Hindlimb unweighting induces changes in the p38MAPK contractile pathway of the rat abdominal aorta

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Hindlimb unweighting induces changes in the p38MAPK contractile pathway of the rat abdominal aorta. J Appl Physiol 107: 121–127, 2009. First published May 14, 2009; doi:10.1152/japplphysiol.00210.2009.—Hindlimb unweighting (HLU) of rats is a model used to mimic the cephalic fluid shift potentially involved in the orthostatic intolerance experienced by astronauts. Certain arteries in these rats exhibit a decreased contractile response to adrenergic agonists. It was shown previously that this may be caused by changes in thick filament regulation (Summers et al., Vascul Pharmacol 48: 208–214, 2008). In the present study, it was hypothesized that HLU also modifies thin filament regulation by effects on p38MAPK and ERK. Abdominal aorta rings from 20-day HLU rats and untreated controls were subjected to phenylephrine and phorbol 12,13-dibutyrate (PDBU) concentration response curves in the presence and absence of two inhibitors: the p38MAPK inhibitor SB-203580 and the MEK inhibitor U-0126. SB-203580 decreased control sensitivity to both agonists, but HLU sensitivity was not significantly affected. U-0126, which blocks enzymes immediately upstream of ERK, affected sensitivity to both agonists equally between control and HLU. Western blot analysis revealed no change in total levels of p38MAPK and its downstream target heat shock protein 27 but did reveal a decrease in phosphorylated levels of both after stimulation with PDBU and phenylephrine after HLU treatment. Neither total ERK nor phosphorylated levels after stimulation were affected by HLU. Total levels of caldesmon, a molecule downstream of both pathways, were decreased, but phosphorylated levels after stimulation were decreased by roughly twice as much. The results of this study demonstrate that HLU downregulates p38MAPK, but not ERK, signaling. In turn, this may decrease actin availability for contraction.

extracellular signal-regulated kinase; phorbol ester; microgravity; orthostatic intolerance; heat shock protein 27

EXPOSURE TO MICROGRAVITY AND subsequent return to a full gravity environment present distinct changes and challenges to the cardiovascular system of astronauts. Hargens and Watenpaugh (12) hypothesized that, during spaceflight, blood is redistributed throughout the body. Specifically, whereas the feet would experience a blood pressure of roughly 200 mmHg on Earth and the cephalic pressure would be 70 mmHg, once in space, the blood would be equally distributed such that both have pressures of 100 mmHg. Thus microgravity causes a marked increase in cephalic pressure and a dramatic reduction in pressure in the lower half of the body. Both pressure changes are thought to contribute to the orthostatic intolerance experienced by space-adapted astronauts return to the gravity of Earth. For example, using the hindlimb unweighted (HLU) rat model to simulate the decreased lower body/caudal blood pressures believed to exist in microgravity, there is evidence that the cerebral vasculature becomes hyperresponsive to vasoconstrictor agents (32, 36), whereas vessels in the lower body become hyporesponsive (9, 25). Thus, the cerebrovascular autoregulatory set point may be shifted to a higher pressure at the very time orthostatic hypotension and reduced brain perfusion occur because of reduced peripheral vasoconstriction and peripheral resistance (17). It was hypothesized that the change in this set point may be related to another common experience of astronauts, that of orthostatic intolerance on return to Earth (2, 20).

Work with the HLU rat largely began in a landmark paper by Delp et al. (9), who found that the abdominal area of rats exposed to HLU for 2 wk exhibited vasoconstrictor hyporesponsiveness. Subsequent studies have shown that all arteries, excluding the cerebrovasculature (32, 35), also exhibit vasoconstrictor hyporesponsiveness in response to HLU (except see Ref. 18). Moreover, in most cases, this effect of HLU can be attributed, at least in part, to hemodynamic mechanisms. For example, arteries in the upper body are exposed to increased pressure and flow (5, 35). In the thoracic aorta (30) and carotid (28) and pulmonary (22) arteries, HLU caused vasoconstrictor hyporesponsiveness via enhancement of endothelium-dependent vasodilator responses. Thus, all of these authors proposed that endothelial nitric oxide expression was increased by the stimulus of the HLU-mediated increase in blood flow (3).

Blood pressure and flow are reduced by HLU in the lower half of the body (5, 35). In this case, it appears that the reduced pressure is important in that it causes a decrease in both medial cross-sectional area and wall thickness. This form of vascular remodeling has been reported in the femoral (4, 19) and gastrocnemius feed (7, 8) arteries and is thought to underlie the vasoconstrictor hyporesponsiveness produced by HLU in these vessels.

The abdominal aorta is among those vessels in the lower half of the body that experience both decreased blood pressure and flow during HLU (5, 23, 35). However, the abdominal aorta exhibits none of the morphological alterations associated with these HLU-induced hemodynamic changes. Specifically, there are no HLU-induced changes in endothelial mechanisms (9, 28), wet or dry weight (25), medial cross-sectional area or wall thickness, material or structural properties (23), or compliance (31) of the abdominal aorta. Thus the mechanisms underlying the HLU-induced vasoconstrictor hyporesponsiveness in this vessel are unknown. In a previous study (29), we demonstrated that HLU impaired RhoA/Rho kinase signaling in the abdominal aorta, leading to a decreased capacity for vasoconstrictor-mediated myosin light-chain phosphorylation. We hypothesized that this biochemical change contributed to the HLU-induced vasoconstrictor hyporesponsiveness. In the present study, we have extended this hypothesis by an analysis of the effects of HLU on α-actin. We hypothesized that HLU causes
vasoconstrictor hyposresponsiveness by reducing the availability of actin for contraction. To address this hypothesis, we explored the effects of simulated microgravity on two major pathways that regulate actin availability, namely, p38 mitogen-activated kinase (p38MAPK) and extracellular signal-related kinase (ERK).

The p38MAPK and ERK thin filament signaling pathways in vascular smooth muscle are complex and only partially understood. Briefly, both p38MAPK (14) and ERK (11) are believed to be activated downstream of adrenergic receptors in vascular tissue, likely through phosphorylation via PKC (1, 27). Once activated, p38MAPK phosphorylates heat shock protein 27 (HSP27), which is directly involved in the conversion of actin from its globular to filamentous form (16). In addition, both ERK and p38MAPK increase actin availability through phosphorylation of caldesmon (13). The unphosphorylated form of caldesmon prevents the cross-bridge cycling of tropomyosin from its globular to filamentous form (16). In addition, both phosphorylation of these proteins after stimulation increased enzymatic activity was demonstrated by decreased vasoconstrictor hyporesponsiveness by reducing the availability of actin for contraction.

To study the potential changes in ERK and p38MAPK pathways that may play a role in the decreased response of HLU rats to adrenergic agonists, the present study takes a two-pronged approach. First, the specific inhibitors U-0126, which blocks MEK, an enzyme immediately upstream of ERK (10), and SB-203580, which blocks p38MAPK (6), were used in the presence of both the adrenergic agonist phenylephrine and the PKC-specific activator phorbol 12,13-dibutyrate (PDBU). Decreased activity in these enzymatic pathways is confirmed when blockade of the pathway has a lesser or complete lack of effect on contraction. The second approach used in this study is the measurement of the total and phosphorylated levels of proteins involved in both the ERK and p38MAPK pathways, including p38MAPK, HSP27, ERK, and heavy caldesmon. Decreased enzymatic activity was demonstrated by decreased phosphorylation of these proteins after stimulation.

MATERIALS AND METHODS

Hindlimb unweighting. All animal experiments were approved by the University of California Irvine Institutional Animal Care and Usage Committee. Age-matched male Wistar rats weighing between 350 and 400 g were randomly assigned to either control (CTL) or HLU treatment groups. The HLU rats were suspended using a tail harness slightly modified from that described in a previous work (25).

Tissue preparation. After 20 days of HLU treatment, both the CTL and HLU rats were anesthetized using isoflurane and euthanized via asanguinolysis through rupture with a metal rod. After aortas were removed, gastrocnemius and soleus muscles from one side of the CTL and HLU samples were carefully removed and weighed to confirm HLU.

Tissue bath. The aortic rings were placed in a tissue bath filled with Krebs solution kept at 37°C and attached via 28-gauge stainless steel triangles through their lumens to a post within the bath and a wire connected to a tension transducer placed above the bath. Using a micrometer adjuster attached to the tension transducer, we slowly stretched the rings to a resting tension of 2.0 g. This resting tension has been previously described as optimal for both the CTL and HLU abdominal aortas (25). Samples were maintained at this tension as they equilibrated for 1 h. Then 100 mM KC1 was added to the tissue baths to determine viability of the aortic rings. Any rings not responding to the KC1 were discarded. The rings were then washed once with fresh Krebs and allowed to relax to their resting tension of 2.0 g over the course of 30 min.

Concentration response curves. For each aortic ring, three sequential cumulative concentration response curves (CRCs) were generated. The first was to phenylephrine (Sigma-Aldrich, St. Louis, MO) in half-log increments from 10−6 to 10−3 M with 3 min between successive drug addition. After this, the aortic rings were washed three times in fresh Krebs solution and re-equilibrated for 60 min. An inhibitor, either 10 μM U-0126 or 10 μM SB-03580 (LC Labs, Woburn, MA), was then added to half the rings, and a second phenylephrine CRC was obtained. Subsequently, all rings were washed three times, the same inhibitor was added to the same rings as in the previous CRC, and all rings were re-equilibrated for 60 min. Then a third CRC was obtained, with PDBU as the agonist. PDBU CRCs were generated from 10−8 to 3 × 10−6 M with 10 min between successive cumulative doses. Each experiment was the average of contractile responses in duplicate rings. In the case of phenylephrine CRCs, paired comparisons were made of contractions in each ring in the absence vs. in the presence of inhibitor. Because PDBU could not be washed out, unpaired comparisons were made between rings in the absence vs. those in the presence of inhibitor.

Western blotting. The preparation of the tissues for Western blotting was described as above in Tissue preparation and Tissue bath using separate sets of abdominal aorta. After 100 mM KC1 was added and aortas were washed and relaxed back to 2 g, 1 μM PDBU or phenylephrine was added to the tissue bath. The ring was allowed to constict until a plateau had been reached in tension, roughly 3 min for phenylephrine and 10 min for PDBU. The tissue was then snap frozen in a slurry made up of 50% (vol/vol) dry ice and 50% acetone containing 10 mM dithiothreitol, 10 mM sodium fluoride, and 1% (wt/vol) of trichloroacetic acid. Two 3-mm rings from the same rat for both CTL and HLU were combined separately for each data point and stored at −80°C until homogenization, within 1 wk.

These two 3-mm rings from CTL and HLU rats were then broken up in glass homogenizers (Kimble Kontes, Vineland, NJ) filled with 125 μl of lysis buffer consisting of 50 mmol/l β-glycerophosphate, 100 μmol/l NaVO3, 2 mmol/l MgCl2, 1 mmol/l EGTA, 0.5% Triton X-100, 1 mmol/l β-dithiothreitol, 20 μmol/l pepstatin, 20 μmol/l leupeptin, 0.1 U/ml aprotinin, and 1 mmol/l phenylmethylsulfonyl fluoride. After complete homogenization, the lysate was spun in a Fisher 235A centrifuge at 14,400 rpm for 15 min at 4°C. The now clarified supernatant was removed and subjected to bicinchoninic acid, appropriate volumes of the sample buffer and sodium dodecyl sulfate, and 10% sodium dodecyl sulfate. Two 3-mm rings from the same rat for both CTL and HLU were combined separately for each data point and stored at −80°C until homogenization, within 1 wk.

One part aqueous sample buffer consisting of 60 mM Tris-HCl, 50% (vol/vol) glycerol, 10% SDS (wt/vol), 14.4 mM 2-mercaptoethanol, and 1% bromphenol blue (wt/vol) was then added to four parts lysate. With the use of the protein concentrations generated from the bicinchoninic acid, appropriate volumes of the sample buffer and lysate mixture from both CTL and HLU tissues, to give equal final protein concentrations, were loaded into 4–20% Bis-Tris Mini-Gels (Invitrogen, Grand Island, NY) and run in Invitrogen’s proprietary Mini-PROTEAN system at 100 V constant voltage.
NuPAGE MOPS SDS running buffer at 200 V for 1 h. Proteins were then transferred to a nitrocellulose membrane (GE Healthcare, Piscataway, NJ) at 0.5 A for 1.5 h on ice. After this, membranes were blocked for 1 h at room temperature in 5% powdered milk (wt/vol) and 2% Tween 20 (vol/vol) PBS solution (T-PBS; Sigma-Aldrich).

Membranes were incubated overnight at 4°C in a primary antibody at a 1:2,000 (except for α-actin, which was 1:20,000) dilution in T-PBS. ERK, p-caldesmon, p38, p-p38, HSP27, and p-HSP27 primary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Heavy caldesmon, GAPDH, and α-actin primary antibodies were from Sigma-Aldrich. p-ERK antibodies were from Cell Signaling Technology (Boston, MA). The following morning, we rinsed each membrane three times at 10-min intervals with T-PBS to remove excess primary antibody; membranes then were exposed to the appropriate secondary antibody (Li-Cor Biosciences, Lincoln, NE) at a 1:20,000 ratio in T-PBS for 1 h at room temperature. We then washed membranes again three times at 10-min intervals with the final wash being Tween 20-free PBS. Blots were read on a Li-Cor Odyssey at an intensity of five, and densitometry analysis was performed using the Li-Cor Odyssey software. GAPDH is a housekeeping protein found to be unchanged by HLU (data not shown) and was thus used as a loading control and correction factor in densitometry.

Statistics. We used Prism (Graphpad, San Diego, CA) for statistical analysis. Contraction response curve points were analyzed using two-way ANOVA. EC50 values were determined in each CRC based on the maximum contraction in that CRC. Western blot densitometry and EC50 results were analyzed with t-tests. P < 0.05 was considered significant in all cases.

RESULTS

Starting body weights, final body weights, and final soleus weights from the 78 rats used in this study were collected to confirm HLU. The initial body weight (g ± SE) of CTL rats was 369.2 ± 8.4 and 364.4 ± 6.2 for HLU rats. The final body weight of CTL rats was 422.0 ± 9.6 and 354.2 ± 5.3 for HLU rats. A difference in final body weight between CTL and HLU following roughly 3 wk of unweighting has been noted previously (10). Final soleus weight (mg ± SE) for CTL rats was 195.6 ± 7.1 and 88.0 ± 6.2 for HLU rats. The soleus weights were significantly different in a t-test (P < 0.05). The significant difference between CTL and HLU groups remained even when soleus weights were corrected by the final body weight (CTL: 0.46 ± 0.01 mg/g vs. HLU: 0.25 ± 0.02 mg/g).

Changes in the contractile response to preincubation with the p38MAPK inhibitor SB-203580 are shown in Fig. 1. Figure 1A shows a shift in CTL abdominal aorta sensitivity to phenylephrine after SB-203580 treatment (pEC50 = −7.32 ± 0.16 vs. 6.29 ± 0.32 after SB-203580; significantly different, P < 0.05). In the HLU animals shown in Fig. 1B, roughly one-third of this shift is observed (−7.32 ± 0.29 vs. −6.83 ± 0.29; P > 0.05). Figure 2 depicts the contractile changes in response to PDBU, a PKC activator, with SB-203580. Similar to with phenylephrine, SB-203580 treatment dramatically decreased the CTL sensitivity to PDBU (Fig. 2A; −7.33 ± 0.09 vs. −6.56 ± 0.19; significantly different, P < 0.05), but this significant shift was not observed in Fig. 2B when HLU rats were used (7.37 ± 0.12 vs. 7.17 ± 0.23; P > 0.05).

The effect of the MEK inhibitor U-0126 was also studied in Figs. 1 and 2. In Fig. 1, U-0126 treatment induced a shift in the sensitivity to phenylephrine in both CTL (Fig. 1A; pEC50 = −7.32 ± 0.16 vs. −6.26 ± 0.19 after U0126; significantly different, P < 0.05) and HLU (Fig. 1B; −7.33 ± 0.29 vs. 6.38 ± 0.24; significantly different, P < 0.05). A shift in sensitivity to PDBU after U-0126 was not observed in Fig. 2 in either CTL (Fig. 2A; −7.33 ± 0.09 vs. 7.33 ± 0.13) or HLU (Fig. 2B; 7.37 ± 0.12 vs. 7.29 ± 0.13) aorta. No significant differences in percent maximal decrease in contractile response due to U-0126 treatment between CTL and HLU tissues was observed in any of the groups. However, HLU aorta did have a significantly decreased maximal response to phenylephrine (3.43 ± 0.28 g in CTL vs. 2.12 ± 0.22 g in HLU) and PDBU (4.10 ± 0.34 g in CTL vs. 2.84 ± 0.29 g in HLU).

Western blot analysis for changes in specific proteins was also carried out in CTL and HLU abdominal aortas. Total levels of p38MAPK were not changed by HLU treatment (Fig. 3), but the amount of phosphorylated (Ser180) p38MAPK was significantly decreased in HLU rat abdominal aorta after 3 min of stimulation with 1 μM of both phenylephrine and PDBU relative to that of CTL. Similarly, total levels of HSP27...
were also not changed by HLU treatment (Fig. 4), and phosphorylated (Ser78) levels were decreased in HLU animals relative to CTL animals after exposure to phenylephrine and PDBU. Unlike phosphorylated p38MAPK and HSP27, all ERK levels were not changed significantly by HLU treatment (Fig. 5), including total phosphorylated (Thr202/Tyr204) after phenylephrine stimulation and phosphorylated after PDBU stimulation. Caldesmon was the only protein showing a decrease in total and phosphorylated levels after stimulation following HLU treatment (Fig. 6). The relative decrease in phosphorylated caldesmon with HLU was greater than the relative decrease of total caldesmon when compared in unpaired t-tests (P = 0.014 and 0.036 for phenylephrine and PDBU, respectively). Finally, α-actin itself was not changed by HLU treatment (Fig. 7).

**DISCUSSION**

The present study demonstrates that changes in second messenger signal regulation may play a role in the deficit in vascular contraction brought about by HLU. Interestingly, and potentially of therapeutic utility, these changes were observed in the p38MAPK but not in the ERK pathway. The well-documented contractile deficit in the rat abdominal aorta after HLU was present in Fig. 1. Moreover, the p38MAPK inhibitor caused a threefold greater decrease in sensitivity to phenylephrine in CTL vs. HLU aorta rings. This supports our hypothesis that HLU decreases signaling through p38MAPK. This difference was even more dramatic in Fig. 2. The present study showed for the first time that HLU causes a contractile deficit to phorbol esters. In addition, SB-203580 decreased sensitivity to direct PKC activation by ninefold in CTL abdominal aorta, with virtually no effect on the HLU sensitivity.

U-0126, a MEK blocker, painted quite a different picture. In Fig. 1, U-0126 right-shifted both CTL and HLU aorta sensitivity to phenylephrine by ~10-fold, indicating no difference in this pathway brought about with HLU treatment. Our results...
are not the first to demonstrate no significant change in vascular phorbol ester response following MEK inhibition. This has been shown previously to be the case in nonpregnant compared with pregnant sheep uterine arteries (33). The lack of HLU-induced change in inhibition by U-0126 may be explained by a finding from Liao et al. (17). In that work, MEK activation induced change in inhibition by U-0126 may be explained by past HLU results from our laboratory. Although at least in our tissues, MEK may not be a part of the contractile pathways activated by PDBU. However, it is also possible that MEK and therefore ERK are activated by proteins other than PKC.

The functional response to the inhibitors was generally supported by Western blot results. In Fig. 3, p38MAPK phosphorylation after stimulation was decreased in HLU abdominal aorta. Although a limitation of the present study was that baseline phosphorylation was not studied, the decrease in phosphorylation after phenylephrine stimulation could possibly be explained by past HLU results from our laboratory.

Previously, we had found that the signal transduction molecule RhoA, which is activated by phenylephrine, is decreased by HLU (29), and recent work has shown that the enzyme immediately activated by RhoA, Rho kinase, is an activator of p38MAPK (34). Thus the decreased levels of RhoA and subsequent decreased activation of Rho kinase could explain the decreased levels of phosphorylated p38MAPK after phenylephrine stimulation.

As would be expected after decreased levels of p38MAPK activation, HSP27 activation via phosphorylation, as shown in Fig. 4, was also less in HLU-treated animals. This finding has multiple potential impacts on the contraction of HLU vascular smooth muscle. Phosphorylated HSP27 no longer dimerizes to form a cap on globular actin. This leads to longer, unbranched chains of filamentous actin needed for smooth muscle contraction (24). Here, because p-HSP27 is decreased in the HLU abdominal aorta, decreased filamentous actin could go some way to explain the vascular contractile deficit seen in the HLU group. In addition, and discussed in detail below, activated HSP27 has its own enzymatic roles that may be involved in smooth muscle contraction.

Figure 1 showed no change in the effect of U-0126, the MEK blocker, against phenylephrine after HLU treatment. This indicates that the MEK/ERK pathway was unchanged by HLU. This idea is supported by Fig. 5 where no differences between CTL and HLU aortas in total ERK or phosphorylation of ERK after phenylephrine were observed. Because MEK blockade in Fig. 2 had no effect on PDBU contraction, it was discussed above that this may demonstrate a lack of activity through MEK brought about by PDBU. However, in Fig. 5, there is some phosphorylation of ERK. This may indicate either a basal level of phosphorylation, which was not examined in this work, or it may indicate a MEK-independent pathway of ERK activation. For example, PKC has been shown to be able to directly phosphorylate ERK (26). Nevertheless, ERK-specific activity does not appear to be altered by HLU.

A surprising result was the change in total heavy caldesmon (Fig. 6). Because heavy caldesmon essentially acts as an inhibitory protein that retards the movement of actinomyosin ATPase along filamentous actin, a potentially greater level could have been hypothesized to result from HLU treatment. The actual reduction in heavy caldesmon may be a compensatory mechanism for the decreased activity in stimulatory

Fig. 5. Sample Western blots using total ERK antibody. A: HLU = 103.5 ± 9.5% (SE) of CTL; p-ERK specific antibody after 3-min exposure to 1 µM phenylephrine. B: HLU = 103.6 ± 8.4% of CTL; p-ERK specific antibody after 10-min exposure to 1 µM PDBU. C: HLU = 106.1 ± 3.7% of CTL and mean data of each from CTL and HLU abdominal aorta. n = 6 pairs for all. GAPDH was used as a loading control.

Fig. 6. Sample Western blots using total heavy caldesmon (h-caldesmon) antibody. A: HLU = 84.7 ± 1.4% (SE) of CTL; p-caldesmon specific antibody after 3-min exposure to 1 µM phenylephrine. B: HLU = 59.8 ± 8.3% of CTL; p-caldesmon-specific antibody after 10-min exposure to 1 µM PDBU. C: HLU = 63.4 ± 8.4% of CTL and mean data of each from CTL and HLU abdominal aorta. *P < 0.05 (n = 6 pairs for all). GAPDH was used as a loading control.
pathways in the HLU rat abdominal aorta. The relative decrease in phosphorylated caldesmon after both phenylephrine and PDBU is likely in part due to the decreased level of total caldesmon. However, when comparing by t-test, the relative decrease in total caldesmon due to HLU treatment vs. the relative decrease in phosphorylated caldesmon due to HLU treatment after stimulation with both agonists, the decrease in phosphorylated caldesmon is significantly greater. This leaves open the possibility that activity of second messenger pathways played a role in the relative difference of phosphorylated caldesmon levels between CTL and HLU after stimulation. The functional results with SB-203580 plus the Western analysis of p-p38MAPK are the basis for stating that HLU treatment reduces p38MAPK activity. The results with caldesmon are consistent with this finding. The absence of a decrease in α-actin in Fig. 7 indicates that the contractile deficit in the HLU rat may be due to changes in second messenger systems and not contractile elements themselves.

In the Introduction, it was discussed that the abdominal aorta is exposed to reduced blood pressure and flow during HLU but exhibits none of the morphological alterations described in other arteries experiencing these same hemodynamic changes. This raises the possibility that the abdominal aorta is unique in its response to hemodynamic change. Alternatively, the differences between the abdominal aorta and other arteries could be only quantitative. For example, it is possible that all arteries exhibit biochemical change first, followed by structural change. Moreover, the time course could be influenced by vessel size. In that case, only biochemical change would be detected at 2 wk of HLU in the large conduit artery, whereas smaller arteries and arterioles could have advanced to overt morphological change in the same time period. Future experiments are required to differentiate between these possibilities.

In conclusion, the present study showed that the defect in adrenergic contractile response resulting from HLU may be due in part to changes in the p38MAPK signal transduction pathway. It further showed, for the first time that the authors are aware, the presence of a contractile pathway not negatively affected by HLU treatment, namely ERK. This presents the intriguing possibility of targeting drugs toward activation of this pathway. In turn, such targeted activation could represent a novel therapeutic intervention for treatment of orthostatic hypotension.

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