ANALYSIS OF THE CONTRACTILE RESPONSE TO SEROTONIN AND TRYPTAMINE OF ISOLATED DOG CEREBRAL, FEMORAL AND MESENTERIC ARTERIES

L. H. Wang FU and Noboru TODA*

Department of Pharmacology, Shiga University of Medical Sciences, Seta, Ohtsu 520-21, Japan

Accepted December 9, 1982

Abstract—In helically-cut strips of cerebral arteries isolated from dogs, serotonin, tryptamine, 5-hydroxytryptophan and tryptophan caused a dose-related contraction. The potency was in the order of serotonin>tryptamine>5-hydroxytryptophan=tryptophan. In femoral arterial strips, only serotonin and tryptamine produced contractions. In cerebral arteries, the dose-response curve for serotonin was shifted to the right and downward by treatment with cinanserin, whereas in femoral and mesenteric arteries, the curves were shifted to the right. The contractile response of cerebral arteries to tryptamine was attenuated by cinanserin in concentrations above 10^{-7} M; however, 10^{-5} M was required to significantly reduce the response of femoral arteries. Phentolamine reduced the contractile response of femoral arteries to tryptamine, but not the response of cerebral arteries. It may be concluded that the different antagonism of cinanserin against the serotonin action on cerebral and femoral arteries is due to the ability of high concentrations of serotonin to induce relaxations of cerebral but not femoral arteries or to the different nature of receptors. Tryptamine appears to elicit contractions of cerebral arteries via a stimulation of tryptamine receptors, but elicit those of femoral arteries via stimulation of both alpha-adrenergic and tryptamine receptors. Whether or not receptors for serotonin and tryptamine are the same was not determined.

Serotonin, a potent constrictor of cerebral arteries (1), is synthesized from dietary tryptophan, which is first hydroxylated to 5-hydroxytryptophan and then decarboxylated. Wooley and Shaw (2) and Allen et al. (3) have studied contractile responses of isolated sheep carotid and dog basilar arteries to a variety of serotonin analogs. Fu and Toda (4) have reported the structure-activity relationship of analogs of 5-hydroxykynurenamine, a serotonin metabolite, in isolated dog cerebral arteries. The existence of specific serotonergic receptors mediating contractions of cerebral blood vessels has been postulated (5-9). Vargaftig and Lefort (10) suggested the presence of two types of serotonergic receptors in dog nasal vessels which mediate vasodilatation and vasoconstriction. The mechanism of action of tryptamine is not necessarily the same as that of serotonin, and different mechanisms of action of these amines may be involved in the genesis of contractions in cerebral and peripheral arteries.

The present study was thus undertaken to compare the effect of serotonin, tryptamine and precursor amino acids on dog cerebral, femoral and mesenteric arteries and to clarify the antagonistic effect of cinanserin, methysergide and phentolamine on arterial contractions induced by serotonin and tryptamine.

* To whom all correspondence should be addressed.
Materials and Methods

Mongrel dogs of either sex, weighing 7 to 15 kg, were anesthetized with ip. injections of 50 mg/kg sodium pentobarbital and were sacrificed by bleeding from the common carotid arteries. The brain was rapidly removed, and basilar and middle cerebral arteries (0.7 to 0.8 mm outside diameter) were isolated. Distal portions of the femoral and mesenteric arteries (0.7 to 0.8 mm) were also isolated. The arteries were cut helically into strips, approximately 20 mm long. The strips were vertically fixed between hooks under a resting tension of 1.5 g (11) in a muscle bath containing the nutrient solution, which was aerated with a mixture of 95% O2 and 5% CO2 and was maintained at 37±0.5°C. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer (Nihonkoden Kogyo Co., Tokyo). The solution had the following composition (mM): Na+, 162.1; K+, 5.4; Ca++, 2.2; Mg++, 1.0; Cl-, 153.9; HCO3-, 20.0; and dextrose, 5.6. The pH of the solution was approximately 7.3. Before starting the experiments, all preparations were allowed to equilibrate in control media for 90 to 120 min, during which time the fluids were replaced every 10 to 15 min.

Isometric contractions were displayed on an ink-writing oscillograph (Sanei Sokki Co., Tokyo). The contractile response to 30 mM K+ was first obtained. The preparations were then washed three times with fresh media and equilibrated for 40 to 50 min. Agonists were added directly to the bathing media in cumulative concentrations. Contractions induced by serotonin or tryptamine relative to those induced by 30 mM K+ are presented. Preparations were exposed for 20 min to blocking agents such as cinanserin, methysergide and phentolamine, before dose-response relationships for serotonin or tryptamine were obtained. PA2 values were obtained by plotting log [dose ratio - 1] against log doses of antagonists. The dose ratio was estimated as a ratio of median effective concentrations (ED50’s) of agonists in the absence and presence of antagonists. The dissociation constant of antagonists (Kb value) was calculated from the equation, 

\[ K_b = \frac{[B]}{\text{dose ratio} - 1} \]

where [B] is the concentration of antagonist (12). Results shown in the text and figures are expressed as the mean values ±S.E.M. Statistical analyses were made using the Student’s t-test.

Drugs used were serotonin creatinine sulfate, tryptamine hydrochloride, 5-hydroxy-dl-tryptophan, l-tryptophan, methysergide bimaleate, cinanserin hydrochloride (Squibb and Sons, Inc., New Jersey), prostaglandin F2α (Ono Pharmaceutical Co., Osaka), dl-norepinephrine hydrochloride, cocaine hydrochloride, bretylium tosylate, dl-propranolol hydrochloride, phentolamine mesylate, aminophylline, atropine sulfate and aspirin.

Results

Contractile responses to serotonin, tryptamine, 5-hydroxytryptophan and tryptophan: The addition of serotonin (10−5 to 2 × 10−6 M), tryptamine (2 × 10−5 to 2 × 10−4 M), 5-hydroxytryptophan (2 × 10−6 to 2 × 10−4 M) and tryptophan (10−5 to 2 × 10−4 M) to helical strips of dog cerebral arteries caused a dose-related contraction. Serotonin in concentrations of 10−5 M or higher produced relaxations (Fig. 1, left). Maximum contractions induced by serotonin (2 × 10−6 M) and tryptamine (2 × 10−4 M) were approximately the same. On the basis of apparent median effective concentrations (ED50’s), serotonin was 1/34 as potent as serotonin ([1.8±0.4] × 10−6 M, N=8, vs. [5.3±1.1] × 10−8 M, N=17). Tryptophan and 5-hydroxytryptophan produced only a slight contraction (Fig. 1, left). The ED50 values of these compounds were [8.4±0.8] × 10−6 M (N=8) and
Serotonin (2 x 10^-8 to 10^-6 M) and tryptamine (2 x 10^-6 to 2 x 10^-4 M) contracted femoral arterial strips in a dose-dependent manner, while tryptophan and 5-hydroxytryptophan even in a high concentration (2 x 10^-4 M) did not evoke contractions (Fig. 1, right). Maximum contractions induced by 10^-5 M serotonin and 2 x 10^-4 M tryptamine did not significantly differ. The average ED50 value of serotonin ([2.6±1.9] x 10^-7 M, N=7) was 1/138 the value of tryptamine ([3.6±1.9] x 10^-5 M, N=3).

Modification by antagonists of the responses to serotonin and tryptamine: In cerebral arterial strips, the dose-response curve for serotonin was shifted to the right and downward by treatment with cinanserin in concentrations of 3 x 10^-8, 10^-7 and 10^-6 M (Fig. 2, left), while the curve in femoral and mesenteric arteries was shifted to the right (Fig. 2, middle and right). Cinanserin alone did not alter the tension. Average pA2 values for femoral and mesenteric arteries were 7.96 and 8.38, respectively, and the slopes of the regression line were -1.02 and -0.88, respectively. Treatment of femoral arteries with methysergide (10^-8, 10^-7 and 10^-6 M) shifted the dose-response curve for serotonin to the right; the pA2 value was 8.21, and the slope of the line was -0.98. Methysergide alone did not alter the tension of femoral arteries.

Contractions of cerebral arteries induced by serotonin in concentrations up to 2 x 10^-6 M, and relaxations seen in concentrations higher than 10^-5 M were not significantly influenced by 10^-6 M propranolol (N=4), 10^-6 M atropine (N=3), 2 x 10^-6 M aminophylline (N=3) and 5 x 10^-6 M aspirin (N=3).

In helical strips of cerebral arteries, treatment with 10^-7 M cinanserin did not significantly alter the response to tryptamine; however, increase in concentrations of
Fig. 2. Modification by cinanserin (CS) of the response to serotonin in cerebral (left figure), femoral (middle) and mesenteric arteries (right). Maximum contractions induced by serotonin in control media were taken as 100%; mean absolute values in cerebral, femoral and mesenteric arteries were 1769±278 mg (N=15), 3662±671 mg (N=14) and 2602±444 mg (N=12), respectively. Numbers in parentheses indicate the number of preparations used.

Fig. 3. Modification by cinanserin (CS) of the contractile response to tryptamine in cerebral (left figure) and femoral arteries (right figure). Maximum contractions induced by tryptamine in control media were taken as 100%; mean absolute values in cerebral and femoral arteries were 1269±154 mg (N=15) and 4230±503 mg (N=13), respectively. a, Significantly different from controls, P<0.001; b, P<0.01; c, P<0.05. Numbers in parentheses indicate the number of preparations used.
cinanserin to $10^{-6}$ and $10^{-5}$ M shifted the dose-response curve to the right (Fig. 3, left). The $K_B$ value estimated from the results with $10^{-5}$ M cinanserin was $6.3 \times 10^{-7}$ M. Treatment of femoral arteries with $10^{-6}$ M cinanserin slightly attenuated the contractile response to tryptamine only in low concentrations ($2 \times 10^{-6}$ and $10^{-5}$ M). Increase in the concentration of cinanserin to $10^{-5}$ M shifted the dose-response curve for tryptamine to the right (Fig. 3, right); the $K_B$ value was $6.8 \times 10^{-6}$ M. Cinanserin ($10^{-6}$ and $10^{-5}$ M) did not alter the contractile response to prostaglandin $F_2\alpha$ of femoral (N=4) and cerebral arteries (N=3).

Treatment for 20 min with $10^{-7}$ M phentolamine did not influence the contraction induced by serotonin in cerebral and femoral arteries nor the response of cerebral arteries to tryptamine (Fig. 4, left). In contrast, phentolamine ($10^{-7}$ M) shifted the dose-response curve for tryptamine to the right in femoral (Fig. 4, right) and mesenteric arteries (N=4). Mean values of the ED50 before and after the treatment with $10^{-7}$ M phentolamine in femoral arteries were $[6.5 \pm 0.5] \times 10^{-5}$ M and $[1.1 \pm 0.1] \times 10^{-4}$ M (N=12), respectively; the values are significantly different (P<0.001). The inhibition was reversed by repeated washing of preparations. In femoral arteries, the contractile response to tryptamine was not reduced by $3 \times 10^{-6}$ M cocaine (N=3) or $2 \times 10^{-5}$ M bretylium (N=3).

**Discussion**

The potency for inducing contractions of helical strips of isolated dog cerebral arteries was in the order of serotonin>tryptamine>5-hydroxytryptophan=tryptophan. Contraction of cerebral arteries induced by kynurenamine were also less than those induced by its 5-hydroxy analogue (4). 5-Hydroxylation of the indole ring appears to play a significant role in raising the contractile potency. Both 5-hydroxytryptophan and tryptophan, having a carboxyl group in the ethylamine side chain, showed only a weak agonistic action: 5-hydroxyindoleacetic acid, in which the ethylamine side chain in serotonin is substituted with acetic acid, did...
not show serotonin-like actions (13). The acidic group in the side chain appears to diminish the action on serotonergic receptors, whereas the hydroxy group in the side chain such as that in 5-hydroxytryptophol (14) retains the ability to contract the arteries.

Treatment of cerebral arteries with cinanserin attenuated the contractile response to serotonin in a dose-related manner as did methysergide, ergotamine and LSD (8), but it did not influence contractions induced by prostaglandin F₂α. The dose-response curve for serotonin was shifted to the right and downward by these antagonists. The lack of a parallel shift in cerebral arteries may be due to the relaxant effect of serotonin in high concentrations (Fig. 1, left) or to different nature of receptors from those in femoral and mesenteric arteries. According to Vargaftig and Lefort (10), dog nasal vessels respond to serotonin with constriction and dilatation, which are suggested to be mediated by two different receptors; the vasodilator response is blocked by cyproheptadine and methysergide. However, methysergide, like cinanserin in the present study, does not elicit a parallel shift of the dose-response curve for serotonin in cerebral arteries, but it attenuates the maximum contraction (8). Coronary vasodilatation mediated by serotonergic receptors has also been reported (15). The possibility of an involvement of β-receptor activation by serotonin (9) was excluded in the present study with isolated dog cerebral arteries since propranolol did not potentiate the serotonin-induced contraction nor inhibited the relaxation induced by high concentrations of serotonin. Further, treatment with atropine, aminophylline and aspirin in concentrations sufficient to significantly attenuate the response to respective agonists (16-18) failed to inhibit the relaxation induced by serotonin, suggesting that muscarinic, adenosine-related and prostaglandin-related mechanisms are not involved.

In contrast to the data on cerebral arteries, dose-response curves for serotonin in femoral and mesenteric arteries were shifted to the right by cinanserin and methysergide. Slopes of the regression line of log [dose ratio -1] against log dose of the antagonists were roughly 1. These findings suggest that the antagonism is competitive. Similar results were obtained in the isolated external maxillary and lingual arteries of cats (9). The pA₂ value of methysergide in dog femoral arteries (8.21) was consistent with the value (8.21) in external maxillary and lingual arteries (9).

The dose-response curve for tryptamine in cerebral arteries was shifted to the right by cinanserin. Tryptamine did not produce relaxations, even when the concentration was raised to 2×10^{-4} M. Treatment with phentolamine did not alter the response to tryptamine. It seems likely that cinanserin competitively antagonizes the action of tryptamine on receptors which mediate the cerebroarterial contraction. There is evidence that receptors for serotonin and tryptamine may be either different or at least differentially accessible (19). This hypothesis is supported by the different ability of cinanserin to antagonize the responses to serotonin (Fig. 2, middle) and tryptamine (Fig. 3, right) in femoral arteries.

In femoral arterial strips, contractions induced by tryptamine were attenuated only slightly by cinanserin, the attenuation being approximately 1/10 that seen in cerebral arteries, but were moderately reduced by 10^{-7} M phentolamine. The tryptamine-induced contraction was not influenced by cocaine in concentrations sufficient to suppress the arterial response to nicotine and tyramine (20, 21) and also not influenced by bretylium in concentrations sufficient to suppress the response to transmural sympathetic nerve stimulation (22). These
findings lead us to conclude that tryptamine appears to act directly on $\alpha$-adrenoceptors and to a lesser extent on tryptamine receptors. Dog cerebral arteries respond to norepinephrine with only a slight contraction (1, 23, 24); therefore, even though tryptamine activates $\alpha$-receptors in cerebral arteries, the induced contraction would be negligible in comparison with the contraction caused by the stimulation of tryptamine receptors.

Acknowledgements: This work was supported in part by Scientific Research Fund 56480102 from the Ministry of Education, Science and Culture of Japan. The authors thank Mr. S. Hayashi for technical assistance.

References

1) Toda, N. and Fujiita, Y.: Responsiveness of isolated cerebral and peripheral arteries to serotonin, norepinephrine and transmural electrical stimulation. Circ. Res. 33, 96–104 (1973)


23) BOHR, D.F., GOULET, P.L. and TAGUINI, A.C., Jr.
Direct tension recording from smooth muscle of resistance vessels from various organs. Angiology 12, 478–485 (1961)