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Short communication

5-Hydroxytryptamine induces endothelium-independent relaxations of sheep pulmonary vein: role of cyclic nucleotide

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Abstract

5-Hydroxytryptamine produced concentration-dependent relaxations in isolated sheep pulmonary vein, which were insensitive to removal of the endothelium. 5-Hydroxytryptamine stimulated concentration-dependent increases of cyclic AMP levels in the pulmonary vein, and there was a significant linear correlation between relaxations elicited by 5-hydroxytryptamine and tissue cyclic AMP formations. The soluble guanylate cyclase inhibitor methylene blue (10 μM) failed to block 5-hydroxytryptamine-induced relaxations. The results suggest that 5-hydroxytryptamine-induced relaxations of sheep pulmonary vein are mediated, at least in part, by increases of tissue cyclic AMP levels.

Keywords: Pulmonary vein, sheep; Vasodilatation; 5-HT (5-hydroxytryptamine, serotonin); Endothelium; Cyclic nucleotide

1. Introduction

It has been well documented that 5-hydroxytryptamine (5-HT) can elicit vasodilatation in certain blood vessels which is either endothelium-dependent or endothelium-independent. 5-HT relaxes the pulmonary vein in sheep (Eyre, 1975). Cocks and Arnold (1992) have demonstrated that 5-HT-induced relaxations of sheep pulmonary vein are endothelium-independent, and are mediated by 5-HT_4 receptors. 5-HT_1-like relaxant receptors were also present in this tissue but 5-HT has a lower affinity at these receptors (Cocks and Arnold, 1992). In a variety of tissues (Dumuis et al., 1988; Craig and Clarke, 1990; Ford et al., 1992), the 5-HT_4 receptor has been shown to be positively coupled to adenylate cyclase and increase tissue cyclic AMP levels, although its role in vascular smooth muscle remains unclear. The purpose of this study was to examine whether tissue cyclic AMP played an important role in mediating the vascular relaxation produced by 5-HT in the pulmonary vein. The finding of a strong correlation between the relaxations elicited by 5-HT and tissue cyclic AMP formations suggests that the 5-HT-induced relaxation in the pulmonary vein is mediated, at least in part, by increases of tissue cyclic AMP levels.

2. Materials and methods

2.1. Tissue preparation

The main pulmonary veins were obtained from mixed breed sheep. Helically cut tissue strips were suspended in 10-ml isolated organ baths and bathed in a modified Krebs' solution at 37° C as previously described (Zhang and Dyer, 1990). The Krebs' solution was oxygenated continuously with 95% O_2-5% CO_2. In some strips, the endothelium was removed mechanically by rubbing the intimal surface of the vessel with moistened tissue paper. This procedure removed over 90% of the endothelium as examined by light microscopy.

2.2. Experimental protocol

Tissues were equilibrated under 1 g of tension for 90 min before initiating the experiment, and tissue re-

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sponses were recorded isotonically. After a 90-min equilibration period, the strips were pre-contracted with 45 mM KCl which produced approximately 50% of the maximum contraction to KCl (150 mM). When the contraction had stabilized, 5-hydroxytryptamine (5-HT) (Sigma Chemical Co., USA) was added cumulatively. Where appropriate, hemoglobin and methylene blue (Sigma Chemical Co., USA) were added 30 min prior to the KCl challenge. The effect of 5-HT on pulmonary vein cyclic AMP levels was determined on strips kept under a constant tension of 1 g weight in the Krebs' solution. After equilibration for 90 min, each strip was exposed to KCl (45 mM) for 5 min, and then to a single dose of 5-HT for 5 min which produced a maximum change in cyclic AMP (preliminary studies not shown). The reactions were stopped by flash freezing tissues in liquid nitrogen. Tissue cyclic AMP content was determined using commercially available kits, and was expressed as pmol per mg vessel protein which was determined by method of Bradford (1976).

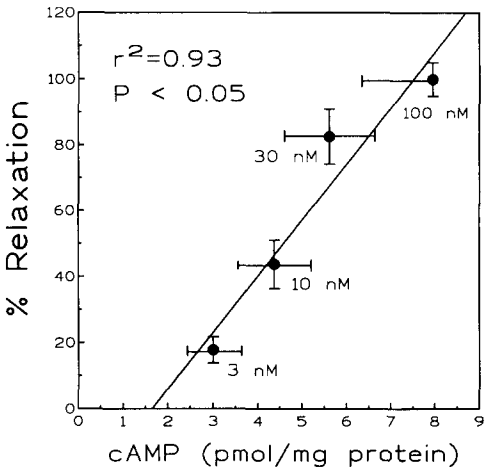

Fig. 2. Correlation of relaxations induced by 5-HT and cyclic AMP formations in sheep pulmonary vein. 5-HT (3–100 nM) stimulated increases of tissue cyclic AMP levels (basal cyclic AMP: 2.08 ± 0.64 pmol/mg protein) and relaxed the pulmonary veins pre-contracted by 45 mM KCl. A significant linear correlation is shown between the relaxation responses and cyclic AMP formations in response to 5-HT. Data are expressed as the means ± standard errors of tissues from 4–7 sheep.

3. Results

3.1. Role of endothelium and cyclic GMP in the 5-HT-induced relaxation

5-HT stimulated concentration-dependent relaxations (pD_2: 8.04 ± 0.8) of the pulmonary vein pre-contracted by 45 mM KCl. The 5-HT-induced relaxation was not altered by removal of the endothelium (pD_2: 8.08 ± 0.6), or by 1 μM hemoglobin (pD_2: 8.05 ± 0.3). Pre-treatment of tissues with the soluble guanylate cyclase inhibitor methylene blue (10 μM) failed to block the 5-HT-induced relaxation (Fig. 1).

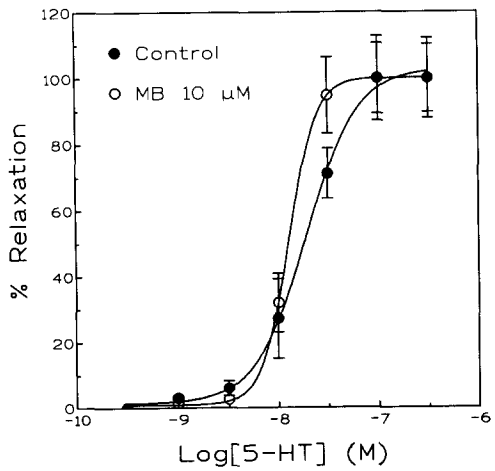

Fig. 1. Effects of methylene blue on 5-HT-induced relaxations of sheep pulmonary vein. Tissue strips were pre-contracted by 45 mM KCl. After the contraction had stabilized, 5-HT was added cumulatively. Responses elicited to 5-HT are expressed as a percentage of the maximum relaxation. Methylene blue (MB) was added 30 min prior to the KCl challenge. KCl-induced contractions were not affected by methylene blue. Data are expressed as the means ± standard errors of tissues from 3–6 sheep.

3.2. Effect of 5-HT on tissue cyclic AMP formation

5-HT stimulated cyclic AMP production in the pulmonary vein in a concentration-dependent manner with an EC_{50} of 0.2 μM. As shown in Fig. 2, there was a significant linear correlation between the relaxation stimulated by 5-HT (3 nM–100 nM) and cyclic AMP formations.

4. Discussion

The major finding of the present study is that in sheep pulmonary vein, 5-HT is positively coupled to adenylate cyclase and stimulates tissue cyclic AMP formation. The finding of a significant correlation between the relaxations and tissue cyclic AMP formations elicited by 5-HT suggests that the 5-HT-induced relaxation in the pulmonary vein is mediated, at least in part, by increases of tissue cyclic AMP levels. These results further support the conclusion that 5-HT_4 receptors mediate relaxations of the sheep pulmonary vein (Cocks and Arnold, 1992).

5-HT_4 receptors have been identified in a variety of tissues including brain (Dumuis et al., 1988), oesophagus (Baxter et al., 1991), ileum (Craig and Clarke,

1990), and heart (Villalon et al., 1990). It has been generally accepted that cyclic AMP is the intracellular second messenger coupled to 5-HT_4 receptors. Many functions associated with 5-HT_4 receptors, including relaxation of the smooth muscle, are thought to be mediated by increases of intracellular cyclic AMP levels (Ford et al., 1992). In the present study, 5-HT stimulated concentration-dependent increases in tissue cyclic AMP with an EC_{50} of 0.2 μM, which is comparable to that (0.12–0.41 μM) reported by other investigators (Bockaert et al., 1990; Ford et al., 1992; Dumuis et al., 1988). In a recent study, Cocks and Arnold (1992) suggested that 5-HT-induced relaxations of sheep pulmonary veins were mediated by 5-HT_4 receptors. Antagonists for 5-HT_1 (methiothepin), 5-HT_2 (ketanserin), and 5-HT_3 (MDL 72222) subtypes failed to block the 5-HT-induced relaxations, but the 5-HT_4 receptor antagonist ICS 205-930 effectively blocked the relaxations elicited by 5-HT. The finding in the present study that 5-HT stimulated cyclic AMP formation suggests that cyclic AMP may play an important role in mediating 5-HT-induced relaxation in this tissue, and reenforces the notion that 5-HT_4 receptors are positively coupled to adenylate cyclase in vascular smooth muscle. The present results confirm and strengthen the results of Cocks and Arnold (1992).

The finding that the 5-HT-induced relaxations were endothelium-independent is in agreement with that by Cocks and Arnold (1992). In the present study, removal of the endothelium did not affect relaxations elicited by 5-HT, suggesting that the endothelium played little role, if any, in the 5-HT-induced relaxations of the pulmonary vein. This is supported by the finding that 5-HT-induced relaxations were not blocked by hemoglobin which effectively blocked acetylcholine-induced endothelium-dependent relaxations in blood vessels (Furchgott, 1984). In addition, the lack of effects of methylene blue suggests that 5-HT-induced relaxations of the pulmonary vein are not mediated by cyclic GMP which is known to mediate endothelium-dependent relaxation of vascular smooth muscle. Methylene blue has been used frequently as a tool to determine whether cyclic GMP is involved in the transduction of agonist responses because it can inhibit the soluble isoforms of guanylate cyclase in smooth muscle (Ignarro et al., 1986).

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