Original Article

Interaction between Serotoninergic-and β-adrenergic Receptors Signaling Pathways in Rat Femoral Artery

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Abstract

Background: Coronary heart disease has been widely studied in cardiovascular research. However, patients with peripheral artery disease (PAD) have worst outcomes compared to those with coronary artery disease. Therefore, pharmacological studies using femoral artery are highly relevant for a better understanding of the pathophysiologic responses of the PAD.

Objective: The aim of this study was to evaluate the pharmacologic properties of the contractile and relaxing agents in rat femoral artery.

Methods: Concentration response curves to the contractile phenylephrine (PE) and serotonin (5-HT) and the relaxing agents isoproterenol (ISO) and forskolin were obtained in isolated rat femoral artery. For relaxing responses, tissues were precontracted with PE or 5-HT.

Results: The order rank potency in femoral artery was 5-HT > PE for contractile responses. In tissues precontracted with 5-HT, relaxing responses to isoproterenol was virtually abolished as compared to PE-contracted tissues. Forskolin, a stimulant of adenylyl cyclase, partially restored the relaxing response to ISO in 5-HT-precontracted tissues.

Conclusion: An interaction between β-adrenergic- and serotoninergic- receptors signaling pathway occurs in femoral artery. Moreover, this study provides a new model to study serotoninergic signaling pathway under normal and pathological conditions which can help understanding clinical outcomes in the PAD. (Arq Bras Cardiol 2012;98(1):29-34)

Keywords: Peripheral arterial disease, femoral artery/physiopathology, receptors, adrenergic/alpha, receptors, adrenergic/beta, receptors, serotonin.

Introduction

Femoral artery is an important branch of the iliac artery that irrigates the lower limb skeletal muscles and peripheral tissues and it is a unique blood vessel with a long conduction, high-flow resistance and a striking relevance for medical interventions[1]. Indeed, approximately 50% of all athero-occlusive disease takes place in the femoral artery which can result in intermittent claudication and critical limb ischemia with significant impairment of patients' daily activity[2]. The incidence of peripheral artery occlusive disease (PAD) has been increasing in the world population and considerable efforts have been made to improve the poor interventional outcomes in long-term treatment. Moreover, factors such as smoking, dyslipidemia, diabetes mellitus, atherosclerosis and advancing age have been implicated in the pathogenesis of the PAD[3,4]. Although most of the many cardiovascular researches have been focused on the coronary heart disease such as advances in surgical interventions and discovery of new pharmacological therapies, patients with PAD have worst outcomes compared to those with coronary artery disease[5]. For that reason, pharmacological studies using femoral artery are highly relevant for a better understanding of clinical and pathophysiologic responses of the PAD.

It is well known that serotonin (5-hydroxytryptamine, 5-HT) plays important biologic functions in the cardiovascular system including platelet aggregation, bradycardia or tachycardia, hypotension or hypertension, and vasodilatation or vasoconstriction[6,7]. The diversity of the functional actions of the 5-HT relates to its receptor subtypes number as well as to the complexity of the signaling pathway involved in their responses. In this regard, at least fifteen 5-HT receptor subtypes have been characterized which are subdivided into seven receptor families according to their pharmacologic properties, amino acid sequences, gene organization, and second messenger coupling pathways[8,9]. The 5-HT_1, 5-HT_2, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 receptor families are coupled to G-proteins, whereas the 5-HT_3 receptors are 5-HT-gated ion channels. Among the 5-HT receptors, 5-HT_1 and 5-HT_2 receptors subtypes play an important role in the cardiovascular system regulation

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where 5-HT_1 receptor family is mostly linked to Gi/o protein, which inhibit cAMP formation, whereas 5-HT_2 receptors family is coupled preferentially via Gq/11 protein leading to the activation of IP3/PKC/cytosolic [Ca^{2+}] signaling pathway[10]. Both 5-HT_1 and 5-HT_2 receptor families have been associated with contraction of vascular smooth muscle and thrombus formation[9,11].

The activation of α- and β-adrenergic receptors by catecholamines also produces a number of functional responses in the cardiovascular system including positive chronotropic responses and vascular tone control[12,13]. The α–adrenergic receptor-mediated vasoconstriction has been widely studied under normal and pathological conditions[14-16], but few studies evaluate the β-adrenergic receptors-mediated vasodilatation[17,18]. In this regard, at least three β-adrenergic receptors subtypes mediate the relaxation responses in vascular tissues, which are coupled to Gs protein leading to the activation of cAMP-dependent protein kinase (PKA) signaling pathway[19].

Either serotoninergic or adrenergic receptors signaling pathways are targets for the treatment of the cardiovascular diseases including heart failure, arterial hypertension, coronary artery disease and PAD[4,20]. Thus, pharmacological studies regarding small arteries and the interactions between drugs involving both serotoninergic and adrenergic receptor signaling pathways are crucial for developing new compounds in a attempt to improve the quality of life of patients with vascular disease. Therefore, the objective of this study was to evaluate the pharmacologic properties of the contractile (5-HT and phenylephrine) and relaxing agents (isoproterenol and forskolin) in the proximal segment of rat femoral artery.

Methods

Animals

This study was approved (protocol: 1307-1) by the Ethical Committee of the Medical School of Universidade de Campinas (UNICAMP). They were individually housed at 26 ± 2 °C with food and water delivered ad libitum on a 12 h light: dark cycle with the lights turned on at 6:00 a.m. Male Wistar rats (407±6g) were stunned by inhalation of CO_2, euthanized by decapitation, and exsanguinated.

Rat isolated femoral artery rings

The femoral artery was quickly removed and placed in chilled Krebs-Henseleit buffer with the following composition (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.17, $CaCl_2.2H_2O$ 2.5, $NaHCO_3$ 25 and glucose 5.6. The proximal segment of the femoral artery was isolated and two rings (approximately 2 mm length) were taken and mounted in a 5 ml organ chamber. A wire myograph for isometric force recording (Danish Myo Technology, model 610M, Aarthus N, Denmark) coupled with an acquisition system with specific software (PowerLab 8/30, LabChart 7, ADInstruments, Sydney-NSW, Austrália). The bathing solution was maintained at 37°C and continuously gassed with 95%-O_2 and 5%-CO_2, with of pH 7.4. The tissues were allowed to equilibrate for 60 minutes under a resting tension of 1 mN[21].

Concentration-response curves to contractile agents

Following the equilibration period, the rings were precontracted with KCl 80 mM to verify the tissue viability and washed with Krebs. The rings were precontracted with phenylephrine (PE: 1 μM) and relaxed to acetylcholine (ACH: 1 μM) to confirm the endothelium integrity. Rings lacking contractile or relaxing responses were disposed of.

To evaluate the contractile responses mediated by serotoninergic and α-adrenergic receptors, cumulative concentration-response curves to 5-hydroxytryptamine (5-HT: 1 nM - 100 μM) and phenylephrine (PE: 1 nM - 100 μM) were constructed in the proximal segment of the femoral artery rings[22].

Concentration-response curves to isoproterenol (1 nM - 10 mM) were obtained in precontracted tissues with PE (1 μM) or 5-HT (10 μM) to further analyze the interactions between β-adrenergic receptors activation and α-adrenergic or serotoninergic receptors. We also performed concentration-response curves to adenylyl cyclase activator, forskolin (1 nM - 100 μM) in femoral rings, to determine whether the drug interactions could be at receptor level or beyond.

All the concentration-response data were evaluated in order to fit into a logistics function in the formula:

$$E = Emax/((1 + (10^c/10^x)^n) + \Phi)$$

where E is the effect of above basal; Emax is the maximum response produced by the agonist; c is the logarithm of the EC_{50}, the concentration of the agonist that produces half-maximal response; x is the logarithm of the concentration of the agonist; the exponential term, n is a curve-fitting parameter that defines the slope of the concentration response line, and Φ is the response observed in the absence of the agonist added. Nonlinear regression analysis was used to determine the parameters Emax and log EC_{50}, by using GraphPad Prism (GraphPad Software Inc., San Diego, CA) with the constraint that $\Phi = 0$. The responses for each agonist are shown as the mean ± SEM of potency (pEC_{50}) and maximal response (Emax). The relaxations were plotted as percentages of the contractions induced by PE or 5-HT and the contractile responses were plotted as percentage of the concentration induced by KCl (80 mM).

Statistical analysis

The data are expressed as mean ± SEM of *n* experiments. Paired or unpaired Student's *t* test was performed using specific software (InStat, GraphPad Software. La Jolla-CA, USA). Values of $p < 0.05$ were considered statistically significant.

Drugs

Acetylcholine chloride, phenylephrine hydrochloride, 5-hydroxytryptamine, forskolin and isoproterenol were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Results

Contractile responses

Both 5-HT and PE produced concentration-dependent contractile responses in femoral artery rings (Figure 1A). The potency of 5-HT was significantly greater compared to the α_1-agonist PE, approximately 20-fold, in the proximal segment of rat femoral artery. No differences were found in the maximal responses for both agonists. The data are summarized in Table 1.

Relaxing responses

In another set of experiments, we evaluate the relaxing responses to β-adrenergic agonist isoproterenol where femoral artery rings were precontracted with PE (10 μM) or 5-HT (1 μM). In PE-precontracted rings, isoproterenol produced concentration-dependent relaxation responses. However, in 5-HT-precontracted rings, isoproterenol evoked a slight relaxing response in femoral artery rings compared to its paired PE-precontracted rings (Figure 1B). To further test the hypothesis that 5-HT-precontracted tissues were affecting at the level of β-adrenergic receptor or beyond (signaling pathway), concentration-response curves to the adenylyl cyclase activator forskolin were obtained in femoral artery rings precontracted with PE (10 μM) or 5-HT (1 μM). In PE-precontracted rings, forskolin produced concentration-dependent relaxation responses. However, a parallel dextral displacement, approximately 9-fold, was observed in the concentration-response curves to forskolin in 5-HT precontracted tissues as compared to its paired PE-precontracted femoral artery rings (Figure 1C). No differences were found in the maximal responses for adenylyl cyclase activator precontracting with both agents. All data are summarized in Table 1.

Discussion

In this study, we observed that the order rank potency in the proximal segment of femoral artery was 5-HT > PE for contractile responses that may reflect a greater density of serotoninergic receptors and/or high effectiveness of its signaling pathway mechanism in this preparation compared to the activation of α-adrenergic receptor/cAMP/PKA downstream pathway. Interestingly, a previous study found a similar rank potency for 5-HT in different artery rings including mesenteric, caudal and basilar[23]. Moreover, the potency for 5-HT found in our study for proximal segment in the femoral artery (6.86) was closely related to those obtained in the basilar artery (6.88) indicating that both preparations present great similarities in pharmacologic properties. Interestingly, clinical evidences have shown that PAD is more common in migraineurs than in controls[24,25]. Altogether, our data showed that femoral artery is an interestingly model to study serotoninergic receptor signaling pathway under normal and pathological conditions such as diabetes mellitus and atherosclerosis.

In this study, we have also found that precontracted tissues with 5-HT virtually abolished the relaxing responses to isoproterenol (10% of maximal responses) that was partially restored by forskolin, a direct-acting stimulant of adenylyl cyclase that bypass β-adrenergic receptors. Therefore, our findings showed an interaction between β-adrenergic- and serotoninergic- receptors signaling pathway in rat femoral artery. In this regard, a previous study demonstrated a cross-talk between β-adrenergic receptor and 5-HT_1 on glutamate release in cerebrocortical nerve terminals[26]. Thus, our data reinforce that the proximal segment of the femoral artery is an interesting vascular tissue to study 5-HT receptors and its complex signaling pathway. However, we cannot ascertain from this study which 5-HT receptor subtype is mediating the contractile response

Table 1 - Potency (pEC50) and maximal responses (Emax) values obtained from concentration-response curves to phenylephrine (PE), 5-hydroxytryptamine (5-HT), isoproterenol (ISO) and forskolin in rat femoral artery rings precontracted with PE or 5-HT

	pEC_{50}	Emax
Contractile responses		
PE	5.50±0.04 (10)	114±2
5-HT	6.86±0.01 (8)*	119±2
Relaxation responses		
ISO (PE-precontracted)	5.33±0.08 (6)	95±2
ISO (5-HT-precontracted)	ND (4)	10±1†
Forskolin (PE-precontracted)	6.70±0.06 (4)	102±1
Forskolin (5-HT-precontracted)	5.74±0.07(5)‡	96±1

*Potency is represented as -log of molar concentration to produce 50% of the maximal response. Data represent the mean ± S.E.M. of 4-10 experiments. ND: not determined. Number of experiments is shown in parentheses. *p < 0.05 compared to PE contractile responses. †p < 0.05 compared to ISO relaxation in PE-precontracted tissues.‡p < 0.05 compared to forskolin relaxation in PE-precontracted tissues.*

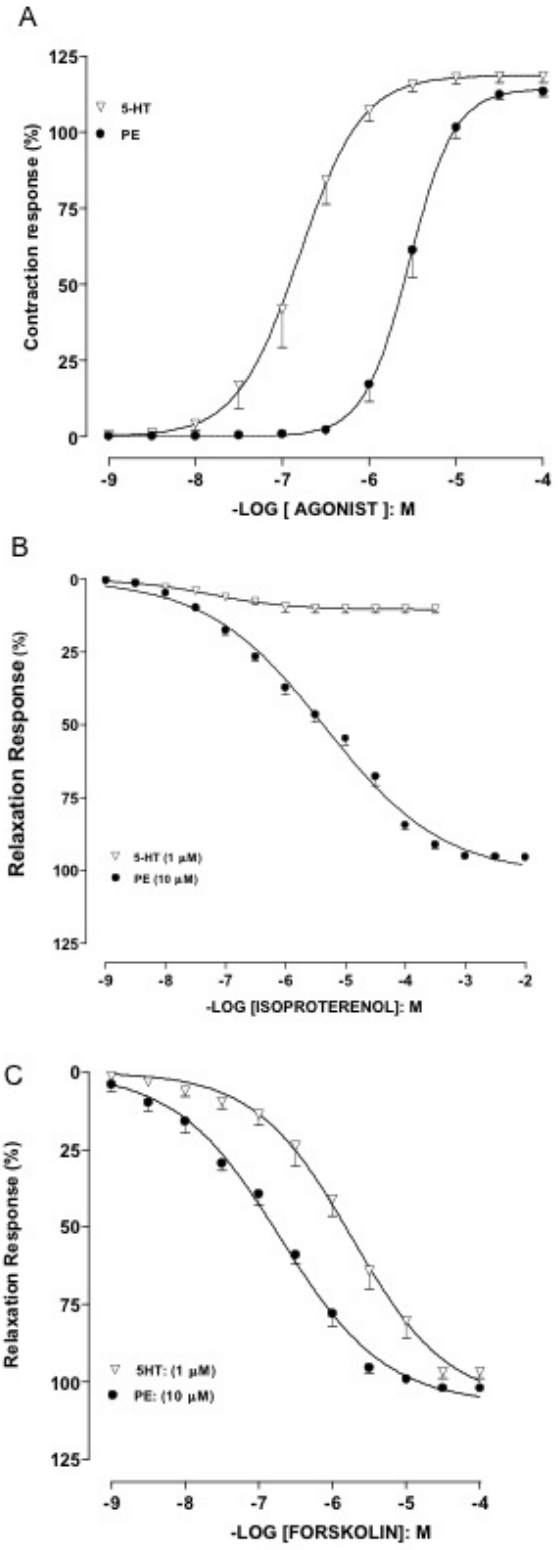

Figure 1 - *Concentration-response curves to phenylephrine (●PE, n = 10) and 5-hydroxytryptamine (▽5-HT, n = 08) in rat femoral artery (panel A). Relaxations induced by isoproterenol precontracted with PE (10 µM, n = 06, ●) or 5-HT (1 µM; n = 4, ▽) in rat femoral artery (panel B). Relaxations induced by forskolin precontracted with PE (10 µM, n = 04, ●) or 5-HT (1 µM; n = 5, ▽) in the rat femoral artery (panel C). Data are means ± SEM.*

and/or which transduction effectors are contributing to the suppression of the β-adrenergic receptor-mediating relaxation in rat femoral artery. Evidence has shown that the 5-HT_1 receptors family is mostly linked to Gi/o, which are pertussis toxin sensitive and negatively coupled with adenylyl cyclase[9]. On the other hand, β-adrenergic receptor activation is coupled to Gs protein which in turn leads to the activation of cAMP/PKA signaling pathway in the cardiovascular system[19]. Thus, the antagonistic cross-talk between 5-HT receptors and β-adrenergic receptor could be occurring at G-protein-coupled receptor as well as at the level of PKA substrates since forskolin partially restored the suppression of the β-adrenergic receptor-mediating relaxation in 5-HT-precontracted rat femoral artery ring. The functional significance of these data may be related to the incidence of vascular disease and 5-HT agonists/5-HT uptake blockers administration in some disorders such migraine and obesity.

Conclusion

In conclusion, our data showed clearly that an interaction between β adrenergic- and serotonergic-receptors signaling pathway occurs in rat femoral artery rings. Moreover, this study provides an interesting perspective to study serotoninergic receptor signaling pathway under normal and pathological conditions in an attempt to improve the clinical outcomes in patients with PAD.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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