Inhibition of protein tyrosine phosphatases unmasks vasoconstriction and potentiates calcium signaling in rat aorta smooth muscle cells in response to an agonist of 5-HT2B receptors BW723C86

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Abstract: In blood vessels, serotonin 5-HT2B receptors mainly mediate relaxation, although their activation by the selective agonist BW723C86 is known to exert contraction of aorta in deoxycorticosterone acetate (DOCA)-salt and N(*omega*)-nitro-L-arginine (L-NAME) hypertensive rats [Russel et al., 2002; Banes et al., 2003] and in mice with type 2 diabetes [Nelson et al., 2012]. The unmasking effect on vasoconstriction can be caused by a shift in the balance of tyrosine phosphorylation in smooth muscle cells (SMC) due to oxidative stress induced inhibition of protein tyrosine phosphatases (PTP). We have demonstrated that BW723C86 which does not cause contraction of rat aorta and mesenteric artery rings, evoked a vasoconstrictor

effect in the presence of PTP inhibitors sodium orthovanadate (Na_3VO_4) or BVT948. BW723C86 induced a weak rise of $[Ca^{2+}]_i$ in the SMC isolated from rat aorta; however, after pre-incubation with Na_3VO_4 the response to BW723C86 increased more than 5-fold. This effect was diminished by protein tyrosine kinase (PTK) inhibitor genistein, inhibitor of Src-family kinases PP2, inhibitor of NADPH-oxidase VAS2870 and completely suppressed by N-acetylcysteine and 5-HT2B receptor antagonist RS127445. Using fluorescent probe DCFH-DA we have shown that Na_3VO_4 induces oxidative stress in SMC. In the presence of Na_3VO_4 BW723C86 considerably increased formation of reactive oxygen species while alone had no appreciable effect on DCFH oxidation. We suggest that oxidative stress causes inhibition of PTP and unmasking of 5-HT2B receptors functional activity.

Keywords: 5-HT2B receptors, smooth muscle cells, protein tyrosine phosphatases, oxidative stress, calcium, hypertension

1. Introduction

Serotonin (5-hydroxytriptamine, 5-HT) plays an important role in regulating embryonic development, the functions of intestine, nervous, cardiovascular and other systems in adult organism. The action of serotonin is mediated by seven types of 5-HT receptors. In blood vessels, 5-HT1B-, 5-HT1D-, 5-HT1F-, 5-HT2A-, 5-HT2B-, 5-HT4- and 5-HT7-receptors have been identified [1,2]. 5-HT2A receptors play the main role in implementation of the vasoconstrictor effect of serotonin [3]. For several vessels contraction was demonstrated in response to 5-HT1B receptors stimulation [3]. 5-HT2B receptors are expressed in endothelial cells and vascular SMC [1,2,4]. Available data suggest that the 5-HT2B receptors mediate vasodilation through activation of NO synthesis [4]. On the other hand, an agonist of 5-HT2B receptors BW723C86 [5] causes constriction of de-endothelized renal arteries [6], suggesting a direct effect on the SMC. Normally BW723C86 has no effect on basal tone of the aorta and mesenteric arteries, however it induces vasoconstriction in DOCA-salt hypertensive rats [7] and L-NAME hypertensive rat [8] as well as in mice with type 2 diabetes [9]. This suggests that 5-HT2B receptors operate as both vasodilators and vasoconstrictors, but normally their contractile effect is not manifested. The purpose of the present study was to find the conditions that lead to unmasking of vasoconstrictor effect of BW723C86. Since it is known that c-Src kinase plays an important role in the contractile action of serotonin [10], and protein tyrosine phosphatases (PTP) are inhibited by reactive oxygen species (ROS) which are increasingly produced in hypertension and diabetes [11], we assumed that an equilibrium shift in the tyrosine phosphatases/tyrosine kinases activities towards

tyrosine phosphorylation should cause unmasking of blood vessels contraction in response to BW723C86. To test this hypothesis, we used sodium orthovanadate (Na_3VO_4) and BVT948, which can induce an elevation of the intracellular concentration of ROS and promote inhibition of PTP through oxidation of SH-groups of cysteine [12,13], i.e. mimic processes that occur in oxidative stress. Calcium is playing a major role in vasoconstriction, so we studied whether inhibition of PTP potentiates elevation of $[Ca^{2+}]_i$ in rat aorta SMC in response to activation of 5-HT2B receptors.

2. Materials and methods

2.1. Reagents.

BW723C86 , RS127445, PP2, PP3, genistein from Tocris; DCFH-DA, Fura-2/AM from Molecular Probes; serotonin, angiotensin II, *N*-Acetyl-L-cysteine, TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl), VAS2870, Na_3VO_4, BVT948 from Sigma-Aldrich.

2.2. Animals

Male Wistar rats weighting 250-300 g were anesthetized with 25% urethane (4 ml/kg) and decapitated. The thoracic part of aorta and superior mesenteric artery were then removed. All manipulations with the animals were performed in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.3. Cell culture

Vascular SMC were derived from aorta of male Wistar rats in an explant method previously described in [14] and cultured in Dulbecco's Modified Eagle Medium (Sigma-Aldrich) containing 10% fetal calf serum.

2.4. Measurement of the mRNA expression of 5-HT2B, 5-HT2AR, 5-HT2C and 5-HT1B receptors

Total RNAs from rat aorta, mesenteric artery and rat aorta SMC was isolated using RNeasy MiniKit (QIAGEN). Reversed transcription was carried out using oligodT-primer. The levels of mRNA expression of 5-HT2B, 5-HT2A and 5- HT1B receptors were measured by real-time PCR. Elongation factor EF1a was used as the endogenous control. The following probes and primer pairs were used for amplification: for 5-HT2A receptors – FAM-TCACCACAGCCGCTTCAACTCCAGA-BHQ1, sense – CGCTATGTCGCCATCCAGAAC, antisense –CAGATATGGTCCACACGGCAATG; for 5-HTR2B receptor – probe FAM-TGCTCTTTGGCTCACTGGCTGCCT-BHQ1, sense –

TGCTGACAAAGGAACGTTTTGG, antisense TTAGGCGTTGAGGTGGCTTG, for 5-HTR2C receptors probe FAM-CGTTCTCATCGGGTCCTTCGTGGCA-BHQ1, sense – GAAAGCAAAGTGTTCGTGAATAAC, antisense – TCCTCCTCGGTGTGACCTC; for 5-HT1B receptors probe FAM-AGCAGTCCAGCACCTCCTCCTCCGC-BHQ1, sense – CACCCTTCTTCTGGCGTCAAG, antisense – CCGTGGAGTAGACCGTGTAGAG, for EF1A – probe FAM-CTGTTCGTGACATGAGGCAGACAGTTG-BHQ1, sense – GACTACCCTCCACTTGGTCGTT, antisense – GCCTTCTTGTCCACGGCTT.

2.5. Registration of aorta and mesenteric artery contraction

Blood vessels, isolated from Wistar rats, were cleaned from connective tissue and cut into rings with a width of 2-3 mm. The rings were mounted on the holders in chambers filled with Krebs-Henseleit solution (37°C) perfused with 95% O_2 / 5% CO_2 and extended with a force of 1 g for aorta and 0.75 g for mesentheric artery. Experiments were performed on a wire myograph (ADInstruments, Australia) using LabChart program for data acquisition and analysis.

2.6. Measurement of free cytoplasmic calcium concentration in SMC

Measurement of $[Ca^{2+}]_i$ was performed on SMC from 2-6 passages grown in 96-well plates. The cells were loaded with 2 μM Fura-2/AM dissolved with 0.02% Pluronic F-127 (Molecular Probes) during one hour incubation at 25°C in a physiological salt solution (PSS) containing NaCl (145 мМ), KCl (5 мМ), $MgCl_2$ (1 мМ), $CaCl_2$ (1 мМ), Hepes (5 мМ), D-glucose (10 мМ), at pH 7.4. The fluorescence was measured in parallel from six wells at excitation wavelengths of 340 and 380 nm and emission wavelength of 505 nm using a Synergy 4 Microplate Reader (BioTek, USA). The increments in $[Ca^{2+}]_i$ are presented as ratios in the fluorescence at 340 and 380 nm.

2.7. Detection of ROS in the smooth-muscle cells

ROS generation in rat aorta SMC was assessed using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) as a probe. The cells grown in 96-well plates were incubated with 2 μM of DCFH-DA during one hour incubation at room temperature. Formation of ROS in the cells was determined by measuring the rate of DCFH oxidation into 2',7'-dichlorofluorescein (DCF). DCF fluorescence was measured at excitation wavelength of 485 ± 20 nm and with emitter bandpass of 527 ± 15 nm. The rate of fluorescence rise was taken as an index ROS generation.

2.8.Statistics

Data are presented as mean±SEM of 6 to 18 measurements. Statistical significance was calculated using MedCalc statistical software according the one-way analysis of variance and Student-Neuman-Keuls test. Differences were considered to be significant at $p<0.05$.

3. Results

3.1. Assessment of 5-HT2 receptors mRNA in rat aorta and mesenteric arteries and in SMC isolated from rat aorta

mRNA of elongation factor EF1a was used as endogenous control and the contents of 5-HT2A, 5-HT2B, 5-HT2C and 5-HT1B receptor mRNA in aorta and superior mesenteric artery of rat and in cultured SMC were calculated based on ΔCT values. In both vessels and in SMC, the mRNA of 5-HT2A receptor is dominating (**Fig.1**). The content of 5-HT2B receptor mRNA relative to mRNA coding 5-HT2A receptor in aorta, mesenteric arteries and SMC was determined as 6.1±1.4, 5.4±0.6 and 36±11.1%, respectively. The relative level of 5-HT2C receptor mRNA in aorta and mesenteric artery was 0.22±0.14% and 0.24±0.03%, respectively, and only trace levels in SMC, 0.012±0.002%. The relative content of 5-HT1B mRNA was 41.3±4.6% and 55.1±4.6% in the aorta and mesenteric artery and 0.74±0.19% in SMC. Thus, 5HT2B receptors are expressed in the rat aorta and mesenteric arteries, and in SMC isolated from rat aorta; the level of their expression in the SMC is comparable with that of 5-HT2A receptors.

3.2. Effects of BW723C86 on the contractility of rat aorta and mesenteric arteries in the presence of PTP inhibitors Na_3VO_4 or BVT948

To investigate whether inhibition of PTP will unmask the vasoconstrictor effect of BW723C86 on isolated blood vessels, experiments were carried out on the rings of rat mesenteric arteries and aorta. There was no vasoconstrictive effect of BW723C86 at concentration range 0.1-100 μM. However, added after PTP inhibitors Na_3VO_4 (500 μM) or BVT948 (0.1 μM), BW723C86 (1 μM) induces contraction of

mesentheric artery rings (**Fig. 2A**). Na_3VO_4 or BVT948 themselves at concentrations 500 and 0.1 μM respectively did not cause contraction. At higher concentrations, 1 mM for Na_3VO_4 and 1 μM for BVT948, each of them induces contraction of mesentheric artery ring without BW723C86.

Effect of Na_3VO_4 on aorta at 500 μM was not consistent, causing either contraction or did not influence the aortic tone. Administration of 1 mM Na_3VO_4 to rat aortic rings resulted in their contraction, the force of which reached a maximum within 10 min. The initial contraction induced by 1 mM Na_3VO_4 was followed by slow relaxation. BW723C86 at 3 μM concentration added to the rings stabilized contraction and constant force was maintained at least for 25 min (**Fig. 2B,C**).

3.3. Effects of orthovanadate and PTK inhibitors on $[Ca^{2+}]_i$ elevation induced by BW723C86 and 5-HT in SMC isolated from rat aorta

A rise of $[Ca^{2+}]_i$ in vascular SMC is a trigger for vessel contraction. Therefore we studied whether inhibition of PTP activity will potentiate calcium signaling via 5-HT2B receptors in SMC. Na_3VO_4 was chosen to evaluate how inhibition of PTP will affect signal transduction from 5-HT2B receptors. Another PTP inhibitor BVT948 was not used in these experiments because its fluorescence overlaps with Fura2 fluorescence. Na_3VO_4 increased elevation of $[Ca^{2+}]_i$ in response to BW723C86 by more than 5 times (**Fig. 3A**). The potentiating effect of Na_3VO_4 on the calcium signal was manifested equally over a concentration range of 100 to 500 μM. Antagonist of 5-HT2B receptors RS127445 suppressed $[Ca^{2+}]_i$ elevation in response to BW723C86 (**Fig. 3B**). Serotonin is a more powerful inducer of $[Ca^{2+}]_i$ elevation in rat aorta SMC compared to BW723C86 (**Fig. 3A**). Na_3VO_4 increased the serotonin-induced elevation

of $[Ca^{2+}]_i$ by 31±11%. It has been shown previously that 5-HT2A receptors mediate elevation of $[Ca^{2+}]_i$ in rat aortic SMC induced by serotonin [15]. In our experiments, RS127445 suppressed by more than 50±8% calcium response to 0.1 μM serotonin (**Fig. 3C**). This indicates that 5-HT2B receptors along with 5-HT2A receptors mediate the action of serotonin on the SMC.

The wide-spectrum PTK inhibitor genistein and Src kinase inhibitor PP2 lowered the $[Ca^{2+}]_i$ increase induced by BW723S86 added after Na_3VO_4 (**Fig. 3B**). Inactive analogue PP3 did not affect the calcium response. PP2 was also shown to inhibit the $[Ca^{2+}]_i$ elevation due to 5-HT (**Fig. 3C**). Thus, Src kinase or other PTK belonging to the Src kinases family is involved in calcium signaling of 5-HT2B receptors.

3.4. ROS formation under the influence of orthovanadate and BW723C86 in SMC

The above data suggest that Na_3VO_4 augments the calcium response upon activation of 5-HT2B receptors through inhibition of PTP. It was previously shown that suppression of PTP activity is the result of oxidation of SH-groups [13]. It is known that orthovanadate can induce formation of H_2O_2 *via* superoxide anion as an intermediate product [16] and H_2O_2 inside the cell is able to oxidize SH-group in active center of PTP [17,18]. We therefore studied the effects of Na_3VO_4 and BW723S86 on accumulation of ROS in the SMC using a cell-permeable probe DCFH-DA, which is cleaved in the cells to DCFH. DCFH can be oxidized by H_2O_2 and to a lesser degree by superoxide anion forming the fluorescent compound 2',7'-dichlorofluorescein (DCF) [19]. The rate of fluorescence rise was taken as an index ROS generation. Without any additives there was a slow elevation in fluorescence,

representing spontaneous ROS formation (**Fig. 4A**). Administration of 300 μM Na_3VO_4 made the curve steeper that indicates increased formation of intracellular ROS. Furthermore, addition of BW723C86 (10 μM) caused a sharp increase in the reaction rate (**Fig. 4B**), thus indicating activation of ROS. Without orthovanadate BW723C86 did not affect the rate of oxidation of DCFH in SMC.

3.5.Suppression of BW723C86-induced $[Ca^{2+}]_i$ elevation by N-acetylcysteine and inhibitor of NADPH-oxidases VAS2870

Since Na_3VO_4 effects are associated with the ROS formation, which can act as signaling molecules in vascular cells, we hypothesized that ROS are involved in potentiating calcium signaling in response to BW723C86. To test this hypothesis, we investigated the effects of antioxidant N-acetylcysteine (N-AcC) and tempol, a membrane-permeable radical scavenger and mimetic of superoxide dismutase (SOD), on BW723C86 induced elevation of $[Ca^{2+}]_i$ in the presence of Na_3VO_4. Preincubation during 10 min with N-AcC almost completely reduced the growth of $[Ca^{2+}]_i$ induced by BW723C86 (**Fig. 4C**). Tempol had no effect on the BW723C86-induced calcium signaling. Since tempol catalytically removes superoxide anions though has no antioxidant activity against hydrogen peroxide [20]. we can eliminate the superoxide anion as a kind of ROS directly involved in the implementation of the potentiating effect of Na_3VO_4. To investigate the role of NADPH-oxidases in realization of the effect of BW723C86 on calcium signaling in SMC we used their selective inhibitor VAS2870 [21,22]. VAS2870 at a concentration of 10 μM inhibited by 79$\pm$4% the increase in $[Ca^{2+}]_i$ that was induced by BW723C86 added after 200 μM Na_3VO_4 (**Fig. 4C**).

4. Discussion

In this work we have demonstrated that silent vasoconstrictive 5-HT2B receptors are present in rat aorta and mesentheric artery and their functional activity is unmasked under the action of PTP inhibitors Na_3VO_4 and BVT948. Our data suggest that this occurs due to potentiating of calcium signaling via these receptors. Elevation of $[Ca^{2+}]_i$. in cultured SMC isolated from rat aorta in response to the agonist of 5-HT2B receptors BW723C86 is almost negligible and is manifested in the presence of Na_3VO_4. 5-HT induced increase in $[Ca^{2+}]_i$ is potentiated by Na_3VO_4 and partially inhibited by 5-HT2B receptor antagonist RS127445. The latter shows that 5-HT-induced elevation of $[Ca^{2+}]_i$ in SMC is mediated both by 5-HT2A and 5-HT2B receptors. According to our knowledge, these is the first demostration of 5-HT2B receptors involvement in calcium signaling in vascular SMC.

Earlier it was shown that orthovanadate increases the level of phosphorylation of proteins on tyrosine residues in SMC by inhibiting PTP [23,24]. We assume that potentiation of calcium signaling via 5-HT2B receptors and unmasking of aorta and mesentheric artery contraction in response to BW723C86 is caused by the shift in the balance of protein tyrosine phosphorylation towards phosphotyrosines. Shifting the balance of protein tyrosine phosphorylation to the initial position should attenuate $[Ca^{2+}]_i$ rise induced by BW873C86 in the presence of Na_3VO_4. Indeed, PTK inhibitors genistein and PP2 decreased calcium response to BW723C86 in SMC, preincubated with Na_3VO_4. The effect of PP2 upon the increase of $[Ca^{2+}]_i$ in the SMC caused by BW723C86 indicates that protein kinase belonging to the Src-kinases family

participates in calcium regulation in these cells through 5-HT2B receptors. Formely it was shown that Src-kinases are involved in signal transduction from angiotensin II receptors in SMC [25].

Na_3VO_4 and BVT948 inhibit PTP as a result of oxidation of SH-group in the active center [12,13]. Our findings suggest that the effect of Na_3VO_4 on calcium metabolism in the SMC is triggered by oxidative stress, because the antioxidant N-acetylcysteine almost completely abolishes the rise of $[Ca^{2+}]_i$ caused by orthovanadate and BW723C86. Activation of the 5-HT2B receptors in the aorta SMC by BW723C86 after exposure to Na_3VO_4 causes an additional substantial increase in the rate of oxidation of DCFH to DCF. We expect that the source of ROS is at least partially a NADPH oxidase since selective NOX inhibitor VAS2870 suppresses the effect of BW723C86 on $[Ca^{2+}]_i$ in the SMC. The product of NOX-catalyzed reaction might be the superoxide anion produced in SMC by isoform NOX1, which is then converted to hydrogen peroxide, or H_2O_2 produced directly by the isoform NOX4 [26]. The superoxide dismutase mimetic tempol, which is known to catalytically remove superoxide anions [20], does not affect the calcium signaling. It follows that the superoxide anion itself does not directly potentiate BW723C86-induced $[Ca^{2+}]_i$ elevation, but it might be an intermediate from which hydrogen peroxide is produced. The formation of hydrogen peroxide in the SMC in response to 5-HT was previously shown with a specific probe HyPer [27]. Our data suggest that 5-HT2B receptors are involved in the implementation of this effect.

The cause of vasoconstrictor action of 5-HT2B receptors at some diseases may be an elevation of their expression in vascular SMC [28], as well as increase in signal

transmission efficiency from these receptors. Our data apparently gives a clue to the understanding of the mechanism of the vasoconstrictive action of 5-HT2B receptors in L-NAME induced hypertension and DOCA-salt hypertensive rats [8,29] and in mouse model of type II diabetes mellitus [9]. Increased intracellular concentration of ROS is a well established pathogenic factor of hypertension and diabetes [11]. We suggest that manifestation of vasoconstrictive action of 5-HT2B receptors in these pathological states occurs due to oxidative stress evoked inhibition of PTP and potentiation of Ca-signaling in vascular SMC via 5-HT2B receptors.

Conflict of interest statement

The authors confirm that there are no conflicts of interest.

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Legends to figures

Fig. 1. Relative contents of mRNA coding 5-HT-receptors in rat aorta, rat mesenteric artery and SMCs from rat aorta. The data are expressed as means ± SE (n=3).

Figure 2. Effects of BW723C86 on the force of rat mesenteric artery (A) and aorta (B,C) rings contraction in the presence of Na_3VO_4 or BVT948. Each curve is a representative of 6 registrations on graph (**A**) and 4 registrations on graph (**B**). On (**C)** the average values of the force of aorta rings contraction induced by Na_3VO_4 25 minutes after adding BW723C86 or vehicle are shown (n=4, *p<0.01). The mean value of the contraction force at the moment of BW723C86 or vehicle adding was 0.88±0.094 g and taken as 100%. The mean values of the force of mesenteric artery ring contraction (**A**) in response to BW723C86 in the presence of Na_3VO_4 or BVT948 were 0.65±0.078 and 0.46±0.044 g, respectively. On (**B)** adding of Na_3VO_4 is shown by white arrow and adding of BW723C86 or vehicle by black arrow. Grey curve depicts contraction in response to Na_3VO_4 in the absence of BW723C86 and black curve – in the presence of BW723C86.

Figure 3. Potentiation of $[Ca^{2+}]_i$ elevation in rat aorta SMCs in response to BW723C86 and 5-HT by Na_3VO_4 and its suppression by protein tyrosine kinase inhibitors and RS127445. (**A)** Kinetics of $[Ca^{2+}]_i$ elevation in SMC in response to BW723C86 (**A**) or 5-HT (**B**) which were added after incubation during 3 min with Na_3VO_4 or vehicle. (**B**) Increments in $[Ca^{2+}]_i$ in response to BW723C86 after incubation with vehicle, Na_3VO_4, Na_3VO_4 + PP3, Na_3VO_4 + PP2, or Na_3VO_4 + RS127445. (**C**) Increments in $[Ca^{2+}]_i$ in response to 5-HT after incubation with vehicle, Na_3VO_4, PP3,

PP2 or RS127445. Effects of BW723C86 and serotonin without these agents were taken as 100%. The cells were incubated with PP3, PP2, genistein, RS127445 or vehicle for 8 min and then with or without Na_3VO_4 for 3 min. Concentration of Na_3VO_4 was 200 μM, concentrations of BW723C86, PP3, PP2, genistein, RS127445 were 10 μM each. The average data of 6-9 independent measurements are expressed as mean ± SEM. On (**B**) *p<0.01 (Na_3VO_4 *vs.* vehicle; Na_3VO_4 *vs.* Na_3VO_4 + genistein or *vs.* Na_3VO_4 + RS12445; Na_3VO_4 + PP2 vs. Na_3VO_4 + PP3). On (**C**) **p<0.05 (Na_3VO_4 *vs.* vehicle and PP2 *vs.* PP3), *p<0.01 (RS127445 *vs.* vehicle).

Figure 4. Generation of reactive oxygen species (ROS) in SMC in response to Na_3VO_4 and Na_3VO_4 with BW723C86 and influence of antioxidants and NADPH oxidase inhibitor VAS2870 on $[Ca^{2+}]_i$ elevation induced by Na_3VO_4 and BW723C86. **(A)** Elevation of DCF fluorescence in SMC after adding of Na_3VO_4 (black arrow) and consequent adding of BW723C86 or vehicle (grey arrow). Black curve depicts effect of Na_3VO_4 with BW723C86. (**B)** The rates of DCF formation after adding of BW723C86 alone, Na_3VO_4 alone or Na_3VO_4 together with BW723C86. Spontaneous generation of ROS was taken as 100%. (**C**) Effects of tempol, N-acetyl cysteine (N-AcC) and VAS2870 on $[Ca^{2+}]_i$ elevation induced by Na_3VO_4 and BW723C86. Concentrations of N-AcC, VAS2870 and tempol were 2 mM, 10 μM and 1 mM, concentrations of Na_3VO_4 was 300 μM in (**A,B**) and 200 μM in (**C**). Each value is an average of 6-18 measurements. The data are expressed as means ± SEM. * p<0.01 (Na_3VO_4 *vs.* control and Na_3VO_4 + BW723C86 *vs.* Na_3VO_4 on **B**; N-AcC and VAS2870 *vs.* control on **C**).

Figure

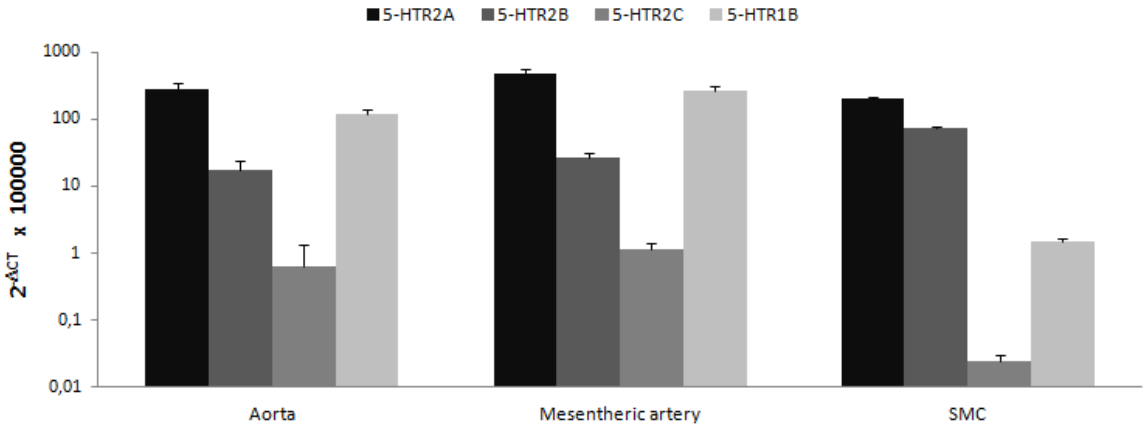
5-HTR2A
5-HTR2B
5-HTR2C
5-HTR1B
1000
100
10
1
0,1
0,01
2-ΔCT x 100000
Aorta
Mesentheric artery
SMC

Fig.1.

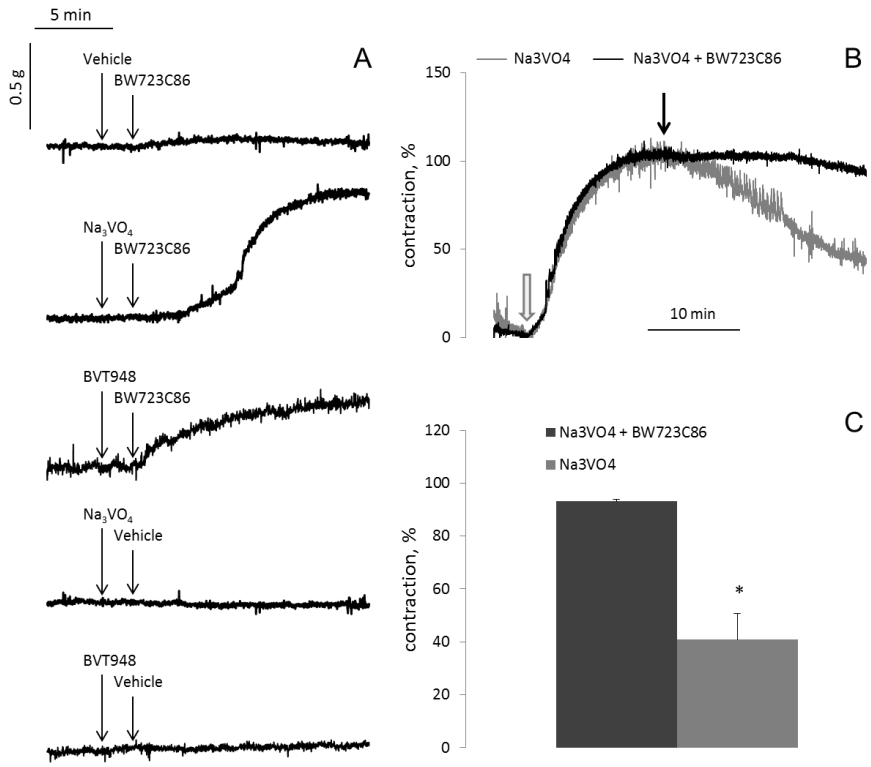
5 min
0.5 g
A
Vehicle
BW723C86
Na3VO4
BW723C86
BVT948
BW723C86
Na3VO4
Vehicle
BVT948
Vehicle
B
Na3VO4
Na3VO4 + BW723C86
contraction, %
150
100
50
0
10 min
C
Na3VO4 + BW723C86
Na3VO4
contraction, %
120
100
80
60
40
20
0
*

Fig.2.

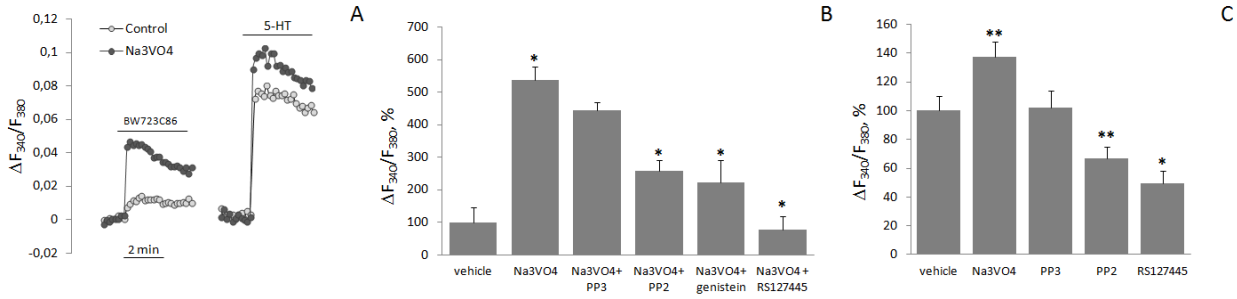
A
B
C
Control
Na3VO4
5-HT
BW723C86
2 min
ΔF340/F380
ΔF340/F380, %
vehicle
Na3VO4
Na3VO4+ PP3
Na3VO4+ PP2
Na3VO4+ genistein
Na3VO4 + RS127445
PP3
PP2
RS127445

Fig.3.

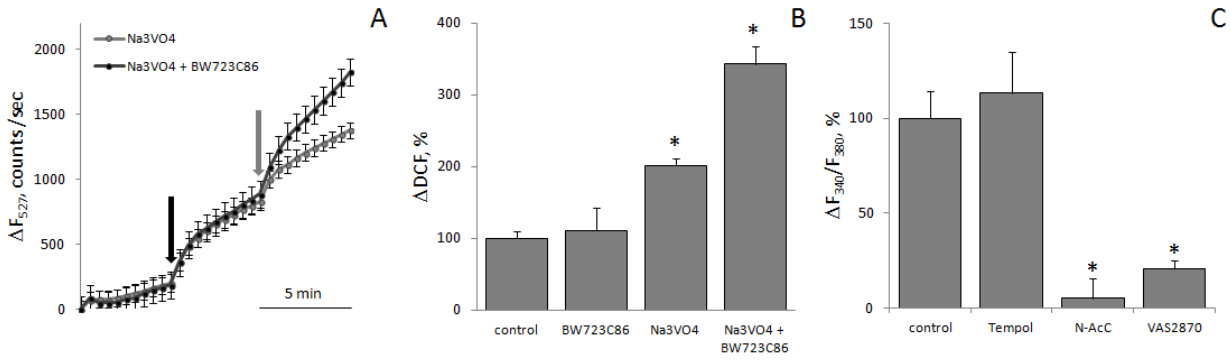
A
Na3VO4
Na3VO4 + BW723C86
ΔF527, counts/sec
2000
1500
1000
500
0
5 min
B
ΔDCF, %
400
300
200
100
0
control
BW723C86
Na3VO4
Na3VO4 + BW723C86
C
ΔF340/F380, %
150
100
50
0
control
Tempol
N-AcC
VAS2870

Fig.4.