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5-Hydroxytryptamine stimulates phosphoinositide hydrolysis and contraction in the ovine umbilical artery

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Abstract

5-Hydroxytryptamine-stimulated contraction and generation of inositol phosphates (InsPs) were investigated in isolated ovine umbilical artery from near term pregnant sheep. 5-Hydroxytryptamine produced a concentration-dependent contraction in the presence or absence of external Ca^{2+}, however, the contractile response in Ca^{2+}-free medium was depressed by 67% ($P < 0.05$). The initial increase of either inositol monophosphate ($InsP_1$) or inositol bisphosphate ($InsP_2$) was not apparent, although a significant rise in both $InsP_1$ and $InsP_2$ was observed at 30 min (107 and 100% over basal level, $P < 0.05$). The generation of inositol trisphosphate ($InsP_3$) was rapid and reached its peak at 60–90 s (35% over basal level, $P < 0.05$), followed by a second rise at 30 min (96% over basal level, $P < 0.05$). The generation of InsPs stimulated by 5-hydroxytryptamine was also concentration-dependent. In agreement with previous contraction studies, the generation of InsPs stimulated by 5-hydroxytryptamine was blocked by 10 nM ketanserin, a specific 5-HT_{2A} receptor antagonist. A temporal study revealed that the generation of $InsP_3$ coincided with the phasic component of the contractile response induced by 5-hydroxytryptamine. Our results suggest that through activation of 5-HT_{2A} receptors, the generation of $InsP_3$ plays a critical role in the contraction induced by 5-hydroxytryptamine in the ovine umbilical artery.

Keywords: 5-HT_{2A} receptor; Inositol 1,4,5-triphosphate; Umbilical artery

1. Introduction

Vascular smooth muscle contracts when neurotransmitters or hormones bind to specific cell surface receptors. The occupancy of certain receptors such as α_1-adrenoceptors (Gu et al., 1991) and 5-HT_{2A} receptors (Roth et al., 1986; Zhang and Hu, 1995) stimulates phospholipase C which then hydrolyses phosphatidylinositol 4,5-bisphosphate, leading to the generation of inositol 1,4,5-trisphosphate ($InsP_3$) in vascular smooth muscle. Ins(1,4,5)P_3 binds to its receptor to release Ca^{2+} from intracellular stores and initiates vasoconstriction (Somlyo et al., 1992). Hence, Ins(1,4,5)P_3 has been suggested to play an important role in pharmacomechanical coupling in smooth muscle (Somlyo and Somlyo, 1994).

The umbilical-placental vessels are devoid of innervation (Fox and Khong, 1990). Therefore, vasoactive factors either carried by blood or produced locally play an important role in the regulation of foetoplacental circulation. 5-Hydroxytryptamine is a potent vasoconstrictor of the umbilical artery (Dyer, 1970; Somlyo et al., 1965). The contractions induced by 5-hydroxytryptamine in the ovine umbilical artery are mediated by 5-HT_{2A} receptors (Zhang and Dyer, 1990). 5-Hydroxytryptamine-stimulated formation of inositol phosphates (InsPs) has been demonstrated in a variety of vascular beds (Berta et al., 1986; Murphy and Garland, 1993; Nakaki et al., 1985), in which both contractions and the generation of InsPs are mediated by activation of 5-HT_{2A} receptors (Cohen and Wittenauer, 1987; Roth et al., 1986; Pauwels et al., 1990; Zhang and Hu, 1995). These observations imply a possible role for Ins(1,4,5)P_3 in 5-hydroxytryptamine-induced vasoconstriction. In fact, the hydrolysis of phosphoinositides and vasoconstriction in response to 5-hydroxytryptamine were closely correlated in the rat aorta (Roth et al., 1986) and ovine uterine artery (Zhang and Hu, 1995). However, a recent study showed that 5-HT_{2A} receptors are not coupled to phospholipase C in guinea-pig trachea (Watts et al.,

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1994). Plasma free 5-hydroxytryptamine in the umbilical artery and the sensitivity of umbilical artery to 5-hydroxytryptamine were increased in preeclamptic women (Taniguchi, 1995), suggesting that 5-hydroxytryptamine may play a role in the pathology of preeclampsia. The mechanism underlying the regulation of umbilical vascular function remains largely unknown. In order to understand the signal transduction pathways in response to 5-hydroxytryptamine in the ovine umbilical artery, the contractile response and phosphoinositide hydrolysis stimulated by 5-hydroxytryptamine were investigated.

2. Materials and methods

2.1. Materials

myo-[^{3}H]inositol (16 Ci/mmol) was from DuPont-NEN (Boston, MA). Dowex AG 1X8 (formate form) and disposable columns (10 ml) were from Bio-Rad Laboratories (Richmond, CA, USA). 5-Hydroxytryptamine creatinine was from Sigma (St. Louis, MO, USA). Ketanserin tartrate was from Janssen Pharmaceutica (Beerse, Belgium).

2.2. Tissue preparation

Mixed breed sheep near term ($\sim$ 140 days of gestation) were anesthetized with an i.v. injection of pentobarbital sodium. Umbilical cords were quickly removed and placed in an oxygenated modified Krebs' solution (pH 7.4) of the following composition (in mM): NaCl, 115.21; KCl, 4.70; $CaCl_2$, 1.80; $MgSO_4$, 1.16; KH_2PO_4, 1.18; $NaHCO_3$, 22.14, dextrose, 7.88; and EDTA, 0.03. The arteries were carefully cleaned free from connective tissue and cut into 0.4–0.5 cm wide ring segments.

2.3. Contraction study

Ring segments of the umbilical artery were suspended in a 10-ml organ bath as described by Isla and Dyer (1990) and isometric tensions were measured with a Grass force transducer. Under 2 g resting tension the tissues were equilibrated for 60 min, and a single or cumulative concentration-response to 5-hydroxytryptamine was obtained in the presence or absence of external Ca^{2+}.

2.4. Inositol phosphates determination

Generation of InsPs was measured by modification of the procedure previously described by Roth et al. (1986). Briefly, the ring segments were equilibrated in Krebs' solution at 37°C for 30 min under a 95% O_2-5% CO_2 atmosphere and then transferred to vials containing 20 μCi/ml of *myo*-[^{3}H]inositol. The incubation was continued for 4 h. After washing 3 times with warm oxygenated Krebs' solution to remove free *myo*-[^{3}H]inositol, LiCl (final concentration 10 mM) was then added to the incubation medium 10 min before the application of the agonist. When an antagonist was used, it was added into the incubation medium 30 min before the application of the agonist. At the end of the incubation, the reaction was terminated by immersing the ring segments in liquid N_2. The tissues were then transferred to 2 ml ice-cold methanol/chloroform/HCl (200:100:1) for approximately 3 min and then were rapidly blotted dry, weighed and homogenized in the same methanol-chloroform-HCl mixture. Chloroform (1 ml) and water (1.1 ml) were added to the homogenates. After standing on ice for 4 h, the homogenates were centrifuged to separate the phases. The upper phase was then added to 1 ml of Dowex AG 1X8 (formate form) resin packed in a disposable column (Bio-Rad). The columns were then washed sequentially with 15 ml of water to elute free *myo*-[^{3}H]inositol, 15 ml of 0.2 mM ammonium formate/0.1 M formic acid to elute inositol monophosphate ($InsP_1$), 15 ml of 0.4 M ammonium formate/0.1 M formic acid to elute inositol bisphosphate ($InsP_2$) and finally 15 ml of 1.0 M ammonium formate/0.1 M formic acid to elute inositol trisphosphate ($InsP_3$). 1 ml of each fraction was then transferred to liquid scintillation vials, and 15 ml scintillant was added and radioactivity was determined by a liquid scintillation spectrophotometry with 40% counting efficiency.

2.5. Statistics

Results are expressed as means $\pm$ S.E. n refers to the number of animals used. A paired or unpaired Student's t-test was used and a P value of < 0.05 was taken as significant.

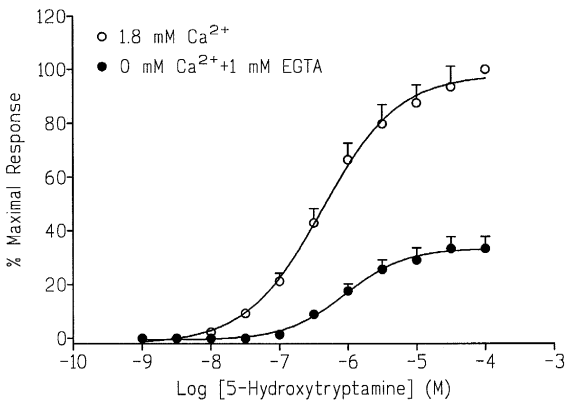

Fig. 1. Concentration–response curve for 5-hydroxytryptamine-induced contraction of the isolated ovine umbilical artery in the absence and presence of external Ca^{2+}. The concentration of 5-hydroxytryptamine was increased by half log increments and isometric tension was measured. The maximal response to 5-hydroxytryptamine was 9.25 $\pm$ 0.66 g. Results are expressed as means $\pm$ S.E. of three individual experiments.

3. Results

3.1. 5-Hydroxytryptamine-stimulated contraction and the time course for the production of inositol phosphates in the ovine umbilical artery

5-Hydroxytryptamine produced a concentration-dependent contraction of the ovine umbilical artery in the presence or absence of external Ca^{2+} (Fig. 1). Contractions induced by 5-hydroxytryptamine were depressed in the Ca^{2+}-free medium, in which Ca^{2+} was replaced by 1 mM EGTA). Although the pD_2 was not changed by removing Ca^{2+} (from 6.28 ± 0.09 to 6.04 ± 0.13), the maximal response for 5-hydroxytryptamine were decreased by 67% ($P < 0.05$). Since the maximal response was obtained at the concentration of 100 μM 5-hydroxytryptamine, 100 μM 5-hydroxytryptamine was chosen for the time-course study of 5-hydroxytryptamine-stimulated generation of InsPs. The effect of the duration of stimulation by 5-hydroxytryptamine on the hydrolysis of phosphoinositides is shown in Fig. 2. Following the application of 5-hydroxytryptamine (100 μM), the initial increases in the generation of $InsP_1$ and $InsP_2$ were not apparent. The rise occurred after 5 min for both $InsP_1$ and $InsP_2$, which were linear with time until 30 min (107% and 100% over basal level at 30 min, respectively, $P < 0.05$). The generation of $InsP_3$ was biphasic in response to stimulation by 5-hydroxytryptamine (Fig. 2C). The generation of $InsP_3$ increased at 5 s and proceeded at a slow rate. A peak of $InsP_3$ generation was reached at 60–90 s (35% over basal level, $P < 0.05$). The generation of $InsP_3$ then declined to the basal level at 2 min. A second rise was seen after 5 min, and at 30 min it reached 96% over basal level ($P < 0.05$). As shown in Fig. 2C, 5-hydroxytryptamine (100 μM) produced a contraction consisting of two components: a phasic contraction followed by a tonic contraction. The time to reach peak tension was 1.8 ± 0.2 min. The contractions in response to 5-hydroxytryptamine coincided with the generation of $InsP_3$, suggesting an important role for $InsP_3$ in 5-hydroxytryptamine-induced contraction of the ovine umbilical artery.

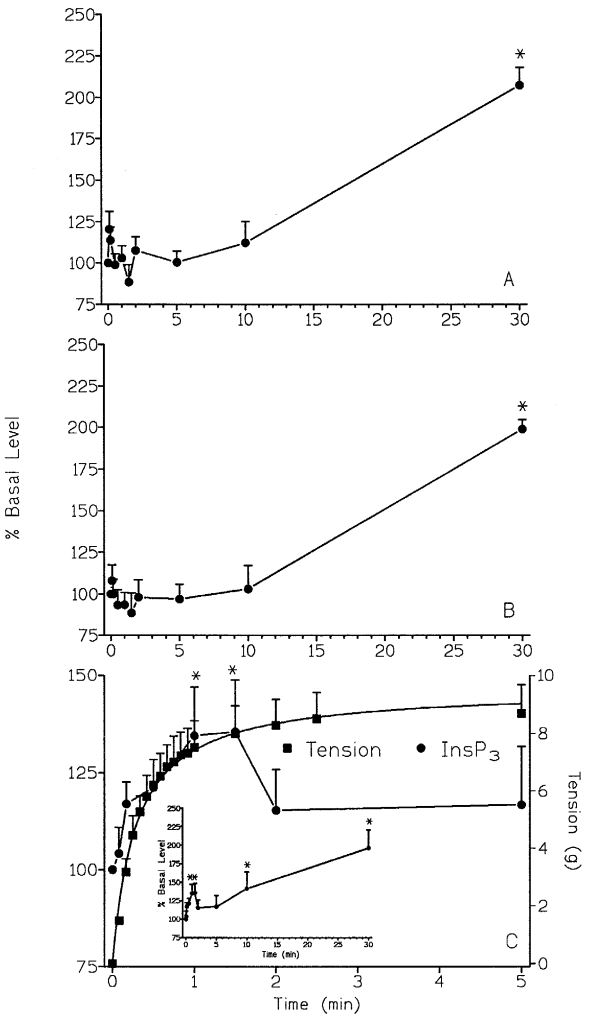

Fig. 2. Time-course of 5-hydroxytryptamine-stimulated production of inositol phosphates in the isolated ovine umbilical artery. Ring segments of the artery were exposed to 100 μM 5-hydroxytryptamine at 37°C for various times. $InsP_1$ (A), $InsP_2$ (B), and $InsP_3$ (C) were separated as described in Section 2 and expressed as cpm. Basal levels of individual inositol phosphates (cpm/mg wet weight) were 144.4 ± 13.3, 89.0 ± 11.7 and 22.5 ± 6.8 for $InsP_1$, $InsP_2$ and $InsP_3$, respectively. In addition to $InsP_3$, the time-course of 100 μM 5-hydroxytryptamine-induced contraction of the umbilical artery is also presented in Fig. 2C ($n = 6$). Inset: time-course (up to 30 min) of 5-hydroxytryptamine-stimulated production of $InsP_3$ in the ovine umbilical artery. Results are expressed as means ± S.E. of five or six individual experiments. * $P < 0.05$ vs. basal level.

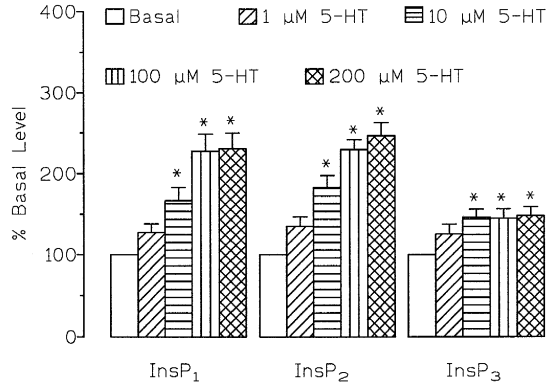

Fig. 3. Concentration–response for 5-hydroxytryptamine (5-HT)-stimulated production of inositol phosphates in the isolated ovine umbilical artery. Ring segments of the artery were exposed to 5-hydroxytryptamine at indicated concentrations (1–200 μM) at 37°C for 30 min. Individual inositol phosphates were separated as described in Section 2 and expressed as cpm. Basal levels of inositol phosphates (cpm/mg wet weight) were 127.8 ± 17.8, 63.7 ± 7.8 and 38.5 ± 9.4 for $InsP_1$, $InsP_2$ and $InsP_3$, respectively. Results are expressed as means ± S.E. of five or six individual experiments. * $P < 0.05$ vs. basal level.

3.2. Concentration-response of 5-hydroxytryptamine-stimulated production of inositol phosphates and effects of ketanserin in the ovine umbilical artery

Since the maximal production of inositol phosphates occurred at 30 min in the ovine umbilical artery, the

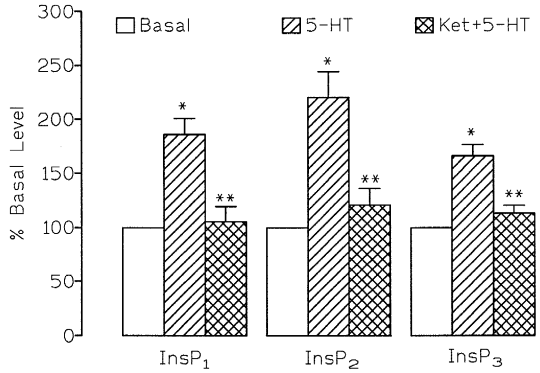

Fig. 4. Effects of ketanserin on 5-hydroxytryptamine (5-HT)-stimulated production of inositol phosphates in the isolated ovine umbilical artery. Ring segments of the artery were exposed to 100 μM 5-hydroxytryptamine at 37°C for 30 min, and ketanserin (10 nM) when used was incubated with the tissues for 30 min before the addition of the agonist. Basal levels of individual inositol phosphates (cpm/mg wet weight) were 117.6 ± 12.6, 60.4 ± 5.0 and 37.5 ± 8.1 for $InsP_1$, $InsP_2$ and $InsP_3$, respectively. Results are expressed as means ± S.E. of five individual experiments. * $P < 0.05$ vs. basal level. ** $P < 0.05$ vs. 5-hydroxytryptamine-stimulated response.

concentration-response of 5-hydroxytryptamine-stimulated generation of InsPs was obtained at this time interval. As shown in Fig. 3, 5-hydroxytryptamine (1–200 μM) stimulated a concentration-dependent increases in the production of $InsP_1$, $InsP_2$ and $InsP_3$. In agreement with the contraction study, in which 5-HT_{2A} receptors mediated the contractile response to 5-hydroxytryptamine (Zhang and Dyer, 1990), the specific 5-HT_{2A} receptor antagonist ketanserin at 10 nM abolished the 5-hydroxytryptamine-stimulated production of $InsP_1$, $InsP_2$ and $InsP_3$ ($InsP_1$: from 186% to 106% over basal level; $InsP_2$: from 221% to 120% over basal level; $InsP_3$: from 166% to 113% over basal level, $P < 0.05$, Fig. 4). This strongly indicates that 5-hydroxytryptamine-stimulated phosphoinositide hydrolysis was mediated by 5-HT_{2A} receptors in the ovine umbilical artery.

4. Discussion

5-Hydroxytryptamine produces vasoconstriction in most isolated vessels through either 5-HT_{2A} or 5-HT_1-like receptors (Martin, 1994). In the isolated ovine umbilical artery, 5-hydroxytryptamine was a potent vasoconstrictor. Previous studies demonstrated that the contractile response to 5-hydroxytryptamine was mediated through 5-HT_{2A} receptors (Zhang and Dyer, 1990). It is generally accepted that 5-HT_{2A} receptors are coupled to phospholipase C (Hoyer et al., 1994). However, 5-HT_{2A} receptors in the guinea-pig trachea were not coupled to phospholipase C (Watts et al., 1994). Little is known about the mechanisms underlying the regulation of umbilical vascular function. Understanding the signal transduction pathways in the umbilical artery will not only increase our knowledge on the regulation of foetoplacental circulation but also provide possible improvement of therapy of diseases associated with pregnancy.

Ca^{2+} plays a pivotal role in pharmacomechanical coupling. An increase in the intracellular Ca^{2+} level initiates vasoconstriction (Somlyo and Somlyo, 1994). Either Ca^{2+} influx from an extracellular environment or Ca^{2+} release from intracellular stores, or both could contribute to the increase in the intracellular Ca^{2+} level. In the absence of external Ca^{2+}, 5-hydroxytryptamine still produced contractions of the ovine umbilical artery, although the contractions were depressed by Ca^{2+} deprivation. Our findings suggest that both Ca^{2+} influx and Ca^{2+} release play a role in 5-hydroxytryptamine-induced contraction of the ovine umbilical artery. Similar findings were observed in the human umbilical artery (Dogan et al., 1991).

Upon occupancy of its receptor by an agonist, a characteristic series biochemical events are initiated. For those receptors coupled to phospholipase C, activation of them results in generation of two second messengers: $InsP_3$ and diacylglycerol (Berridge, 1993). $InsP_3$ releases Ca^{2+} from intracellular stores and initiates vasoconstriction (Somlyo et al., 1992), whereas diacylglycerol may play a role in the maintenance of contraction (Rasmussen et al., 1987). Agonist-induced vasoconstriction in the absence of external Ca^{2+} is believed to be the result of $InsP_3$-induced Ca^{2+} release from intracellular stores (Minneman, 1988). Contraction of the ovine umbilical artery by 5-hydroxytryptamine in a Ca^{2+}-free medium implies the possible role of $InsP_3$.

Measurement of phosphoinositide turnover could serve as a useful means for monitoring the functional effects of 5-HT_{2A} receptors (Hoyer et al., 1994). Therefore, the generation of InPs in response to 5-hydroxytryptamine in the ovine umbilical artery was determined. Similar to the findings in other vascular beds (Berta et al., 1986; Murphy and Garland, 1993; Nakaki et al., 1985; Zhang and Hu, 1995), 5-hydroxytryptamine stimulated the generation of InsPs in the ovine umbilical artery. As a general rule, the generation of second messenger(s) should precede or coincide with the cellular response upon stimulation of the receptors. We compared the time course of 5-hydroxytryptamine-stimulated $InsP_3$ and contractile response. The temporal studies revealed that the generation of $InsP_3$ coincided with the contraction in response to 5-hydroxytryptamine in the ovine umbilical artery. This finding is in close agreement with those seen in the rat tail artery (Gu et al., 1991) and in the ovine uterine artery (Zhang and Hu, 1995), and confirms the suggestion that $InsP_3$ functions as a causative factor in agonist-induced vasoconstriction (Somlyo and Somlyo, 1994). Due to the inability of anion exchange chromatography to separate Ins(1,4,5)P_3 from Ins(1,3,4)P_3, $InsP_3$ measured in the present study could consist of these two isoforms. Current knowledge indicate that only Ins(1,4,5)P_3 has the ability to release Ca^{2+} from intracellular stores (Berridge, 1993). However, Ins(1,3,4)P_3 is an enzymatic metabolite of Ins(1,4,5)P_3 (Majerus, 1992)

and $InsP_3$ measured in the present study can be considered as an index of the production of $Ins(1,4,5)P_3$.

The receptor subtype involved in 5-hydroxytryptamine-stimulated generation of InsPs in the ovine umbilical artery was also investigated. Similar to the observation in a previous contraction study (Zhang and Dyer, 1990), ketanserin, a specific 5-HT_{2A} receptor antagonist (Hoyer et al., 1994), blocked 5-hydroxytryptamine-stimulated phosphoinositide hydrolysis. This observation suggests that 5-hydroxytryptamine stimulates the hydrolysis of phosphoinositides as a consequence of activation of 5-HT_{2A} receptors in the ovine umbilical artery. Our finding is in agreement with those observations in the rat aorta (Cohen and Wittenauer, 1987; Roth et al., 1986), rat portal vein (Cohen and Wittenauer, 1987), A7r5 cells (Doyle et al., 1986), and ovine uterine artery (Zhang and Hu, 1995), in which 5-HT_{2A} receptors mediate the hydrolysis of phosphoinositides. In the human umbilical artery smooth muscle cells, 5-hydroxytryptamine also stimulated the production of InsPs (Hawley et al., 1995). Although both 5-HT_{2A} and 5-HT_1-like receptors are present in the human umbilical artery (MacLennan et al., 1989), recent findings suggest that 5-HT_1-like receptors are not coupled to phospholipase C (Murphy and Garland, 1993; Seager et al., 1994).

In summary, we demonstrated that 5-hydroxytryptamine induced vasoconstriction of the ovine umbilical artery, using both extracellular and intracellular Ca^{2+} sources. 5-Hydroxytryptamine stimulated phosphoinositide hydrolysis, which was blocked by ketanserin, a specific 5-HT_{2A} receptor antagonist. 5-Hydroxytryptamine-stimulated generation of $InsP_3$ coincided with the contractile response. Our findings suggest that 5-hydroxytryptamine-induced vasoconstriction of the ovine umbilical artery was mediated by activation of 5-HT_{2A} receptors. The generation of $InsP_3$ played an important role in 5-hydroxytryptamine-induced contractions.

Acknowledgements

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