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European Journal of Pharmacology 313 (1996) 97–102

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Norepinephrine attenuates serotonin inhibition of pial venous tone

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Received 21 March 1996; revised 20 June 1996; accepted 25 June 1996

Abstract

Serotonin (5-hydroxytryptamine, 5-HT) has been shown to inhibit the rhythmic constrictions, accompanied by an increase in cAMP synthesis, in porcine pial veins. Since porcine pial veins contain predominant postsynaptic α_2-adrenoceptors which are coupled to G_i-protein, the possibility that the inhibitory effect of 5-HT is antagonized by norepinephrine was examined pharmacologically, using tissue bath techniques. The results indicated that norepinephrine (0.1–1 μM) attenuated 5-HT-induced inhibition of rhythmic constriction. This effect of norepinephrine was mimicked by clonidine (an α_2-adrenoceptor agonist), but not by methoxamine (an α_1-adrenoceptor agonist). Furthermore, the effect of norepinephrine was prevented by yohimbine (an α_2-adrenoceptor antagonist) and pertussis toxin, but was not prevented by prazosin (an α_1-adrenoceptor antagonist). In parallel studies, the basal concentration of cAMP and that induced by 5-HT in the pial veins were inhibited by norepinephrine (0.3 μM). These results are consistent with the previous findings that 5-HT-induced inhibition of rhythmic constriction in the porcine pial veins is associated with an increase in vascular cAMP synthesis and suggest that norepinephrine attenuates 5-HT-induced inhibition of rhythmic constriction in part by negatively coupling to adenylate cyclase via α_2-adrenoceptors.

Keywords: Rhythmic constriction; 5-HT (5-hydroxytryptamine, serotonin); cAMP; Norepinephrine; Pial vein; (Porcine)

1. Introduction

We have demonstrated that isolated porcine pial veins exhibit rhythmic constriction (Lee and Sunagane, 1989; Lee et al., 1994; Ueno et al., 1995). The constrictions were inhibited by serotonin (5-hydroxytryptamine, 5-HT) via activation of 5-HT_1-like receptors on the smooth muscle cells (Lee et al., 1994). Biochemical and pharmacological studies further demonstrated that 5-HT-induced inhibition of rhythmic constriction and vascular tone in porcine pial veins was mediated by an increase in synthesis of vascular cAMP, but not cGMP (Ueno et al., 1995). In the presence of norepinephrine, however, 5-HT-induced relaxation and inhibition of rhythmic constriction were attenuated (Lee and Sunagane, 1989). The porcine pial veins are innervated by dense adrenergic nerves, and contain predominant postsynaptic α_2-adrenoceptors which mediate norepinephrine-induced constriction (Asada and Lee, 1992). Since α_2-adrenoceptors have been reported to be negatively coupled to adenylate cyclase (Dohlman et al., 1987; Wright et al., 1995), the possibility that norepinephrine attenuation of 5-HT-induced inhibition of rhythmic constriction in the porcine pial veins is due to inhibition of cAMP synthesis through the activation of α_2-adrenoceptors was therefore examined.

2. Materials and methods

Fresh heads of adult pigs of either sex were obtained from a local packing company. Brains were removed immediately and placed in Krebs bicarbonate solution equilibrated with 95% O_2 and 5% CO_2 at room temperature. The composition of the solution was (in mM) 144.2 Na^+, 4.9 K^+, 1.6 Ca^{2+}, 1.2 Mg^{2+}, 126.7 Cl^-, 1.19 SO_4^{2-}, 25.0 HCO_3^-, 11.1 dextrose, 0.01 ascorbic acid and 0.023 EDTA (pH 7.4).

2.1. Measurement of vascular tone

The in vitro tissue bath techniques were used to measure changes in the venous wall tension (Lee et al., 1994). Pial veins were dissected and cleaned of surrounding tissues using a dissecting microscope. The pial venous ring segment (4 mm long; 250–400 μm o.d.) was cannulated with a stainless steel rod and a platinum wire, and was

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PII S0014-2999(96)00506-7

mounted horizontally in a plastic bath containing 5 ml of Krebs solution at 37°C and gassed with 95% O_2 and 5% CO_2. Changes in isometric tension were measured by Gould Statham UC-2 transducers and recorded on a Grass polygraph (Lee et al., 1994). A resting tension of 75 m g was applied, and the tissues were equilibrated for an additional 60 min. An active muscle tone of approximately 0.6 g was then induced in each ring segment by U-46619, a thromboxane A_2 analog. The induction of active muscle tone always results in rhythmic constriction (Lee and Sunagane, 1989). Concentration-response relationships for 5-HT inhibition of rhythmic constriction were obtained by a cumulative technique. In venous rings pre-incubated with norepinephrine, clonidine or methoxamine, which raised the resting tone to variable degrees, the active muscle tones were raised to 0.6 g by adding U-46619 (0.1–1 μM).

In order to determine the effect of pertussis toxin on norepinephrine blockade of 5-HT inhibition of rhythmic constriction, the venous rings were incubated with pertussis toxin (100 ng/ml) according to Flavahan et al. (1991) for 2 h, after obtaining the concentration-response relationship for 5-HT inhibition of rhythmic constriction in pial veins in the presence of active muscle tone induced by U-46619 (0.1–1 μM). At the end of incubation, the pertussis toxin was removed by washing with fresh Krebs solution. 10 min later, norepinephrine (0.3 μM) was added and the active muscle tone was raised to approximately 0.6 g by adding U-46619 (0.1–1 μM). 5-HT concentration-response relationship was repeated. At the end of each experiment, papaverine (300 μM) was given to achieve maximum inhibition (Lee et al., 1994). The 5-HT-induced inhibition of rhythmic constriction was calculated as percentage of the maximum inhibition induced by papaverine. The concentrations that produced 50% maximum inhibition (IC_{50}) with 95% confidence intervals were calculated (Fleming et al., 1972).

2.2. Measurement of cAMP contents

Longitudinally stretched pial venous preparations were used to simultaneously measure the changes in tension and cyclic nucleotide levels according to our previous report (Ueno et al., 1995). Veins (1 cm long) with each end tied with a string (6-0 silk; Ethicon) were mounted vertically in a 10 ml tissue bath filled with Krebs solution at 37°C. One string was anchored to the bottom of the tissue bath while the other string was connected to a Gould Statham UC-2 transducer for recording changes in isometric force. A resting tension of 75 m g was applied (Lee et al., 1994). The tissues were incubated with Krebs solution at 37°C equilibrated with 95% O_2 and 5% CO_2 for 30 min. The tissues were then contracted with U-46619 (1 μM) in the presence of indomethacin (10 μM; to eliminate possible influence of prostanoids), and 3-isobutyl-1-methylxanthine (IBMX, 1 μM to inhibit phosphodiesterase). 10 min later, 5-HT at specified concentration was added to inhibit the U-46619-induced venous rhythmic constriction. To study effect of norepinephrine on 5-HT-inhibition of venous rhythmic constriction, norepinephrine (0.3 μM) was added 10 min before applications of 5-HT. 1 min after addition of 5-HT and during active inhibition/relaxation, the veins were quickly removed from tissue baths and frozen in liquid nitrogen. The frozen tissues were weighed and homogenized in 50 mM sodium acetate buffer (pH 4.0) and centrifuged (3000 rpm for 20 min). Supernatant fractions were lyophilized, reconstituted in sodium acetate buffer (pH 4.75), acetylated and radioimmunoassayed for cyclic nucleotide (Shirasaki et al., 1988) with kits purchased from Biomedical Technologies (Stoughton, MA, USA).

2.3. Statistical methods and drugs used

The data were computed as means ± S.E.M. and were evaluated statistically by Student's t-test for paired or

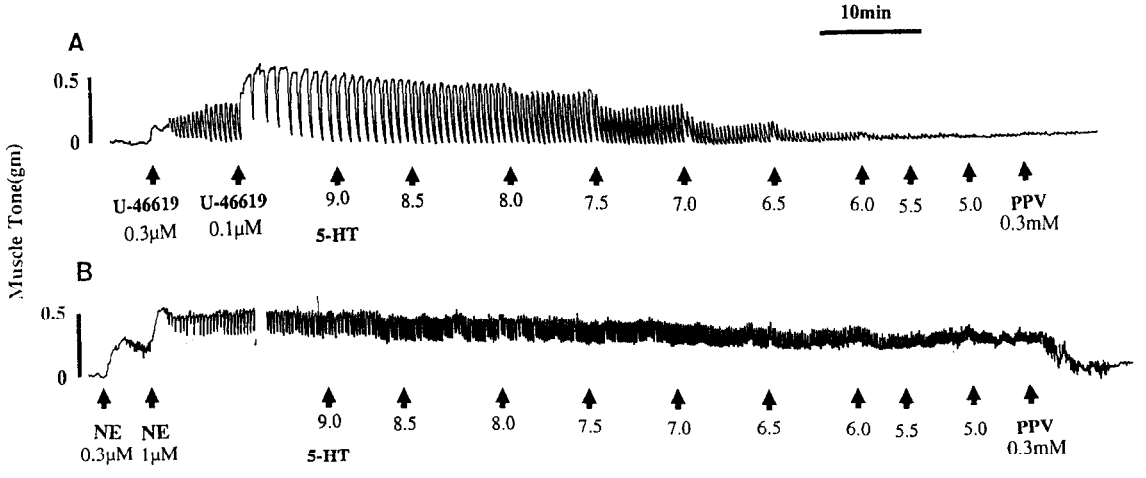

Fig. 1. Rhythmic constrictions in isolated porine pial venous rings in the presence of active muscle tone induced by U-46619 (A) and norepinephrine (B). 5-HT inhibits rhythmic constriction concentration dependently in the presence of U-46619, but only minimally affected that in the presence of norpinephrine. Numbers with arrows indicate negative log molar concentrations of 5-HT in bath. PPV, papaverine.

Table 1
The effects of various drugs on 5-HT-induced inhibition of RC

	IC_{50} (M) [a]	Maximum [b] relaxation (% ± S.E.M.)	n
5-HT (control)	$2.16\ (1.09-4.31) \times 10^{-8}$	97.69 ± 1.19	14
5-HT + indomethacin 3 μM	$1.40\ (0.50-3.90) \times 10^{-8}$	97.33 ± 2.14	8
5-HT + NE 0.1 μM	$2.70\ (0.60-12.30) \times 10^{-8}$	65.14 ± 5.85 *	5
5-HT + NE 0.3 μM	$6.70\ (1.80-25.00) \times 10^{-8}$ *	53.74 ± 7.09 *	5
5-HT + NE 1 μM	$8.50\ (3.70-19.30) \times 10^{-7}$ *	11.49 ± 3.28 *	5
5-HT + methoxamine 1 μM	$3.85\ (0.88-16.83) \times 10^{-8}$	89.55 ± 3.46	5
5-HT + clonidine 0.1 μM	$1.07\ (0.77-1.50) \times 10^{-7}$ *	67.98 ± 5.58 *	8
5-HT + NE 0.3 μM + prazosin 0.3 μM [c]	$3.09\ (0.14-68.90) \times 10^{-7}$ *	88.88 ± 6.92 *	7
5-HT + NE 0.3 μM + yohimbine 0.3 μM [c]	$9.20\ (1.16-73.65) \times 10^{-8}$	100.00 ± 0.00	5
5-HT + NE 0.3 μM + PTx 0.1 μg/ml	$1.58\ (0.51-4.87) \times 10^{-8}$ #	83.92 ± 5.62 #	6

RC, rhythmic constriction; 5-HT, serotonin; NE, norepinephrine; PTx, pertussis toxin; n, number of experiments.
[a] Concentrations of 5-HT producing 50% maximum inhibition (IC_{50}) are geometric means with 95% confidence intervals.
[b] Percentage of papaverine-induced maximum relaxation.
[c] In the presence of ketanserin (0.1 μM).
* $P < 0.05$ vs. control and # $P < 0.05$ vs. norepinephrine (0.3 μM) indicate significant differences (unpaired Student's t-test).

unpaired samples, as appropriate. The following drugs were used: U46619 (Upjohn, Kalamazoo, MI, USA), *l*-norepinephrine bitartrate, serotonin creatinine sulfate, clonidine hydrochloride, yohimbine hydrochloride, methoxamine, ketanserin, pertussis toxin, indomethacin, and 3-isobutyl-1-methylxanthine (all from Sigma, St Louis, MO, USA), and prazosin hydrochloride (Pfizer, Brooklyn, NY, USA).

3. Results

3.1. Effects of norepinephrine, clonidine and methoxamine on 5-HT inhibition of spontaneous rhythmic constriction

In the presence of active muscle tone induced by U46619 (0.3 μM) and norepinephrine (1 μM), the porcine pial venous rings exhibited rhythmic constriction (Fig. 1). The rhythmic constriction, in the presence of U-46619, were inhibited by 5-HT concentration-dependently; a result consistent to our previous findings (Lee and Sunagane, 1989; Lee et al., 1994; Ueno et al., 1995). This inhibition was not affected by indomethacin (3 μM, $n = 5$) (Table 1). The 5-HT inhibition of rhythmic constriction, however, was diminished or absent in the presence of norepinephrine (Fig. 1B). Attenuation of 5-HT inhibition of rhythmic constriction by norepinephrine is concentration-dependent with maximum effect of norepinephrine at 1 μM (Fig. 1B, Fig. 2, Table 1). 5-HT inhibition of rhythmic constriction was also blocked by clonidine (0.3 μM), but was not by methoxamine (1 μM) as judged by IC_{50} values and maximum inhibitory responses (Fig. 3, Table 1).

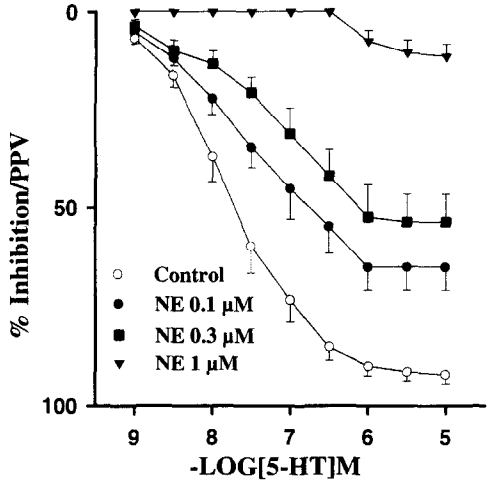

Fig. 2. 5-HT-induced inhibition of rhythmic constriction in porcine pial veins in the presence of U-46619-induced active muscle tone was concentration-dependently blocked by norepinephrine. Each point represents mean ± S.E.M. ($n = 5$) of percentage of maximum inhibition induced by PPV (papaverine; 300 μM).

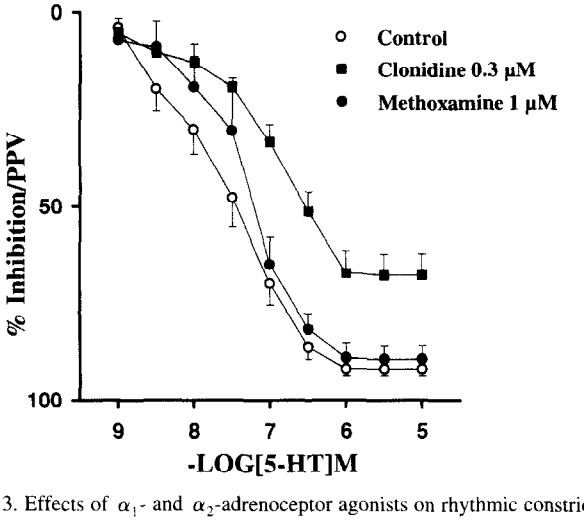

Fig. 3. Effects of α_1- and α_2-adrenoceptor agonists on rhythmic constriction in porcine pial veins in the presence of active muscle tone induced by U-46619. 5-HT-induced inhibition of rhythmic constriction was only partially blocked by clonidine (0.3 μM), but not by methoxamine (1 μM). Each point represents mean ± S.E.M. ($n = 5$) of percentage of maximum inhibition induced by PPV (papaverine; 300 μM).

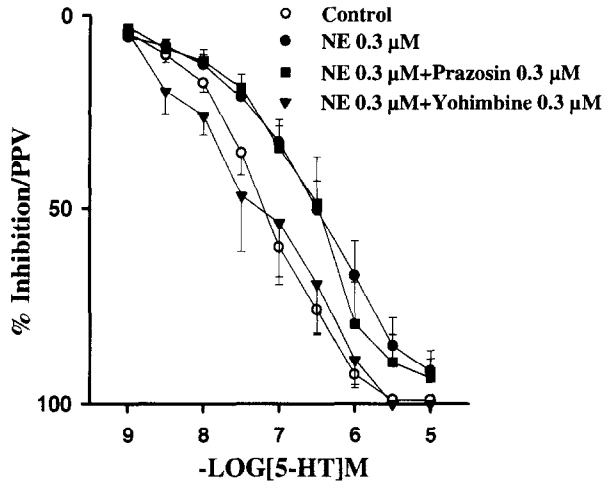

Fig. 4. Effects of α_1- and α_2-adrenoceptor antagonists on the norepinephrine blockade of 5-HT inhibition of rhythmic constriction in porcine pial veins in the presence of ketanserin (0.1 μM) and active muscle tone induced by U-46619. Norepinephrine blockade of 5-HT inhibition of rhythmic constriction was prevented by yohimbine (0.3 μM), but not by prazosin (0.3 μM). Each point represents mean ± S.E.M. ($n = 5$) of percentage of maximum inhibition induced by PPV (papaverine; 300 μM).

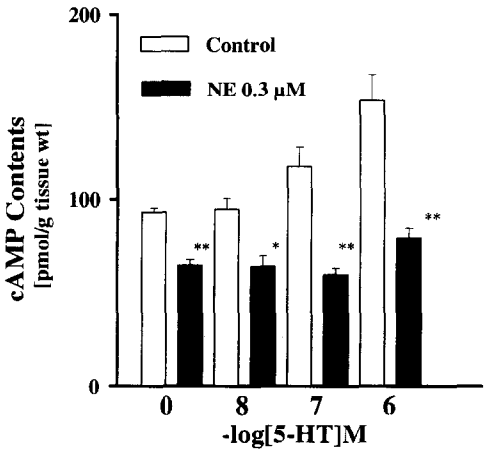

Fig. 6. Effects of 5-HT and norepinephrine on cAMP contents in porcine pial veins. Open columns indicate the reported data (Ueno et al., 1995) that vascular cAMP contents in porcine pial veins are increased by 5-HT in a concentration-dependent manner. On the other hand, in the presence of norepinephrine (0.3 μM), the 5-HT-induced increase of cAMP contents was inhibited (solid columns). Each point represents mean ± S.E.M. ($n = 4$). * $P < 0.02$ and ** $P < 0.01$ indicate significantly different from the respective control.

3.2. Effects of α-adrenoceptor antagonists

In the presence of ketanserin (0.1 μM) and in the presence of active muscle tone induced by U-46619 (0.3 μM), the norepinephrine attenuation of maximum 5-HT inhibition of rhythmic constriction was significantly smaller than that in the absence of ketanserin, but the IC_{50} values and maximum inhibition were still significantly different from the control values. The norepinephrine attenuation effect in the presence of ketanserin was prevented by yohimbine (0.3 μM), but was not affected by prazosin (0.3 μM) (Fig. 4, Table 1).

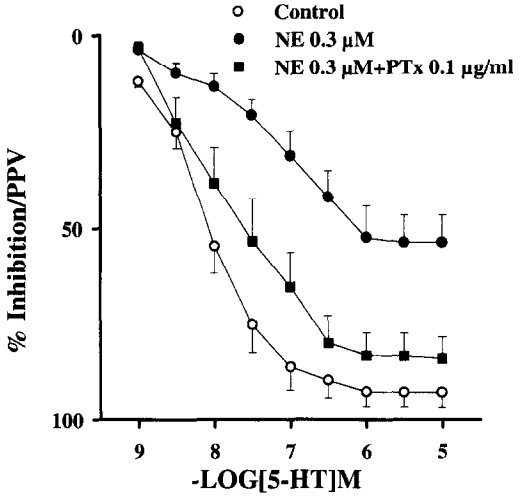

Fig. 5. Effect of pertussis toxin (PTx) on the norepinephrine blockade of 5-HT inhibition of rhythmic constriction in porcine pial veins. PTx (100 ng/ml) significantly reversed the blocking effect of norepinephrine. Each point represents mean ± S.E.M. ($n = 5$) of percentage of maximum inhibition induced by PPV (papaverine; 300 μM).

3.3. Effects of pertussis toxin

In venous rings precontracted with U-46619 (0.3 μM), pertussis toxin (100 ng/ml) incubated for 2 h did not significantly affect the vessel tone or rhythmic constriction. This concentration of pertussis toxin, however, attenuated the norepinephrine blockade of 5-HT-induced inhibition of rhythmic constriction (Fig. 5, Table 1).

3.4. Effects of norepinephrine on cAMP contents

In the presence of U46619 (0.3 μM)-induced active muscle tone and rhythmic constriction, the basal cAMP contents in porcine pial veins were significantly decreased by norepinephrine (0.3 μM). This concentration of norepinephrine also inhibited the synthesis of cAMP induced by 5-HT (0.01–1 μM) measured 1 min after its addition (Ueno et al., 1995) (Fig. 6).

4. Discussion

We have reported that 5-HT induces relaxation and inhibition of rhythmic constriction in porcine pial veins by activating vascular cAMP synthesis (Lee et al., 1994; Ueno et al., 1995). This effect of 5-HT was not resulted from releasing nitric oxide from endothelial cells (Lee et al., 1994). It is not due to release of endothelial prostanoids either, since 5-HT inhibition of rhythmic constriction is not affected by indomethacin. This is consistent with our previous reports that 5-HT inhibition of rhythmic constriction in porcine pial veins still exists in the presence of indomethacin (Lee et al., 1994; Ueno et al., 1995). These

results support our original findings that 5-HT inhibits rhythmic constriction and venous tone by activating 5-HT receptors on the vascular smooth muscle cells (Lee and Sunagane, 1989; Lee et al., 1994).

The present study, for the first time, demonstrated that 5-HT-induced inhibition of rhythmic constriction in the porcine pial veins was attenuated by norepinephrine in a concentration-dependent manner. This effect of norepinephrine was mimicked by clonidine, but not by methoxamine. In addition, this norepinephrine effect was prevented by yohimbine, but not by prazosin. These results suggest that norepinephrine attenuation of 5-HT inhibition of rhythmic constriction is mediated by α_2-adrenoceptors. It should be noted that in examining effects of yohimbine and prazosin on norepinephrine effect, pial veins were pretreated with ketanserin in order to eliminate the potential involvement of α_1-adrenoceptors and 5-HT_2 receptors (Leysen et al., 1981). These two types of receptors have been shown to play a small vasomotor role in this vasculature (Asada and Lee, 1992; Lee et al., 1994). In the presence of ketanserin, attenuation by norepinephrine of 5-HT-induced maximum inhibition of spontaneous rhythmic constriction, however, was significantly decreased (Fig. 4). The amplifying vasoconstricting effects between 5-HT_2 receptors and α-adrenoceptors in other vascular beds have been reported (De La Lande, 1990; Shimamoto et al., 1994). It appears that in porcine pial veins norepinephrine may amplify the vasoconstricting effects of 5-HT via 5-HT_2 receptors, thus attenuating the 5-HT inhibition (via 5-HT_1-like receptors) of venous tone and rhythmic constriction (Lee et al., 1994). Blocking 5-HT_2 receptors by ketanserin therefore decreased the attenuating effect of norepinephrine on 5-HT inhibition of rhythmic constriction.

The involvement of α_2-adrenoceptors in norepinephrine blockade of 5-HT-induced inhibition of rhythmic constriction and venous tone is further supported by the finding that the norepinephrine attenuating effect was prevented by pertussis toxin pretreatment. This toxin did not affect the basal tone or rhythmic constriction, suggesting the specifity of this toxin in blocking norepinephrine effect. It is well known that α_2-adrenoceptors are linked in an inhibitory fashion to adenylate cyclase via the pertussis toxin-sensitive G_i-proteins in many cell types (Dohlman et al., 1987), and that porcine pial veins contain predominantly α_2-adrenoceptors (Asada and Lee, 1992). Furthermore, the basal level and 5-HT-induced synthesis of cAMP in pial veins were inhibited by norepinephrine at the concentration which significantly inhibited the rhythmic constriction. Together, these results suggest that the α_2-adrenoceptors which mediate norepinephrine blockade of 5-HT inhibition of rhythmic constriction and venous tone are coupled to G_i-proteins.

Results of our preliminary studies have indicated that 5-HT-induced inhibition of rhythmic constriction is blocked by ergotamine, which is an effective antimigraine drug (Saxena and Den Boer, 1991). This blockade by ergotamine is prevented by yohimbine (3×10^{-7} M), suggesting that ergotamine acts via α_2-adrenoceptors. This is consistent with the report that several clinically effective antimigraine drugs such as ergotamine inhibit adenylate cyclase (Deliganis and Peroutka, 1991) or bind to the receptors that are negatively coupled to adenylate cyclase via 5-HT receptors (Humphrey et al., 1988; Sumner and Humphrey, 1990).

In summary, results of the present study demonstrate that 5-HT inhibition of porcine pial venous rhythmic constriction is attenuated by norepinephrine. This effect of norepinephrine is mediated by α_2-adrenoceptors coupled to pertussis toxin-sensitive G-proteins, possibly the G_i-protein. These results are consistent with our previous findings that 5-HT inhibition of porcine pial venous tone and rhythmic constriction is mediated by cAMP synthesis. They further suggest that the dense adrenergic innervation in porcine pial veins plays a role in regulating pial venous circulation.

Acknowledgements

This work was supported by grants from NIH (Nos. HL27763 and HL47574), AHA/IHA (No. 91010850), Fraternal order of Eagles foundation and Southern Illinois University School of Medicine. We thank Mrs. Dawn Melcher for preparing the manuscript.

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