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RESPONSIVENESS TO VASOACTIVE AGENTS OF CEREBRAL AND MESENTERIC ARTERIES ISOLATED FROM CONTROL AND RESERPINE-TREATED DOGS

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1 Pretreatment of dogs for 20 to 24 h before the start of experiments with reserpine (0.5 mg/kg) depleted noradrenaline from cerebral and mesenteric arteries, the diminution being greater in the latter arteries.

2 Contractile responses of helically-cut strips of cerebral and mesenteric arteries to noradrenaline were unaffected by pretreatment with reserpine. Tyramine-induced contractions of mesenteric arteries were markedly attenuated by reserpine-pretreatment, whereas the contraction of cerebral arteries was not influenced. The contractile response of mesenteric arteries to transmural nerve stimulation or nicotine was abolished by reserpine-pretreatment, but the relaxation induced by nicotine of cerebral arteries contracted with prostaglandin $F_{2\alpha}$ was not affected. Pretreatment with reserpine attenuated the contractions of mesenteric arteries induced by angiotensin II, but did not alter the response of cerebral arteries to 5-hydroxytryptamine.

3 In prostaglandin-contracted cerebral and mesenteric arterial strips, relaxant effects of acetylcholine, isoprenaline and K^+ were not significantly influenced by reserpine-pretreatment.

4 It appears that tyramine and nicotine do not release noradrenaline from dog cerebral arteries in amounts sufficient to cause significant contractions. Attenuation of the response to angiotensin II by pretreatment with reserpine is not the result of depletion of noradrenaline from the mesenteric arterial wall but may be due to interference with the mechanism specific to actions of angiotensin II.

Introduction

Reserpine, one of commonly used anti-hypertensive agents, is widely known to deplete noradrenaline from adrenergic nerves. According to the histochemical study by Rosenblum (1973), reserpine depletes noradrenaline less readily from nerves supplying cerebral blood vessels of the rat than from nerves to extracerebral vessels. However, alterations in the function of cerebral vasculatures induced by pretreatment with reserpine as compared to those with extra-cerebral vasculatures have not been clarified.

It has been demonstrated that responses of dog cerebral arteries to nicotine, tyramine and 5-hydroxytryptamine are not related to a release of endogenous noradrenaline (Toda, 1975; 1976a; Toda, Hayashi, Fu & Nagasaka, 1976; Toda, Hayashi & Hattori, 1978b), while the responses of peripheral arteries to nicotine and tyramine are mainly mediated by released noradrenaline. Studies on isolated arteries from which endogenous noradrenaline is depleted may provide further information concerning the mechanism of action of these amines in different arteries.

Thus, the aim of the present study was to compare the noradrenaline-depleting action of reserpine in dog cerebral and mesenteric arteries, to determine responses to vasoactive agents of cerebral arteries from reserpine-treated dogs, and to clarify different influences of pretreatment with reserpine on the responsiveness of cerebral and mesenteric arteries. Responses of arteries isolated from control dogs were also obtained for comparison.

Methods

Mongrel dogs of either sex, weighing 8 to 16 kg, were anaesthetized with intraperitoneal injections of sodium pentobarbitone in a dose of 50 mg/kg, and were killed by bleeding from common carotid arteries. Twenty dogs were pretreated 20 to 24 h before the start of experiments with intramuscular injections of 0.5 mg/kg reserpine. The brain and distal portion of the superior mesenteric artery (0.6 to 0.8 mm outside diameter) were rapidly removed. Basilar and middle cerebral arteries (0.6 to 0.8 mm) were isolated from the brain. The arteries were cut helically into strips,

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the length being approximately 20 mm. The specimen was fixed vertically between hooks in the muscle bath containing the nutrient solution, which was aerated with a mixture of 95% O_2 and 5% CO_2, and was maintained at 37 ± 0.5°C. The hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer (Nihonkoden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 1.5 g, which has been reported to be optimal for inducing the maximum contraction (Toda, Hatano & Hayashi, 1978a). Constituents of the solution were as follows (mM): Na^+ 162.1, K^+ 5.4, Ca^{2+} 2.2, Mg^{2+} 1.0, Cl^- 159.0, HCO_3^- 14.9, and dextrose 5.6. The pH of the solution was 7.2 to 7.3. Osmotic adjustment was not made when K^+ up to 30 mM was added. Before the start of experiments, preparations were allowed to equilibrate for 90 to 120 min in the bathing media, during which time the fluids were replaced every 10 to 15 min.

Isometric contractions and relaxations were displayed on an ink-writing oscillograph (Sanei Sokki Co., Tokyo, Japan). Contractile responses to 30 mM K^+ were first obtained in all preparations. The K^+-induced contraction was not significantly influenced by pretreatment with reserpine; mean absolute values obtained with control and reserpine-pretreated cerebral arteries were 1628 ± 126 mg (n = 29) and 1810 ± 189 mg (n = 20), respectively, and those with the mesenteric arteries were 2561 ± 288 mg (n = 16) and 2526 ± 227 mg (n = 14), respectively. After the preparations were repeatedly washed and equilibrated for 40 to 50 min in control media, dose-response curves were obtained by adding drugs, including noradrenaline, tyramine, 5-hydroxytryptamine, acetylcholine and isoprenaline, directly to the bathing media in cumulative concentrations. Responses to a single dose of nicotine (10^{-4} M) or angiotensin II (10^{-7} M) were obtained. Tachyphylaxis developed with repeated trials of these drugs; therefore, responses in the first series of experiments were compared in arteries obtained from control and reserpine-treated dogs. Arterial strips were contracted with prostaglandin $F_{2\alpha}$ (5×10^{-7} to 2×10^{-6} M) before acetylcholine, isoprenaline, nicotine or small amounts of K^+ (5 mM) were added. At the end of experiments with vasodilators, papaverine in a concentration of 10^{-4} M which is sufficient to cause the maximum relaxation (Toda, 1974a) was added, and relaxations relative to those induced by papaverine were examined.

Mesenteric arterial strips (15 from control dogs and 13 from reserpine-treated dogs) were placed between a pair of platinum stimulating electrodes (Toda, 1971). The gaps between the electrodes and the strip were wide enough to allow undisturbed contraction and yet sufficiently narrow to permit stimulation of intramural nerves effectively. The arterial strips were transmurally stimulated by a train of 0.3 ms square pulses of supramaximal intensity, at frequencies of 2, 5, 20, 50 and 100 Hz for 100, 40, 10, 4 and 2 s, respectively. Thus, the total number of stimulus pulses was kept constant (200 pulses). Electrical pulses were delivered from an electronic stimulator (Nihonkoden Kogyo Co.).

Posterior cerebral arteries, posterior communicating arteries of the circulus arteriosus cerebri (Willis) and superior mesenteric arteries close to the portion used for the study with tension recordings were rapidly isolated, cleaned, blotted and weighed. The arteries were homogenized in 0.4 N perchloric acid for the assay of noradrenaline and adrenaline. These amines were extracted by the method of Anton & Sayre (1962) with minor modification and assayed fluorometrically.

Results shown in the text, figures and tables are expressed as mean values ± s.e. mean. Statistical analyses were made using Student's t test. Drugs used were reserpine (Apoplon, Daiichi Pharmaceutical Co., Tokyo), (±)-noradrenaline hydrochloride, tyramine hydrochloride, 5-hydroxytryptamine creatinine sulphate, nicotine, angiotensin II, acetylcholine chloride, (±)-isoprenaline hydrochloride, phentolamine mesylate, tetrodotoxin (Sankyo Co., Tokyo) and prostaglandin $F_{2\alpha}$ (Ono Pharmaceutical Co., Osaka).

Results

Noradrenaline content of cerebral and mesenteric arteries

Dog cerebral and mesenteric arteries contained similar amounts of noradrenaline. Average values of the noradrenaline content and the wet weight of cerebral arteries were 3.21 ± 0.34 μg/g wet tissue wt. and 26.7 ± 2.4 mg (n = 11), respectively, and those in mesenteric arteries were 3.57 ± 0.53 μg/g and 33.0 ± 4.8 mg (n = 11), respectively. Measurable amounts of adrenaline were not detected in these arteries. Pretreatment of dogs for 20 to 24 h before experiments with 0.5 mg/kg reserpine diminished the noradrenaline content in cerebral and mesenteric arteries to 0.21 ± 0.06 μg/g tissue wt. and 0.10 ± 0.03 μg/g, respectively, the diminution being greater in mesenteric (97.2%) than in cerebral arteries (93.5%).

Contractile responses

Noradrenaline: The addition of noradrenaline in concentrations ranging from 2×10^{-8} to 5×10^{-5} M caused a dose-related contraction of dog cerebral and mesenteric arterial strips. The contraction of mesenteric arteries was greater than that of cerebral arteries (Figure 1). Dose-response curves for noradrenaline in

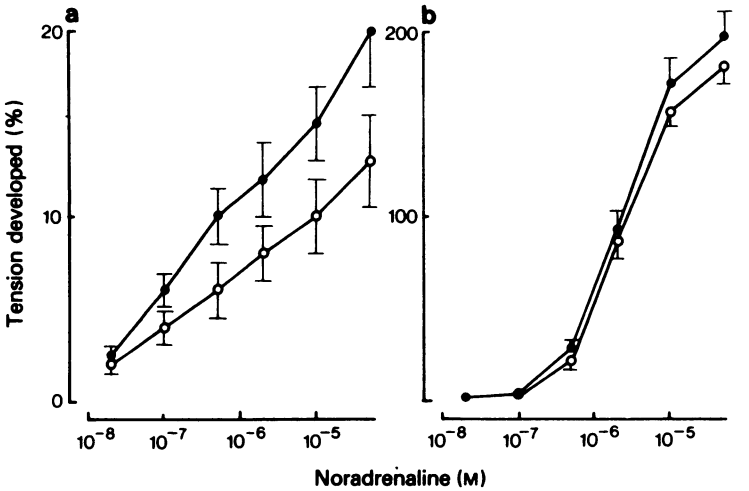

Figure 1 Dose-response curves for noradrenaline in cerebral (a) and mesenteric arteries (b) isolated from control (●) and reserpine-treated (○) dogs. Contractions induced by 30 mM K^+ were taken as 100%; mean absolute values in cerebral and mesenteric arteries are shown in Table 1. In (a) 29 control and 20 preparations from reserpine-treated dogs were used; in (b) 16 control and 14 reserpine-pretreated preparations were used.

cerebral and mesenteric arteries were not significantly influenced by pretreatment of dogs with reserpine. Noradrenaline-induced contractions relative to those induced by 30 mM K^+ are shown in Figure 1. Absolute values of the contraction induced by the maximum concentration of noradrenaline were not significantly different in arteries from control and reserpine-treated dogs (Table 1).

Tyramine: Pretreatment of dogs with reserpine attenuated the contractile response of mesenteric arterial strips to tyramine but did not significantly inhibit the response of cerebral arteries (Figure 2).

Transmural stimulation: Transmural electrical stimulation applied to mesenteric arterial strips at frequencies of 2 to 20 Hz elicited a frequency-dependent, transient contraction, and further increase in the frequency to 100 Hz reduced the response. The frequency-response curve was suppressed by reserpine-pretreatment: contractile responses at 2, 5 and 20 Hz in control preparations were 8.9 ± 1.7, 16.8 ± 2.7 and 38.7 ± 4.0% (n = 15), respectively, relative to those induced by 30 mM K^+, and those in arteries from reserpine-treated dogs were 0.6 ± 0.2, 1.5 ± 0.5, and 4.4 ± 1.0% (n = 13), respectively. The response of control mesenteric arteries to transmural stimulation

Table 1 Mean absolute values of contractions obtained in preparations from control and reserpine-treated dogs

		Control		*Reserpine-pretreatment*		
Artery	*Drug and dose*	n	*Contraction (mg)*	n	*Contraction (mg)*	P<
Cerebral	Noradrenaline 5×10^{-5} M	29	336 ± 49	20	207 ± 29	NS
	Tyramine 10^{-4} M	27	371 ± 62	27	360 ± 67	NS
	5-Hydroxytryptamine 2×10^{-6} M	15	2020 ± 235	21	1792 ± 123	NS
Mesenteric	Noradrenaline 5×10^{-5} M	16	4716 ± 469	14	4378 ± 291	NS
	Tyramine 10^{-4} M	15	1812 ± 267	15	172 ± 50	0.001
	Transmural stimulation 20 Hz	15	1040 ± 178	13	103 ± 21	0.001
	Nicotine 10^{-4} M	13	565 ± 180	11	21 ± 9	0.02
	Angiotensin II 10^{-7} M	15	2684 ± 269	22	1976 ± 200	0.05

n number of preparations used. NS, not significant.

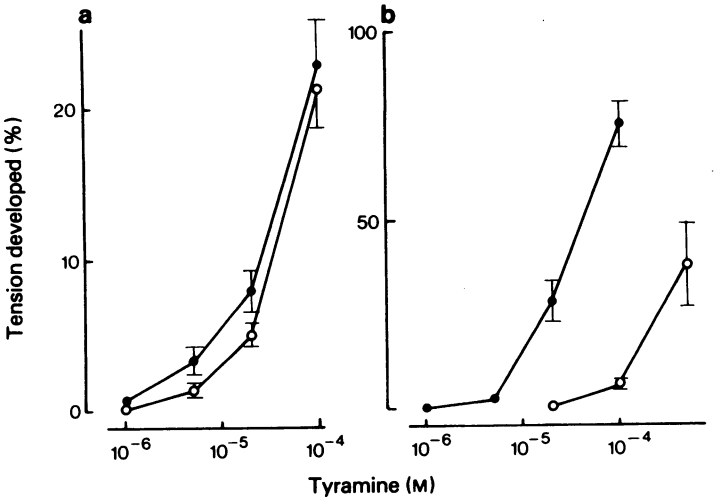

Figure 2 Dose-response curves for tyramine in cerebral (a) and mesenteric arteries (b) isolated from control (●) and reserpine-treated (○) dogs. Contractions induced by 30 mM K^+ were taken as 100%; mean absolute values in cerebral arteries from control and reserpine-treated dogs were 1634 ± 127 mg (n = 27) and 1856 ± 181 mg (n = 27), respectively (a), and those in mesenteric arteries from control and reserpine-treated dogs were 2475 ± 307 mg (n = 15) and 2512 ± 212 mg (n = 15), respectively (b).

was abolished by 10^{-6} M phentolamine or 10^{-7} M tetrodotoxin. In all of the 11 cerebral arterial strips from control dogs, the same frequency range of electrical stimulation failed to cause any significant contractions.

Nicotine: The addition of 10^{-4} M nicotine relaxed the basilar and middle cerebral arteries contracted with prostaglandin $F_{2\alpha}$, the relaxation being significantly greater in the latter arteries (13.4 ± 3.3 vs 46.7 ± 5.6%, n = 10, relative to relaxations induced by 10^{-4} M papaverine; $P < 0.01$). The nicotine-induced relaxation was not significantly altered by pretreatment of dogs with reserpine. In mesenteric arterial strips, nicotine in a concentration of 10^{-4} M caused a contraction (565 ± 180 mg, n = 13), which was abolished by pretreatment with reserpine.

Angiotensin II: Angiotensin II in a concentration of 10^{-7} M, sufficient to cause a maximum contraction (Toda, Hayashi & Miyazaki, 1978c), contracted cerebral arteries only slightly and this action was unaffected by reserpine pretreatment. In contrast, the same concentration of angiotensin II elicited a marked contraction of mesenteric arteries (127 ± 13.2%, n = 15, relative to contractions induced by 30 mM K^+) and was significantly attenuated to 74.8 ± 7.1% (n = 22, $P < 0.01$) by pretreatment with reserpine. The mean absolute value of angiotensin-induced contractions of mesenteric arteries from reserpine-pretreated dogs was also significantly smaller than that with control dogs (Table 1).

5-Hydroxytryptamine: Dog cerebral arterial strips responded to 5-hydroxytryptamine with a large contraction. Neither the 5-hydroxytryptamine-induced contractions relative to those induced by 30 mM K^+ nor the absolute maximum contractions induced by 2×10^{-6} M 5-hydroxytryptamine were altered by pretreatment with reserpine (Table 1).

Relaxation responses

Acetylcholine: Relaxations induced by acetylcholine were slightly different in basilar and middle cerebral arteries contracted with prostaglandin $F_{2\alpha}$; only basilar arteries responded to low concentrations (10^{-7} and 10^{-6} M) of acetylcholine (Toda, 1979). Relaxations induced by acetylcholine of basilar and mesenteric arteries were unaffected by pretreatment of dogs with reserpine.

Isoprenaline: The relaxant effect of isoprenaline on both cerebral and mesenteric arteries isolated from 15 control and 18 reserpine-treated dogs did not differ significantly.

Potassium: Relaxations of cerebral arteries induced by 5 mM K^+ have been postulated to derive from stimulation of the electrogenic Na^+ pump (Toda, 1974b;

1976b). The K^+-induced relaxations of basilar and middle cerebral arteries were not significantly different. Reserpine-pretreatment failed to alter the relaxation.

Discussion

Pretreatment of dogs for 20 to 24 h before experiments with reserpine diminished the content of noradrenaline in cerebral and mesenteric arteries, the diminution being less in cerebral arteries. A similar tendency has been demonstrated in a histochemical study on rat cerebral and femoral vessels and portal veins (Rosenblum, 1973). The amine-depleting action of reserpine is different in short and long adrenergic neurones (Owman & Sjöberg, 1967) and also in differently-activated adrenergic nerves (Weiner, Perkins & Sidman, 1962; Hertting, Axelrod & Patrick, 1962). Adrenergic nerves to cerebral pial arteries are supplied from cervical sympathetic ganglia; thus, the cerebral arteries used in the present study as well as mesenteric are innervated by long adrenergic neurones. Therefore, less depletion of noradrenaline from cerebral pial arteries may derive from a lesser degree of tonic activity of adrenergic nerves to cerebral arteries than to peripheral arteries, as postulated from studies on sympathetic denervation in dogs, cats and monkeys (Heistad, Marcus & Gross, 1978) and α-methyl tyrosine-treated rats (Rosenblum & Chen, 1977).

Despite a marked decrease in the noradrenaline content and a diminution of the contractile response of mesenteric arteries to tyramine and transmural electrical stimulation following pretreatment with reserpine, the response of cerebral arteries to tyramine was unaffected, suggesting that an indirect, noradrenaline-mediated action is not significantly involved in the cerebroarterial contraction induced by tyramine. The same conclusion has been drawn from results with dog cerebral arterial strips treated with cocaine (Toda *et al.*, 1978b).

It has been demonstrated that the vascular sensitivity to noradrenaline is increased (Macmillan, Smith & Jacobson, 1962; Baum, 1963), unaffected (Abboud & Eckstein, 1964) or decreased (Withrington & Zaimis, 1961) by pretreatment with reserpine. The reserpine-induced sensitivity change is dependent on extracellular concentrations of Ca^{2+} (Pegram & Carrier, 1969), doses of reserpine (Pegram & Carrier, 1969) and period of reserpine-pretreatment (Fleming & Trendelenburg, 1961; Trendelenburg & Weiner, 1962). In the present study, the content of noradrenaline in cerebral and mesenteric arteries was diminished by pretreatment of dogs for 20 to 24 h beforehand with injections of 0.5 mg/kg reserpine. However, in these arteries which were exposed to solutions containing 2.2 mM Ca^{2+}, no supersensitivity to noradrenaline developed. Lack of the supersensitivity in the present study is not associated with an augmentation of relaxation mediated by β-receptor activation, since isoprenaline-induced relaxations were unaffected by pretreatment with reserpine.

Pretreatment with reserpine decreases the Ca^{2+} content of vascular tissue of rabbits (Carrier & Shibata, 1967). However, in mesenteric or cerebral arteries isolated from control and reserpine-treated dogs, absolute and relative contractions induced by noradrenaline, 5-hydroxytryptamine and K^+ were not significantly different. Contractility and Ca^{2+} availability of the arterial smooth muscles may not be affected by such an acute pretreatment of dogs with reserpine. Further, the data obtained in the present study and those with phentolamine and cocaine (Toda *et al.*, 1976) indicate that 5-hydroxytryptamine-induced contractions in dog cerebral arteries are not related to the α-adrenergic mechanism via a release of noradrenaline but to a direct stimulation of tryptamine receptors.

Nicotine-induced relaxations seen in basilar and middle cerebral arteries were neither potentiated nor inhibited by pretreatment with reserpine. This finding indicates that nicotine does not appear to release noradrenaline from adrenergic nerves in a sufficient amount to cause significant contractions, which possibly counteract the relaxant effect of nicotine, and that the β-adrenergic mechanism is not involved in the nicotinic action. The latter has been postulated from experiments showing unresponsiveness of cerebroarterial relaxations induced by nicotine to an effective concentration of sotalol (Toda, 1975). Relaxations induced by nicotine were significantly greater in middle cerebral arteries than in basilar, suggesting uneven distribution of, or different responsiveness to, proposed vasodilator substance(s) which is released from dog cerebral arteries by nicotine (Toda, 1975).

Regional differences in the arterial response to angiotensin II have been demonstrated (Toda & Miyazaki, 1978), and the present study revealed that reserpine-pretreatment attenuated the contractile response of mesenteric arteries to angiotensin II. Arterial contractions induced by the peptide in a concentration used here are not influenced by 10^{-6} M phentolamine; thus, the α-adrenergic mechanism is not involved (Toda, *et al.*, 1978c). Therefore, the attenuation of the responses to the peptide is not due to the diminution by reserpine of noradrenaline content but possibly to interference with the mechanism specific to actions of angiotensin II, since the other vasoconstrictor agents, including K^+, noradrenaline and 5-hydroxytryptamine, caused similar contractions in the arteries isolated from control and reserpine-pretreated dogs. Whether or not such an action of reserpine is related to the hypotensive effect remains to be ascertained.

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