ELSEVIER

European Journal of Pharmacology 434 (2002) 133–139

ejp

www.elsevier.com/locate/ejphar

Severe impairment of CGRP-induced hypotension in vivo and vasorelaxation in vitro in elderly rats

Gabriel H.H. Chan, Ronald R. Fiscus*

Department of Physiology, Faculty of Medicine, Epithelial Cell Biology Research Center, BMSB room 507, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China

The Center for Gerontology and Geriatrics, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China

Received 11 June 2001; received in revised form 31 October 2001; accepted 20 November 2001

Abstract

The aim of this study was to investigate the effects of aging on hypotension in vivo and vasorelaxation in vitro induced by calcitonin gene-related peptide (CGRP), using young (3 months old) and elderly (20 and 28 months old) Sprague–Dawley rats. Vasorelaxant responses were measured in isolated rings of rat thoracic aorta and rat caudal artery, which show endothelium-dependent and endothelium-independent responses to CGRP, respectively. The CGRP-induced vasorelaxations were significantly diminished in 28-month-old male rats in both aorta (39.3% of responses in young controls at 10 nM CGRP) and caudal artery (28.5% of responses in young controls at 10 nM CGRP). Acetylcholine caused vasorelaxations in aortic rings of young male rats, but vasocontractions in aortic rings of 28-month-old male rats. Hypotension induced by CGRP was significantly diminished in both 20-month-old male rats (47.7% of young controls) and 20-month-old female rats (34.4% of young controls). Moreover, ovariectomy, known to decrease CGRP-induced hypotension in young female rats, did not further decrease hypotension to CGRP in elderly female rats. In conclusion, vasorelaxant responses in vitro and hypotensive responses in vivo induced by the neuropeptide CGRP are severely impaired in elderly rats as compared to young rats. The data suggest that the vasodilatory responses to CGRP in both large arteries and the small resistance-sized arteries regulating arterial blood pressure are damaged or down-regulated by the aging process. © 2002 Published by Elsevier Science B.V.

Keywords: CGRP (calcitonin gene-related peptide); Blood pressure; Gender; Aging; Ovariectomy; Artery

1. Introduction

CGRP, a 37-amino acid neuropeptide discovered in 1983 from alternate encoding of the calcitonin gene (Rosenfeld et al., 1983), is one of the most potent endogenous vasodilators known (Wimalawansa, 1996). The peptide is thought to be involved in the regulation of peripheral vascular tone and regional organ blood flows in normal physiological conditions through endothelium-dependent or endothelium-independent mechanisms, depending on the type of blood vessel (Brain et al., 1985; Edvinsson et al., 1985; Shoji et al., 1987; Thom et al., 1987; for a review, see Fiscus, 1988). The endothelium-dependent vasorelaxant responses to CGRP in rat aorta are associated with activation of both cAMP and cGMP signal transduction pathways (Fiscus, 1988; Fiscus et al., 1991; Wang et al., 1991; Gray and Marshall, 1992). Both cAMP and cGMP responses, as well as vasorelaxations, to CGRP are dependent on the release of nitric oxide (NO) from the endothelium (Fiscus et al., 1991; Gray and Marshall, 1992). Furthermore, CGRP acts synergistically with NO (Fiscus et al., 1994) as well as with other agents that elevated cGMP levels (e.g. brain natriuretic peptide (Fiscus et al., 1998; Lu and Fiscus, 1999) to cause vasorelaxations in aortic rings and elevations of cAMP levels in aortic smooth muscle cells.

CGRP is thought to inhibit the development of atherosclerosis by inhibiting the proliferation of vascular smooth muscle cells (Li et al., 1997). Interestingly, this anti-proliferative effect of CGRP is also synergistically enhanced by NO (Wang et al., 1999). Also, CGRP is likely involved in blood pressure regulation, because gene deletion of CGRP results in hypertension (Gangula et al., 2000). Thus, loss of CGRP-induced responses in blood vessels, as for example during aging, may result in increased risks of developing atherosclerosis and hypertension.

* Corresponding author. Department of Physiology, Faculty of Medicine, BMSB Room 507, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong. Tel.: +852-2609-6780; fax: +852-2603-5022.

E-mail address: ronfiscus@cuhk.edu.hk (R.R. Fiscus).

0014-2999/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0014-2999(01)01545-X

Aging has been reported to impair vasodilation induced by the β-adrenoceptor agonist isoproterenol (Docherty, 1990). However, vasodilatory responses to endothelial-dependent agents, such as acetylcholine, have been reported to increase, decrease or not change with aging (Docherty, 1990; Mayhan et al., 1990; Vargas et al., 1997). Recent reports have shown a decrease in acetylcholine-induced vasodilations in aorta of old versus young rats, despite an increase in the protein levels and activity of the endothelial form of nitric oxide synthase (eNOS) in the aortic endothelial cells of the old rats (Matz et al., 2000; Van der Loo et al., 2000). The diminished vasorelaxation response to acetylcholine in aorta of older rats was attributed to enhanced production of superoxide, which combines with NO to form peroxynitrite and thus diminishes the vasorelaxant effects of the endothelium-derived NO (Van der Loo et al., 2000).

Slight decreases in the vasodilatory responses to CGRP have been reported for isolated mesenteric arterial beds of 7.5-month-old Wistar–Kyoto (WKY) rats as compared to 2-month-old WKY rats (Kawasaki et al., 1990, 1991). A larger decrease in the CGRP-induced vasodilations was observed in isolated mesenteric arterial beds of 18-month-old WKY rats (Amerini et al., 1994). However, another report, using isolated mesenteric arterial beds of male Fisher 344 rats, found no change with aging in the CGRP-induced vasodilations (Li and Duckles, 1993). In addition, a decrease in vasorelaxant responses to CGRP has been reported in isolated coronary arteries (Corr et al., 1991) and isolated basilar arteries (Brizzolara et al., 1994) of 12-month-old female rabbits as compared to 4-month-old female rabbits. However, in isolated coronary arteries from humans of different ages, no changes have been found in CGRP-induced vasorelaxations (Opgaard et al., 2000). Thus, aging-related changes in CGRP-induced vasorelaxations are not consistently observed, possibly reflecting difference in the type of blood vessels (and whether these vessels respond to CGRP in an endothelium-dependent or endothelium-independent manner) or species/strain used for the aging studies. Furthermore, no previous report has shown if the aging process causes changes in CGRP-induced hypotensive responses in vivo, which would involve the effects of CGRP on the small resistance-sized arteries/arterioles regulating arterial blood pressure.

The present study determines if the hypotensive responses to CGRP, given in a wide range of doses, are altered in elderly (20 months old) male and female rats, as compared to young (3 months old) controls. Furthermore, the present study determines if isolated vascular rings of male rats of more advancing age (28 months old) have altered vasorelaxant responses to CGRP. Rat aortic rings, which respond to CGRP in an endothelium-dependent manner (Brain et al., 1985), and rat caudal arterial rings, which respond to CGRP in an endothelium-independent manner (Fiscus et al., 1992), were used. For comparison, we also determined if acetylcholine-induced vasorelaxations are altered in the same aortic rings. Because ovariectomy in young female rats causes diminished hypotensive responses to CGRP in vivo (unpublished data from our laboratory), we determined if ovariectomy causes further loss of CGRP-induced hypotension in the elderly female rats.

2. Methods

2.1. Tissue preparation of the vascular rings

Studies were performed in healthy young (3 months old) and old (28 months old) Sprague–Dawley male rats. Female rats were not included, because mammary sarcomas were found in most of the 28-month-old female rats. The male rats were killed by cervical dislocation and their thoracic aortae and caudal arteries were removed and placed in modified Krebs–Ringer–Bicarbonate (KRB) solution containing 118.5 mM NaCl, 4.74 mM KCl, 1.18 mM $MgSO_4$, 1.18 mM KH_2PO_4, 2.5 mM $CaCl_2$, 24.9 mM $NaHCO_3$, 10 mM glucose, and 0.03 mM EDTA, and aerated with 95% O_2 plus 5% CO_2. The aortae were cut into rings of 3 mm long after trimming off the adhering connective tissue and fat. Extreme care was used in handling the vascular rings in order to avoid damage to the endothelium. The rings were carefully put on two parallel stainless steel rods and were then suspended in organ baths containing 5 ml of KRB bubbled with 95% O_2 plus 5% CO_2 at 37 °C and were set under a resting tension of 1 g, an optimal preload used in our previous studies (Fiscus et al., 1991, 1994, 1998). The KRB was replaced every 10 min during a 30-min equilibration period. Vasorelaxant responses were measured using force transducers (Grass, Model FT-03) and recorded on a PowerLab system (ADInstruments, PowerLab 8sp).

2.2. Vasorelaxation studies in vitro

The aortic and caudal arterial rings were equilibrated over 30 min with replacement of the KRB solution with fresh, bubbled KRB solution every 10 min. Complete concentration–response curves for phenylephrine-induced contractions were conducted on each vascular ring and the concentration of phenylephrine that gave 50% of maximum contraction was determined to be 1×10^{-7} M in both aortic and caudal arterial rings. Phenylephrine at 1×10^{-7} M was then used for all subsequent experiments to induce stable precontractions prior to the addition of either acetylcholine or CGRP. The contractile response to phenylephrine in the young and elderly vascular rings were not significantly different for both aortic rings and caudal arterial rings (e.g. maximum response to phenylephrine was 2.0 ± 0.3 g for aortic rings of young rats and 1.8 ± 0.3 g for aortic rings of elderly rats). CGRP was added in a cumulative manner to the aortic rings in a range from 1×10^{-10} to 1×10^{-7} M and to caudal arterial rings in a range from 1×10^{-10} to 3×10^{-7} M. Each incremental concentration of CGRP was added after the response to the previous concentration had reached a stable plateau.

After the cumulative additions of CGRP, the aortic rings were washed with fresh, bubbled KRB solution every 10 min over a 30-min recovery period. The aortic rings were precontracted with phenylephrine (1×10^{-7} M), and after reaching a stable contraction, acetylcholine (1×10^{-10} to 3×10^{-4} M) was added in a cumulative manner.

2.3. Animal preparation for in vivo studies

The experiments were conducted with healthy 3-month-old and 20-month-old (female and male) Sprague–Dawley rats. Female rats were included in this part of the study, because, unlike in the 28-month-old female rats described above, there was no evidence of mammary sarcoma in the 20-month-old female rats. Some of the female animals were ovariectomized bilaterally under ketamine/xylazine (90/10 mg/kg, i.p.) anesthesia when they were 4 months old. Each group consisted of at least five rats. The rats were housed in 12:12 hour light/dark cycle and were fed with standard chow with water to drink ad libitum.

The rats were anaesthetized with urethane/alpha-chloralose (1000/100 mg/kg, i.p.) and were kept under complete anesthesia throughout the experiment, which included the surgical procedure for cannulation, the 30-min recovery period and the measurement of arterial blood pressure following administration of CGRP. Cannulas filled with sterile heparinized saline (100 U/ml) were inserted into the left carotid artery and the right jugular vein. The rats were allowed to recover from the surgical procedure for 30 min before the measurement of blood pressure. The blood pressure was mea sured from the cannula in the carotid artery using a pressure transducer connecting to a Grass Polygraph recorder (Model 7), similar to previous study in our laboratory (Wang et al., 1992).

2.4. Measurement of hypotensive responses to CGRP

The initial mean arterial pressures (MAPs) were not significantly different in the different groups of rats. The initial MAPs were: 74.3 ± 12.5 for young female rats, 64.6 ± 14.4 for young male rats, 58.0 ± 3.8 for elderly female rats and 63.2 ± 6.8 for elderly male rats. CGRP, dissolved in sterile saline with 0.1% BSA, was administered as a bolus injection through the cannula in the left jugular vein. The doses of the CGRP injections were started at 0.001 nmol/kg and were increased by half log doses. The vehicle (i.e. sterile saline plus 0.1% BSA) had no effect on arterial blood pressure in any of the animals. The arterial blood pressures in all rats were continuously monitored for 10 min after each dose of CGRP, and then the next higher dose of CGRP was administered. Peak responses to CGRP were recorded at 2 min after CGRP injection. We also conducted time controls, in which the vehicle was injected seven times at 10-min intervals (identical to the CGRP-treated group except without CGRP in the vehicle). The blood pressures remained stable throughout this entire period of time in the time controls.

2.5. Drugs

Acetylcholine, alpha-chloralose, phenylephrine, urethane, bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). The rat form of alpha-CGRP was purchased from Phoenix Pharmaceuticals (Mountain View, CA, USA). The ketamine/xylazine was obtained from our Laboratory Animal Service Center.

2.6. Statistical analyses

All values are expressed as mean ± standard error of mean (SEM). The significant differences between the young and aged groups in the isolated tissue experiments were analyzed using unpaired *t*-test. Graphpad Prism (GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego, CA, USA, http://www.graphpad.com) was used as the statistical package for the *t*-tests as well as for generating the graphs for both in vitro and in vivo studies. Significant differences between the hypotensive effects of CGRP in the elderly group of rats and the young controls were analyzed using two-way ANOVA with one factor repeated measures and preplanned contrasts (Super ANOVA, v1.11, Macintosh). A P value of less than 0.05 was considered to be significant. The mean values were obtained from at least five animals per treatment group.

2.7. Animal statement

The treatment of laboratory animals and the experimental protocols of the study adhered to guidelines of The Chinese University of Hong Kong and were approved by the Animal Research Ethics Committee of The Chinese University of Hong Kong.

3. Results

3.1. Effect of aging on CGRP-induced vasorelaxations in rings of thoracic aorta and caudal artery

In endothelium-intact aortic rings of 28-month-old male rats, there was a dramatic reduction of the CGRP-induced vasorelaxations at all concentrations of CGRP, as compared to young controls. Fig. 1 (Panel A) shows that CGRP-induced vasorelaxations in aortic rings were significantly less in the elderly rats as compared to the young ones (e.g. at 10 nM CGRP, relaxant responses in elderly aorta were only 39.3% of responses in young aorta).

In caudal arterial rings, there was also a dramatic reduction of the CGRP-induced vasorelaxations in the elderly group as compared with the young group. Fig. 1 (Panel B) shows that caudal arterial rings from elderly rats have only 28.5% of the relaxant response of young controls following the addition of 10 nM CGRP.

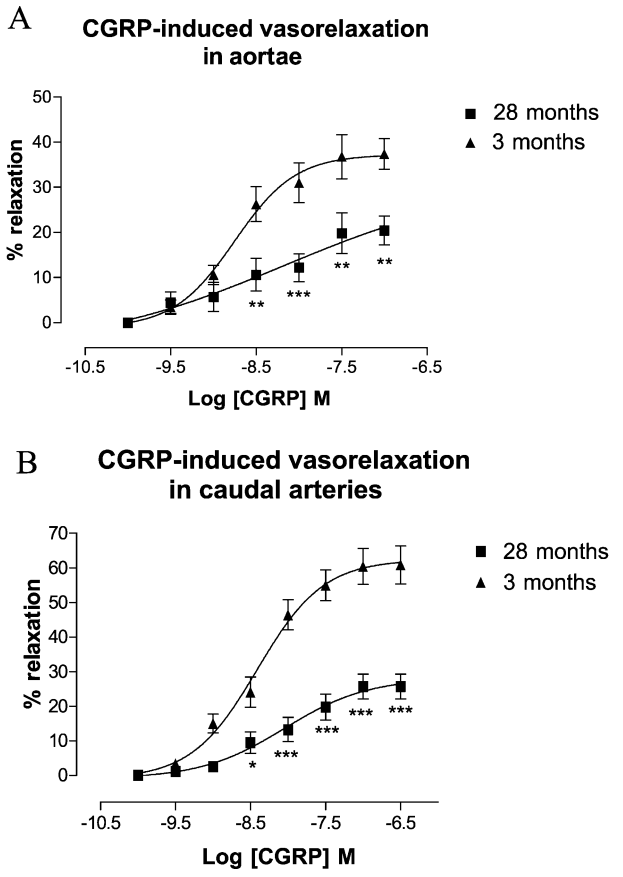

Fig. 1. Concentration–response relationship of CGRP-induced vasorelaxations in rat isolated aortic rings (Panel A) and caudal arterial rings (Panel B) from 3-month-old and 28-month-old male rats. The aortic and caudal arterial rings were precontracted with phenylephrine (1×10^{-7} M) and CGRP was added at increasing concentrations from 1×10^{-10} to 1×10^{-7} M (aorta) or 3×10^{-7} M (caudal artery). The data represent the mean ± SEM of responses in six aortic rings and six caudal arteries taken from six different rats. Responses in rings from elderly (28 months old) male rats were significantly less than in rings from young (3 months old) male rats. $^{**}P<0.01$, $^{***}P<0.001$.

3.2. The effect of age on acetylcholine-induced responses in aortic rings

Fig. 2 shows the responses induced by acetylcholine (1×10^{-10} to 3×10^{-4} M) in the aortic rings of 3-month-old and 28-month-old male rats. Acetylcholine (1×10^{-9} to 1×10^{-7} M) caused concentration-dependent relaxations in aortic rings from young rats. No relaxations were observed in the aortic rings from old rats. Instead, the aortic rings of the old rats contracted in response to acetylcholine at the higher concentrations, starting at 3×10^{-7} M.

3.3. CGRP-induced hypotension in young female and male rats

Fig. 3 (Panel A) shows the complete dose–response curves of CGRP-induced hypotension in young female and male rats. There were no significant differences between CGRP-induced hypotensive responses in the two groups.

3.4. CGRP-induced hypotension in elderly female and male rats

The CGRP-induced hypotensive responses in aged female rats were significantly ($P<0.001$) smaller than those in young female rats (Fig. 3, Panel B). The hypotensive responses to CGRP, at a dose of 1 nmol/kg, in the elderly female rats were reduced to 34.4% of responses in the young control female rats. There was also a significant ($P<0.001$) decrease in the CGRP-induced hypotensive responses in elderly male rats compared to young male rats (Fig. 3, Panel C). The hypotensive responses to CGRP (1 nmol/kg) were reduced to 47.7% of responses in the young control male rats. The degree of this aging-related impairment of CGRP-induced hypotension appeared to be very similar in female and male rats (compare Panels B and C in Fig. 3). Thus, there was no evidence of a gender difference in the damaging effects of aging on the vascular responses to CGRP.

3.5. CGRP-induced hypotension in elderly female rats with ovariectomy

The 20-month-old female rats that had been ovariectomized at 4 months of age showed the same level of impaired hypotensive responses to CGRP as the non-ovariectomized 20-month-old female rats (data not shown). Thus, removal of the ovaries at 4 months did not cause further decrease in the hypotensive responses to CGRP measured in the 20-month-old rats.

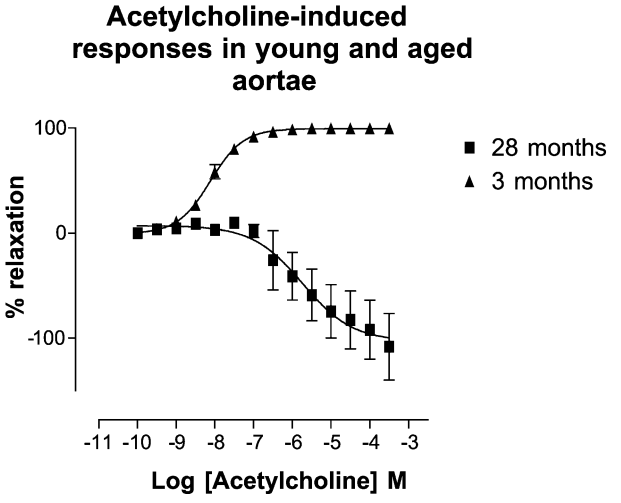

Fig. 2. Concentration–response relationship of acetylcholine-induced responses in endothelium-intact aortic rings of rats of 3 and 28 months of age. The aortic rings were precontracted with phenylephrine (1×10^{-7} M). Acetylcholine caused vasorelaxations in the rings of the young (3 months old) male rats, but vasoconstrictions in the elderly (28 months old) male rats. The data represent the mean ± SEM of responses in six aortic rings taken from six different rats.

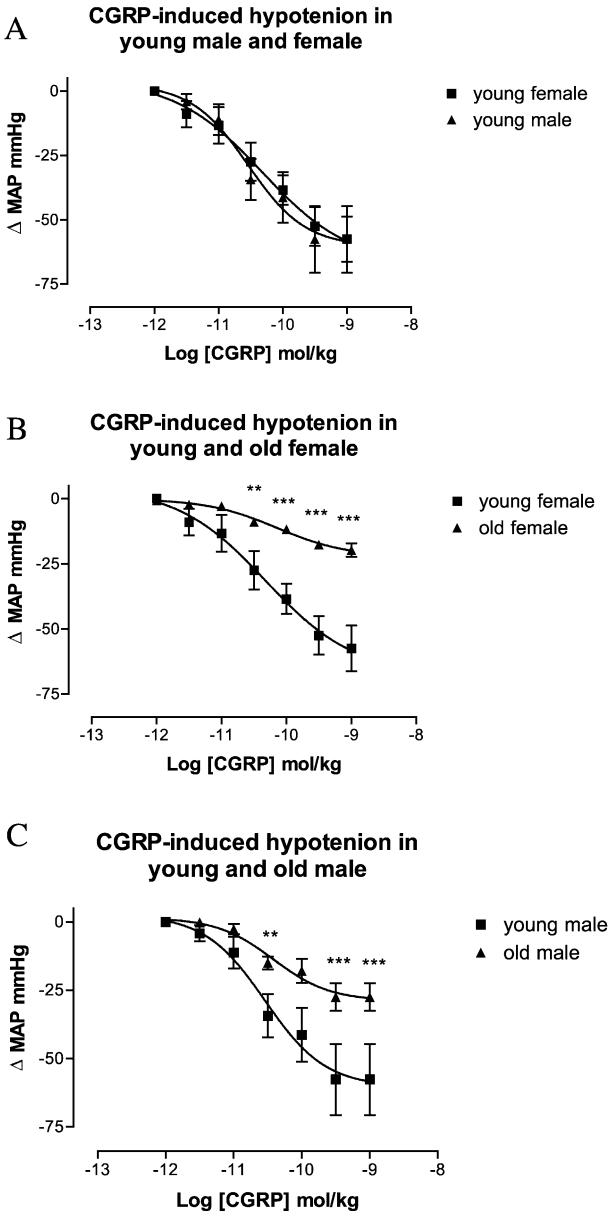

Fig. 3. (Panel A) Comparison of CGRP-induced hypotensive responses in young female and male rats. There were no significance differences between the responses in the two groups. The data represent the mean ± SEM of responses in five to seven different rats. (Panel B) Comparison of CGRP-induced hypotensive responses in 3-month-old (young) and 20-month-old (old) female rats. The responses in the elderly female rats were severely impaired compared to young female rats. Hypotension induced by CGRP at the highest dose (1 nmol/kg) in the elderly female rats was only 34.4% of the control response in young female rats. The data represent the mean ± SEM of responses in five to seven different rats. $**P<0.001$, $***P<0.001$. (Panel C) Comparison of CGRP-induced hypotensive responses in 3-month-old (young) and 20-month-old (old) male rats. The responses in the elderly male rats were severely impaired compared to young male rats. Hypotension induced by CGRP at the highest dose (1 nmol/kg) in the elderly male rats was only 47.7% of the control response in young male rats. The data represent the mean ± SEM of responses in five to seven different rats. $**P<0.01$, $***P<0.001$.

4. Discussion

In the present study, we determined if the CGRP-induced vasorelaxations of arteries in vitro and the CGRP-induced hypotensive responses in vivo are altered in elderly rats as compared to young rats. In addition, we determined if the aging process effects CGRP-induced hypotension differently in female and male rats and if long-term ovariectomy diminishes the hypotensive response to CGRP in elderly female rats. The present study shows that the vasorelaxant responses to CGRP are severely impaired in isolated aortic rings and caudal arteries of 28-month-old male rats as compared to 3-month-old male rats. From the in vivo studies, we found severe impairment of the CGRP-induced hypotension in 20-month-old rats of both genders, but this impairment of the hypotension was not further diminished by the combination of old age and ovariectomy in the female rats.

Nitric oxide (NO), released from the endothelial cells, exerts vasorelaxant effects in almost all types of arteries (Vanhoutte, 2000). Blocking this effect of NO in arterial rings in vitro is known to cause loss of vasorelaxations induced by many different endothelium-dependent vasodilators (Fiscus, 1988; Vanhoutte, 2000), including CGRP in rat aortic rings (Fiscus et al., 1991; Gray and Marshall, 1992). Blocking vasorelaxant effects of NO in vivo causes hypertensive responses (Takahashi et al., 1995), indicating the importance of NO in blood pressure regulation. In general, the level of NO released from endothelial cells is thought to decrease with aging (Tschudi et al., 1996; Vanhoutte, 2000). However, the endothelium- and NO-dependent vasorelaxant responses to acetylcholine are reported to decrease, not change or even increase in arteries of aged animals (Docherty, 1990; Mayhan et al., 1990). Thus, it is not clear if there is a consistent loss of endothelium/NO-dependent vasodilation during the aging process. Our results show that acetylcholine causes vasorelaxations in aortic rings of young rats but vasocontractions in aortic rings of elderly 28-month-old rats, indicating that there was severe depression of the NO pathway in the aortic rings of the elderly animals. This loss of NO lead to an unmasking of the direct vasocontractile actions of acetylcholine in aortic rings of elderly Sprague–Dawley rats of the present study. Similar age-related contractions to acetylcholine have been reported previously for isolated aorta of elderly Wistar–Kyoto rats (Koga et al., 1989). A clear decrease in the endothelial production of NO in response to acetylcholine has been documented in two recently published studies using rat aorta (Matz et al., 2000; Van der Loo et al., 2000).

The decrease in NO production with aging may also diminish the CGRP-induced vasorelaxant responses, which are known to be synergistically enhanced by NO in aortic rings (Fiscus et al, 1994) and cultured vascular smooth muscle cells (Lu and Fiscus, 1999). The results of the present study indicate that there is a significant and severe impairment of the vasorelaxant responses to CGRP in vitro and of the hypotensive responses to CGRP in vivo in the elderly rats, as compared to the young controls. This impairment may result,

in part, from the aging-related decrease in NO production. However, the present study also shows that caudal arterial rings, which respond to CGRP independent of endothelium-derived NO (Fiscus et al., 1992), also showed similar impairment of the CGRP-induced vasorelaxation. Thus, aging appears to also damage other pathways (independent of NO) that are responsible for the CGRP-induced vasorelaxations.

Aging has been reported to cause a decrease in CGRP-induced vasorelaxations in isolated basilar arteries of female rabbits but not male rabbits (Brizzolara et al., 1994). The gender difference between the two aged groups suggested that CGRP-induced responses may be influenced by sex steroid hormones during aging. In female rats, ''senile deviations of the estrous cycle'' appear, characterized by spontaneous repetitive pseudopregnancies, permanent diestrus, with decreases in estrogen levels during aging, or constant estrus (Lefevre and McClintock, 1988; Okatani et al., 1999). However, other studies have reported that estrogen levels remain unchanged during aging in female rats (Tschudi et al., 1996; Wight et al., 2000). Thus, it remains unclear whether changes in estrogen levels have contributed to the changes in CGRP-induced responses in the elderly female rats. In the present study, we found that both male and female elderly rats had similar impairment of the CGRP-induced hypotension, suggesting that aging-related changes other than decreases in estrogen levels are likely responsible for the major part of the impairment.

The elevation of blood glucose levels during diabetes mellitus is known to cause glycosylation of proteins (e.g. collagen and receptors) in the wall of arteries, leading to damage and loss of vascular responses (Fiscus and Ming, 2000). Even normal levels of blood glucose are thought to cause similar glycosylation of proteins, but at a slower rate. Over time, this can lead to changes in receptor function and subsequent impairment of vascular responses. Thus, in addition to decreased release of NO from the endothelium, the glycoslyation of CGRP receptors or other proteins involved in the signal transduction pathway of CGRP-induced vasodilation may be responsible, in part, for the severe aging-related impairment of the CGRP-induced vasorelaxant and hypotensive responses. Further studies are needed to determine the exact cause of the decreases in the vascular responses to CGRP during aging.

In conclusion, the results of the present study indicate that both endothelium-dependent relaxations in aortic rings and endothelium-independent relaxations in caudal arterial rings induced by CGRP are greatly decreased in 28-month-old male rats as compared to 3-month-old male rats. Furthermore, CGRP-induced hypotensive responses in vivo are also severely impaired in 20-month-old rats, both female and male, as compared to young controls. Thus, aging appears to damage or down-regulate the vascular responses to CGRP in both large arteries and small resistance-sized arteries/arterioles regulating arterial blood pressure. Ovariectomy at 4 months of age in the female rats did not cause further impairment of the hypotensive responses to CGRP in the elderly female rats.

Acknowledgements

This work was supported by a Direct Grant for Research from the Research Grants Council of Hong Kong awarded to R.R. Fiscus and by departmental funding for partial research support of graduate students in the Department of Physiology, Faculty of Medicine, The Chinese University of Hong Kong, to G.H.H. Chan. We would like to give special thanks to Dr. Anthony James, Director of the Laboratory Animal Services Center at The Chinese University of Hong Kong, and the other staff of this center for their helpful technical assistance.

References

Amerini, S., Mantelli, L., Filippi, S., Ledda, F., 1994. Effects of aging and hypertension on vasorelaxant activity of calcitonin gene-related peptide: a comparison with other vasodilator agents. J. Cardiovasc. Pharmacol. 23, 432–437.

Brain, S.D., Williams, T.J., Tippins, J.R., Morris, H.R., MacIntyre, I., 1985. Calcitonin gene-related peptide is a potent vasodilator. Nature 313, 54–56.

Brizzolara, A.L., Stewart-Lee, A., Burnstock, G., 1994. Responses of rabbit basilar arteries to vasoconstrictor and vasodilator agents: the effects of atherosclerosis, age and sex. J. Vasc. Res. 31, 106–113.

Corr, L.A., Poole-Wilson, P., Burnstock, G., 1991. Responses of coronary arteries to neurotransmitters: changes with sexual maturity in the female rabbit. J. Cardiovasc. Pharmacol. 18, 144–150.

Docherty, J.R., 1990. Cardiovascular responses in ageing: a review. Pharmacol. Rev. 42, 103–125.

Edvinsson, L., Fredholm, B.B., Hamel, E., Jansen, I., Verrecchia, C., 1985. Perivascular peptides relax cerebral arteries concomitant with stimulation of cyclic adenosine monophosphate accumulation or release of an endothelium-derived relaxing factor in the cat. Neurosci. Lett. 58, 213–217.

Fiscus, R.R., 1988. Molecular mechanisms of endothelium-mediated vasodilation. Sem. Thromb. Hemostasis 14, 12–22 Suppl.

Fiscus, R.R., Ming, S.K., 2000. Biology of ageing. In: Pang, W.S., Teoh, M.K., Ming, S.K. Medicine and Surgery in the Elderly Person. Armour Publishing, Singapore, pp. 29–42.

Fiscus, R.R., Zhou, H.L., Wang, X., Han, C., Ali, S., Joyce, C.D., Murad, F., 1991. Calcitonin gene-related peptide (CGRP)-induced cyclic AMP, cyclic GMP and vasorelaxant responses in rat thoracic aorta are antagonized by blockers of endothelium-derived relaxant factor (EDRF). Neuropeptides 20, 133–143.

Fiscus, R.R., Wang, X., Hao, H., 1992. hCGRP8-37 antagonizes vasodilations and cAMP responses to rat CGRP in rat caudal artery. Ann. N.Y. Acad. Sci. 657, 513–515.

Fiscus, R.R., Hao, H., Wang, X., Arden, W.A., Diana, J.N., 1994. Nitroglycerin (exogenous nitric oxide) substitutes for endothelium-derived nitric oxide in potentiating vasorelaxations and cyclic AMP elevations induced by calcitonin gene-related peptide (CGRP) in rat aorta. Neuropeptides 26, 133–144.

Fiscus, R.R., Lu, L., Tu, A.W.K., Hao, H., Yang, L., Wang, X., 1998. Brain natriuretic peptide enhances the endothelium-independent cAMP and vasorelaxant responses of calcitonin gene-related peptide in rat aorta. Neuropeptides 32, 499–509.

Gangula, P.R., Zhao, H., Supowit, S.C., Wimalawansa, S.J., Dipette, D., Westlund, K.N., Gagel, R.F., Yallampalli, C., 2000. Increased blood pressure in alpha-calcitonin gene-related peptide/calcitonin gene knockout mice. Hypertension 35, 470–475.

Gray, D.W., Marshall, I., 1992. Human alpha-calcitonin gene-related peptide stimulates adenylate cyclase and guanylate cyclase and relaxes rat thoracic aorta by releasing nitric oxide. Br. J. Pharmacol. 107, 691–696.

Kawasaki, H., Saito, A., Takasaki, K., 1990. Age-related decrease of calcitonin gene-related peptide-containing vasodilator innervation in the mesenteric resistance vessel of the spontaneously hypertensive rat. Circ. Res. 67, 733–743.

Kawasaki, H., Saito, A., Goto, K., Takasaki, K., 1991. Age-related changes in calcitonin gene-related peptide (CGRP)-mediated neurogenic vasodilation of the mesenteric resistance vessel in SHR. Clin. Exp. Hypertens., Part A 13, 745–754.

Koga, T., Takata, Y., Kobayashi, K., Takishita, S., Yamashita, Y., Fujishima, M., 1989. Age and hypertension promote endothelium-dependent contractions to acetylcholine in the aorta of the rat. Hypertension 14, 542–548.

Lefevre, J., McClintock, M.K., 1988. Reproductive senescence in female rats: a longitudinal study of individual differences in estrous cycles and behavior. Biol. Reprod. 38, 780–789.

Li, Y., Duckles, S.P., 1993. Effect of age on vascular content of calcitonin gene-related peptide and mesenteric vasodilator nerve activity in the rat. Eur. J. Pharmacol. 236, 373–378.

Li, Y., Fiscus, R.R., Wu, J., Yang, L., Wang, X., 1997. The antiproliferative effects of calcitonin gene-related peptide in different passages of cultured vascular smooth muscle cells. Neuropeptides 31, 503–509.

Lu, L.F., Fiscus, R.R., 1999. Nitric oxide donors enhance calcitonin gene-related peptide-induced elevations of cyclic AMP in vascular smooth muscle cells. Eur. J. Pharmacol. 376, 307–314.

Matz, R.L., de Sotomayor, M.A., Schott, C., Stoclet, J.C., Andriantsitohaina, R., 2000. Vascular bed heterogeneity in age-related endothelial dysfunction with respect to NO and eicosanoids. Br. J. Pharmcol. 131, 303–311.

Mayhan, W.G., Faraci, F.M., Baumbach, G.L., Heistad, D.D., 1990. Effects of aging on responses of cerebral arterioles. Am. J. Physiol. 258, H1138–H1143.

Okatani, Y., Morioka, N., Hayashi, K., 1999. Changes in nocturnal pineal Melatonin synthesis during the perimenopausal period: relation to estrogen levels in female rats. J. Pineal. Res. 27, 65–72.

Opgaard, O.S., Ytterberg, H.T., Saxena, P.R., Edvinsson, L., 2000. Influence of ageing on vasomotor responses of human epicardial coronary arteries. Pharmacol. Toxicol. 86, 183–191.

Rosenfeld, M.G., Mermod, J.J., Amara, S.G., Swanson, L.W., Sawehendo, P.E., River, J., Vale, W.W., Evans, R.M., 1983. Production of a novel neuropeptide encoded by calcitonin gene via tissue specific RNA processing. Nature 304, 129–135.

Shoji, T., Ishihara, H., Ishikawa, T., Saito, A., Goto, K., 1987. Vasodilating effects of human and rat calcitonin gene-related peptides in isolated porcine coronary arteries. Naunyn-Schmiedeberg's Arch. Pharmacol. 336, 438–444.

Takahashi, H., Hara, K., Komiyama, Y., Masuda, M., Murakami, T., Nishimura, M., Nambu, A., Yoshimura, M., 1995. Mechanism of hypertension induced by chronic inhibition of nitric oxide in rats. Hypertens. Res. 18, 319–324.

Thom, S.M., Hughes, A.D., Goldberg, P., Martin, G., Schachter, M., Sever, P.S., 1987. The actions of calcitonin gene-related peptide and vasoactive intestinal peptide as vasodilators in man in vivo and in vitro. Br. J. Clin. Pharmacol. 24, 139–144.

Tschudi, M.R., Barton, M., Bersinger, N.A., Moreau, P., Cosentino, F., Noll, G., Malinski, T., Luscher, T.F., 1996. Effect of age on kinetics of nitric oxide release in rat aorta and pulmonary artery. J. Clin. Invest. 98, 899–905.

Van der Loo, B., Labugger, R., Skepper, J.N., Bachschmid, M., Powell, J.M., Palacios-Callender, M., Erusalimsky, J.D., Quaschning, T., Malinski, T., Gugi, D., Ullrich, V., Lüscher, T.F., 2000. Enhanced peroxynitrite formation is associated with vascular aging. J. Exp. Med. 192, 1731–1744.

Vanhoutte, P.M., 2000. Say no to ET. J. Auton. Nerv. Syst. 81, 271–277.

Vargas, F., Osuna, A., Fernandez-Rivas, A., 1997. Abnormal renal vascular reactivity to acetylcholine and nitroprusside in aging rats. Gen. Pharmacol. 28, 133–137.

Wang, X., Han, C., Fiscus, R.R., 1991. Calcitonin gene-related peptide (CGRP) causes endothelium-dependent cyclic AMP, cyclic GMP and vasorelaxant responses in rat abdominal aorta. Neuropeptides 20, 115–124.

Wang, X., Jones, S.B., Zhou, Z.Z., Han, C., Fiscus, R.R., 1992. Calcitonin gene-related peptide (CGRP) and Neuropeptide Y(NPY) levels are elevated in plasma and decreased in vena cava during endotoxin shock in the rat. Circulatory Shock 36, 21–30.

Wang, X., Wang, W., Li, Y., Bai, Y., Fiscus, R.R., 1999. Mechanism of SNAP potentiating antiproliferative effect of calcitonin gene-related peptide in cultured vascular smooth muscle cells. J. Mol. Cell. Cardiol. 31, 1599–1606.

Wight, E., Kung, C.F., Moreau, P., Takase, H., Bersinger, N.A., Lüscher, T.F., 2000. Aging, serum estradiol levels, and pregnancy differentially affect vascular reactivity of the rat uterine artery. J. Soc. Gynecol. Invest. 7, 106–113.

Wimalawansa, S.J., 1996. Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiology, and therapeutic potentials. Endocr. Rev. 17, 533–582.