Effects of 5-hydroxykynurenamine, a metabolite of 5-hydroxytryptamine, on guinea-pig isolated trachea

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5-Hydroxykynurenamine (5-HK) was first detected in mouse urine and was referred to as mousamine (Makino 1961; Hasegawa & Makino 1968). However, the synthetic pathway of this compound remains unclear. Recently, an enzyme was isolated from various organs of mammals, including brain, lung and small intestine, that catalysed the oxygenative ring cleavage of 5-hydroxytryptamine (5-HT) to produce *N*-formyl-5-hydroxykynurenamine which is hydrolysed to 5-HK by formamidase (Hirata & Hayaishi 1972; Hayaishi et al 1975; Shimizu et al 1978). These authors suggested that this process may be one of major pathways of 5-HT metabolism. The present study was undertaken to observe the effects of 5-HK on isolated guinea-pig trachea especially its modification of the action of 5-HT.

The trachea from male Hartley strain guinea-pigs, 250–300 g, were cut spirally into strips (3 cm × 1·5 mm) suspended in a bioassay glass jacket and superfused at the rate of 20 ml min^{-1} with a Krebs-Henseleit solution, pH 7·4, at 37 °C aerated with 5% CO_2 in oxygen. A load of 0·5 g was applied to the tissue which was allowed to equilibrate for 1 h. Drugs were infused by an infusion pump (Harvard Apparatus, U.S.A.) at the rate of 0·97 ml min^{-1} for 1 min. Contractions of tracheal strips were recorded via an isotonic transducer (ME Commercial, Tokyo) and polyrecorder. The degree of contraction was expressed as the contraction index which consisted of the area (cm^2) encircled by the baseline and the response curve.

5-HK dihydrochloride monohydrate kindly donated by Drs T. Tokuyama (Osaka City University, Osaka, Japan) and S. Senoh (Central Research Institute, Suntory Ltd., Osaka) was synthesized as follows: melatonin was oxidized with sodium metaiodate in aqueous methanol containing sodium acetate, hydrolysed by boiling in HCl, and recrystallized three times from methanol. Purity was assessed by thin-layer chromatography and gas chromatography-mass spectrometry. The content of 5-HT in the 5-HK preparation was <0·01% as determined by the method of Curzon & Green (1970). Acetylcholine hydrochloride (Daiichi Co., Ltd. Tokyo) and methysergide bimaleate (Sandoz) were used. All other chemicals were of a reagent grade.

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5-HT, 5 × 10^{-9} to 5 × 10^{-7} M, and 5-HK, 5 × 10^{-8} to 5 × 10^{-6} M produced contractions in a dose-dependent manner (Fig. 1). 5-HK did not induce a contraction with a dose less than 5 × 10^{-10} M. Contractile responses to both 5-HK and 5-HT were blocked by methysergide, 10^{-6} M. With continuous infusion of 5-HK (5 × 10^{-8} M), the dose-response curve of 5-HT moved to the right and downward (Fig. 2B) while that of acetylcholine showed no significant shift (Fig. 2A). With different doses of 5-HK (2·5 × 10^{-11} to 2·5 × 10^{-5} M) contractile responses to 5-HT (5 × 10^{-4} M) were markedly attenuated in a dose-dependent manner (Fig. 3), 50% inhibition by 5-HK was at approximately 10^{-9} M, suggesting high affinity of 5-HK for the tracheal receptors. 10 min after infusion of 2·5 × 10^{-4} M 5-HK for 1 min, the strips failed to respond to 5-HT.

Thus, 5-hydroxykynurenamine produced dose-dependent contractions in guinea-pig tracheal strips, the potency being approximately 1/100 that of 5-HT. The dose-response curve of 5-HK almost paralled that of 5-HT and methysergide blocked contractile responses to 5-HK as it did those to 5-HT. In strips treated with a high concentration of 5-HK, the responses to both 5-HK and 5-HT were undetectable. The dose-response curve of 5-HT moved to the right and down with continuous infusion of a low concentration of 5-HK

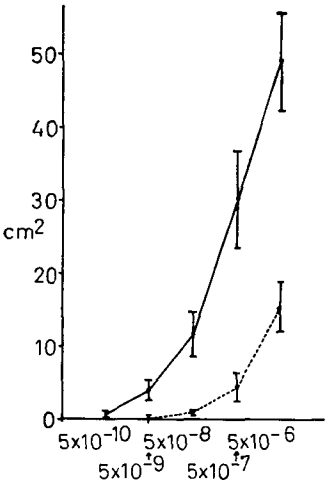

FIG. 1. Dose-response curves of 5-HT and 5-HK. Contractile responses were expressed as the contraction indices (C.I.) (ordinate) which consisted of the area (cm^2) encircled by the baseline and the response curve. Abscissa: Dose (M). —5-HT; - - - 5-HK.

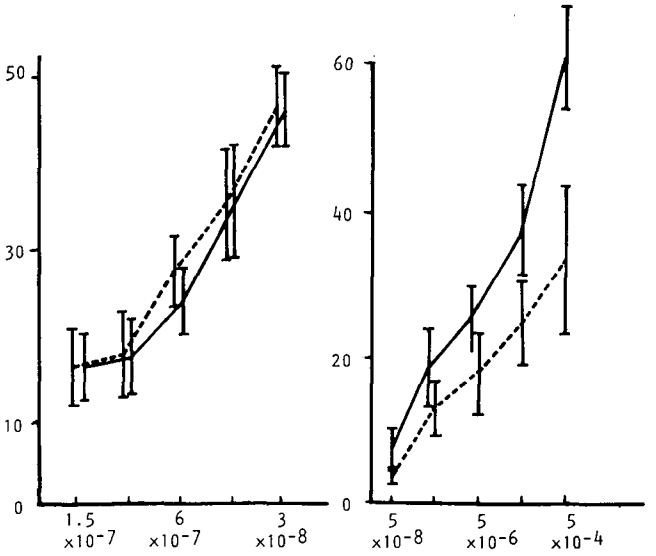

FIG. 2. Modification of contractile responses to acetylcholine (A) and 5-HT (B) by continuous infusion of 5×10^{-8} M 5-HK. —without - - - - - with 5-HK (5×10^{-8} M). Ordinate: C.I. (cm^2). Abscissa: Dose (M).

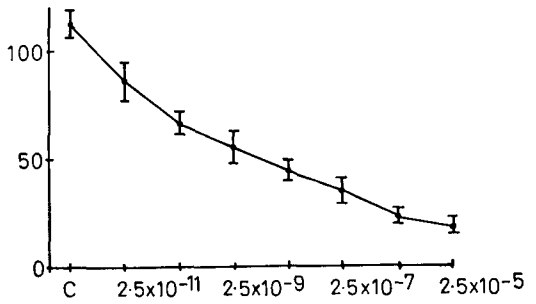

FIG. 3. Modification of the contractile response to 5-HT (5×10^{-4} M) with different doses of 5-HK ($2{\cdot}5 \times 10^{-11}$ to $2{\cdot}5 \times 10^{-5}$ M). Ordinate: C.I. (cm^2). Abscissa: Dose (M). C = control.

(5×10^{-8} M), while that of acetylcholine was unchanged. These results, taken with the previous findings on dog basilar arteries (Toda et al 1974) and bovine platelets (Okuma et al 1976), suggest that 5-HK and 5-HT share receptors in various tissues and that the effect of 5-HT is specifically antagonized by its metabolite, 5-HK.

The conversion of 5-HT to the 5-HK is catalysed by indoleamine-2,3-dioxygenase (Shimizu et al 1978), and the enzyme activity is high in pulmonary tissues as well as in small intestine. Thus, in case of a carcinoid syndrome or drug intoxication, this conversion might be defence mechanism against exposure to unusually concentrations of 5-HT, a potent vaso- and broncho-constrictors.

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