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Effects of 5-Hydroxykynurenamine, a New Serotonin Metabolite, on Isolated Dog Basilar Arteries

(serotonin/amine/antagonist/artery)

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ABSTRACT **Serotonin and 5-hydroxykynurenamine caused dose-related contractions in the spiral strips of dog basilar arteries. The potency of 5-hydroxykynurenamine was approximately 1/100 that of serotonin, the former frequently causing a transient relaxation preceding the contraction. The contractile responses to 5-hydroxykynurenamine and serotonin were attenuated by methysergide. Treatment with 5-hydroxykynurenamine inhibited the response to serotonin, and this inhibitory effect was not completely reversed by removal of 5-hydroxykynurenamine from the bathing medium. The contractile response to K^+ was only slightly attenuated by high concentrations of 5-hydroxykynurenamine. It appears that 5-hydroxykynurenamine and serotonin share receptors in dog basilar arteries and that the effect of serotonin is specifically antagonized by 5-hydroxykynurenamine.**

5-Hydroxykynurenamine (5-HK) was first found in mouse urine by Makino (1), who suggested that it was biologically active (2). Recently, Hayaishi and Hirata (3, 4) isolated from rabbit brain and intestine a new tryptophan 2,3-dioxygenase which catalyzes the conversion of serotonin to 5-hydroxyformylkynurenamine, the latter being hydrolyzed to 5-HK by formamidase. They suggested that this process may be one of the major pathways of serotonin metabolism in the body. The present study was undertaken to determine and compare the effects and investigate interactions of 5-HK and serotonin on isolated dog basilar arteries, which are sensitive specifically to serotonin (5).

MATERIALS AND METHODS

Twenty-one mongrel dogs of both sexes were used. The method used has been described (6). Basilar arteries (0.3–0.5 mm outer diameter) were cut spirally into strips and then fixed vertically under a resting tension of 1.5 g in a muscle bath containing modified Ringers' solution. The solution was maintained at 37 ± 0.5° and gased with 95% O_2 and 5% CO_2. The upper end of the strips was connected to the lever of a force-displacement transducer.

5-HK hydrobromide was synthesized as follows. Melatonin was oxidized with sodium metaiodate in aqueous methanol containing sodium acetate, to give 3-acetamido-2′-formamido-5′-methoxypropiophenone which was hydrolyzed by boiling in hydrobromic acid. 5-HK hydrobromide was used unless otherwise mentioned. Serotonin creatinine sulfate (Wako chemicals), methysergide bimaleate (Deseril®, Sandoz), and *dl*-propranolol hydrochloride (Sumitomo) were used. 5-HK and serotonin were added directly to the bathing medium in cumulative concentrations at the time when the dose–response curve was obtained.

Abbreviation: 5-HK, 5-hydroxykynurenamine.

RESULTS

Serotonin in concentrations ranging from 10^{-9} to 10^{-5} M caused a dose-related increase in the tension of basilar arterial strips (Fig. 1*A*). Contractile responses to serotonin were reproducible five times when preparations were repeatedly washed and equilibrated in normal solutions for 40–60 min. 5-HK (5×10^{-7} to 5×10^{-5} M) caused slowly developing contractions in a dose-dependent manner (Fig. 1*B*). Dose-response curves of 5-HK and serotonin are compared in Fig. 2. The maximum tension increment caused by 5-HK was approximately 70% the increment by serotonin. Median effective doses of 5-HK and serotonin were 8.4×10^{-6} M and 8.6×10^{-8} M, respectively. Contractile responses to 5-HK and serotonin were markedly attenuated by treatment with 10^{-6} M methysergide.

In five out of eight arterial strips in which the dose-response curve of 5-HK was obtained, the amine produced a transient relaxation preceding the contraction (Fig. 1*B*). Mean values of the relaxation induced by 2×10^{-6}, 10^{-5}, and 5×10^{-5} M 5-HK were 54 ± 24 mg ($n = 3$), 112 ± 30 mg ($n = 5$), and 184 ± 82 mg ($n = 3$), respectively. The relaxation was not influenced by 10^{-6} M propranolol. Further increases in the concentration of 5-HK to 2×10^{-4} M caused a marked, persistent relaxation.

In strips exposed to 10^{-5} M 5-HK for 15–20 min, the dose-response curve of serotonin was moved to the right and downward. The preparations were repeatedly washed with drug-free solutions and allowed to relax to their initial levels of tension. In these preparations the inhibition induced by 5-HK was not completely reversed (Fig. 3*A*). Increasing the concentration of 5-HK to 5×10^{-5} M increased the tension until the maximum tension increment was attained within 10 min. Five out of eight preparations relaxed to approximately their initial levels of tension within 30 min. The dose-response curve of serotonin was obtained after the tension had been stabilized. The response to serotonin was markedly inhibited and the inhibition was slightly reversed by removal of 5-HK from the bathing media (Fig. 3*B*). Further restoration of the effect of serotonin was obtained after additional wash of the preparations. Arterial strips, when treated for 15 min with 2×10^{-4} M 5-HK and repeatedly washed, failed to respond not only to serotonin but also to 5-HK.

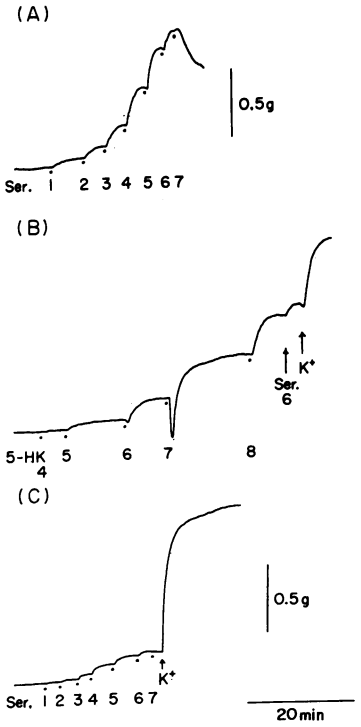

FIG. 1. Responses of a dog basilar artery to serotonin (*Ser.*), 5-HK, and K^+. *1–8:* concentrations of 10^{-9}, 5×10^{-9}, 2×10^{-8}, 10^{-7}, 5×10^{-7}, 2×10^{-6}, 10^{-5}, and 5×10^{-5} M, respectively. Concentration of K^+: 25 mM. The preparation was washed five to seven times with fresh solutions between *A* and *B*, and *B* and *C*.

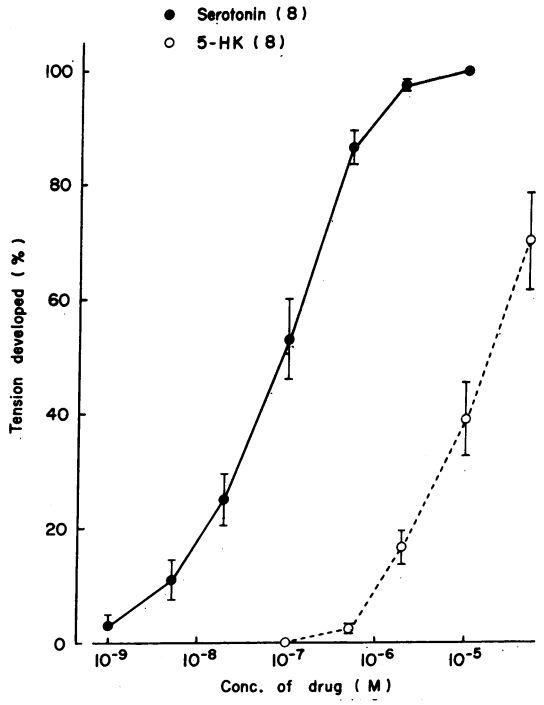

FIG. 2. Dose-response curves of 5-HK and serotonin. Contractile responses to 10^{-5} M serotonin were taken as 100% (mean contraction, 518 ± 96 mg, $n = 8$). Responses to serotonin and 5-HK were obtained with the same preparations. Figures in parentheses indicate the number of preparations used.

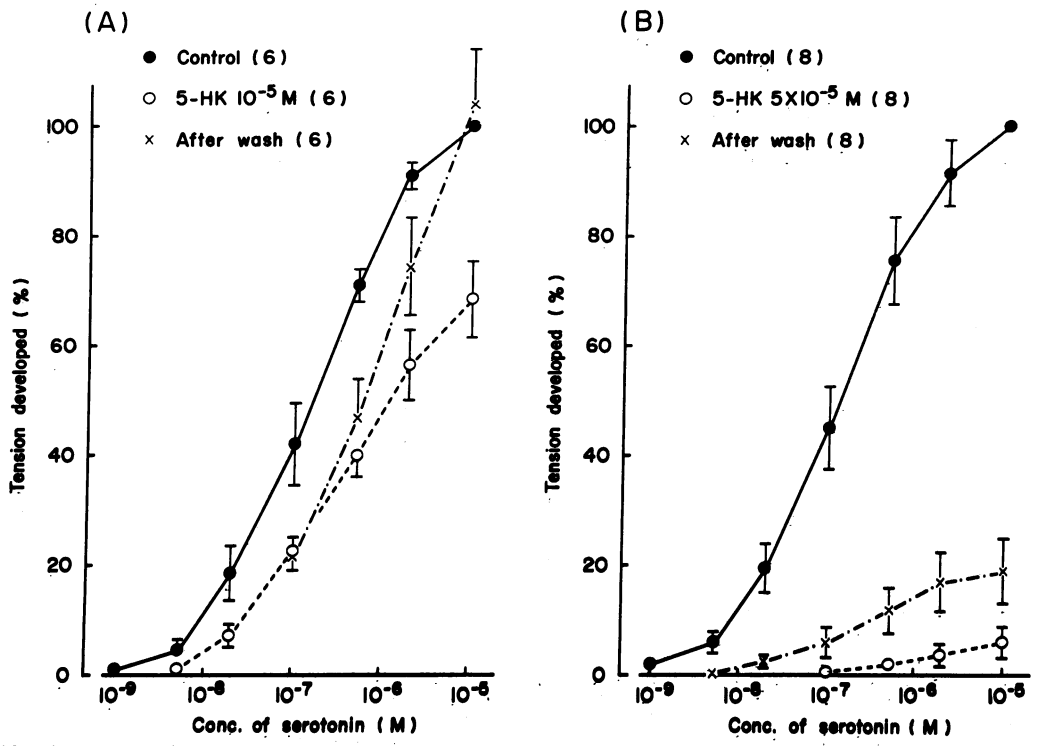

FIG. 3. Modification by 5-HK of the contractile response to serotonin. Responses to 10^{-5} M serotonin in normal solutions were taken as 100%. Mean contractions were 756 ± 102 mg ($n = 6$) for *A* and 876 ± 93 mg ($n = 8$) for *B*. After wash: preparations were washed five to seven times and equilibrated for 40–60 min in normal solutions. Figures in parentheses indicate the number of preparations used. Tension increments by 10^{-5} and 5×10^{-5} M 5-HK before serotonin was added averaged 396 ± 78 mg ($n = 6$) and 142 ± 104 mg ($n = 8$) (see *text*), respectively.

Contractile responses of strips to a submaximum concentration (25 mM) of K^+ (mean contraction, 734 ± 98 mg, $n = 9$) were not influenced by 10^{-5} M 5-HK but were attenuated 25.1 ± 4.6% by 5×10^{-5} M 5-HK. This attenuation was completely reversed by removal of 5-HK from the bathing media. Fig. 1 demonstrates a typical experiment in that K^+ produced similar contractions before 5-HK addition (1.03-g contraction, not shown in the figure) and after removal of 5-HK (1.10 g, Fig. 1*B*), despite a marked difference in responses to serotonin (compare Fig. 1*A* and *B*).

Arterial strips did not respond to NaBr in concentrations up to 10^{-4} M. No significant difference in the effects of 5-HK hydrobromide and 5-HK hydrochloride was observed.

DISCUSSION

5-Hydroxykynurenamine caused dose-dependent contractions in dog basilar arteries, the potency being 1/100 that of serotonin. The dose-response curve of 5-HK almost paralleled that of serotonin. Contractile responses to 5-HK were blocked by methysergide, a serotonin antagonist, as were the responses to serotonin. Further, in preparations treated with high concentrations of 5-HK, the responses to both 5-HK and serotonin were diminished. These findings support the hypothesis that 5-HK and serotonin share receptors in dog basilar arteries.

In contrast to serotonin, 5-HK frequently caused a transient relaxation followed by contraction. Similar relaxation induced by low concentrations of K^+ (5–10 mM) has been demonstrated in isolated human and dog basilar arteries (5). A beta-adrenergic mechanism is not involved in the genesis of the relaxation, since propranolol, a beta-adrenergic blocking agent, did not inhibit the relaxation and isoproterenol, a beta agonist, elicited no or only a slight relaxation of basilar arteries even when high concentrations were applied (Toda, unpublished data). Acetylcholine does not produce relaxation (7) but rather causes contraction in isolated cerebral arteries (8).

The dose-response curve of serotonin was moved to the right after removal of 10^{-5} M 5-HK from the bathing medium. Increasing the concentration of 5-HK to 5×10^{-5} M suppressed the serotonin dose-response curve. It appears that 5-HK elicits a block of serotonin receptors which is not easily reversible and that the effect of serotonin is competitively antagonized by the lower concentration of 5-HK but is noncompetitively antagonized at higher concentrations. The fact that 5-HK in concentrations sufficient to markedly attenuate the effect of serotonin produced no or only a slight decrease in the contractile response to K^+ suggests a specific antagonism of 5-HK to serotonin. Whether or not the response to other stimulating agents, such as norepinephrine and histamine, is also specifically antagonized by 5-HK has yet to be determined.

Serotonin is present mainly in the brain, intestine, and blood platelets of mammals, and it has been postulated that it plays a physiologically important role in maintaining the regulatory functions of the brain and intestine (9). If 5-HK can be considered as a physiological metabolic product in tissues where the activities of the metabolizing enzymes are high, excitability of cells in these tissues would be altered when there is an excess accumulation of 5-HK.

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