~~Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) Induces Location- and Age-Dependent Relaxations of Arteries~~Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) Induces Relaxations of Peripheral and Cerebral Arteries, which are Impaired Differently by Aging

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Abstract

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a well-known neuropeptide, which also has vasomotor effects. However, little is known regarding its age-related and organ-specific vasomotor effects. We hypothesized that the vasomotor effects of PACAP depend on the tissue origin of the vessels and aging substantially modulates its actions. Thus, carotid (CA) and basilar arteries (BA) were isolated from young (2 months old), middle age (12 months old), and old (30 months old) rats. Their vasomotor responses were measured with an isometric myograph (DMT610M) in response to cumulative concentrations of PACAP1-38 (10^{-9}–10^{-6} M). PACAP1-38 induced (1) ~~a~~ significantly greater concentration-dependent relaxations in CA compared to that of BA of young, middle age, and old rats; (2) relaxations of CA significantly decreased, whereas ~~it~~ theydid not change substantially in BA, as a function of age; (3) sodium nitroprusside (SNP)-induced relaxation did not change after PACAP1-38 administration in any conditions; and (4) inhibition of PAC1 receptors by selective PAC1 receptor blocker (PACAP6-38) completely diminished the responses to PACAP in all age groups of BA and CA. In conclusion, these findings suggest that PACAP1-38 has greater vasomotor effect in CA than that in BA, whereas aging has less effect on PACAP-induced relaxation of cerebral arteries and BA than that in peripheral arteries and CA suggesting that the ~~vasomotor role of~~relaxation to PACAP is maintained in cerebral arteries even in old age.

Keywords

PACAP1-38
Vascular tone
PAC1 receptor

Isolated carotid and basilar arteries
Aging

Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a well-known neuropeptide with widespread organ and tissue distribution. The two biologically active forms (PACAP1-38 and PACAP1-27) are important neuroendocrine regulators of many biological systems, including the cardiovascular system (Okazaki et al. 1992; Suzuki et al. 1993; Somogyvári-Vigh and Reglődi 2004). The vascular actions of PACAP1-38 are more effective compared to those of PACAP1-27 (Huang et al. 1992; Okazaki et al. 1992). The vasodilator activity of PACAP has been documented in vessels of various organs (Nandha et al. 1991; Ross-Ascuitto et al. 1993) via three specific receptors (PAC1R and VPAC1R/VPAC2R), both of which are highly expressed in the cerebral and peripheral blood vessels (Fahrenkrug et al. 2000; Nandha et al. 1991). They are localized in the smooth muscle cells of cerebral arteries and arterioles (Fahrenkrug et al. 2000; Amenta et al. 1991). In vivo studies on beagle dogs described that intravenous infusion of very low doses (0.01–10 pmol/min) of PACAP induced a concentration-dependent increase in blood flow in human skin and a concomitant decrease in systemic blood pressure (Cardell et al. 1991; Nandha et al. 1991; Naruse et al. 1993). High doses (>3 nmol) of PACAP elicited a biphasic effect: a transient hypotensive response, followed by a sustained period of hypertension (Ishizuka et al. 1992; Cardell et al. 1991). It was suggested that the action of PACAP on the vascular tone could be ascribed both to a direct relaxant effect and an indirect vasopressor action mediated through the release of endogenous catecholamines (Cardell et al. 1991). In vivo studies of cats showed that PACAP administration had a pressor effect in the hindquarters and PACAP increased pulmonary vascular resistance, which was explained by the systemic release of catecholamines from the adrenal gland (Minkes et al. 1992; Moller and Sundler 1996; Nogi et al. 1997). In vitro isolated vessel studies (Whalen et al. 1999) described that the dilator effects (measured by isotonic myograph) of PACAP were due to its actions on the small arterioles, rather than the release of adrenal catecholamines. These vessels were isolated, and this vasodilation—increasing the diameter—may not involve the release of endothelium-derived nitric oxide

(NO) (Vaudry et al. 2000). Previous studies have also shown that PACAP receptors are present in various organs (Cardell et al. 1991) and also in vessels, including intracerebral (Vaudry et al. 2000) and extracerebral arteries (Miyata et al. 1998; Amenta et al. 1991), mesenteric, porcine coronary arteries (Cardell et al. 1991), pulmonary vascular bed of rats (Minkes et al. 1992; Cheng et al. 1993), and stem villous and intramyometrial arteries of humans (Steenstrup et al. 1996). In spite of the numerous studies on the vascular functions of PACAP, region-specific vasomotor actions are not well-characterized. Aging has ~~also~~ been shown to greatly affect the vasomotor function of vessels, which potentially contributes to organ dysfunction (Tripathy et al 2010, Vamos et al. 2013, Ivic et al 2012). We thought that it would be especially important to elucidate the effects of healthy aging on PACAP-induced vasomotor regulation of basilar arteries, as PACAP is a neuropeptide with potential vasomotor regulatory effect in the cerebrovascular system.

In the present study, we hypothesized that PACAP has region-specific vasomotor effects, which are substantially modulated by aging. Thus, we characterized the concentration-dependent relaxations of carotid and basilar arteries isolated from young (2 months old), middle age (12 months old), and old (30 months old) rats to PACAP in the absence and presence of PACAP (PAC1) receptor inhibitor.

Materials and Methods

Animals

For these experiments, young (2 months old), middle age (12 months old), and old (30 months old) male Wistar-Kyoto rats (WKY, $N = 8$, $n = 16$/per group of age) were used ("N" for number of rats and "n" for number of vessels). All procedures in the experiments were approved in accordance with the general rules for animal protection in science work, a 2010 European Directive on ethical issues (European Communities Council) Directive 2010/63/ECC and Ethical Committee of the University for the Protection of Animals in Research and approved by the same committee. These rules agree with the regulation of the BA02/2000-8/2008 directive.

Isolation and Preparation of Carotid and Basilar Arteries

The vessels were isolated as previously described (Vamos et al. 2013; Toth et al. 2011). In brief, animals were anesthetized by intraperitoneal ketamin and decapitated immediately. The common carotid arteries (CA) and basilar arteries (BA) were isolated from the rats, then cleaned and prepared under an Olympus operation microscope, and quickly transferred into ice-cold (4 °C) oxygenated (95 % O_2, 5 % CO_2) Krebs' buffer solution as described previously (Vamos et al. 2013; Bagi et al. 2008). Then, BA and CA were dissected into 5-mm rings and canulated by wires.

Isolation Vessel Measurements

Each artery was positioned between two stainless steel wires (diameter 0.04 mm) in a 5-mL organ bath of a small-vessel myograph (DMT 610-M, Danish Myo Technology, Aarhus, Denmark). Isometric tension generated by the vessels was continuously measured, and the software Myodaq 2.01 M610+ was used for data acquisition and display. At the beginning of experiments, the length tension curve— (normalized to 2.0 g—) was obtained, and the vessels were allowed to stabilize for 60 min according to experimental protocols (Vamos et al. 2013). The bath solution was continuously oxygenated with a gas mixture of 95 % O_2 plus 5 % CO_2 and kept at 36.8 °C (pH 7.4).

Administration of Vasoactive Agents

Vessels were allowed to stabilize for 60 min; then, to establish a tone, 60 mM KCl was administered (Vamos et al. 2013). Vasomotor function of vessels was studied in response to cumulative concentration of PACAP1-38, synthetized as previously described (Jozsa et al. 2005), from 10^{-9} to10^{-6} mol/L. The intact vasomotor function of smooth muscle was verified at the end of the experiment by dilation to sodium nitroprusside (SNP; 10^{-8} to 10^{-5} mol/L). The age-dependent changes in the vasomotor activity were measured by the difference of PACAP1-38-induced isometric relaxation of BA and CA between 12- and 30-, 2- and 30-, and 2- and 12-month-old rats. Same protocols were repeated with presence of PAC1 receptor blocker, PACAP6-38 (10^{-7} M).

Statistical Analysis

Experimental results are presented as mean ± S.E.M. Statistical analysis was performed by two-way ANOVA. To determine which groups differ from the others, we have used the one-way ANOVA (Holm-Sidek, Tukey, Student-Newman-Keuls) multiple comparison procedure by SPSS 11.0 for Windows software. p Values <0.05 were considered to be statistically significant. Figures were made by Sigma Plot 12.0 for Windows software.

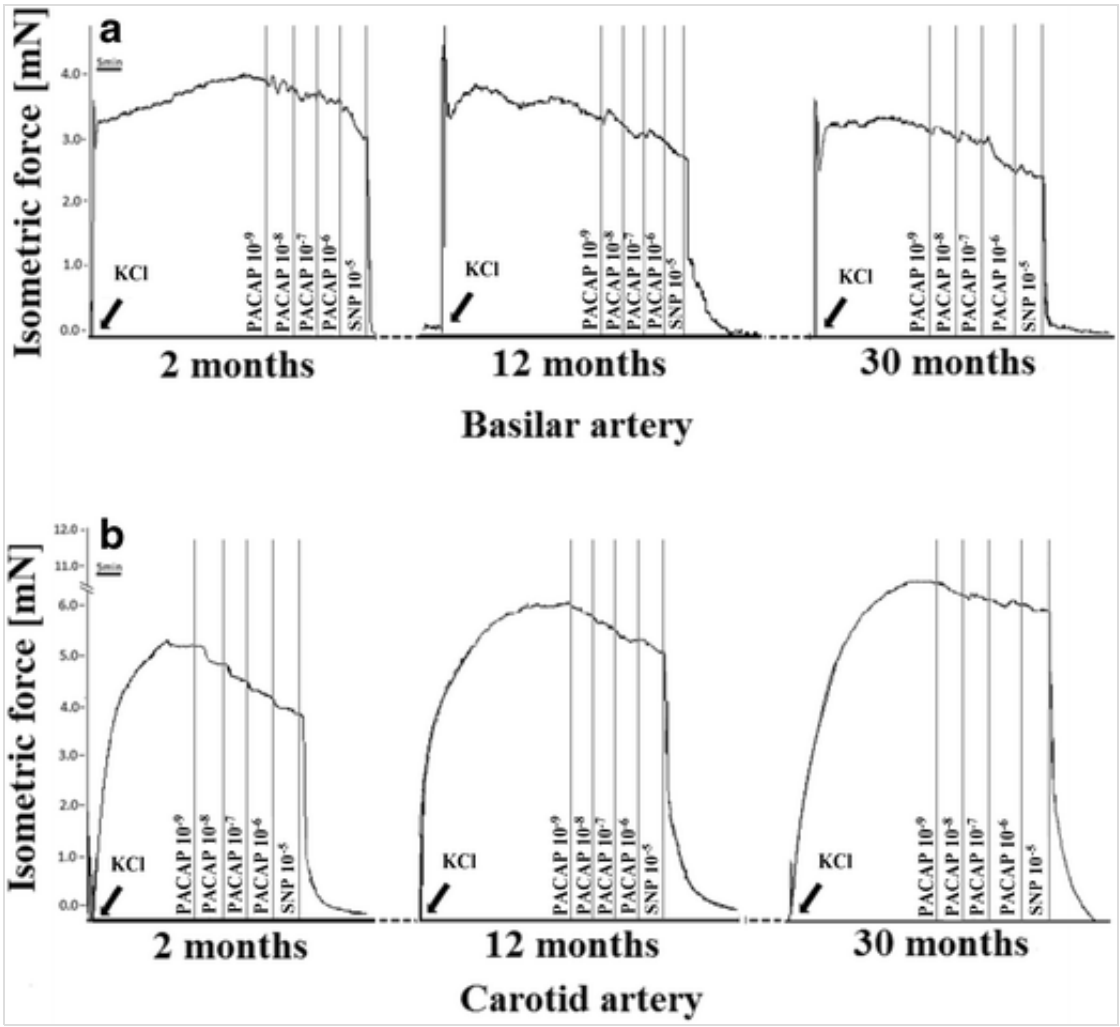
Results

Effect of Cumulative Concentration-Dependent Administration of PACAP1-38 on the Relaxation of Basilar and Carotid Arteries as a Function of Age

Original records (Fig. 1, panel a) show the relaxation of BA to the cumulative doses of PACAP1-38. The relaxations did not change substantially from young (2 months old—2 m), middle (12 months old—12 m), and old age (30 months old—30 m). Steady-state values are the following: 2 m 10^{-9} M 5.17 mN, 10^{-8} M 4.7 mN, 10^{-7} M 4.58 mN, 10^{-6} M 4.56 mN; 12 m 10^{-9} M 4.56 mN, 10^{-8} M 4.35 mN, 10^{-7} M 4.11 mN, 10^{-6} M 3.99 mN; 30 m 10^{-9} M 4.29 mN, 10^{-8} M 4.05 mN, 10^{-6} M 3.94 mN, 10^{-6} M 3.84 mN.

Fig. 1

Effect of cumulative concentrations-dependent administration of PACAP1-38 on the relaxation of basilar and carotid arteries as a function of age (original figure). *Black arrow* representing administration of KCl (60 mM); PACAP was administrated in sequential order: 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M. Last administration was SNP 10^{-5} M.

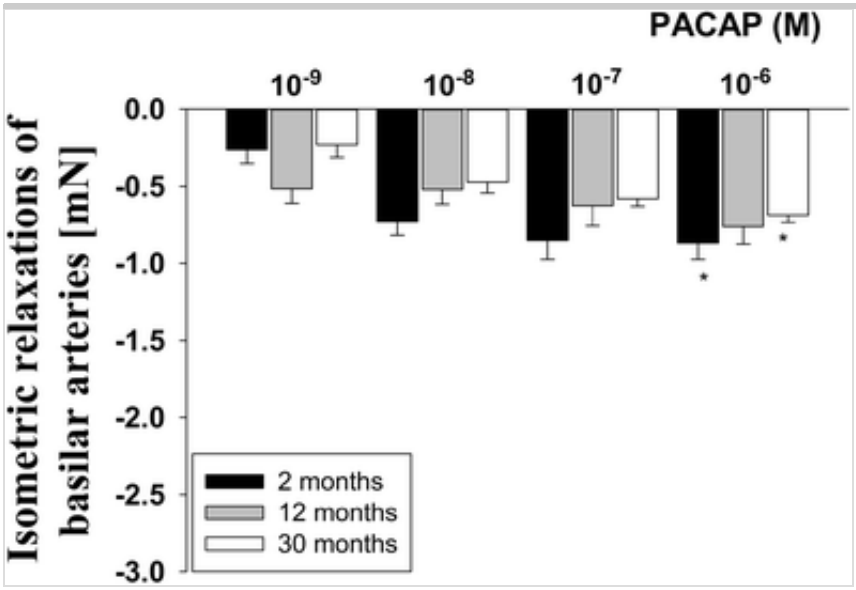


Original records (Fig. 1, panel b) show the relaxations of CA to the cumulative doses of PACAP1-38. It shows that the relaxations substantially decreased from young (2 m), middle (12 m), and old age (30 m). Steady-state values are the following: 2 m 10^{-9} M 5.71 mN, 10^{-8} M 5 mN, 10^{-7} M 4.31 mN, 10^{-6} M 3.78 mN; 12 m 10^{-9} M 6.21 mN, 10^{-8} M 5.99 mN, 10^{-7} M 5.39 mN, 10^{-6} M 4.88 mN; 30 m 10^{-9} M 11.21 mN, 10^{-8} M 10.77 mN, 10^{-7} M 10.28 mN, 10^{-6} M 9.55 mN.

Summary data (Fig. 2) show that the relaxations of BA to the cumulative administration of PACAP1-38 significantly, but not substantially, decreased from young (2 m), middle age (12 m), and old (30 m) age (2 m 10^{-9} M −0.26 ± 0.09 mN, 10^{-8} M −0.73 ± 0.08 mN, 10^{-7} M −0.85 ± 0.12 mN, 10^{-6} M −0.86 ± 0.1 mN; 12 m 10^{-9} M −0.24 ± 0.09 mN, 10^{-8} M −0.42 ± 0.09 mN, 10^{-7} M −0.52 ± 0.13 mN, 10^{-6} M −0.76 ± 0.11 mN; 30 m 10^{-9} M −0.23 ± 0.08 mN, 10^{-8} M −0.47 ± 0.07 mN, 10^{-7} M −0.58 ± 0.04 mN, 10^{-6} M −0.68 ± 0.06 mN, $N = 8$, $n = 16$, $p < 0.05$).

Fig. 2

Effect of cumulative concentration-dependent administration of PACAP1-38 on the relaxation of basilar artery as a function of age (summary data). Data are means ± standard error of the mean; $*p < 0.05$ indicates significant changes between the PACAP 10^{-6} and 10^{-9} ($N = 8$, $n = 16$).

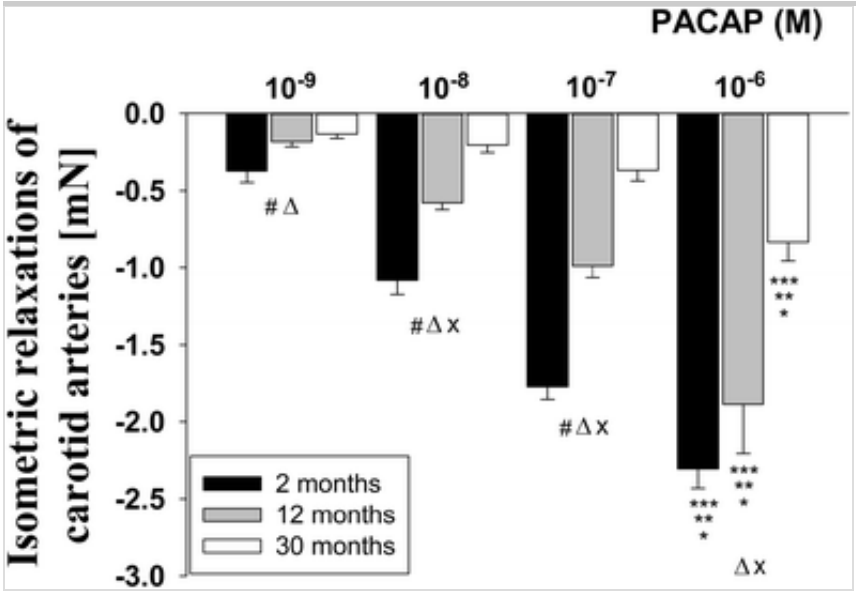


Summary data (Fig. 3) show that relaxations of CA to the cumulative doses of PACAP1-38 significantly and substantially decreased from young (2 m) and middle age (12 m) to old (30 m) age (2 m 10^{-9} M −0.37 ± 0.07 mN, 10^{-8} M −1.08 ± 0.06 mN, 10^{-7} M −1.77 ± 0.08 mN, 10^{-6} M −2.3 ± 0.12 mN; 12 m 10^{-9} M −0.18 ± 0.03 mN, 10^{-8} M −0.57 ± 0.04 mN, 10^{-7} M −0.98 ± 0.07 mN, 10^{-6} M −1.88 ± 0.31 mN; 30 m 10^{-9} M −0.13 ± 0.02 mN, 10^{-8} M −0.2 ± 0.04 mN, 10^{-7} M −0.36 ± 0.06 mN, 10^{-6} M −0.83 ± 0.12 mN, $N = 8$, $n = 16$, $p < 0.05$).

Fig. 3

Effect of cumulative concentration-dependent administration of PACAP1-38 on the relaxation of carotid artery as a function of age (summary data). Data are means ± standard error of the mean; $*p < 0.05$ indicates significant changes between the PACAP 10^{-6} and 10^{-9} ($N = 8$, $n = 16$), $**p < 0.05$ indicates significant changes between the PACAP 10^{-6} and 10^{-8} ($N = 8$, $n = 16$), $***p < 0.05$ indicates significant changes between the PACAP 10^{-6} and 10^{-7} ($N = 8$, $n = 16$). $\#p < 0.05$ indicates significant changes between 2 and 12 months of age groups ($N = 8$, $n = 16$). $\Delta p < 0.05$ indicates significant changes between 2 and

30 months of age groups ($N = 8$, $n = 16$). X$p < 0.05$ indicates significant changes between 12 and 30 months of age groups ($N = 8$, $n = 16$).

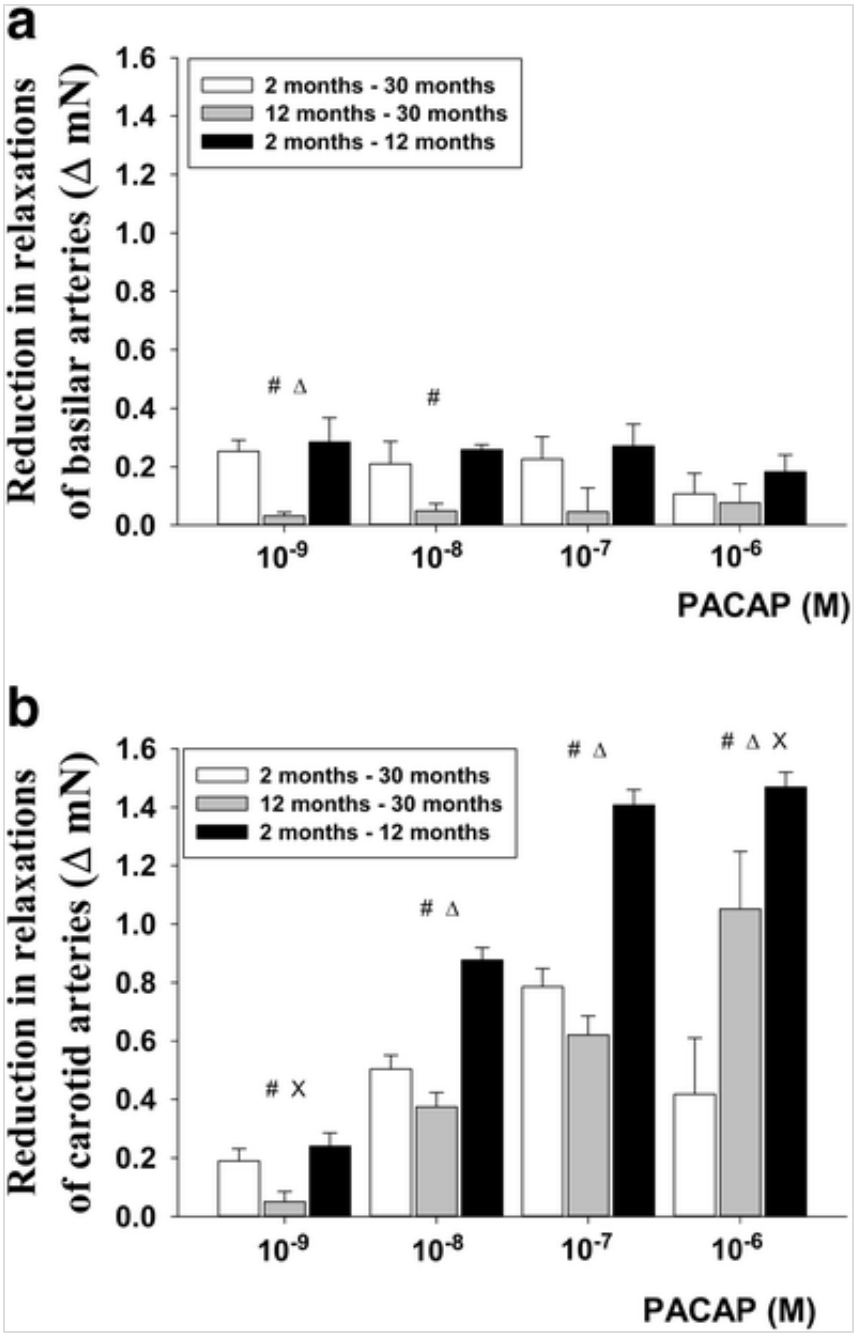


Age-Dependent Differences in PACAP1-38-Induced Relaxation of Basilar and Carotid Arteries

To indicate the age-dependent changes in PACAP1-38-induced relaxations, we calculated the difference between the responses of BA and CA of 30-, 12-, and 2-month-old rats. Summary data in Fig. 4a show that in BA of various age groups, there were slight but significant reduction in relaxations to the lower concentrations (30–2 m 10^{-9} M 0.03 ± 0.08 mN, 10^{-8} M 0.25 ± 0.02 mN, 10^{-7} M 0.27 ± 0.07 mN, $p < 0.05$), whereas there were no differences in relaxations to higher concentrations of PACAP1-38 (30–12 m 10^{-9} M 0.28 ± 0.01 mN, 10^{-8} M 0.05 ± 0.02 mN, 10^{-7} M 0.05 ± 0.08 mN, 10^{-6} M 0.07 ± 0.06 mN; 12–2 m 10^{-9} M 0.25 ± 0.04 mN, 10^{-8} M 0.21 ± 0.07 mN, 10^{-7} M 0.22 ± 0.08 mN, 10–6 M 0.10 ± 0.07 mN, $N = 8$, $n = 16$).

Fig. 4

a, **b** Age-dependent differences in PACAP1-38-induced relaxations of basilar and carotid arteries (BA and CA). Data are means ± standard error of the mean; #$p <$ 0.05 indicates significant changes between the delta's of 2 m–30 m and 12 m–30 m ($N = 8$, $n = 16$). Δ$p < 0.05$ indicates significant changes between the delta's of 2 m–30 m and 2 m–12 m ($N = 8$, $n = 16$). X$p < 0.05$ indicates significant changes between the delta's of 12 m–30 m and 2 m–12 m ($N = 8$, $n = 16$).



Summary data in Fig. 4b show that in responses of CA of various age groups, there were significant and substantial reductions in relaxations as a function of age (30–2 m 10^{-9} M 0.24 ± 0.04 mN, 10^{-8} M 0.88 ± 0.04 mN, 10^{-7} M 1.4 ± 0.05 mN, 10^{-6} M 1.46 ± 0.05 mN; 30–12 m 10^{-9} M 0.05 ± 0.03 mN, 10^{-8} M 0.37 ± 0.05 mN, 10^{-7} M 0.62 ± 0.06 mN, 10^{-6} M 1.05 ± 0.19 mN; 12–2 m 10^{-9} M 0.18 ± 0.04 mN, 10^{-8} M 0.5 ± 0.04 mN, 10^{-7} M 0.78 ± 0.06 mN, 10^{-6} M 0.41 ± 0.19 mN, $N = 8$, $n = 16$, $p < 0.05$). That is, aging reduced relaxations to PACAP more in CA than in BA (Fig. 4a, b).

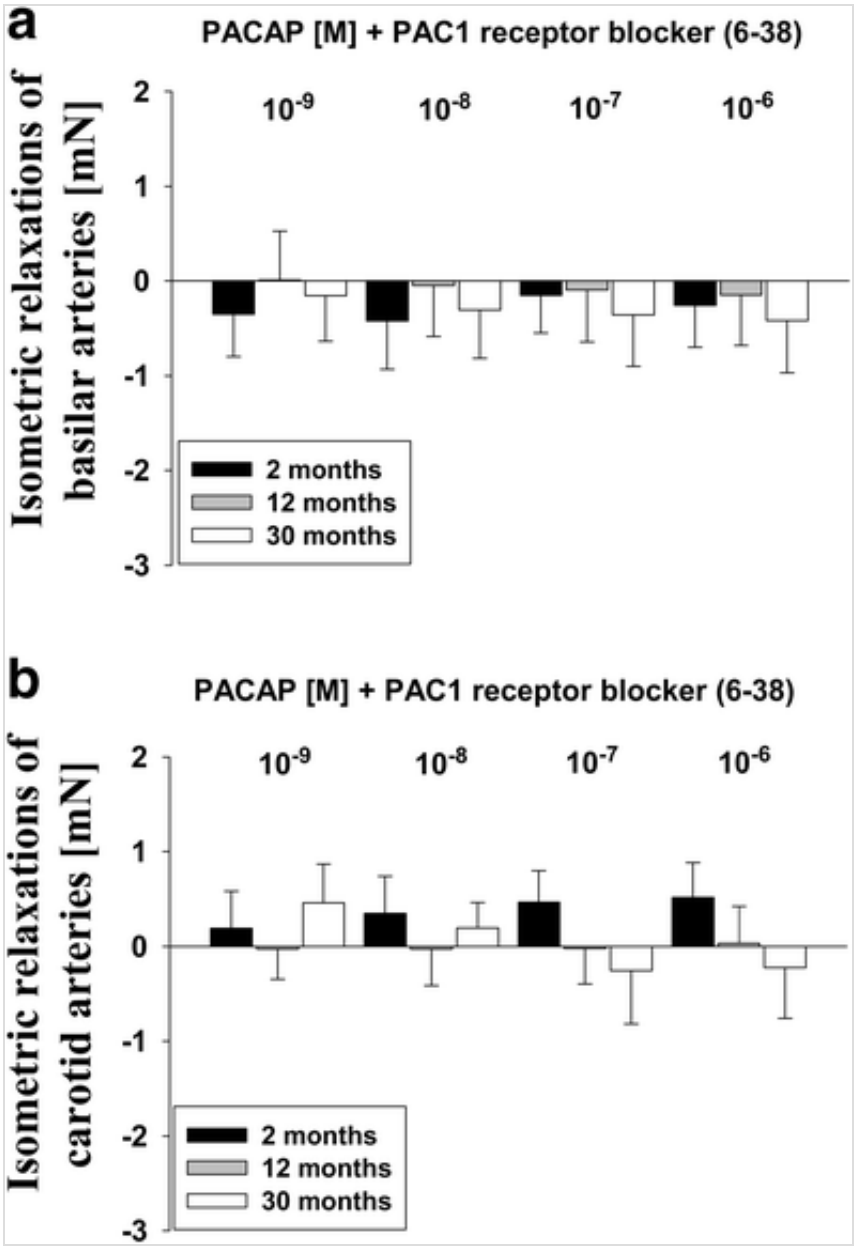
Effect of Cumulative Concentration-Dependent

Administration of PACAP1-38 on the Relaxation of Basilar and Carotid Arteries in Presence of PAC1 Receptor Blocker (6–38) as a Function of Age

Summary data in Fig. 5a show that in the presence of PAC1 receptor blocker (10^{-7} M), there was no change in vasomotor response of BA to the cumulative administration of PACAP1-38 that was not significant, increased from young (2 m), middle age (12 m), and old (30 m) age (2 m 10^{-9} M −0.35 ± 0.44 mN, 10^{-8} M −0.42 ± 0.51 mN, 10^{-7} M −0.15 ± 0.39 mN, 10^{-6} M −0.25 ± 0.44 mN; 12 m 10^{-9} M 0.01 ± 0.51 mN, 10^{-8} M −0.05 ± 0.53 mN, 10^{-7} M −0.09 ± 0.54 mN, 10^{-6} M −0.14 ± 0.53 mN; 30 m 10^{-9} M −0.15 ± 0.47 mN, 10^{-8} M −0.3 ± 0.51 mN, 10^{-7} M −0.35 ± 0.54 mN, 10^{-6} M −0.41 ± 0.55 mN, $N = 8$, $n = 16$, $p < 0.05$).

Fig. 5

a, **b** Effect of cumulative concentration-dependent administration of PACAP1-38 on the relaxation of carotid artery in the presence of PAC1 receptor blocker (6–38) as a function of age (summary data). Data are means ± standard error of the mean ($N = 8$, $n = 16$).



Summary data in Fig. 5b show that in the presence of PAC1 receptor blocker (10^{-7} M), there was no change in vasomotor response of BA to the cumulative administration of PACAP1-38 that was not significant, increased from young (2 m), middle age (12 m), and old (30 m) age (2 m 10^{-9} M 0.19 ± 0.39 mN, 10^{-8} M 0.35 ± 0.39 mN, 10^{-7} M −0.46 ± 0.33 mN, 10^{-6} M 0.51 ± 0.36 mN; 12 m 10^{-9} M −0.02 ± 0.32 mN, 10^{-8} M −0.02 ± 0.39 mN, 10^{-7} M −0.02 ± 0.37 mN, 10^{-6} M 0.03 ± 0.38 mN; 30 m 10^{-9} M 0.46 ± 0.4 mN, 10^{-8} M 0.19 ± 0.26 mN, 10^{-7} M −0.25 ± 0.56 mN, 10^{-6} M −0.22 ± 0.53 mN, $N = 8$, $n = 16$, $p < 0.05$).

The Nitric Oxide Donor, Sodium Nitroprusside-Induced Relaxations of Basilar and Carotid Arteries as a Function of

Age

As the original figures show, SNP induced complete relaxations of BA and CA in each age group, without differences (Fig. 1). Summary data show no significant differences (BA 2 m 98 ± 2 %, 12 m 97 ± 3 % 30 m 99 ± 1 % vs; CA 2 m 96 ± 4 %, 12 m 98 ± 2 %, 30 m 99 ± 1 %, $N = 8$, $n = 16$, $p > 0.05$, NS).

Discussion

The novel findings of the present study are the following: (1) PACAP1-38 induced a significantly greater relaxation in isolated CA than that in BA of young and old rats; (2) the PACAP1-38-induced relaxation of CA significantly decreased as a function of age, whereas it did not change substantially in BA; (3) the magnitude of SNP-induced relaxation did not change after PACAP1-38 administration and was not affected by age; and (4) selective PAC1 receptor blocker completely diminished PACAP1-38-induced vasomotor response in all age groups of CA and BA. Collectively, these findings suggest that PACAP1-38 has greater vasomotor effect in CA than that in BA and that PACAP-induced signaling does not interfere with cGMP-mediated relaxation. Furthermore, aging elicits major impairment in relaxations of peripheral carotid arteries ~~and CA~~, whereas aging has no substantial effect on PACAP-induced relaxations of basilar artery ~~BA~~, suggesting that the vasomotor role of PACAP is maintained in cerebral arteries even in old age.

PACAP-Induced Vasomotor Responses of Various Tissues

The polypeptide PACAP has been shown to be a multifunctional molecule having several biological regulatory roles (Vaudry et al. 2000; Tamas et al. 2012). For example, PACAP has various hormonal functions in all endocrine glands; it influences motility and secretion in the gastrointestinal and respiratory tracts and regulates urogenital physiology (Vaudry et al. 2000; Cardell et al. 1991; Cheng et al. 1993; Ishizuka et al. 1992; Botz et al. 2013). In addition, it has been revealed that PACAP has potent vasomotor effects (Suzuki et al. 1993; Nandha et al. 1991; Hagi et al. 2008). Several human studies demonstrate that PACAP exerts cardiovascular actions partially by the decrease in mean arterial blood pressure and an increase in blood flow, measured

invasively on beagle dog femoral arteries (Naruse et al. 1993; Whalen et al. 1999). Previous studies have shown that vessel walls are richly innervated by PACAP-containing fibers, a high density of PACAP binding sites is present in arteries (Erdling et al. 2013; Baun et al. 2011), and PACAP is widely distributed in 1- to 4-month-old rat brain vessels (Erdling et al. 2013). ~~The blocked vasomotor activity suggests that PACAP 1-38 acts on PAC1 receptor, which elicits vasorelaxation in a concentration- and age-dependent manner. The receptor activity is similar in cerebral and extracerebral arteries also.~~

Studies performed in isolated aortae and coeliac arteries ~~frmm~~ from rabbit showed that PACAP elicits relaxation, likely via increasing cAMP level in the smooth muscle (Wilson and Warren 1993). Interestingly, there are no data regarding the vasomotor effects of PACAP in cerebral and peripheral vessels of old rats. On the basis of ~~many~~ previous studies showing that aging impairs vasomotor function (Ivic et al, 2012, Vamos et al. 2013Tripathy et al, 2010), we hypothesized that the PACAP-induced region-specific vasomotor effects are substantially modulated by aging. Because PACAP can have multiple effects in a complex organ, we aimed to characterize the vasomotor responses of PACAP1-38 in isolated rat BS and CA in young, middle age, and old of rats.

PACAP1-38-Induced Relaxation in Carotid Arteries

In the present study, we found that PACAP1-38 induced concentration-dependent and substantial relaxations of CA of young rats ~~to the old one~~, which were reduced in that of the older rats (Fig 1/b and Fig 3). Others have found similar results: PACAP1-38 elicits relaxant effect on rabbit aorta and mesenteric arteries, and also on human and porcine coronary arteries (Warren et al. 1991; Kastner et al. 1995). Comparing the magnitude of relaxation within a single species (rabbit), more prominent response was found in the macrovessels than the microcirculation (Wilson and Warren 1993). It was particularly effective in skin microcirculation measured in vivo with a laser Doppler flow probe and shows a particularly sensitive vasodilator response to PACAP (Lissbrant et al. 1999). The PACAP1-38-induced relaxation was more prominent in rabbit coeliac arteries compared to our findings in CA (Wilson and Warren 1993). There are a number of possible explanations for the previous findings that PACAP induced different relaxations in vessels of different origins, such as differences in the animal used or origin of vessel or methods used. Thus, in the

present study, we used one species (rats) and isolated arteries from two different origin to reduce uncertainties in the interpretation of results and allowing us to discern the effect of age on the PACAP-induced vasomotor effects. Interestingly, relaxations were significantly greater in CA than those in BA, and PACAP-induced relaxations were significantly reduced in CA of older rats. Our findings with CA are important, because previous studies showed that these arteries mirror changes that occur in the coronary arteries ~~(Vogel et al. 1999)~~.(Rothwell et al. 2001).

PACAP-Induced Relaxations of Basilar Arteries

We found that PACAP1-38 elicited substantially smaller relaxations in BA compared to CA, suggesting that PACAP1-38 is ~~acting primarily on CA~~more effective in peripheral than cerebal arteries.. In previous studies (Amenta et al. 1991; Fahrenkrug et al. 2000; Erdling et al. 2013), the vascular effects of PACAP1-38 were investigated in segments of rat middle cerebral artery (MCA) by pressurized arteriography and in a wire myograph. The authors have found the same magnitude of concentration-dependent relaxations of the MCA, in arteries of rat dura, whereas in human meningeal arteries, PACAP1-38 induced weak relaxations (~~Arsalan et al. 2013;~~ Baun et al. 2011). Others described that cerebellar arteries isolated from the brain surface were ~1,000-fold less sensitive to PACAP than the middle meningeal arteries (Syed et al. 2012) showing important region specificity. In the present study, we also found thatdecreases in PACAP1-38-induced relaxation in BA ~~decreases~~were less in old age than in CA. The magnitude of changes of relaxations was assessed by subtracting the responses of 2-month-old rats from those of 30-month-old animals. We found a greater decrease in the magnitude of response in CA, but not in BA, suggesting that aging affects more the responses of peripheral carotid arteries ~~and CA~~ than those of the cerebral arteries, such as BA.

AQ1

AQ2

PACAP-Induced Vasomotor Molecular Signaling

~~Our findings~~The findings of the present study help to further elucidate the region-specific PACAP-induced vasomotor responses and their changes in the healthy aging. It has been demonstrated that the wall of blood vessels is richly

innervated by PACAP-containing fibers and a high density of PACAP binding sites is present in arteries (Vaudry et al. 2000). Also, it has been shown that PAC1 receptors are present and located in the cytoplasm of smooth muscle cells (Erdling et al. 2013).In the present study PACAP 6-38 inhibited the vasorelaxations suggesting that PACAP 1-38 acts on PAC1 receptor and that the activity of receptors are similar in cerebral and extracerebral arteries. The present findings of our studies show that PACAP-induced signaling does not ~~interfere with the direct relaxant cGMP-mediated~~ involve cGMP in mediation of relaxation of arteries and aging specifically affects PACAP responses because SNP-induced responses were not affected by aging.

As shown by previous studies, in arterial vessels, the PACAP-induced dilator effect is mediated by activating PAC1 receptor on the smooth muscle cell, followed by activation of adenylate cyclase (Erdling et al. 2013; Tamas et al. 2012). A previous study (Erdling et al. 2013) suggests that activation is via the stimulation of cAMP production in blood vessels, which modulates L-type calcium channels in vascular smooth muscle cells through the activation of both protein kinase A (PKA) and protein kinase C (PKC) mechanisms resulting in vasodilation (Chik et al. 1996). PACAP also stimulates the release of the prostaglandin $PGF_{2\alpha}$ in the wall of the arteries but does not affect other cyclooxygenase metabolites (Huang et al. 1992; Kastner et al. 1995). Others found that PACAP relaxes human pulmonary arteries by opening of KATP and KCa channels (Bruch et al, 1998).The possible involvement of the endothelium in the vasodilator activity of PACAP is still not clear: Some reports indicate that the relaxant effect of PACAP on the rabbit aorta and coronary arteries is endothelium-independent (Warren et al. 1991; Kastner et al. 1995), whereas another study reveals that removal of the vascular endothelium abolishes the ~~dilatory~~dilator response induced by PACAP in guinea pig pulmonary arteries (Cardell et al. 1991). More importantly, the effect of age on the PACAP-induced vasomotor responses are not known.

Potential Roles of PACAP1-38 Signaling Eliciting Relaxation as a Function of Age

Previous studies described age-related changes in PACAP immunoreactivity and its protein levels in the gerbil hippocampus (Tripathy et al. 2010). However, the effect of age on the PACAP-induced vasomotor activity is not

known. In the present study, we observed that the PACAP1-38-induced vasomotor response shows an age and vessel location dependency. These findings suggest that aging may induce vessel-specific changes in the functional availability and/or density of PAC1 receptor. This could be due to the altered receptor mRNA and protein expression in older age compared to the younger rats or different activation and internalization of the receptors and/or their subcellular pathways (cAMP, PKA, PKC, or MLCK) (Boni et al. 2009).

Indeed, most previous studies have found that PAC1 receptors possess a unique feature: The density and distribution of these proteins change as a function of age. In the present study, we have found that inhibition of PACAP receptors (Fig. 5a, b) completely blocked relaxation to PACAP in all age groups, suggesting that primarily PAC1 receptors are involved in the signaling. Nevertheless, more studies are necessary to further reveal the vasomotor effects of PACAP in various age groups. It is important to elucidate whether it has protective or deleterious effects, since previous studies assigned clinical significance for PACAP in headache due to prolonged dilation of cerebral arteries to PACAP (Amin et al. 2012). In contrast, other studies demonstrated increased vascular contractile activity in older age (Ivic et al. 2012), when relaxations to PACAP was less affected.

In conclusion, the novel findings of the present study suggest that PACAP1-38 has a greater dilator effect in peripheral carotid arteries ~~and CA~~ than that in cerebral basilar arteries ~~and BA~~. Furthermore, aging elicits major impairment in the PACAP-induced relaxation of peripheral carotid arteries ~~and CA~~, whereas aging has no substantial effect on the relaxation of cerebral basilar arteries ~~and BA~~, suggesting that the ~~vasomotor role of~~relaxations to PACAP is maintained in cerebral arteries even in old age.

Acknowledgments

Hungarian National Science Research Fund (OTKA) K 108444, K104984, Developing Competitiveness of Universities in the South Transdanubian Region, “Identification of new biomarkers…”, SROP-4.2.2.A-11/1/KONV-2012–0017 and “Complex examination of neuropeptide…” SROP-4.2.2.A-11/1/KONV-2012-0024 and SROP-4.2.4.A/2-11-1-2012-0001 “National Excellence Program”, Arimura Foundation, MTA Lendület Program and

KTIA_NAP_13-1-2013-0001, Hungarian Brain Research Program—Grant No. KTIA_13_NAP-A-III/5 and Hungarian Hypertension Society (MHT) 2013/2014.

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