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Extraneuronal serotonin accumulation in peripheral arteries of the rat

S. Fukuda*, C. Su† and T.J.-F. Lee

Department of Pharmacology, Southern Illinois University, School of Medicine, Springfield (Illinois 62708, USA), 31 January 1986

Summary. Accumulations of serotonin (5-HT) and norepinephrine (NE) were compared in control and 6-hydroxydopamine (6-OHDA) pretreated rat aorta, mesenteric and tail arteries. The distribution of these amines was corrected by subtracting tissue uptake of tritiated sorbitol in the extracellular space. 5-HT greatly accumulated both in control and 6-OHDA pretreated arteries. In contrast, NE accumulation in mesenteric and tail arteries was substantially decreased after 6-OHDA treatment. In the aorta 6-OHDA pretreatment did not affect the accumulation of both amines. These findings suggest that 5-HT accumulation in these arteries is mainly extraneuronal, and NE mainly neuronal. Since the accumulation of 5-HT in the aorta was not influenced by pretreatment with 10 μM NE, the extraneuronal uptake mechanisms for 5-HT and NE appear to be different.
Key words. Serotonin; uptake; vascular smooth muscle; rat.

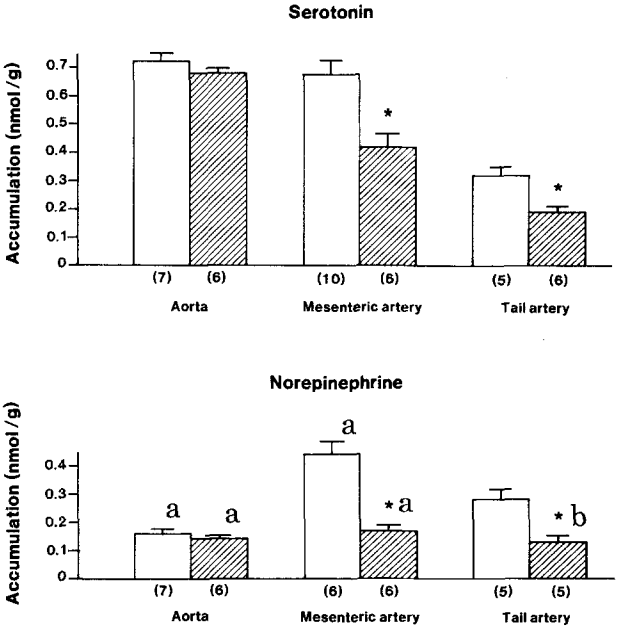
Among vasoactive substances, serotonin (5-HT) has been considered to be one of the most active vasocontractile substances[1]. Its inactivation such as by uptake into specific sites therefore will play an important role in determining the vascular tone. Unlike norepinephrine (NE) which is primarily taken up into sympathetic neuronal tissues in most vascular beds[2], 5-HT uptake into neuronal[3] and/or extraneuronal tissue[4] remains unclear. We have, therefore, examined and compared the accumulation of 5-HT and NE in several control and sympathetically denervated vascular tissues of the rat.

Materials and methods. Male Sprague-Dawley rats weighing 280–330 g were anesthetized with intraperitonial pentobarbital (50 mg/kg), and heparin (2 mg/kg) was injected via inferior vena cava. The aortic, mesenteric and tail arterial preparations were dissected out and kept in a modified Krebs solution bubbled with 95% O_2 and 5% CO_2. The composition of the Krebs solution at 37 °C was as follows (mM): NaCl 122.0; KCl 5.2; $CaCl_2$ 2.4; $MgSO_4$ 1.2; $NaHCO_3$ 25.6; KH_2PO_4 1.2; disodium EDTA 0.03; ascorbic acid 0.1; dextrose 11.0 (pH 7.4). After removing excess surrounding tissues, the specimen was cut open to remove any blood clots and incubated with 0.1 mM pargyline for 30 min. It was then washed with fresh Krebs solution three times every 10 min, and cut into 4-mm-long segments. The tissues were incubated for 5 min with 2 ml Krebs solution containing 0.1 μM [^{3}H]-5-HT or [^{3}H]-NE (16.9 Ci/mmol and 34.0 Ci/mmol, respectively, New England Nuclear, Boston, MA). After incubation, the tissues were blotted between two pieces of filter paper moistened with two drops of Krebs solution and immediately weighed using a Perkin-Elmer electrobalance (AD-2Z). The tissues were digested with 0.5 ml BTS-450 (Beckman) at 37 °C in closed vials. After addition of 4.0 ml of Ready Solv NA (Beckman) to the vials, radioactivity was measured with Beckman liquid scintillation counter (LS-5800). For chemical denervation using 6-OHDA, the tissues were incubated in Krebs solution containing 0.1 mM 6-OHDA for 30 min followed by incubation in 6-OHDA-free Krebs solution for another h at 37 °C[5,6]. The complete adrenergic denervation was confirmed by the disappearance of catecholamine fluorescence[6]. In [^{3}H]-NE uptake experiments, 0.1 mM U-0512, an inhibitor of catechol-O-methyl transferase, was added to the incubation medium.

For determination of extracellular space, tissues were incubated in Krebs solution (37 °C) containing 0.5 μM [^{3}H]-sorbitol (24.0 Ci/mmol, New England Nuclear, Boston, MA) for 5 min. The tissues were then blotted, weighed and digested as described above. The distribution of 5-HT in extracellular space was corrected by subtracting [^{3}H]-sorbital uptake.

The data were expressed as means ± SEM. For the statistical analysis of the data, Student's t-test for unpaired observation was used. P-values less than 0.05 were considered to be significant.

Results. Results of the present study demonstrate that 5-HT is greatly accumulated in the rat aorta, mesenteric and tail arteries (fig.). The accumulations of 5-HT in 6-OHDA pretreated mesenteric and tail arteries remains greater than 50% of the control arteries. The accumulations of 5-HT in 6-OHDA pretreated mesenteric and tail arteries were 61.7 and 59.8% of their respective control arteries. 6-OHDA pretreatment, however, did not affect the 5-HT accumulation in the aorta. Substantial NE accumulation was also found in control mesenteric and tail arteries. Accumulations of NE in aorta however were little. The accumulations of NE in 6-OHDA treated mesenteric and tail arteries were less than 50% (38.2 and 46.5%, respectively) of those in intact arteries. The accumulation of NE in the aorta was not affected by 6-OHDA pretreatment. The accumulations of 5-HT in aorta and mesenteric artery were greater than those of NE in the respective control and 6-OHDA-pretreated arteries (fig.). The accumulations of 5-HT in control and 6-OHDA-pretreated aortae were not influenced by pretreatment of 10 μM NE.



Effects of adrenergic denervation with 6-OHDA on accumulations of 5-HT and NE (0.1 μM, respectively) in rat aortae, mesenteric and tail arteries. Distribution of these amines in the extracellular space was corrected by substracting [^{3}H]-sorbitol uptake. Open column: control, hatched column: 6-OHDA pretreated. *indicates statistically significant from control tissues ($p < 0.01$). [a] ($p < 0.01$); [b] ($p < 0.05$) significantly smaller than the respective values for 5-HT. Parentheses indicate number of experiments.

The extracellular space measured by [^{3}H]-sorbitol in intact and 6-OHDA-treated arteries were 0.50 ± 0.02 ($n = 6$) and 0.47 ± 0.01 ml/g ($n = 5$) in aorta, 0.50 ± 0.01 ($n = 8$) and 0.49 ± 0.01 ml/g ($n = 8$) in mesenteric arteries and 0.48 ± 0.02 ($n = 4$) and 0.47 ± 0.01 ml/g ($n = 5$) in tail arteries, respectively.

Discussion. The exact sites of 5-HT accumulation in vascular walls still remain controversial. It has been reported that 5-HT is taken up by nerve tissues in dog cerebral arteries and saphenous vein[3]. Iwasawa and Gillis[4] reported that the uptake sites of 5-HT and NE are pharmacologically different in rabbit perfused lung tissues. In contrast, Paiva et al.[7] reported that the distribution and uptake sites of 5-HT and NE are essentially the same in the dog saphenous vein. Results of the present study show that in the rat aorta, mesenteric and tail arteries 5-HT accumulation occurs in both neuronal and extraneuronal tissue, but to a greater extent in the latter. This is based on the observation that more than 50% of 5-HT accumulation remains in the adrenergically denervated aorta, mesenteric and tail arteries. Furthermore, adrenergic denervation using 6-OHDA did not significantly affect the accumulation of both 5-HT and NE in the aorta which is known to receive sparse or no sympathetic innervation[8], indicating that accumulation of both amines in the aorta is primarily extraneuronal. In contrast, adrenergic denervation results in less than 50% of NE content in the mesenteric and tail arteries, indicating that NE mainly accumulates in neuronal tissues in these arteries.

The primary finding of the present study is that a greater accumulation of 5-HT occurs in extraneuronal tissue than neuronal tissues in the aorta (sparse adrenergic innervation), mesenteric (moderate adrenergic innervation)[9] and tail arteries (dense adrenergic innervation)[10] of the rat. Preliminary results indicate that removal of endothelial cells in the aorta only slightly inhibits 5-HT accumulation, suggesting that extraneuronal accumulation of this amine probably occurs in medial muscle layers. This is supported by the finding from autoradiographic studies at light microscopic level that 5-HT accumulation is largely associated with smooth muscle cells in the rat aorta (Fukuda et al., unpublished observation). The greater extraneuronal uptake of 5-HT therefore emphasizes the significant role of the vascular smooth muscle in regulating the hemostatic and hemodynamic effects of 5-HT in the rat.

The exact mechanism of extraneuronal accumulation of 5-HT remains unclear. Since 5-HT-accumulation in the denervated aorta was not affected by pretreatment with NE, and the accumulation of 5-HT is much higher (4.7 times) than that of NE in the aorta, the mechanism of extraneuronal accumulation of 5-HT is probably different from that of NE.

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* Present address: Department of Anesthesiology, Yamaguchi University, School of Medicine, 1144 Yogushi, Ube, Yamaguchi 755, Japan.
† Deceased August 1985.

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Serotonin metabolism in the CNS in cerebellar ataxic mice

K. Ohsugi, K. Adachi and K. Ando

Department of Neurology, National Center for Nervous, Mental and Muscular Disorders, 4-1-1, Ogawahigashi, Kodaira, Tokyo 187 (Japan), 31 December 1985

Summary. The metabolism of 5-hydroxytryptamine (5-HT) in the CNS was investigated in four kinds of morphologically different ataxic mice; reeler, staggerer, weaver and Purkinje cell degeneration mutants, and in hypocerebellar mice experimentally produced by injection of cytosine arabinoside. 5-HT and 5-hydroxyidoleacetic acid concentrations and tryptophan hydroxylase (TrpOH) activity were measured in the cerebrum, cerebellum and brain stem, respectively. TrpOH activity was significantly reduced only in the reeler mouse. The enhancements of the cerebellar 5-HT metabolism observed in the ataxic mice other than the reeler were supposed to be pseudo-enhancements subsequent to the cerebellar hypoplasia.
Key words. Serotonin metabolism; tryptophan hydroxylase; cerebellar ataxic mice.