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Alterations with age of the response to vasodilator agents in isolated mesenteric arteries of the beagle

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1 Responses to vasodilator agents were compared in helical strips of mesenteric arteries from beagles of different ages (30 days, 3 months, 2 years and 12 years old), precontracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Relaxations induced by isoprenaline and adenosine were related inversely to age, whereas those induced by PGI_2 and K^+ (5 mM) did not alter with age.

2 Acetylcholine-induced relaxations were less in the arteries from the aged (12 year old) beagles than in those from the younger beagles. Histamine-induced relaxations related directly to age between 30 days and 2 years. Acetylcholine-induced relaxation was reversed to a contraction in infant (30 day old) beagle arteries by removal of the endothelium, and significantly attenuted in the older beagle arteries. In adult (2 year old) and aged beagle arteries, removal of the endothelium attenuated the histamine-induced relaxation. Treatment with indomethacin reversed the histamine-induced relaxation to a marked contraction in aged beagle arteries.

3 It may be concluded that responses mediated by ß-adrenoceptors and P_1-purinoceptors are less in aged mesenteric arteries than in those from the younger beagles, whereas responses mediated by PGI_2 receptors and the electrogenic Na^+ pump do not alter with age. The release of endothelium-derived relaxing factor(s) by acetylcholine appears to be inversely related to age. However, the release of PGI_2 possibly caused by histamine may be less in infant beagle mesenteric arteries than in those from the older beagles.

Introduction

Functional, biochemical and morphological changes occur in the vascular wall with increasing age (Altura & Altura, 1977; Berkowitz, 1978; Fleisch, 1980). Vascular responses to chemical substances alter differently with age in a variety of vessels from the same animal and in the same vessel from various animals. Relaxation induced by isoprenaline is related inversely to age in rabbit and rat isolated aortae (Fleisch *et al.*, 1970) and pulmonary arteries (Fleisch & Hooker, 1976; O'Donnell & Wanstall, 1984) and rabbit basilar arteries (Toda & Hayashi, 1979). However, the amine-induced relaxation is greater in cerebral arteries from 3 year old beagles than in those from the younger beagles (Toda *et al.*, 1986), and does not alter with age in rat jugular veins (Duckles & Hulbert, 1986).

The necessary role of endothelial cells in the response to some vasodilator agents, including acetylcholine, histamine, bradykinin, Ca^{2+}-ionophore, substance P, arachidonic acid, ATP and ADP, is widely recognized (Furchgott, 1984). Acetylcholine-induced relaxation is postulated to be mediated by a vasodilator factor(s) released from endothelial cells (Furchgott & Zawadzki, 1980). Histamine relaxes dog mesenteric arteries, due partly to the release of prostaglandin I_2 (PGI_2) from endothelium (Toda *et al.*, 1982; Toda, 1984) and relaxes rat aortae and guinea-pig pulmonary arteries as a result of the release of endothelium-derived relaxing factor(s) (Van de Voorde & Leusen 1983; Satoh & Inui, 1984). However, whether or not endothelial functions alter with age has not been clarified.

In the present study the response, to vasodilator agents of mesenteric arteries isolated from beagles of different ages (from 30 days to 12 years) was investigated to determine age-related changes in endothelial cell function in the arteries stimulated by acetylcholine and histamine.

Methods

Beagles of either sex, 30 days (28–35 days) ($n = 7$), 3 months (80–110 days) ($n = 12$), 2 years (10–40 months) ($n = 13$), and 12 years (135–161 months) old

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($n = 7$), were used. The animals were anaesthetized with intravenous injections of sodium pentobarbitone and killed by bleeding from the common carotid arteries. Distal portions of the superior mesenteric artery were isolated. The arteries were helically cut into strips approximately 20 mm long. The cross-sectional area of the strips was estimated by the wet weight/length, a calculation that assumes a tissue density of 1; mean values in 30 day, 3 month, 2 year, and 12 year old beagles were 0.116 ± 0.008 ($n = 28$), 0.272 ± 0.013 ($n = 36$), 0.366 ± 0.019 ($n = 34$), and $0.470 \pm 0.026\,mm^2$ ($n = 26$), respectively. The preparation was vertically fixed between hooks in a muscle bath containing the nutrient solution which was aerated with a mixture of 95% O_2 and 5% CO_2 and maintained at 37 ± 0.3 °C. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer (Nihonkohden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 1.5 g for arteries from 2 and 12 year old beagles, which is optimal for producing the maximum contraction in mesenteric arteries from adult mongrel dogs (Toda *et al.*, 1978). On the basis of the ratio of cross-sectional areas, resting tensions for the arteries from 30 day and 3 month old beagles were adjusted to 0.5 and 1.0 g, respectively. Constituents of the bathing solution were (mM): NaCl 120, KCl 5.4, $NaHCO_3$ 25.0, $CaCl_2$ 2.2, $MgCl_2$ 1.0, and dextrose 5.6. The pH of the solution was 7.35–7.41. The strips were allowed to equilibrate for 40–80 min in the bathing medium, during which time the medium was replaced every 10–15 min.

Isometric contractions and relaxations were recorded on an ink-writing oscillograph (Nihonkohden Kogyo Co.). The contractile response to 30 mM K^+ was first obtained, and then preparations were repeatedly washed and equilibrated in control medium. The preparations were partially precontracted with $PGF_{2\alpha}$ (10^{-7} to 2×10^{-6} M); the contraction was between 15 and 45% of the contraction induced by 30 mM K^+. Isoprenaline, adenosine, PGI_2, K^+, acetylcholine and histamine were added directly to the bathing medium in cumulative concentrations. At the end of each experiment, papaverine (10^{-4} M) was added to produce maximal relaxation. The papaverine-induced relaxation and the K^+ (30 mM)-induced contraction were taken as 100% for relaxant and contractile responses to test drugs, respectively. In some preparations, the intimal surface was gently rubbed with a cotton pellet, as described by Furchgott & Zawadzki (1980). Preparations were treated for 20 to 30 min with blocking agents, before the response to test drugs was obtained.

The results shown in the text and figures are expressed as mean values ± s.e. mean. Statistical analyses were made by use of Tukey's method after one-way analysis of variance or Student's unpaired *t* test. Drugs used were: (±)-isoprenaline hydrochloride, indomethacin (Sigma Chemical Co., St Louis, USA); histamine dihydrochloride (Kanto Chemical Co., Tokyo, Japan), adenosine (Kohjin Co., Tokyo); $PGF_{2\alpha}$, PGI_2 (Ono Pharmaceutical Co., Osaka, Japan); ouabain octahydrate, atropine sulphate (E. Merk, Darmstadt, GFR);(±)-propranolol hydrochloride (Sumitomo Pharmaceutical Co., Osaka), acetylcholine chloride, papaverine hydrochloride (Daiichi Parmaceutical Co., Tokyo);(+)-chlorpheniramine maleate (Shionogi Pharmaceutical Co., Osaka).

Results

Responses to isoprenaline, adenosine, prostaglandin I_2 and K^+

In mesenteric arterial strips, obtained from 30 day (infant), 3 month (juvenile), 2 year (adult) and 12 year old (aged) beagles precontracted with $PGF_{2\alpha}$, the addition of isoprenaline (10^{-9} to 10^{-6} M) produced a concentration-related relaxation (Figure 1a). Increasing the amine concentration to 10^{-5} M caused contractions from the level of relaxation induced at 10^{-6} M. The relaxant response tended to be related inversley to age; there was a significant difference between the relaxation of the infant and aged beagle arteries. Mean values of the maximal relaxation at 10^{-6} M isoprenaline in the arteries from 30 day, 3 month, 2 year, and 12 year old beagles relative to those induced by 10^{-4} M papaverine, were $91.7 \pm 3.0\%$ ($n = 7$), $81.3 \pm 3.6\%$ ($= 12$), $79.6 \pm 3.3\%$ ($n = 9$), and $61.3 \pm 8.2\%$ ($n = 6$), respectively (the values at 30 day versus 3 month old, and 30 day versus 12 year old were significantly different, Figure 1), and EC_{50} (apparent median effective concentration) values were 1.44 ± 0.71 ($n = 7$), 2.78 ± 0.89 ($n = 12$), 3.03 ± 1.54 ($n = 9$) and $2.84 \pm 0.96 \times 10^{-8}$ M ($n = 6$), respectively (not significantly different). The amine-induced relaxation was suppressed by treatment with 10^{-6} M propranolol.

Mesenteric arteries precontracted with $PGF_{2\alpha}$ responded to adenosine (10^{-7} to 10^{-4} M) with a concentration-related relaxation (Figure 1b). The relaxant responses were related inversely to age, except that the responses in the juvenile and adult beagle arteries did not differ. Mean values of the apparent EC_{50} of adenosine in the 30 day, 3 month, 2 year and 12 year old beagle arteries were 1.34 ± 0.29 ($n = 12$), 2.71 ± 0.55 ($n = 13$), 2.14 ± 0.64 ($n = 13$) $7.86 \pm 1.96 \times 10^{-7}$ M ($n = 7$), respectively; on the basis of Tukey's method, the values obtained from 12 year old arteries versus those of other ages were significantly different ($P < 0.01$). PGI_2 (10^{-9} to 10^{-6} M) relaxed $PGF_{2\alpha}$-contracted mesenteric arteries dose-dependently. The relaxant responses in beagles

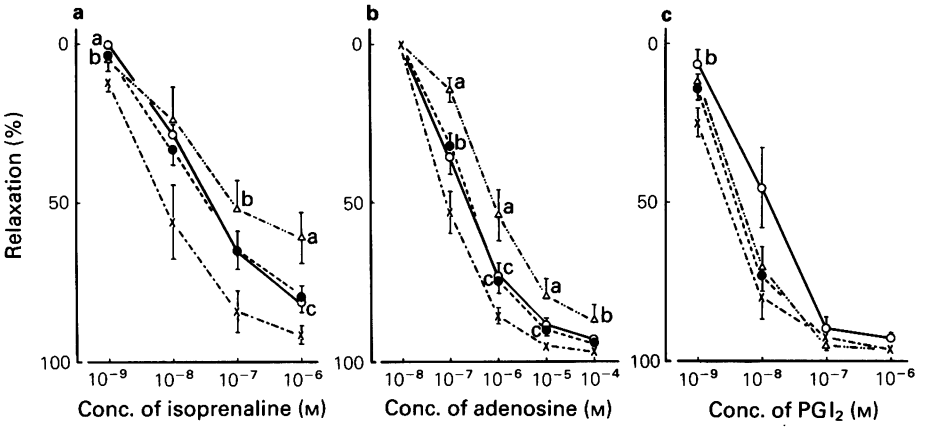

Figure 1 Concentration-response curves for the relaxation induced by isoprenaline (a), adenosine (b), and prostaglandin I_2 (PGI_2, c) of mesenteric arterial strips, from beagles of different ages, partially contracted with $PGF_{2\alpha}$. Relaxation induced by 10^{-4} M papaverine was taken as 100%. Mean absolute values at 30 days (X), 3 months (O), 2 years (●), and 12 years (Δ) old were 192 ± 28 mg (n = 7), 288 ± 19 mg (n = 12), 335 ± 42 mg (n = 9), and 426 ± 88 mg (n = 6), respectively, in experiments with isoprenaline; 264 ± 37 mg (n = 12), 297 ± 22 mg (n = 13), 257 ± 36 mg (n = 13), and 260 ± 37 mg (n = 7), respectively, in experiments with adenosine; 221 ± 69 mg (n = 6), 288 ± 59 mg (n = 4), 336 ± 42 mg (n = 7), and 335 ± 43 mg (n = 11), respectively, in experiments with PGI_2. Significantly different from values at 30 days old, $^aP < 0.01$; $^bP < 0.05$. Significantly different from values at 12 years old, $^cP < 0.05$ (Tukey's method after one-way analysis of variance). Vertical lines represent s.e.mean.

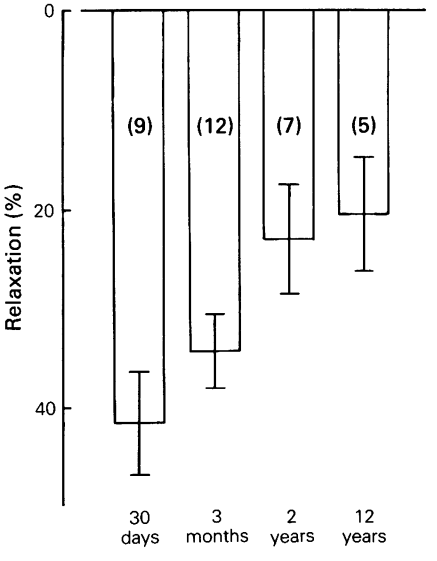

Figure 2 Relaxant responses induced by 5 mM K^+ of mesenteric arteries, from beagles of different ages, partially contracted with prostaglandin $F_{2\alpha}$. Relaxation induced by 10^{-4} M papaverine was taken as 100%. Mean absolute values at 30 days, 3 months, 2 years, and 12 years old were 222 ± 57 mg (n = 9), 219 ± 38 mg (n = 12), 365 ± 50 mg (n = 7), and 366 ± 56 mg (n = 5), respectively. Vertical line represent s.e.mean.

of different ages did not differ significantly (Figure 1c). Apparent EC_{50} values in 30 day, 3 month, 2 year and 12 year old beagles were 4.05 ± 1.86 (n = 6), 13.2 ± 5.20 (n = 4), 5.27 ± 1.58 (n = 7) and 6.16 ± 1.68 × 10^{-9} M (n = 11), respectively, the differences being statistically insignificant.

The addition of K^+ in a low concentration (5 mM), sufficient to cause a maximal relaxation in cerebral arteries from mongrel dogs (Toda, 1976), relaxed the mesenteric arterial strips contracted with $PGF_{2\alpha}$. The K^+-induced relaxation did not differ significantly between beagles of differents ages (Figure 2). Treatment with 10^{-6} M ouabain abolished the relaxation induced by K^+.

Responses to acetylcholine and histamine

Mesenteric arterial strips partially contracted with $PGF_{2\alpha}$ responded to acetylcholine (ACh, 10^{-8} to 10^{-4} M) with a concentration-dependent relaxation. In the arteries from infant, juvenile, and adult beagles, acetylcholine produced similar magnitudes of relaxation, which were significantly greater than the responses of the aged beagle arteries to 10^{-7}–10^{-5} M ACh (Figure 3a).

In the $PGF_{2\alpha}$-precontracted arteries from infant beagles, histamine (2 × 10^{-8} to 10^{-5} M) produced relaxations (19 to 67% of maximal relaxation) in 4 out of 12 preparations, and slight or moderate contrac-

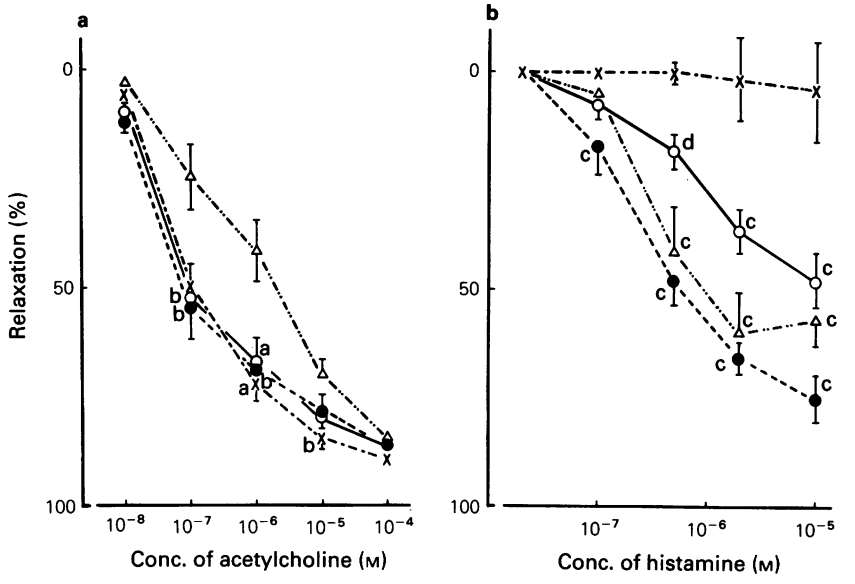

Figure 3 Concentration-response curves for acetylcholine (a)-and histamine (b)-induced relaxation of mesenteric arteries, from beagles of different ages, partially contracted with prostaglandin $F_{2\alpha}$. Relaxation induced by 10^{-4} M papaverine was taken as 100%. Mean absolute values at 30 days (X), 3 months (O), 2 years (●), and 12 years (Δ) old were 246 ± 31 mg ($n = 11$), 302 ± 27 mg ($n = 12$), 406 ± 42 mg ($n = 9$), and 289 ± 32 mg ($n = 11$), respectively, in experiments with acetylcholine; and 189 ± 19 mg ($n = 12$), 282 ± 41 mg ($n = 9$), 295 ± 37 ($n = 9$), and 288 = 43 mg ($n = 9$), respectively, in experiments with histamine. Significantly different from values at 12 years old, $^{a}P < 0.01$; $^{b}P < 0.05$. Significantly different from values at 30 days old, $^{c}P < 0.01$; $^{d}P < 0.05$ (Tukey's method after one-way analysis of variance). Vertical lines represent s.e. mean.

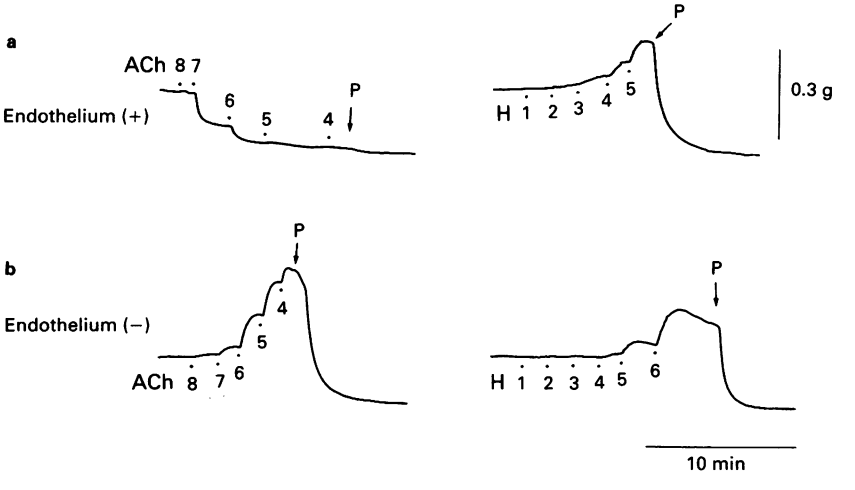

Figure 4 Responses to acetylcholine (ACh) and histamine (H) of mesenteric arterial strips, from a 30 day old beagle, with (a) and without (b) endothelium, partially contracted with prostaglandin $F_{2\alpha}$. Concentrations of ACh, 8–4 = 10^{-8} to 10^{-4} M, respectively. Concentrations of histamine from 1 to 6 = 2×10^{-8}, 10^{-7}, 5×10^{-7}, 2×10^{-6}, 10^{-5} and 5×10^{-5} M, respectively. P = 10^{-4} M papaverine.

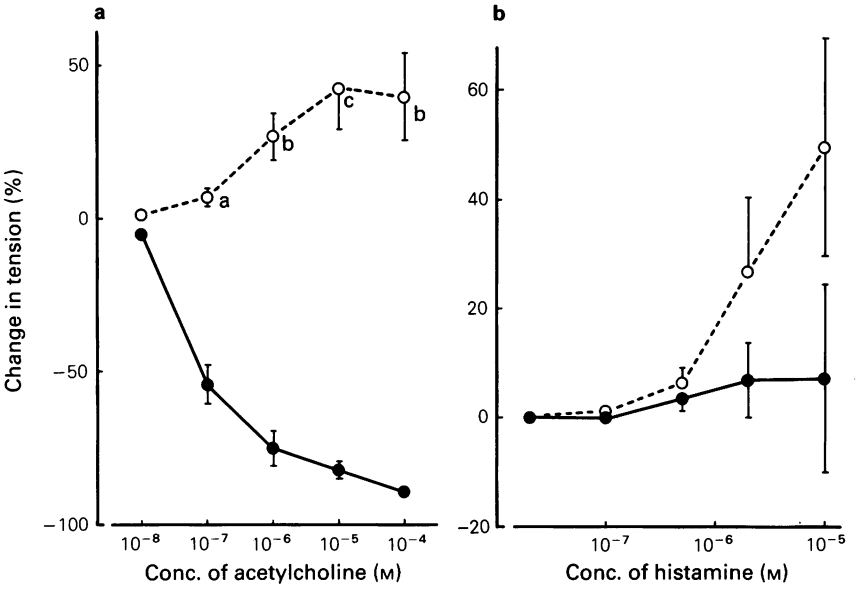

Figure 5 Concentration-response curves for the effects of acetylcholine (a) and histamine (b) on mesenteric arterial strips, from 5 beagles of 30 days old, with (●) and without endothelium (○). Preparations were partially contracted with prostaglandin $F_{2\alpha}$ before the addition of acetylcholine or histamine. Contraction induced by 30 mM K^+ was taken as 100%. The mean absolute value in strips without endothelium was 431 ± 82 mg ($n = 5$) in experiments with acetylcholine, and the values in strips with and without endothelium were 402 ± 59 mg ($n = 5$) and 431 ± 82 mg ($n = 5$), respectively, in experiments with histamine. Relaxation induced by 10^{-4} M papaverine was taken as 100%; the mean absolute value in strips with endothelium was 206 ± 25 mg ($n = 5$), in experiments with acetylcholine. Significantly different from values in strip with endothelium, $^aP < 0.001$; $^bP < 0.01$; $^cP < 0.05$ (Student's unpaired t test). Vertical lines represent s.e.mean.

tions (8 to 52% of maximal contraction) in the remaining 8. In the arteries from the older beagles, histamine (10^{-7} to 10^{-5} M) elicited a concentration-dependent relaxation. The average relaxant response was related directly to age between 30 days and 2 years (Figure 3b). Histamine relaxed the arteries from aged beagles to a similar extent to those from 2 year old beagles.

In mesenteric arteries from infant beagles, removal of the endothelium reversed the acetylcholine (10^{-7} to 10^{-4} M)-induced relaxation to a contraction, but did not significantly affect the contractile response to histamine (5×10^{-7} to 10^{-5} M) (Figure 4). Quantitative data obtained from 5 pairs of experiments are shown in Figure 5. In the arteries from 2 year old beagles, the relaxation induced by acetylcholine (10^{-8} to 10^{-5} M) was abolished or reversed to a contraction by removal of the endothelium (Figure 6a). Histamine (5×10^{-7} to 2×10^{-6} M)-induced relaxations were significantly attenuated in the arteries with damaged endothelium (Figure 6b). In mesenteric arteries from aged beagles, acetylcholine (10^{-7} to 10^{-5} M) elicited a transient contraction followed by a moderate relaxation, as demonstrated in Figure 7a. The contraction relative to that induced by 30 mM K^+ was not significantly affected by removal of the endothelium, whereas the relaxant response was significantly attenuated (Figure 8a). The contractions and relaxations were suppressed by treatment with 10^{-7} M atropine. Histamine (10^{-7} to 10^{-5} M) caused a slight contraction or a transient contraction followed by a rapidly-developing relaxation (Figure 7b) in arteries from aged beagles. In the arteries with damaged endothelium the relaxant response was abolished almost completely, although the contractile response was not affected (Figure 8b). Histamine-induced contractions were abolished by treatment with 10^{-6} M chlorpheniramine ($n = 4$). Relaxation of the aged beagle arteries induced by histamine was abolished, and contractions by the amine were markedly potentiated by treatment with 10^{-6} M indomethacin (Figure 9). Similar results were obtained in 2 additional preparations.

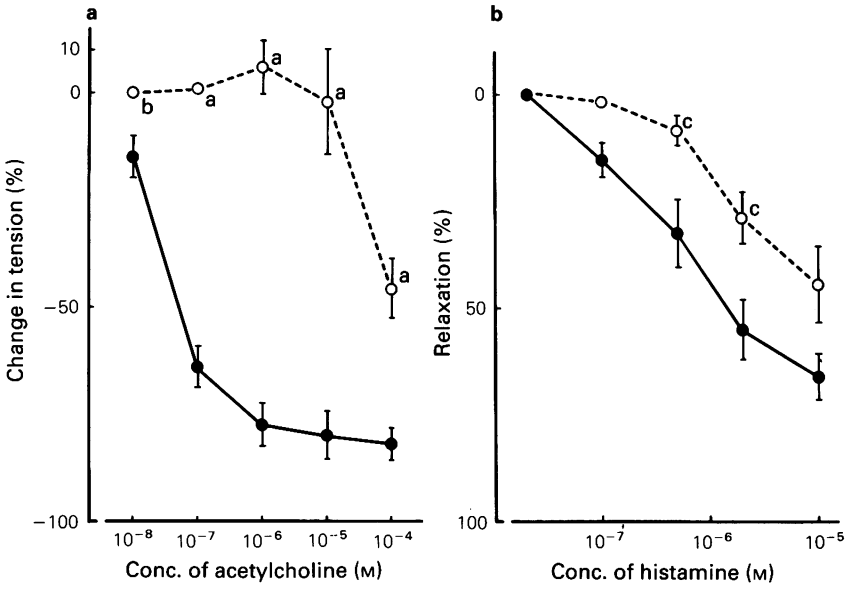

Figure 6 Concentration-response curves for the effects of acetylcholine (a) and histamine (b) on mesenteric arteries, from 2 year old beagles, with (●) and without endothelium (○) partially contracted with prostaglandin $F_{2\alpha}$. Contractions induced by 30 mM K^+ were taken as 100%. Mean absolute value in strips without endothelium was 2337 ± 528 mg ($n = 8$) in experiments with acetylcholine. Relaxation induced by 10^{-4} M papaverine was taken as 100%. The mean absolute values in strips with and without endothelium were 375 ± 49 mg ($n = 8$) and 435 ± 44 mg ($n = 8$), respectively, in experiments with acetycholine; and 490 ± 62 mg ($n = 8$) and 524 ± 39 mg ($n = 8$), respectively, in experiments with histamine. Significantly different from values in strips with endothelium, $^aP < 0.001$; $^bP < 0.01$; $^cP < 0.02$ (Student's unpaired t test). Vertical lines represent s.e.mean.

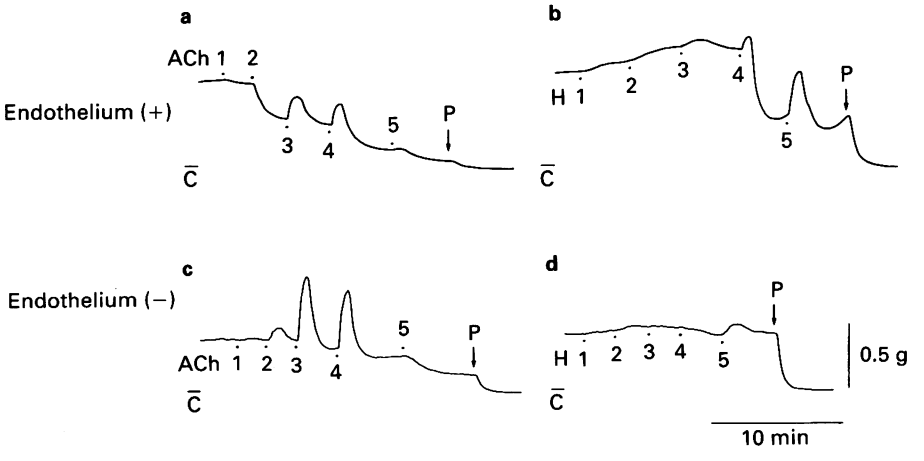

Figure 7 Responses to acetylcholine (ACh) and histamine (H) of mesenteric arterial strips, from a 12 year old beagle, with (a and b) and without endothelium (c and d). Horizontal lines (C) represent the level before the addition of prostaglandin $F_{2\alpha}$. Concentrations of acetylcholine from 1 to 5 represent 10^{-8}, 10^{-6}, 10^{-5}, and 10^{-4} M, respectively. Concentrations of histamine from 1 to 5 represent 2×10^{-8}, 10^{-7}, 5×10^{-7}, 2×10^{-6} and 10^{-5} M, respectively. P = 10^{-4} M papaverine.

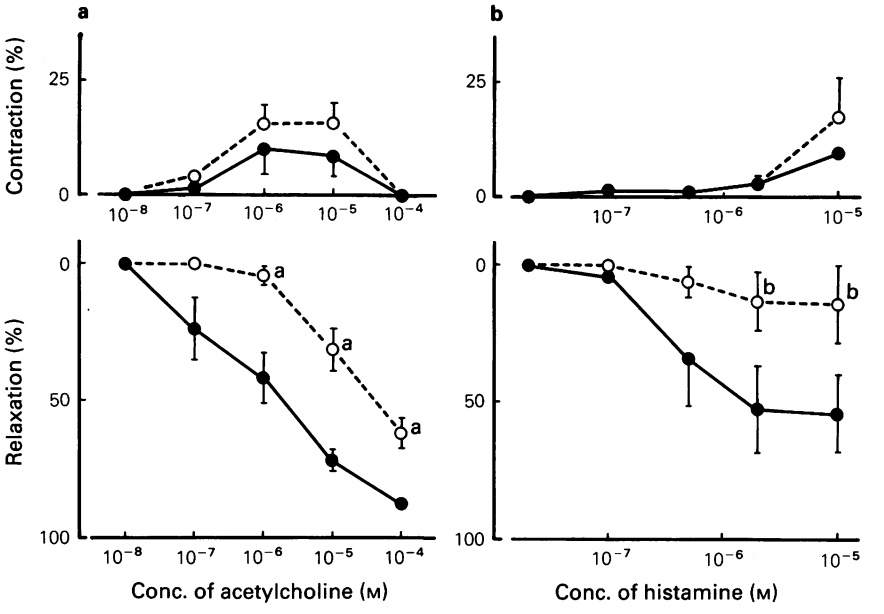

Figure 8 Concentration-response curves for the effects of acetylcholine (a) and histamine (b) in mesenteric arteries, from 5 beagles of 12 years old, with (●) and without endothelium (○) partially contracted with prostaglandin $F_{2\alpha}$. Contraction induced by 30 mM K^+ was taken as 100%. Mean absolute values in strips with and without endothelium were 3104 ± 721 mg ($n = 5$) and 2376 ± 76 mg ($n = 5$), respectively. Relaxation induced by 10^{-4} M papaverine was taken as 100%. Mean absolute values in strips with and without endothelium were 283 ± 48 mg ($n = 5$) and 253 ± 60 mg ($n = 5$) respectively, in experiments with acetylcholine; 276 ± 51 mg ($n = 5$) and 266 ± 40 mg ($n = 5$), respectively, in experiments with histamine. Significantly different from values in strips with endothelium, $^aP < 0.01$; $^bP < 0.05$ (Student's unpaired t test). Vertical lines represent s.e.mean.

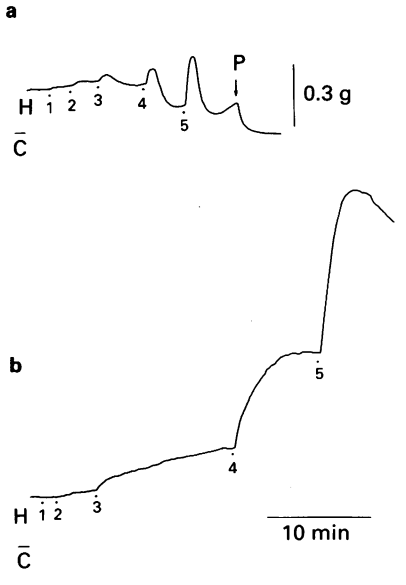

Figure 9 Responses to histamine (H) of a mesenteric arterial strip, from a 12 year old beagle, before (a) and after (b) treatment with indomethacin 10^{-6} M. The strip was partially precontracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Horizontal lines (C) represent the level before the addition of $PGF_{2\alpha}$. Concentrations of histamine from 1 to 5 represent 2×10^{-8}, 10^{-7}, 5×10^{-7}, 2×10^{-6} and 10^{-5} M, respectively. $P = 10^{-4}$ M papaverine.

Discussion

Relaxant responses of mesenteric arteries to isoprenaline were inversely related to age of beagles from 30 days to 12 years, although the responses did not differ between arteries from 3 month and 2 year old beagles. An age-related reduction in the amine-induced relaxation has been demonstrated in rabbit and rat isolated aortae (Fleisch *et al.*, 1970) and in pulmonary arteries (Fleish & Hooker, 1976; O'Donnell & Wanstall, 1984) as well as in perfused mesenteric terminal arterioles of

the rabbit (Altura & Altura, 1977). On the other hand, PGI_2-induced relaxations did not differ significantly between mesenteric arteries from beagles of different ages. As the isoprenaline-induced relaxations were suppressed by treatment with propronolol, these relaxations were supposed to be mediated by stimulation of ß-adrenoceptors. In mesenteric arteries from juvenile, adult and aged beagles, contractile responses mediated by α-adrenoceptors did not differ; however, they were less than those in the infant beagle arteries (Toda *et al.*, unpublished data). Therefore, it seems unlikely that the age-related reduction in isoprenaline-induced relaxation is due to a non-selective decrease in the ability of vascular smooth muscle to relax with increasing age, or to an increase in the contractile response mediated by α-adrenocpetors. Since the maximal relaxation induced by isoprenaline was less in the aged beagle arteries than in the arteries from the younger beagles, whereas the EC_{50} values did not differ, it appears that the sensitivity or the quantity of ß-adrenoceptors is less in the aged beagle arteries, but the affinity of the receptors does not differ. These findings are supported by previous studies that have demonstrated a decrease in the ß-adrenoceptor binding capacity in the rat brain (Greenberg & Weiss, 1978) and human lymphocyte (Schocken & Roth, 1977) with advanced age. Maggi *et al.* (1979) have also suggested that an age-related decrease in ß-adrenoceptor binding in the human brain is due to a reduction in the number of receptor sites rather than to a change in receptor affinity.

Relaxant responses to adenosine are reportedly suppressed by treatment with aminophylline, and mediated by P_1-purinoceptors (Toda *et al.*, 1982). The maximal relaxations induced by adenosine did not differ in mesenteric arteries from beagles of different ages, but the EC_{50} value of 12 year old arteries was greater than the values of arteries of other ages, suggesting that in aged beagle arteries the affinity of P_1-purinoceptors is lower than in the arteries from beagles of younger ages. An age-related decrease in adenosine-induced relaxations has also been observed in isolated aortae from 30 to 360 days old rabbits (Hayashi & Toda, 1978). Adenosine 3', 5'-cyclic monophosphate (cyclic AMP) has been proposed as a mediator for the relexation of vascular smooth muscle elicited by isoprenaline (Triner *et al.*, 1971; Volicer & Hynie, 1971; Andersson, 1973), adenosine (Kukovetz *et al.*, 1978) and PGI_2 (Kukovetz *et al.*, 1979). According to Cohen & Berkowitz (1974), vascular relaxation in response to cyclic AMP is decreased with increasing animal age. They have also demonstrated that isoprenaline increases cyclic AMP levels in aortae from rats of different ages to a similar extent, and suggested that diminished aortic relaxation with age is not associated with a reduced ability of the ß-agonist to increase aortic cyclic AMP level (Cohen *et al.*, 1977). On the other hand, Ericsson (1972) has postulated that the reduced relaxant action of isoprenaline in aortic strips from old rats is due both to a reduced formation of cyclic AMP and to a decreased sensitivity to exogenous cyclic AMP. In the present study, relaxant responses to PGI_2 did not differ between mesenteric arteries from beagles of different ages, whereas those elicited by isoprenaline and adenosine were decreased in the aged beagle arteries. If ß-adrenoceptors, P_1-purinoceptors and PGI_2 receptors are assumed to share the same adenylate cyclase, which intracellularly generates cyclic AMP responsible for vascular relaxations, an age-related decrease in the response to isoprenaline and adenosine would be associated with reduced function of their receptors, thereby diminishing the production of cyclic AMP. In order to clarify the mechanism, it has to be determined whether or not the cyclic AMP production by these agonists and the responsiveness of the arteries to cyclic AMP are altered with age.

The addition of a small amount of K^+ relaxed beagle mesenteric arteries contracted with $PGF_{2\alpha}$, the relaxation being abolished by treatment with ouabain. The K^+-induced relaxation has been postulated to be mediated by an activation of the electrogenic Na^+ pump in arterial smooth muscle cell membranes (Toda, 1976). Relaxant responses to K^+ did not differ significantly between mesenteric arteries from beagles of different ages as well as in beagle cerebral arteries (Toda *et al.*, 1986). Therefore, functions of the Na^+ pump may mature by 30 days after birth and persist without significant deterioration until 12 years old.

In mesenteric arteries from beagles of 30 days to 2 years old, relaxant responses to acetylcholine did not differ. The relaxations were reversed to contractions in the infant beagle arteries by removal of the endothelium and were abolished or reversed to contractions in the adult beagle arteries. On the other hand, the arteries from aged beagles responded to acetylcholine with a transient contraction followed by a moderate relaxation. The relaxant response to acetylcholine (10^{-7} to 10^{-5} M) was significantly less than that seen in the arteries from the younger beagles. Removal of the endothelium markedly attenuated the relaxation, but did not significantly affect the contraction. Atropine suppressed the contractile and relaxant responses. These results may indicated that: (1) the acetylcholine-induced relaxation is mediated mainly by a vasodilator factor(s) released from endothelial cells due to stimulation of their muscarinic receptors, as postulated by Furchgott & Zawadzki (1980). (2) Acetylcholine contracts mesenteric arteries by stimulation of muscarinic receptors on arterial smooth muscle. (3) Mechanisms underlying acetylcholine-induced contractions have a more predominant function in the infant beagle arteries than in the older beagle arteries, which is suggested on the basis of the contractile

response in the denuded arteries. Further, endothelium-dependent relaxations may be related inversely to age from 30 days to 12 years, since the difference in the responses of mesenteric arteries with and without endothelium (Figures 5a, 6a and 8a) decreased with increasing age. In infant beagle mesenteric arteries, moderate contractions appear to be masked by marked relaxations. Hollenberg *et al.*, (1974) have also observed a greater dilatation of renal vasculature to acetylcholine in 20 year old healthy volunteers than in older subjects (up to 70 years old).

Contractions induced by histamine in infant and aged beagle arteries were abolished by treatment with chlorpheniramine, but were not affected by removal of endothelium, suggesting that the contraction is mediated by histamine H_1-receptors in arterial smooth muscle, as previously postulated in cerebral (Konishi *et al.*, 1981) and mesenteric arteries (Toda *et al.*, 1982) from adult mongrel dogs. Relaxations induced by histamine were attenuated by removal of endothelium. In aged beagle mesenteric arteries, the relaxant response was abolished or reversed to a contraction by treatment with indomethacin. These results may indicate that histamine-induced relaxations in the beagle arteries are dependent on the release of PGI_2, at least in part, from endothelium. On the basis of pharmacological analyses in mesenteric arteries of adult mongrel dogs, histamine-induced relaxations are postulated to derive from stimulation of H_2-receptors in smooth muscle and from stimulation of H_1-receptors in endothelial cells, which results in the release of PGI_2 (Toda *et al.*, 1982; Toda, 1984). Despite the fact that PGI_2-induced relaxations did not alter significantly with age, relaxations induced by histamine were related directly to age from 30 days to 2 years. H_2-receptor activity in the rabbit aorta is reportedly diminished as the animal matures (Holl & Mokler, 1982). If this is true in the case of beagle mesenteric arteries, it may be concluded that the release of PGI_2 by histamine from the arterial wall is increased with advancing age, and PGI_2 in an amount sufficient to mask the contractile action of histamine on arterial smooth muscle is not released in the infant beagle arteries. Whether this is due to a paucity of H_1-receptors in the endothelium or a decrease in the amount of PGI_2 released remains to be determined.

In infant beagle mesenteric arteries, acetylcholine produced endothelium-dependent, marked relaxant responses, whereas histamine produced contractions (Figure 4). In aged beagle arteries, acetylcholine-induced relaxations were less than those in the younger beagle arteries, whereas, in the same arterial strips, histamine elicited an endothelium-dependent, marked relaxation (Figure 7). The release of endothelium-derived relaxing factor(s) by acetylcholine appears to be greater, and the histamine-induced release of PGI_2 less in infant beagle mesenteric arteries than in arteries from the older beagles.

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