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Cyclopiazonic acid alters serotonin-induced responses in rat thoracic aorta

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ABSTRACT

We previously showed that endothelin A (ET_A) receptor antagonist BQ-123 partially inhibited cyclopiazonic acid (CPA)-enhanced endothelin-1 (ET-1)-induced contractions suggesting enhancement of ET_A receptor internalization in caveolar structures by sarco/endoplasmic reticulum Ca^{+2} ATPase (SERCA) blockade. Since serotonin (5-Hydroxytryptamine, 5-HT) receptors are reported to be localized on caveolar membranes, we investigated whether SERCA inhibition affects 5-HT-induced responses and 5-HT receptor antagonism. For this purpose, vascular responses were measured in thoracic aorta segments from male Wistar albino rats using isolated tissue experiments. Data showed that CPA inhibits 5-HT- and PE-induced contractions in intact vessels while potentiating those in endothelium-denuded. Furthermore, non-selective 5-HT receptor blocker methysergide partially inhibited CPA-induced 5-HT contractions. However, α_1-adrenergic receptor antagonist prazosin totally inhibited CPA-potentiated PE contractions. We suggest that SERCA inhibition results in 5-HT receptor internalization similar to ET_A receptors possibly through protein kinase C activation by increased subsarcolemmal Ca^{2+} levels, eventually preventing 5-HT receptor antagonism.

1. Introduction

Store-operated Ca^{2+} entry (SOCE) takes part in the regulation of intracellular Ca^{+2} ($[Ca^{2+}]_i$) in vascular smooth muscle [1] and endothelial cells as well [2,3]. SOCE is activated by intracellular Ca^{+2} store (sarcoplasmic reticulum, SR) depletion either by agonist induced inositol 1,4,5-trisphosphate (IP_3) formation or SR Ca^{+2} ATPase (SERCA) inhibitors such as thapsigargin and cyclopiazonic acid (CPA). IP_3-induced Ca^{2+} release from CPA-sensitive stores also contributes to endothelin (ET-1)- and serotonin (5-Hydroxytryptamine, 5-HT)-induced contractions [19–21]. In the presence of endothelium, SOCE leads to endothelium-dependent vascular relaxations via endothelial $[Ca^{2+}]_i$ elevation [4], endothelial nitric oxide synthase (eNOS) activation and nitric oxide release [5,6]. eNOS activity is suppressed by caveolin-1, a caveolar scaffolding protein [7], and induced by Ca^{2+}-calmodulin [8]. Endothelial $[Ca^{2+}]_i$ elevation results in the displacement of eNOS from caveolae followed by NO release [9]. On the other hand, SOCE mediates $[Ca^{2+}]_i$ elevation and vascular contraction in the absence of endothelium [10–12].

Prolonged elevation of $[Ca^{2+}]_i$ results in cellular damage especially in cardiac and cerebral ischemia [13]. In these pathological events, SERCA plays an essential role in $[Ca^{2+}]_i$ regulation by pumping Ca^{2+} into SR [14]. Changes in SERCA expression have been associated with cardiovascular diseases [15,16]. SERCA2 mRNA levels and SR Ca^{2+} uptake decreased in post-myocardial infarction failing rat hearts [17] and in hypertrophic cardiac myocytes [18].

5-HT plays important roles in physiological processes such as platelet aggregation, proliferation, gastrointestinal motility, and smooth muscle contraction and in pathological conditions as well. Although 5-HT is an established vasoconstrictor in vitro, its in vivo effects on blood pressure are not clarified yet. Circulating free 5-HT levels were elevated in hypertension [22] and 5-HT-induced vascular contractions significantly enhanced in stroke-prone spontaneously hypertensive rats [23]. Furthermore, higher 5-HT levels were found to be associated with cardiac events and coronary artery disease [24]. Controversial effects of 5-HT in the regulation of vascular tone and hypertension have also been reviewed recently [25]. 5-HT is also involved in migraine pathogenesis and selective $5\text{-}HT_{1B}$ and $5\text{-}HT_{1D}$ receptor agonists, triptans, used for acute treatment of migraine headaches [26,27]. Furthermore, the $5\text{-}HT_{2A}$ receptors are widely distributed in the brain [28] and are therapeutically important in the treatment of depression.

$5\text{-}HT_{2A}$, a G protein-coupled receptor (GPCR), mediates 5-HT contractions in rat thoracic aorta [29] and pulmonary arteries [30]. It activates phospholipase C via Gq resulting in IP_3 and DAG formation, which induces protein kinase C (PKC) and Ca^{2+} entry through voltage-operated Ca^{2+} (VOC) channels. In human pulmonary artery, Ca^{+2} entry via VOC and SOC channels, Ca^{2+} release from SR and Ca^{2+} sensitization of the contractile apparatus contribute to 5-HT-induced responses [21]. In rat thoracic aorta, tyrosine kinase and p38 MAPK pathways are also involved in 5-HT-induced contractions [31].

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The localization of 5-HT_{2A} receptors in caveolae was shown in a number of cell types including vascular smooth muscle cells [32,33]. Caveolae, plasmalemmal microdomains characterized by caveolin-1 presence [34], regulate GPCR signaling as well as spatiotemporal regulation of $[Ca^{2+}]_i$ [9,35]. Caveolar integrity is essential for 5-HT-induced contractions and proper 5-HT_{2A} receptor signaling in smooth muscle cells [36,37]. Furthermore, disruption of caveolae has been shown to significantly reduce SOCE [38–40]. Transient receptor potential canonical (TRPC)1 is an important component of SOC channel [41,42] and a purported regulator of SOCE [43]. It is known that co-localization of TRPC1 with caveolin-1 is essential for SOCE activation [38,39,44]. In addition to TRPC1, another purported regulator of SOCE, STIM1, was shown to be scaffolded by lipid raft domains [45].

We previously showed that depletion of SR Ca^{2+} by 10 μM CPA did not affect the resting tone in the presence of indomethacin in endothelium-denuded rat thoracic aorta [12,20]. However, 10 μM CPA potentiated ET-1- and PE-induced vascular contractions [20]. In our previous report, CPA-enhanced PE contractions were abolished by α_1-AR selective antagonist prazosin whereas ET_A receptor antagonist BQ-123 partially inhibited CPA-enhanced ET-1 contractions suggesting that ET_A receptor internalization in caveolar structures is enhanced by SERCA blockade [20].

We hypothesized that 5-HT-mediated contractions and 5-HT receptor antagonism are affected by SERCA blockade. The potentiation of 5-HT contractile responses and any decrease in 5-HT receptor antagonism by SERCA downregulation may contribute to cardiovascular and cerebral pathologies. Therefore, we investigated the effects of SERCA downregulation simulated by SERCA blocker CPA, on 5-HT- and PE-induced contractions as well as on 5-HT and PE receptor antagonism in rat thoracic aorta.

2. Methods

2.1. Animals and isolated tissue experiments

All animal experiments were approved by the Institution's Committee on Animal Use in Research and Education, Ege University. Rats (300–350 g, male Wistar albino) were maintained in appropriate conditions with ad libitum access to food and water, standard temperature and humidity, and a 12-hour light/dark cycle. Animals were asphyxiated with CO_2. The thoracic aorta was removed, cleaned from extraneous fatty tissue and dissected into 3 mm rings. Endothelium was removed by rubbing the luminal side of the vessel with a cotton thread. A detailed protocol of isolated tissue experiments was given previously [20]. Changes in isometric force were recorded via data acquisition system (BioPac Systems, MP100A-CE). Force development was normalized to cross sectional area [force (in mN) / cross sectional area (in mm^2, F/CSA) = (change in force × circumference) / 2 × wet wt].

2.2. Chemicals

All chemicals were from Sigma and dissolved in appropriate solvents as given: TRIM (PubChem CID: 1359) (10^{-2} M) in 0.9% NaCl; 5-HT (PubChem CID: 160436) (10^{-2} M) in distilled water (DW); PE (PubChem CID: 5284443) (10^{-1} M) in DW; CPA (PubChem CID: 54695722) (10^{-1} M) in dimethylsulfoxide (DMSO); indomethacin (PubChem CID: 3715) (10^{-2} M) in EtOH; verapamil HCl (10^{-2} M) in DW, methysergide (PubChem CID:5281073) (10^{-2} M) in DMSO; and prazosin (PubChem CID:68546) (10^{-2} M) in EtOH. Final DMSO and EtOH concentrations did not exceed 0.1% to avoid vehicle's vasorelaxant effect [46].

2.3. Data analysis

Data analyses as well as graphical presentations were done by using GraphPad Prism5. The least squares method was used for non-linear curve-fitting. The results were given as mean ± standard error of the mean. "n" represents the number of animals used. The significance of differences was evaluated by the Student's *t*-test for two groups and one-way ANOVA with post-hoc Newman–Keuls test for multiple comparisons. $P < 0.05$ was considered significant.

3. Results

In order to determine optimum agonist concentration, concentration–response curves were obtained in the presence of increasing 5-HT and PE concentrations in endothelium-denuded rat thoracic aorta in the presence of 10 μM indomethacin, a nonspecific cyclooxygenase inhibitor, to eliminate endogenous prostanoid synthesis (Fig. 1). The EC_{50} values (in nM) were 546.90 ± 12.80 and 47.97 ± 3.99 for 5-HT and PE, respectively. Based on these data, 5-HT at 300 nM was used in endothelium-denuded vessels. Although the EC_{50} value for PE was approximately 50 nM, in order to obtain contractions similar to that of 5-HT (approximately 10 mN), PE at 300 nM was used in endothelium-denuded vessels based on our previous study [20]. Since agonists at 300 nM concentration did not induce any measurable contractions in the presence of endothelium, a semi-logarithmic higher agonist concentration (1 μM) yields a measurable force development in intact vessels.

A selective SERCA inhibitor, CPA was used to mimic SERCA downregulation. CPA at 10 μM that reportedly depletes SR Ca^{2+} [47] was

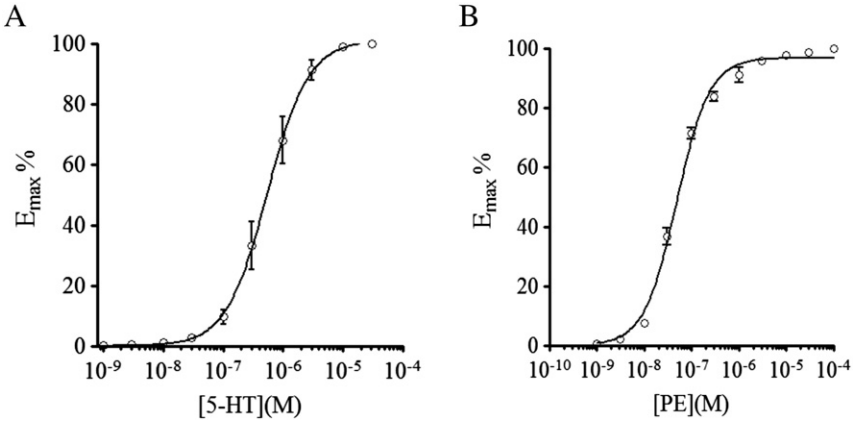

Fig. 1. Concentration–response curves of 5-HT (A) and PE (B). Concentration–response curves were constructed for 5-HT and PE in endothelium-denuded rat thoracic aorta in the presence of 10 μM indomethacin (n = 3–4).