Responsiveness to Vasoconstrictor and Dilator Agents of Senescent Beagle Cerebral Arteries

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Abstract—With advancing age from 2 to 12 years, contractions of isolated beagle cerebral arteries mediated by histamine H_1 and serotonin receptors increased, whereas those induced by noradrenaline and angiotensin II did not differ. Relaxations by vasodilator substances, such as prostaglandin (PG) I_2 , isoproterenol, adenosine and K^+ (5 mM), and by stimulation of vasodilator nerves in the adult and senescent beagle arteries did not significantly differ.

Vascular reactivity to chemical stimuli alters with age. Such an alteration in senescent animals would be caused by histological and biochemical impairments in blood vessel cells, which may in turn be hastened by changes in the reactivity to physiological substances. Some information is available concerning the reactivity of aged rat aortae (1); however, functional characteristics in arteries of certain regions from senescent animals have not been determined. Therefore, in the present study, responsiveness to vasoconstrictor and dilator substances was compared in cerebral arteries isolated from beagles of about 2 and 12 years old; their life-span is approximately 12 to 15 years.

Adult (10 to 40 months old, $n=16$) and senile beagles (135 to 161 months old, $n=7$) of both sexes were anesthetized with sodium thiopental and sacrificed by bleeding. Basilar and middle cerebral arteries were rapidly removed and cut into helical strips of about 20 mm long. The specimen was vertically fixed in a muscle bath containing the modified Ringer-Locke solution aerated with 95% O_2 and 5% CO_2 , and the tension developed was isometrically recorded. The resting tension was adjusted to 1.5 g. Mean values of the cross sectional area of the strips, estimated by an equation of wet weight/length, in the

arteries from adult and senile beagles were 0.223 ± 0.013 ($n=17$) and 0.289 ± 0.022 mm² ($n=20$, $P<0.02$), respectively, and those of the K^+ (30 mM)-induced contraction per sectional area were 5017 ± 756.1 and 3219 ± 326.7 mg/mm² ($P<0.05$), respectively. Detailed experimental procedures have been described in the previous report (2). The data from 2 year-old beagles were partly obtained from previous experiments (2). Statistical comparisons were made using Student's unpaired t -test.

Contractions induced by noradrenaline (2×10^{-8} to 10^{-5} M) relative to those induced by 30 mM K^+ did not differ in cerebral arterial strips obtained from adult and senile beagles (Fig. 1, left). Increase in the amine concentration to 5×10^{-5} M produced a slight or no additional contraction. Serotonin-induced contractions normalized by the K^+ -induced contraction were greater in the arteries from senile beagles than in those from adult beagles (Fig. 1, middle). Mean values of $-\log$ of the apparent median effective concentration (EC₅₀) were 7.54 ± 0.12 ($n=11$) and 7.21 ± 0.07 ($n=25$, $P<0.02$), respectively. The contraction was attenuated by treatment with 10^{-8} M methysergide ($n=3$) and 10^{-7} M ketanserin ($n=3$). Histamine contracted cerebral arterial strips dose-dependently; the contraction in the senile beagle arteries was markedly greater than that in the adult beagle arteries (Fig. 1, right). Increase in the con-

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Fig. 1. Concentration-contractile response curves for noradrenaline (left figure), serotonin (middle) and histamine (right) in cerebral arterial strips from adult and senile beagles. Contractions induced by 30 mM K^+ were taken as 100%. Vertical bars represent S.E. * Significantly different from the value of adult beagles, $P < 0.001$; b $P < 0.01$; c $P < 0.05$. Numbers in parentheses indicate the number of preparations used.

centration to 2×10^{-4} M did not produce additional contractions. The $-log$ EC_{50} values were 5.94 ± 0.22 ($n=8$) and 4.95 ± 0.09 ($n=17$), respectively, the difference being statistically significant ($P < 0.001$). Treatment with 10^{-6} M chlorpheniramine suppressed the contractile response to the amine ($n=4$), whereas cimetidine (10^{-5} M) did not alter the response ($n=4$). Angiotensin II in a concentration (10^{-7} M) sufficient to elicit the maximum contraction produced a slight, transient contraction. The contractions relative to those induced by 30 mM K^+ in adult ($22.9 \pm 6.69\%$, $n=9$) and senile beagles ($30.5 \pm 10.5\%$, $n=8$) did not significantly differ.

In beagle cerebral arteries partially contracted with prostaglandin (PG) $F_{2\alpha}$ (2×10^{-7} to 10^{-6} M), the addition of PGI_2 (10^{-8} to 10^{-6} M), adenosine (10^{-7} to 10^{-4} M), or isoproterenol (10^{-8} to 10^{-5} M) caused a dose-related relaxation. The magnitude of relaxations relative to those induced by 10^{-4} M papaverine in the adult and senescent beagle arteries did not significantly differ (Fig. 2). The isoproterenol-induced relaxation was suppressed by 10^{-6} M propranolol. Nicotine

(10^{-4} M)-induced relaxations were abolished by treatment with 10^{-5} M hexamethonium; the relaxation in cerebral arteries from adult and senile beagles did not differ (33.7 ± 3.81 , $n=15$, and $25.3 \pm 4.68\%$, $n=16$, respectively, relative to papaverine-induced relaxations). Relaxant responses to 5 mM K^+ in the adult and senile beagle arteries averaged 59.1 ± 4.23 ($n=15$) and $57.1 \pm 4.19\%$ ($n=16$), respectively, the difference being not significant. The K^+ -induced relaxation was abolished by treatment with 2×10^{-7} M ouabain.

Contractions of cerebral arteries induced by serotonin, normalized by the response to 30 mM K^+ , were potentiated with advancing age from 2 to 12 years. The contraction was suppressed by treatment with ketanserin and methysergide. Serotonin $5-HT_1$ and $5-HT_2$ receptors may be involved in the contraction, although receptor binding studies have led to contradictory conclusions (3, 4). The maximum contraction was greater, and the apparent EC_{50} value was less in the senile beagle arteries than in the arteries from adult beagles, suggesting that the efficiency and the affinity of serotonergic receptors are

Fig. 2. Concentration-relaxant response curves for PGI₂ (left figure), adenosine (middle) and isoproterenol (right) in cerebral arteries from adult senile beagles, partially contracted with PGF_{2α}. Relaxations induced by 10⁻⁴ M papaverine were taken as 100%. Numbers of preparations used are indicated in parentheses. Vertical bars represent the S.E.

increased in the aged beagle arteries. In contrast, in rabbit aortic strips, age-related decrease in the serotonin-induced contraction was observed (5).

Histamine-induced contractions were also increased in cerebral arteries from senile beagles, and the apparent EC₅₀ value significantly decreased. In the isolated rabbit aorta, histamine H₂ receptors are postulated to be diminished as the animal matures (6). However, in beagle cerebral arteries, the age-related increase in the contractile response does not appear to be due to loss of H₂ receptor activity, but rather to increased affinity of H₁ receptors to histamine, since histamine-induced contractions were markedly reduced by treatment with chlorpheniramine but were not potentiated by cimetidine. There was no difference in the contractile response to noradrenaline and angiotensin II in the arteries from adult and senile beagles. Therefore, susceptibility of serotonin and histamine receptors responsible for cerebroarterial contractions is expected to selectively increase in senescent beagles.

Relaxations induced by PGI₂, adenosine and isoproterenol did not differ in the arteries from adult and senescent beagles. Age-dependent decrease in the isoproterenol-

induced relaxation has been recognized in aortae and arteries isolated from various species (7). Relaxations of rabbit basilar arteries by isoproterenol are also related inversely to age from 15 days to 1 year (8); however, the responses of beagle cerebral arteries did not alter with age from 30 days to 12 years (2).

Nicotine produces cerebroarterial relaxations, possibly due to stimulation of vasodilator nerves (9). The relaxant response to nicotine did not alter with age, suggesting that the nerve function is sustained well in the senile beagle arteries. Relaxations induced by 5 mM K⁺ were abolished by treatment with ouabain and are postulated to derive from an activation of the electrogenic Na⁺ pump (10). Cerebral arteries from 2 and 12 year-old beagles responded to the ion with similar magnitudes of relaxation. In rabbit basilar arteries, the K⁺-induced relaxation increases with age from 15 days to 3 months; however, the response did not alter in the arteries from the older rabbits (8). Susceptibility of membrane ATPase to K⁺ under the experimental conditions used does not appear to alter in cerebral arteries from aged dogs and rabbits.

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