-salt hypertensive rats (Mangiarua et al., 1989).

Apart from cAMP, the traditional mediator of the effects of β-adrenoceptor agonists, isoproterenol elicits a relaxation that is endothelium-dependent and mediated by NO/cGMP (Bradley et al., 2000). Isoproterenol, therefore, provides a better tool than an agonist that uses a single mechanism for evaluating the effects of salt loading on different vascular relaxant mechanisms. This is underscored by recent demonstrations of cross talk between many signalling pathways including the cAMP and cGMP pathways (White et al., 2000; Murthy, 2001). These experiments, therefore, evaluated the effects of high salt diet on cAMP-mediated relaxation responses to isoproterenol and the associated signaling pathways. Our data suggest that salt loading impaired the cAMP- and NO-mediated components of isoproterenol-induced relaxation of rat aortic ring and highlights the complex mechanisms involved in vascular reactivity following ingestion of a high salt diet.

2. Materials and methods

2.1. Drugs

Forskolin, 1*H*-[1,2,4]oxadiazolol [4,3,-α]quinoxalin-1-one (ODQ) and (*N*-[2-((*p*-bromocinnamyl)aminoethyl]-5-isoquinolinesulfonamide hydrochloride (H-89) were dissolved in dimethylsulfoxide (DMSO) to prepare stock solutions (100 μM) from which aliquots were added to Krebs buffer giving a final DMSO concentration not greater 1%. Indomethacin was dissolved in 2.5% sodium carbonate while L-NAME was dissolved in normal saline to give stock solutions of (100 μM) and both were prepared on each experimental day. All drugs used were obtained from Sigma (St. Louis, MO, USA) except H-89, which was obtained from Calbiochem (La Jolla, CA, USA).

2.2. Protocol

Experiments were carried out on weanling (4 weeks old, 172 ± 2 g) male Sprague–Dawley rats (Harlan-Teklad, Madison, WI). These rats were kept on a normal rat diet (0.4% NaCl) for 2 weeks for acclimatization, after which they were divided into two groups. One group continued on normal diet while the other group was placed on a high salt diet containing 8% NaCl for the next 5–6 weeks. At the end of the feeding period, the rats were euthanized (sodium pentobarbital, 100 mg kg^{-1}, i.p.) and the thoracic aorta was quickly excised and placed in ice-cold (4 °C) Krebs solution of this composition (mM): NaCl, 119; $NaHCO_3$, 25; KCl, 4.7; $CaCl_2$, 1.6; $MgSO_4 \cdot 7H_2O$, 1.17; KH_2PO_4, 1.18; and glucose, 5.5, pH 7.4. The aorta was then cut into 3 mm ring segments and mounted in a 10-ml jacketed organ bath (World Precision Instruments, Sarasota, FL, USA) containing warmed (37 °C), oxygenated (5% CO_2–95% O_2) Krebs solution to which indomethacin (10 μM) was added to block endogenous production of prostanoids. The ring was suspended in the bath solution and attached to an isometric force transducer, model FORT 10 (WPI). The force transducer was connected via a transbridge TBM-4 (World Precision Instruments) coupled to a data-acquisition system (DataQ Instruments, Akron, OH, USA) for recording of isometric tension developed. The rings were subjected to a resting tension of 2 g and allowed to equilibrate for a period of 90 min while being rinsed every 15 min. During this period, the rings were subjected to two challenges of 100 nM phenylephrine 30 min apart.

At the end of the equilibration period, relaxation responses to cumulative doses of isoproterenol (1 nM to 10 μM) were determined in aortic rings that were preconstricted to 70–80% of maximal contraction with 100 or 70 nM phenylephrine in rats on normal or high salt diets, respectively. Phenylephrine concentrations were adjusted under different conditions as needed to achieve the desired preconstricted vascular tone. Unless otherwise stated, all experiments were conducted in endothelium-intact aortic rings, the presence of endothelium being ascertained by a full relaxation to acetylcholine (10 μM). In all experiments, a drug-tissue contact time of at least 3 min was allowed for each concentration of agonist. In order to reduce the effect of photodegradation on isoproterenol, the tests were performed in a darkened room dimly lit by a distantly placed sodium lamp. At the end of the tests, the rings were thoroughly rinsed, left for at least 30 min before other agonists were evaluated. In evaluating the effects of inhibitors, they were added to the buffer for 30 min before redetermining the concentration–response relationship to isoproterenol. The effects of antagonists were evaluated by comparing the responses to isoproterenol between vehicle- and inhibitor-treated aortic rings.

In order to analyse the relaxation mechanisms, isoproterenol responses were determined before and after the addition of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 100 μM) an inhibitor of nitric oxide synthase (NOS), ODQ (10 μM), an inhibitor of soluble guanylate cyclase, propranolol (10 μM), a β-adrenoceptor antagonist, or H-89 (8 μM), an inhibitor of protein kinase A (PKA). The efficacy of H-89 as a PKA inhibitor at this concentration was ascertained by its ability to selectively blunt the relaxation induced by 8-Bromo-cAMP (8-Br-cAMP). For each inhibitor, one ring from one vessel was used. As L-NAME enhanced the contraction produced by phenylephrine, concentration of phenylephrine used in this set of experiments was reduced to 70 nM in rats on normal diet or 55 nM in rats on high salt diet to produce the required 70–80% increase in tone of the aorta.

Additional experiments were also carried out using other agonists. For these experiments, cumulative dose responses were determined for forskolin (1 nM to 10 μM), an activator of adenylate cyclase, 8-Br-cAMP (1 nM to 100 μM), a cell-permeable analog of cAMP, a second messenger for isoproterenol, or sodium nitroprusside (0.1–100 nM), a donor of NO. The responses to sodium nitroprusside were determined in the presence of indomethacin (10 μM) and L-NAME (100 μM) so as to prevent any effects from prostanoids and endothelial NO. In order to avoid any carry-over effects between these agonists and the inhibitors, one ring was used for each drug.

2.3. Statistical analysis

Relaxation response at each dose of an agonist was calculated as the percentage change of tension from that obtained following PE-induced contraction and the concentration–response curves were plotted. Area under the curve was determined for each curve by the trapezoidal rule using a regression analysis using a statistical software package (GraphPad Prism, version 3.0). Differences between rats on normal or high salt diet were compared using repeated measures analysis of variance (ANOVA) followed by the Student–Newman–Keuls' post hoc test. In some cases, Student's *t*-test for unpaired data was used for comparison between the means of two groups using an average of all data points on each curve. *P* values <0.05 were considered significant.

3. Results

At the end of the 5- to 6-week feeding period, the rats on normal diet weighed more than those on high salt (321 ± 3 g, $n=26$, versus 297 ± 3 g, $n=24$). However, in a randomly selected number of rats from both groups, systolic blood pressure was greater in rats on high salt diet (149 ± 2 mm Hg, $n=9$) than that obtained in rats on normal diet (123 ± 2 mm Hg, $n=9$).

3.1. Relaxation responses to isoproterenol

The tension developed to 100 nM phenylephrine in endothelium-intact aortic rings used in these experiments was ~51% greater in rats on high salt diet (1.77 ± 0.10 g, $n=15$, versus 1.17 ± 0.07 g, $n=15$). The relaxation responses to isoproterenol in aortic rings from rats fed a normal diet ($n=12$) and those on high salt diet ($n=12$) are shown in Fig. 1A. Relaxation responses were evaluated in aortic rings from rats on normal or high salt diets that were challenged with 100 or 70 nM phenylephrine, respectively, producing tensions of 1.21 ± 0.05 or 1.18 ± 0.06 g, respectively. Isoproterenol elicited a dose-dependent relaxation of the rat aortic ring preconstricted with phenylphrine. The relaxation was significantly attenuated in rings from rats on high salt diet ($60 \pm 4\%$, $P<0.01$). The threshold concentrations for the effect of isoproterenol in rats on normal or high salt diets are 10 and 100 nM, respectively. At the highest concentration (10 μM), isoproterenol-induced relaxation was greater in rats on normal diet ($83.0 \pm 2.9\%$ versus $45.1 \pm 3.0\%$, $P<0.01$). Propranolol (10 μM) abolished the responses in both groups of rats ($n=3$ per group) uncovering a 5–8% vasoconstriction at higher concentrations (>1 μM), Fig. 1B.

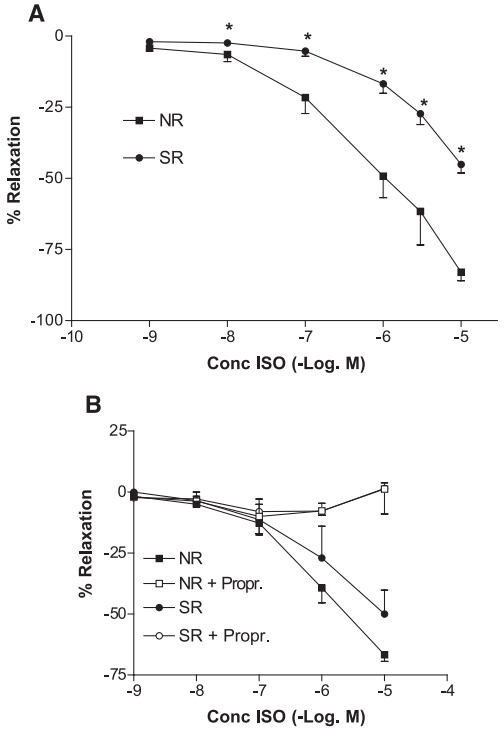

Fig. 1. Dose–response relationship to isoproterenol (ISO) in aorta of rats on normal diet (■; NR, $n=12$) or 8% NaCl diet (●; SR, $n=12$) (A). Relaxation responses to isoproterenol before and after 10 μM propranolol (+Prop) in NR (□; $n=3$) and SR (○; $n=3$) (B). Responses were significantly attenuated in SR. *$P<0.01$ versus NR.

3.2. Responses to isoproterenol as affected by NO and cGMP

The effect of inhibition of NOS with L-NAME (10^{-4} M) on the relaxation response to isoproterenol in rats on normal or high salt diet is shown in Fig. 2A. In the rings ($n=6$) from rats on high salt diet, L-NAME had no effect on the relaxation response ($7 \pm 7\%$) whereas in rats on normal diet ($n=7$), addition of L-NAME resulted in a significant attenuation of the relaxation at concentrations of isoproterenol >0.1 μM ($64 \pm 13\%$, $P<0.001$). This inhibition is such that the difference that hitherto existed between both groups of rats was obliterated.

The relaxation response to sodium nitroprusside was not different between aortic rings from rats on normal ($n=5$) or high salt diet ($n=5$) ($P>0.05$) (Fig. 2B). ODQ (10 μM), an inhibitor of soluble guanylate cyclase, abolished the relaxation induced by sodium nitroprusside (3 nM) in rats on normal ($n=3$) and high salt diet ($n=3$). ODQ also inhibited the relaxation produced by isoproterenol in both groups of rats (Fig. 3). This inhibition was similar in magnitude for rats on normal or high salt diet being: $59 \pm 16\%$ ($P<0.01$) in rats on high salt diet, a value that was not different from an inhibition of $50 \pm 11\%$ ($P<0.01$) that was obtained in rats on normal diet. Inhibition by ODQ of isoproterenol relaxation was such that there was no difference in response between ODQ-treated aortic rings from rats on normal diet and that from untreated aortic rings from high salt diet rats. However, ODQ further blunted the reduced relaxation to isoproterenol in aortic rings from rats on high salt diet by $59 \pm 16\%$ ($P<0.01$).

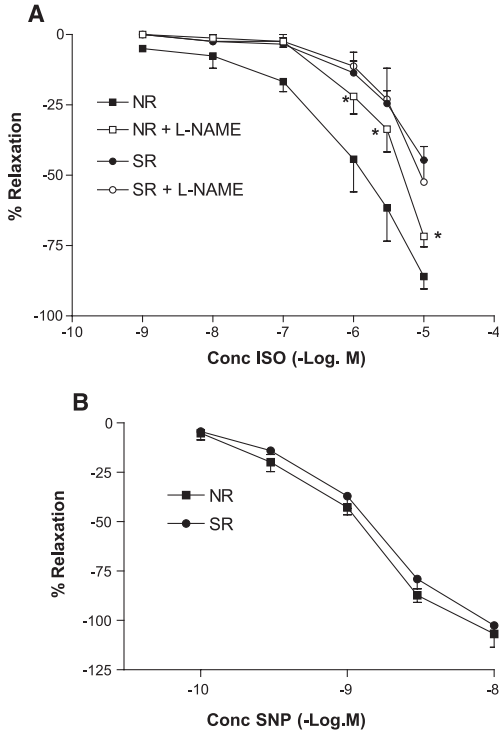

Fig. 2. Relaxation responses to isoproterenol (ISO) in untreated thoracic aortic rings from NR in the absence (■) or presence (□) of L-NAME (100 μM) or in rings from SR in the absence (●) or presence (○) of L-NAME (A). Responses to sodium nitroprusside (SNP) in NR (■, $n=6$) or SR (●, $n=6$) (B). *$P<0.01$ versus NR.

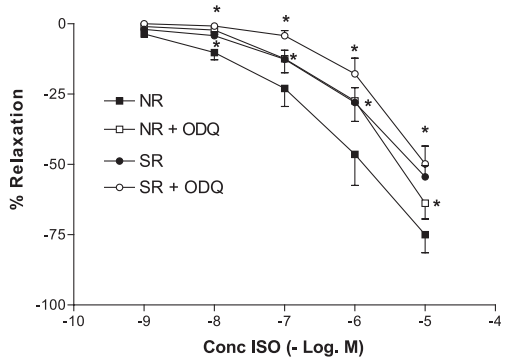

Fig. 3. Responses to isoproterenol (ISO) before or after ODQ (10 μM) in NR (□; $n=5$) and SR (○; $n=5$). Responses in ODQ-treated vessels were compared with that in untreated vessels from NR (■) or SR (●). *$P<0.01$ versus NR or SR.

3.3. Responses of aortic rings to forskolin and 8-Br-cAMP: effect of inhibition of NO production

The relaxation responses to forskolin and 8-Br-cAMP are presented in Fig. 4. Compared to rats on normal diet ($n=7$), the overall relaxation response to forskolin (1 nM to 1 μM) was lower in aortic rings from rats on high salt diet ($34 \pm 5\%$, $P<0.01$; $n=6$). Relaxation elicited by 8-Br-cAMP (1 nM to 10 μM) was $50 \pm 4\%$ lower in rats on high salt diet ($n=6$) compared with rats on normal diet ($n=6$), ($P<0.01$). The weaker response to 8-Br-cAMP (exogenous)

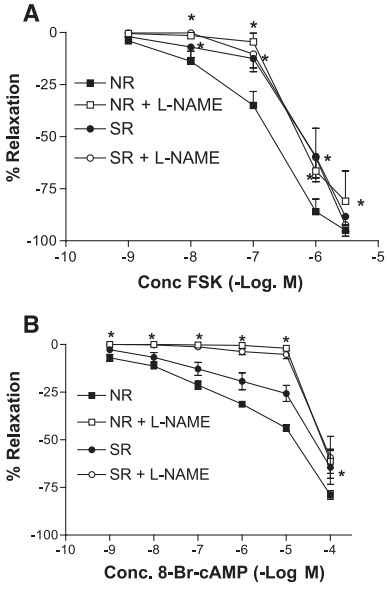

Fig. 4. Dose–response relaxation to forskolin (FSK, A) or 8-Bromo-cAMP (8-Br-cAMP; B) in untreated vessels from NR (■; $n=5–6$) and SR (●; $n=5–6$) or in L-NAME-treated vessels harvested from NR (□) or SR (○). Responses to FSK were attenuated in SR. *$P<0.01$ versus NR or SR.

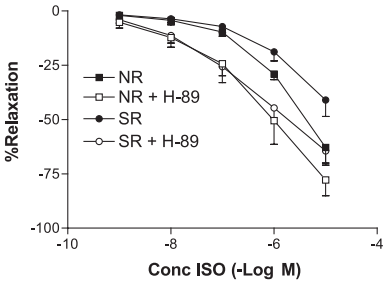

Fig. 5. Relaxation responses to isoproterenol (ISO) before and after addition of the protein kinase A inhibitor, H-89 (8 μM), in NR (□; $n=6$) or SR (○; $n=6$).

in rats on high salt diet is similar to that seen with forskolin (Fig. 4A) and isoproterenol (Fig. 2A) (endogenous cAMP). Fig. 4 also presents data demonstrating a role for NO in cAMP-mediated relaxation of the rat aorta. L-NAME (100 μM) attenuated forskolin-induced relaxation (63 ± 12%, $P<0.01$) in aortic rings from rats on normal diet ($n=5$) but not in rats on high salt diet ($n=5$) (Fig. 4A), as did removal of the endothelium (data not shown). As observed with isoproterenol in Fig. 2A, L-NAME abolished the difference in forskolin-induced relaxation between rats on normal or high salt diet. By contrast, L-NAME attenuated the relaxation responses elicited by 8-Br-cAMP in both groups of rats ($n=4$ per group) (Fig. 4B).

3.4. Responses of aortic rings to isoproterenol as affected by inhibition of PKA

cAMP-induced vasodilation is mediated via a transduction cascade involving cAMP-dependent protein kinase (White et al., 2000) and this pathway may be affected in rats fed high salt. However, H-89 (8 μM), an inhibitor of PKA, attenuated the relaxation response to 8-Br-cAMP (100 μM), a PKA activator (52 ± 8%, $P<0.01$; $n=5$), but did not inhibit the response to isoproterenol in rats on normal or high salt diet. If anything, the responses were enhanced comparably in aortic rings from both groups of rats (Fig. 5).

4. Discussion

The present study demonstrates that high salt diet impaired β-adrenoceptor-mediated relaxation responses to isoproterenol, as well as relaxation to forskolin, and 8-Br-cAMP, but not sodium nitroprusside in the thoracic aorta of Sprague–Dawley rats. The impaired relaxation in the salt-loaded rats to isoproterenol appears to be exclusively linked to impairment of NO production inasmuch as L-NAME, the NO inhibitor, abolished the difference between rats on normal or high salt diet. In addition, L-NAME did not produce additional inhibition of isoproterenol relaxation in rats on high salt diet but inhibition of soluble guanylate cyclase with ODQ did.

Attenuation by L-NAME of isoproterenol relaxation in rats fed a normal diet suggests that NO production is a major component of the relaxant effect in the rat aorta and is consistent with some reports that demonstrated that isoproterenol relaxation is partly endothelium-dependent and is mediated by NO (Bradley et al., 2000; Toyoshima et al., 1998). However, this result is at variance with that reported in spontaneously hypertensive rat where high salt diet was shown not to affect the endothelial-NOS/NO pathway but impaired the guanylate cyclase/cGMP pathway (Kagota et al., 2001). The lack of a difference in sodium nitroprusside-induced relaxation between rats on normal or high salt diet and ODQ inhibition of isoproterenol relaxation in both groups of rats in the present study does not support an impairment of the cGMP pathway but demonstrates that NO relaxation is not always linked to activation of the cGMP pathway. Until recently, it has generally been assumed that the actions of NO are solely mediated via activation of soluble guanylate cyclase which increases the levels of cGMP. This conclusion is supported by the finding that endothelium-derived NO and NO donors increase cGMP levels in vascular tissues and that inhibitors of soluble guanylate cyclase attenuate the vasodilatory response to NO in many vessels (Martin et al., 1985; Wanstall et al., 2001). Further support for this hypothesis was provided by the observation that the effects of L-NAME on arterial pressure and renal function can be reversed by 8-Br-cGMP (Lahera et al., 1993). However, this scheme for NO-induced vasodilation has been questioned because in a variety of vascular tissues, NO acts via both cGMP-dependent and cGMP-independent mechanisms (Bolotina et al., 1994; Trottier et al., 1998). The abolition by L-NAME of the difference in isoproterenol relaxation between rats on normal or high salt diet, the failure of L-NAME to produce further inhibition of isoproterenol relaxation in rats on high salt diet, and the lack of difference in sodium nitroprusside relaxation between both groups of rats may at first suggest that the cGMP pathway is not involved in high salt-induced impairment of NO-induced relaxation in the rat aorta. However, considering that ODQ inhibition of the relaxation elicited by isoproterenol was of similar magnitude in rats on normal or high salt diet and that ODQ abolished the relaxation by sodium nitroprusside in both groups of rats invalidates such a notion. In view of the presence of residual NO as suggested by Cohen et al. (1997), our data imply that L-NAME alone may not completely inhibit NO synthesis. It is also possible that in the presence of high salt, NO or any other soluble guanylate cyclase activator, e.g. hydrogen peroxide or carbon monoxide, may contribute to L-NAME-insensitive relaxation.

The effects of salt loading on the cAMP system and the involvement of NO was evaluated next since increase in cAMP production by isoproterenol, the traditional mechanism for β-adrenoceptor-mediated responses in various tissues, was linked to NO production (Bradley et al., 2000; Toyoshima et al., 1998). Data from this study demonstrate

that salt loading indeed affects the cAMP system in as much as salt loading diminished relaxation induced by isoproterenol and forskolin, activators of adenyl cyclase, and 8-Br-cAMP, the cell permeable analog of cAMP and second messenger for isoproterenol. In rats on normal diet, inhibition of NO production attenuated the relaxation of the aorta produced by isoproterenol, 8-Br-cAMP, and forskolin. Similar observations were made by Toyoshima et al. (1998) in which they demonstrated that relaxant responses of the rat aorta to cAMP-elevating vasodilators are mediated, in part, by NO production. However, in rats on high salt diet, L-NAME attenuated only the relaxation by 8-Br-cAMP. The reason for this effect is not clear but may reflect differences in the mechanisms of action of exogenous and endogenously generated cAMP (Satake et al., 1996; Zhu et al., 2002). We speculate that under normal conditions, activation of the NOS is a common pathway linked to relaxation induced by cAMP coming from endogenous or exogenous sources. However, under conditions of high salt, it appears that there is a selective impairment of endogenous cAMP production via the adenylate cyclase pathway in the aorta thereby eliminating the coupling to stimulation of NO production in favour of alternative dilator pathways. As isoproterenol and forskolin activate potassium channels (Vedernikov et al., 2000; Zhu et al., 2002), it is possible that activation of potassium channels may be an alternative dilator mechanism in this study. This hypothesis is consistent with the demonstration that activation of potassium channels becomes a prominent dilator mechanism under pathological conditions (Nishikawa et al., 2000) as may be provided by salt loading in this study.

In order to demonstrate if high salt affected downstream events to cAMP, the effects of salt loading were evaluated with respect to the involvement of cAMP-dependent PKA. cAMP is known to phosphorylate cAMP-dependent proteins for downstream cellular signalling and a PKA mechanism has been linked to NO production (Cornwell et al., 1998). However, various studies have demonstrated that cAMP may produce its effects through PKA-dependent and -independent pathways (White et al., 2001; Decker et al., 2002). In the present study, H-89, an antagonist of PKA, tended to enhance the relaxant responses to isoproterenol but this did not reach statistically significant levels. This thus rules out a role for PKA in these responses and is in agreement with the demonstration of a PKA-independent mechanism in isoproterenol-induced relaxation in the rat mesenteric artery (White et al., 2001) or the ovine pulmonary vasculature (Gao and Raj, 2002). However, the observations that the effects of endogenous cAMP (isoproterenol and forskolin) were attenuated by inhibition of NO, but not by PKA inhibition, whereas the effects of exogenous cAMP (8-Br-cAMP) were attenuated by both inhibition of NO and PKA, suggest that differences exist in the signal transduction mechanisms associated with endogenous and exogenous cAMP. For example, iberiotoxin, an inhibitor of large conductance Ca^{2+}-activated potassium channels (BK_{Ca}), attenuated isoproterenol—but not dibutyryl cAMP-induced relaxation of the rat aorta, whereas aminopyridine, another BK_{Ca} inhibitor, attenuated the relaxation by both agents (Satake et al., 1996). The lack of activation by PKA by endogenous cAMP in this study is in agreement with other studies that demonstrated lack of PKA activation by activators of adenylate cyclase (Cornwell et al., 1998; White et al., 2001). Thus, PGE_2 elevation of cAMP and activation of BK_{Ca} channels in the human coronary artery was mimicked by forskolin and cAMP analogues but the effects of PGE_2 and forskolin were attenuated by inhibition of protein kinase G but not PKA (Zhu et al., 2002). Taken together, these data highlight the complexity of the signal transduction mechanisms involving cAMP and cGMP systems. Other studies have also suggested a complicated signaling process involving cAMP and the cGMP systems (Cornwell et al., 1998; White et al., 2000; Murthy, 2001). For example, "cross-activation" of the cGMP-dependent protein kinase by cAMP was suggested to be a key element in the signal transduction cascade of cAMP-induced vasodilation (White et al., 2000; Decker et al., 2002) and vice versa (Lincoln et al., 1990).

Increased blood pressure in rats on high salt diet in this study can of itself contribute to endothelial dysfunction and modulate vascular responses in response to high salt. Moreover, the loss of body weight and the resultant dehydration can also modulate vascular responses. However, the lack of a difference in sodium nitroprusside-induced relaxation between rats on normal or high salt diet rules out the possibility of salt-induced nonspecific effects or changes in hemodynamic parameters brought about by the increased vascular tone or the increase in blood pressure.

In conclusion, high salt diet fed to Sprague–Dawley rats resulted in reduced relaxation response to isoproterenol as well as other cAMP-elevating agents. The mechanisms involved in this attenuation include the suppression of NOS activity and attenuation of the adenylate cyclase/cyclic AMP pathway that is PKA-independent. This study highlights that complicated mechanisms are involved in salt loading induced changes in cAMP and cGMP systems and their effects on vascular reactivity.

Acknowledgements

This study was supported by the National Institutes of Health Grants HL59884 and UH1 HL03674. AOO is an Established Investigator of the American Heart Association (Award #0040119N). The facilities of the Research Center in the Minority Institution's program at Texas Southern University were used for these studies.

References

Adegunloye, B.J., Sofola, O.A., 1997. Effect of high dietary salt loading and high calcium diet on vascular smooth muscle responses and endothelial function in rats. Clin. Exp. Pharmacol. Physiol. 24, 814–818.

Bolotina, V.M., Najibi, S., Palacino, J.J., Pagano, P.J., Cohen, R.A., 1994.

Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. Nature 368, 850–853.

Bragulat, E., de la Sierra, A., Antonio, M.T., Coca, A., 2001. Endothelial dysfunction in salt-sensitive essential hypertension. Hypertension 37, 444–448.

Bradley, L., Shaw, A.M., MacDonald, A., 2000. Role of endothelium/nitric oxide in atypical B-adrenoceptor-mediated relaxation in rat isolated aorta. Eur. J. Pharmacol. 398, 285–296.

Cohen, R.A., Plane, F., Najibi, S., Huk, I., malinski, T., Garland, C.J., 1997. Nitric oxide is the mediator of both endothelium-dependent relaxation and hyperpolarization of the rabbit carotid artery. Proc. Natl. Acad. Sci. U. S. A. 94, 4193–4198.

Cornwell, T.L., Arnold, E., Boerth, N.J., Lincoln, T.M., 1998. Inhibition of smooth muscle cell growth by nitric oxide and activation of cAMP-dependent protein kinase by cGMP. Am. J. Physiol. 267, 1405–1413.

Decker, E.D., Heemskerk, J.W.M., Gorter, G., van der Vuurst, H., Donath, J., Kroner, C., Mikoshiba, K., Akkerman, J.N., 2002. Cyclic AMP raises intracellular calcium in human megakaryocytes independent of protein kinase A. Arterioscler. Thromb. Vasc. Biol. 22, 179–186.

Frisbee, J.C., Sylvester, F.A., Lombard, J.H., 2001. High salt diet impairs hypoxia-induced cAMP production and hyperpolarisation in rat skeletal muscle vessels. Am. J. Physiol. 281, H1808–H1815.

Gao, Y., Raj, J.U., 2002. Effects of SQ 22536, an adenyl cyclase inhibitor, on isoproterenol-induced cyclic AMP elevation and relaxation in newborn ovine pulmonary veins. Eur. J. Pharmacol. 436, 227–233.

Giardina, J.B., Green, G.M., Rinewalt, A.N., Granger, J.P., Khalil, R.A., 2001. Role of endothelin-β receptors in enhancing endothelium-dependent nitric oxide-mediated vascular relaxation during high salt diet. Hypertension 37, 516–523.

Hayakawa, H., Coffee, K., Raij, L., 1999. Endothelial dysfunction and cardiorenal injury in experimental salt-sensitive hypertension: effects of anti-hypertensive therapy. Circulation 96, 2407–2413.

Intersalt, 1988. An international study on electrolyte excretion and blood pressure. Results of 24 hour urinary sodium and potassium excretion. Br. Med. J. 237, 319–328.

Kagota, S., Tamashiro, A., Yamaguchi, Y., Suguira, R., Kuno, T., Nakamura, K., Kunitomo, M., 2001. Downregulation of vascular soluble guanylate cyclase by high salt intake in spontaneously hypertensive rats. Br. J. Pharmacol. 134, 737–744.

Lahera, V., Navarro, J., Biondi, M.L., Ruilope, L.M., Romero, J.C., 1993. Exogenous cGMP prevents decrease in diuresis and natriuresis induced by inhibition of NO synthesis. Am. J. Physiol. 264, F344–F347.

Lenda, D.M., Sauls, B.A., Boegehold, M.A., 2000. Reactive oxygen species may contribute to reduced endothelium-dependent dilation in rats fed a high salt diet. Am. J. Physiol. 279, H7–H14.

Lincoln, T.M., Cornwell, T.L., Taylor, A.E., 1990. cGMP-dependent protein kinase mediates the reduction of Ca^{2+} by cAMP in vascular smooth muscle cells. Am. J. Physiol. 258, C399–C407.

Mangiarua, E.I., Wright, G.L., McCumbee, W.D., 1989. Aortic cyclic AMP levels in hypertensive rats. Clin. Exp. Hypertens. 11, 75–87.

Martin, W., Villani, G.M., Jothianandan, D., Furchgott, R.F., 1985. Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. J. Pharmacol. Exp. Ther. 232, 708–716.

Miyajima, E., Bunag, R.D., 1985. Dietary salt loading produces baroreflex impairment and mild hypertension in rats. Am. J. Physiol. 249, H278–H284.

Murthy, K.S., 2001. cAMP inhibits IP_3-dependent Ca^{2+} release by preferential activation of cGMP-primed PKG. Am. J. Physiol. 281, G1238–G1245.

Nava, E., Luscher, T.F., 1995. Endothelium-derived vasoactive factors in hypertension: nitric oxide and endothelin. J. Hypertens. 13, S39–S48.

Nishida, Y., Ding, J., Zhou, M., Chen, Q., Murakami, H., Wu, X., Kosaka, H., 1998. Role of nitric oxide in vascular hyperesponsiveness to norepinephrine in hypertensive Dahl rats. J. Hypertens. 16, 1611–1618.

Nishikawa, Y., Stepp, D.W., Chilian, W.M., 2000. Nitric oxide exerts feedback inhibition on EDHF-induced coronary arteriolar dilation in vivo. Am. J. Physiol. 79, H459–H465.

Obiefuna, P.C.M., Ebeigbe, A.B., Sofola, O.A., Aloamaka, P.C., 1991a. Altered responses of aortic smooth muscle from Sprague Dawley rats with salt-induced hypertension. Clin. Exp. Pharmacol. Physiol. 18, 813–818.

Obiefuna, P.C.M., Sofola, O.A., Ebeigbe, A.B., 1991b. Dietary salt loading attenuates endothelium-dependent relaxation to histamine but not to acetylcholine. Exp. Physiol. 76, 135–138.

Sacks, F.M., Svetkey, L.P., Vollman, W.M., Appel, L.J., Bray, G.A., Harsha, D., Obarnanek, E., Conlin, P.R., Miller, E.R., Simons-Morton, D.G., Karanja, N., Lin, P.H., 2000. Effects on blood pressure of reduced dietary sodium and dietary approaches to stop hypertension (DASH) diet. N. Engl. J. Med. 344, 3–10.

Satake, N., Shibata, M., Shibata, S., 1996. The inhibitory effects of iberiotoxin and 4-aminopyridine on the relaxation induced by beta 1- and beta 2-adrenoceptor activation in rat aortic rings. Br. J. Pharmacol. 119, 505–510.

Sofola, O.A., Obiefuna, P.C.M., Adegunloye, B.J., 1993. Contractile responses of normotensive rat aorta to serum from salt loaded Sprague Dawley rats. Pflugers Arch., Eur. J. Physiol. 423, 161–163.

Stamler, J., Rose, G., Elliot, P., Dyer, A., Marmont, M., Kestelot, H., Stamler, R., 1991. Findings of the international co-operative intersalt study. Hypertension 17 (Suppl. I), I-9–I-15.

Toyoshima, H., Nasa, Y., Hashizume, Y., Koseki, Y., Isayama, Y., Kohaska, Y., Yamada, T., Takeo, S., 1998. Modulation of cAMP-mediated vasorelaxation by endothelial nitric oxide and basal cGMP in vascular snooth muscle cell. J. Cardiovasc. Pharmacol. 32, 543–551.

Trottier, G., Triggle, C.R., O'Neill, S.K., Loutzenhiser, R., 1998. Cyclic GMP-dependent and cyclic GMP-independent actions of nitric oxide on the renal afferent arteriole. Br. J. Pharmacol. 125, 563–569.

Vedernikov, Y.P., Syal, A.S., Okawa, T., Saade, G.R., Garfield, R.E., 2000. Adenylate cyclase and potassium channels are involved in forskolin- and 1,9-dideoxyforskolin-induced inhibition of pregnant uterus contractility. Am. J. Obstet. Gynecol. 182, 620–624.

Wanstall, J.C., Jeffrey, T.K., Gambino, A., Lovren, F., Triggle, C.R., 2001. Vascular smooth muscle relaxation mediated by nitric oxide donors: a comparison with acetylcholine, nitric oxide and nitroxyl ion. Br. J. Pharmacol. 134, 463–472.

White, R.E., Kryman, J.P., El-Mowafy, A.M., Han, G., Carrier, G.O., 2000. cAMP-dependent vasodilators cross-activate the cGMP-dependent protein kinase to stimulate BK(Ca) channel activity in coronary artery smooth muscle cells. Circ. Res. 86, 897–905.

White, R., Bottrill, F.E., Siau, D., Hiley, C.R., 2001. Protein kinase A-dependent and -independent effects of isoproterenol in rat isolated mesenteric artery: interactions with levcromakalim. J. Pharmacol. Exp. Ther. 298, 917–923.

Wu, X., Totvanen, J., Hutri-Kahonen, N., Kahonen, M., Makyen, J., Korpella, R., Ruskoaho, H., Karjala, K., Porsti, I., 1998. Comparison of the effect of supplementation with whey mineral and potassium on arterial tone in experimental hypertension. Cardiovasc. Res. 40, 364–374.

Zhu, S., Han, G., White, R.E., 2002. PGE_2 action in human coronary artery smooth muscle: role of potassium channels and signalling cross-talk. J. Vasc. Res. 39, 477–488.