Nitric oxide and receptors for VIP and PACAP in cutaneous active vasodilation during heat stress in humans

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Kellogg DL Jr, Zhao JL, Wu Y, Johnson JM. Nitric oxide and receptors for VIP and PACAP in cutaneous active vasodilation during heat stress in humans. J Appl Physiol 113: 1512–1518, 2012.—VPAC2 receptors sensitive to vasoactive intestinal polypeptide (VIP) and pituitary adenyl cyclase activating polypeptide (PACAP), PAC1 receptors sensitive to PACAP, and nitric oxide (NO) generation by NO synthase (NOS) are all implicated in cutaneous active vasodilation (AVD) through incompletely defined mechanisms. We hypothesized that VPAC2/PAC1 receptor activation and NO are synergistic and interdependent in AVD and tested our hypothesis by examining the effects of VPAC2/PAC1 receptor blockade with and without NO inhibition during heat stress. The VPAC2/PAC1 antagonist, pituitary adenylate cyclase activating peptide 6–38 (PACAP6–38) and the NOS inhibitor, 7-nitro-L-arginine methyl ester (L-NAME), or Ringer’s solution administered by intradermal microdialysis. PACAP6–38, L-NAME, a combination of PACAP6–38 and L-NAME, or Ringer’s solution alone were perfused at four separate sites. Skin blood flow was monitored by laser-Doppler flowmetry at each site. Body temperature was controlled with water-perfused suits. Blood pressure was monitored by Finapres, and cutaneous vascular conductance (CVC) calculated (CVC = laser-Doppler flowmetry/mean arterial pressure). The protocol began with a 5- to 10-min baseline period without antagonist perfusion, followed by perfusion of PACAP6–38, L-NAME, or combination PACAP6–38 and L-NAME at the different sites in normothermia (45 min), followed by 3 min of whole body cooling. Whole body heating was then performed to induce heat stress and activate AVD. Finally, 58 mM sodium nitroprusside were perfused at all sites to effect maximal vasodilation for normalization of blood flow data. No significant differences in CVC (normalized to maximum) were found among Ringer’s PACAP6–38, L-NAME, or combined antagonists sites during normothermia (P > 0.05 among sites) or cold stress (P > 0.05 among sites). CVC responses at all treated sites were attenuated during AVD (P < 0.05 vs. Ringer’s). Attenuation was greater at L-NAME and combined PACAP6–38 and L-NAME-treated sites than at PACAP6–38 sites (P > 0.05). Because responses did not differ between L-NAME and combined treatment sites (P > 0.05), we conclude that VPAC2/PAC1 receptors require NO in series to effect AVD.

laser-Doppler flowmetry; VPAC2; PAC1; thermoregulation; microdialysis

THE CUTANEOUS CIRCULATION is a major effector of human thermoregulatory reflex responses. During heat stress, elevated internal temperature and skin temperature (Tsk) lead to a reflex cutaneous vasodilation, while, during cold stress, reduced internal temperature and/or Tsk lead to reflex cutaneous vasoconstriction. Under normothermic conditions, skin blood flow (SkBF) over the body surface averages ~300 ml/min; however, the absolute flow to the skin can vary from nearly zero during periods of maximal vasoconstriction in cold stress to as much as 8 l/min distributed over the body surface during maximal vasodilation in heat stress (35).

In nonglabrous skin (limbs, head, and trunk), reflex changes in SkBF are mediated by two branches of the sympathetic nervous system: noradrenergic vasoconstrictor nerves and active vasodilator nerves (9, 15, 34). In normothermia, cutaneous arterioles receive little neural activity, hence they are near basal tone. During cold stress, falling internal temperature and Tsk initiate a thermoregulatory reflex to conserve body heat by increasing noradrenergic vasoconstrictor activity to decrease SkBF. Conversely, during heat stress, rising skin and internal temperatures initiate a thermoregulatory reflex to facilitate heat loss. The initial response to heat stress is the abolition of any extant vasoconstrictor activity. As body heating continues, internal temperature reaches a threshold value at which active vasodilator neural activity to the cutaneous arterioles is enhanced, causing increases in SkBF to deliver heat to the body surface for dissipation to the environment in conjunction with the evaporation of sweat. Overall, the active vasodilator system is responsible for 80–95% of the elevation in SkBF that accompanies heat stress (15, 35).

The mechanisms whereby active vasodilator nerves increase SkBF involve cotransmission by sympathetic cholinergic nerves (19) and the release of acetylcholine and one or more cotransmitters (2, 15, 25, 27, 47, 48). Among the cotransmitter candidates for the active vasodilator system are the neuropeptides, vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP). Both are found in skin and can be colocalized with acetylcholine (32, 41, 42). VIP and PACAP mediate their effects through the G-protein-coupled receptors VPAC1, VPAC2, and PAC1 (10). VPAC1, VPAC2, and PAC1 receptors all have been found in human nonglabrous skin, although VPAC1 receptors have been reported to be sparse in number (26). Work in our laboratory showed that antagonism of VPAC2 and PAC1 receptors in forearm skin significantly attenuated the increases in SkBF during thermoregulatory reflex responses to whole body heat stress; thus activation of one or both of these receptors contributes significantly to active vasodilation (AVD) in nonglabrous skin (25). Work by Wilkins et al. (44) showed that during AVD, nitric oxide (NO) generated by NO synthase (NOS) plays both direct vasodilator and synergistic roles in AVD,
leading to enhanced increases in SkBF during heat stress. These results suggest that NO may enhance VPAC2 and/or PAC1 responses during AVD.

Work by Wilkins et al. (43) found that skin vasodilation in response to exogenous VIP, administered by intradermal microdialysis, involves both NO-dependent and NO-independent mechanisms. These results suggest that VIP and/or PACAP38 could mediate a portion of cutaneous AVD through inducing NO generation. In addition, they found that the NO-independent component of the exogenous VIP-induced vasodilation was not augmented when administered in combination with exogenous NO supplementation and NOS antagonism, suggesting that NO and VIP do not have synergistic postsynaptic interactions in human skin.

The foregoing suggests that VIP and/or PACAP38 activation of the VPAC/PAC1 receptors could effect cutaneous vasodilation through several possible mechanisms. Since several studies have concluded that 1) NO via NOS is involved in AVD (12, 18, 20–23, 38, 39, 44, 45, 48); 2) cotransmitters from cholinergic nerves are important contributors to AVD (2, 19, 43, 47); and 3) participation by VPAC2/PAC1 receptors is involved in AVD (25), we tested the hypothesis that VPAC2/PAC1 receptors effect AVD synergistically with NO-dependent mechanisms. We tested our hypothesis by examining the consequences of VPAC2/PAC1 receptor antagonism alone, NOS antagonism alone, and combined VPAC2/PAC1 receptor and NOS antagonism on cutaneous AVD induced by whole body heat stress.

METHODS

All subjects gave their written, informed consent to participate in these studies, which conformed to the standards of the Declaration of Helsinki. The University of Texas Health Science Center at San Antonio Institutional Review Board approved all procedures. Subjects were in good health, were nonsmokers, and took no prescription medications. Subjects did not use over-the-counter medications or caffeinated beverages on the day of the study.

Eight volunteer subjects participated in the experiments (3 men and 5 women) with an average age of 33.2 ± 4.1 yr. Their average weight was 61.1 ± 4.7 kg and average height was 167.4 ± 3.3 cm. Menstrual phase of the female subjects was not assessed.

For these experiments, SkBF was monitored by laser-Doppler flowmetry (LDF; MoorLab, Moor Instruments, Devon, UK) from a small volume of skin (~1 mm³) over each microdialysis probe. LDF measurements are specific to skin, being unfluenced by blood flow in the underlying skeletal muscle (37). Pulse rate and mean arterial pressure (MAP) were monitored continuously from a finger by the Penaz method with a Finapres Blood Pressure Monitor (31) (Finapres BP Monitor, Ohmeda, Madison, WI).

To induce thermoregulatory reflexes, subjects wore a tube-lined suit that was used to control TΔs by perfusion with water of different temperatures. By varying the temperature of the water, periods of normothermia, cold stress, and heat stress were evoked (16, 36). To lower TΔs and induce cold stress, the suit was perfused with cold water (18°C); warm water (48°C) was perfused to increase TΔs to 39°C to induce heat stress. Subjects wore a water-impermeable plastic garment over the tube-lined suit to insulate them from the room environment and prevent sweat evaporation. The tube-lined suit and water-impermeable garment covered the entire body except for the forearms, where LDF measurements were made, as well as the head, hands, and feet.

NOS was antagonized with N°°-nitro-l-arginine methyl ester (l-NAME) (AXXORA LCC, San Diego, CA), while VPAC2 and PAC1 receptors were antagonized by administration of PACAP6–38 (25). These agents were administered separately and in combination by intradermal microdialysis to allow direct, local administration of the antagonists into the interstitial space of small areas of skin. This approach permitted local monitoring of SkBF from an untreated control site and adjacent locally treated sites within each subject without confounding systemic effects.

For the protocol, four microdialysis probes were inserted into forearm skin. The probes were made from polyimide tubing with a 1-cm length of capillary microdialysis membrane (regenerated cellulose, 200-μm diameter, molecular cutoff 20 kDa). Each probe was reinforced with stainless steel wire in the lumen of the membrane and tubing. Each skin microdialysis site was anesthetized with ice before probe insertion to obviate confounding trauma effects attendant to probe placement (11). Following ice application, a 25-gauge needle was inserted into the dermis with sterile technique. A microdialysis probe was threaded through the needle lumen, which was then withdrawn, leaving with the microdialysis probe in place (17). Following insertion, all microdialysis probes were perfused with Ringer’s solution at 5 μl/min with a syringe pump. Subjects then waited 140 min or more for resolution of insertion trauma (19).

After arriving in the laboratory in the morning of the study, subjects were garbed and instrumented for intradermal microdialysis as outlined above. Subjects were then placed in a supine position and instrumented to monitor LDF at all microdialysis sites. Finally, a Finapres cuff was placed on a finger for pulse rate and MAP measurements. Internal temperature was monitored with a thermocouple in the sublingual sulcus [oral temperature (Tor)]. TΔs was measured as a weighted electrical average from six thermocouples taped on the skin surface of the calf, thigh, abdomen, chest, and upper and lower back (40).

Data collection began with a 5- to 10-min normothermic control period during which TΔs was maintained at 34°C. Initially, all microdialysis sites were perfused with Ringer’s solution at 5 μl/min. Following this normothermic period, the perfusates were changed to 100 μM PACAP6–38 in Ringer’s solution at one site, 10 mM l-NAME in Ringer’s solution at a second site, and a combination of 100 μM PACAP6–38 and 10 mM l-NAME in Ringer’s solution at a third site. l-NAME (10 mM) was chosen based on multiple previously published reports that established the adequacy of the concentration on blocking NO generation by NOS (12–14, 17, 18, 27–29, 44, 45, 47, 48). PACAP6–38 (100 μM) was chosen based on our prior studies with this agent that showed this concentration to consistently and significantly attenuate PACAP38-induced cutaneous vascular conductance (CVC) increase from 89 ± 7%max to 59 ± 3%max without effects on baseline values (25). The perfusate at a fourth microdialysis site was maintained with Ringer’s solution alone as a control. Perfusion rates at all sites were maintained at 5 μl/min. These solutions were perfused for 40–45 min to achieve local antagonism in the skin. Cold stress was then initiated by perfusing the tube-lined suit with cold water for 3 min to verify the absence of unanticipated neurovascular drug effects, after which TΔs was raised and subjects returned to normothermia in 2–3 min. TΔs was then increased to 38–39°C by perfusing the suit with warm water and held at that temperature for 35–50 min to induce whole body heat stress and active thermoregulatory vasodilation. Following heat stress, TΔs was lowered to terminate heat stress and return subjects to normothermia. All microdialysis sites were then perfused with 58 mM sodium nitroprusside to effect maximal vasodilation at each site (23–25, 28). CVC values were normalized to these maximal levels for data analysis. The protocol and examples of results from two subjects are illustrated in Fig. 1.

Data are presented as means ± SE. For data analysis, CVC values were calculated (CVC = LDF/MAP) and normalized to their respective maxima, as achieved with sodium nitroprusside administration at each site (23–25). CVC data normalization permitted comparisons among sites, both within and among subjects. The vasomotor responses to heat stress were also analyzed by comparing the TΔs thresholds for the initial increase in CVC at the different microdialysis
sites. The level of $T_{tr}$ at which a sustained increase in CVC began after $T_{sk}$ had been increased to 38°C was defined as the $T_{tr}$ threshold for the onset of vasodilation. For each microdialysis site, $T_{tr}$ thresholds were chosen from graphs of CVC vs. time by an investigator ignorant of the conditions, subjects, and antagonist treatment. To test for effects of the treatments on the slope of the CVC increase as a function of $T_{tr}$, skin temperature was increased to evoke heat stress for ~40 min. Following heat stress, the subject was cooled, and the perfusate at all microdialysis sites changed to 58 mM sodium nitroprusside (SNP) in Ringer’s to evoke maximal vasodilation. CVC, cutaneous vascular conductance.

**RESULTS**

In normothermia, when all microdialysis sites were perfused with Ringer’s alone, CVC values did not differ among sites ($P > 0.05$). Changing the perfusates at three different sites to PACAP6–38, L-NAME, and a combination of PACAP6–38 plus L-NAME did not significantly alter CVC. After 40–45 min of perfusion with these antagonists, skin temperature was lowered to produce a 3-min period of cold stress (CS). After a return to normothermia, skin temperature was increased to evoke heat stress for ~40 min. Following heat stress, the subject was cooled, and the perfusate at all microdialysis sites changed to 58 mM sodium nitroprusside (SNP) in Ringer’s to evoke maximal vasodilation. CVC, cutaneous vascular conductance.
During cold stress, CVC decreased at all sites. By the final minute of cold stress, CVC had decreased to 9 ± 2%max at untreated sites, 10 ± 2%max at PACAP6–38-treated sites, 8 ± 2%max at l-NAME-treated sites, and 7 ± 1%max at sites treated with the combination PACAP6–38 and l-NAME. These values did not differ significantly among sites (P > 0.05) and verified the absence of unanticipated neurovascular drug or intradermal microdialysis effects.

\( T_{aw} \) increased from 36.38 ± 0.13°C in normothermia to 37.36 ± 0.12°C at the peak of heat stress (P < 0.01). During whole body heating, the initial increase in CVC began when \( T_{aw} \) reached 36.64 ± 0.12°C at untreated sites, 36.64 ± 0.10°C at PACAP6–38-treated sites, 36.68 ± 0.12°C at l-NAME-treated sites, and 36.66 ± 0.11°C at sites treated with combined PACAP6–38 and l-NAME. These \( T_{aw} \) threshold values for the initiation of AVD were not statistically different among sites (P > 0.05).

In response to whole body heating, CVC increased at all sites as \( T_{aw} \) rose (P < 0.05, normothermia vs. peak heat stress). During the initial rising phase of CVC in heat stress, the rate of CVC increase in relation to \( T_{aw} \) was 72.1 ± 11.1%max/°C at untreated sites, 69.2 ± 11.4%max/°C at PACAP6–38-treated sites, 67.8 ± 8.2%max/°C at l-NAME-treated sites, and 59.7 ± 17.3 ± 0.11%max/°C at sites treated with combined PACAP6–38 and l-NAME. These CVC-\( T_{aw} \) relationships for the initial rising phase of AVD with rising \( T_{aw} \) were not statistically different among sites (P > 0.30).

As whole body heating continued, CVC increased and reached a plateau in most subjects by the final 3 min of heat stress to 72 ± 7%max at untreated sites, 57 ± 5%max at PACAP6–38-treated sites, 34 ± 4%max at l-NAME-treated sites, and 33 ± 4%max at sites treated with the combination of PACAP6–38 and l-NAME. CVC increases at sites treated with PACAP6–38, l-NAME, or the combination of PACAP6–38 and l-NAME were attenuated compared with CVC increases at untreated sites (P < 0.05). Attenuation of CVC responses at PACAP6–38-treated sites was less than the attenuation achieved by l-NAME alone or with the combination of PACAP6–38 plus l-NAME (P < 0.05, l-NAME or PACAP6–38 plus l-NAME vs. PACAP6–38 alone). Responses did not differ significantly between sites treated with l-NAME and those treated with the combination of PACAP6–38 and l-NAME (P > 0.05 between sites), CVC results from periods of whole body cold and heat stress are summarized in Fig. 2.

MAP averaged 80 ± 2 mmHg, and pulse rate averaged 56 ± 2 beats/min in normothermia. During the final 3 min of heat stress, MAP averaged 79 ± 3 mmHg, and pulse rate averaged 81 ± 4 beats/min. MAP values from normothermia and the final 3 min of heat stress did not differ significantly (P > 0.05); however, pulse rate increased significantly (P < 0.01, normothermia vs. heat stress).

**DISCUSSION**

We found that combined VPAC2/PAC1 and NOS antagonism attenuated AVD to the same extent as NOS antagonism alone, but more than VPAC2/PAC1 antagonism alone. Unique to our study is the conclusion that the VPAC2/PAC1 receptor contribution to AVD is dependent on the presence of functional NOS. In addition, our results strongly suggest that these mechanisms are activated in series rather than in parallel; however, our results do not indicate the order of that series relationship.

The rationale for our study came from the following: 1) our prior finding that agonist activation of VPAC2/PAC1 receptors by VIP or PACAP38 mediates a portion of AVD during whole body heat stress in humans (2, 25); and 2) the finding by Wilkins et al. that exogenous VIP can effect vasodilation in skin by mechanisms involving VIP-induced NOS and NO generation, without postjunctional synergy (43). In addition, studies show that, during AVD, NO-dependent mechanisms are synergistic with other AVD effector mechanisms, although whether this involves prejunctural and/or postjunctural mechanisms is unknown (44). These findings led us to address the relationship between VPAC2/PAC1 receptor activation and NO-dependent mechanisms during AVD so as to define how these two systems interact by administering the VPAC2/PAC1 antagonist, PACAP6–38, and the NOS antagonist, l-NAME, both separately and in combination. We reasoned that, if combined VPAC2/PAC1 and NOS antagonism attenuated AVD to an extent similar to antagonism of the two systems separately, that would indicate that VPAC2/PAC1 receptor activation in AVD mediates increases in SkBF through NO-dependent mechanisms. Alternatively, if combined PACAP6–38 and l-NAME blockade produced greater attenuation than observed with either of the antagonists separately, these results would support the views that 1) VPAC2/PAC1 activation during AVD mediates SkBF increases, at least in part, through NO-independent vasodilation; or 2) VPAC2/PAC1 receptor activation and NO interact synergistically during AVD. Since combined VPAC2/PAC1 and NOS antagonism has no greater effect than antagonism of the systems separately, we conclude that VPAC2/PAC1 receptor activation effects increases in SkBF...
through NO-dependent mechanisms during AVD. Furthermore, the lack of additional inhibition by combined PACAP6–38 and L-NAME suggests that PACAP6–38 exerts its inhibition via NOS or at least an L-NAME-sensitive pathway. Assuming the PACAP6–38 is fully blocking the VPAC2 and PAC1 receptors, then the data imply that there is an additional mechanism for stimulating NOS besides those receptors, perhaps involving VPAC1 receptors and/or any of several other NOS-dependent pathways (47, 48).

At present, we are unable to define whether VPAC2 and/or PAC1 receptors interact with NOS separately or together due to the complexity of this neuropeptide system and the limited receptor specificity of available receptor antagonists. To wit, both VIP and PACAP38 occur in cutaneous cholinergic sympathetic nerves (3, 7, 30, 41, 42). The presence of two potential agonists restricts us from reaching any firm conclusions as to whether VIP, PACAP38, or both are the involved agonist(s). Further complications derive from the fact that both of these neuropeptides are agonists with high affinities for VPAC1 and VPAC2 receptors (10). In the case of PAC1 receptors, PACAP38 is a high-affinity agonist, while VIP is a low-affinity agonist. All three receptors occur in human nonglabrous skin, although VPAC1 receptors occur sparsely (26). Antagonists available to study these receptors are problematic. For example, VIP10–28 is a strong VPAC1 antagonist, but has only weak VPAC2 antagonism (8). PACAP6–38 is a strong VPAC2 and PAC1 antagonist, but has only weak, if any, VPAC1-blocking activity (25). These complexities leave us with the present dichotomous conclusion that VIP and/or PACAP38 act through VPAC2 and/or PAC1 receptors and require NO in series to cause AVD.

Our results also suggest that other NO-dependent pathways, in addition to VPAC2/PAC1 receptor activation, participate in AVD. This interpretation arises from our observation that NOS antagonism alone has a significantly greater inhibitory effect on AVD than VPAC2/PAC1 antagonism alone. Assuming that the difference is not explained by incomplete blockade, it suggests that something in addition to VPAC2/PAC1 receptor activation stimulates NO production during AVD; otherwise, AVD attenuation by L-NAME blockade of NOS and AVD attenuation by PACAP6–38 blockade of VPAC2/PAC1 receptors would have caused similar reductions in AVD. While it is possible that some of the greater inhibitory effect of L-NAME compared with PACAP6–38 is due to blockade of basal NO production rather than by factors secreted during AVD, the observation that L-NAME did not significantly affect baseline CVC argues for the former.

Although AVD was attenuated by all antagonist treatments, no changes were noted in the T_or at which AVD began. Increases in the internal temperature thresholds for AVD initiation have been reported in several studies using muscarinic antagonists (19, 33). Most (12, 17, 21–23), but not all (48), studies of NOS antagonism did not find an effect of NOS blockade on the T_or threshold. Our prior study with VPAC2/PAC1 receptor antagonism during AVD found no effect on the T_or threshold (25). We also observed that the slopes of the CVC relationship with T_or did not differ among treatments during the initial rapidly rising phase of AVD. The overall significance of these findings is unclear; however, they suggest that NOS and VPAC2/PAC1 activity play more important mechanistic roles in the latter stages of heat stress than during the initiation of AVD. These results also suggest an early NO-independent role for acetylcholine and muscarinic receptors, consistent with the observation that atropine delays, but does not abolish, AVD (19).

While effects of VPAC2/PAC1 and NOS antagonism were manifest during whole body heat stress, no alterations in the cutaneous vasoconstrictor response to whole body cooling were found. This result indicates that none of our antagonist treatments altered the cutaneous noradrenergic vasoconstrictor nerves, nor did they alter responsiveness of skin vascular smooth muscle.

The implication of the present study that VPAC2/PAC1 receptor activation by endogenously released agonists mediates skin vasodilation through NO-dependent mechanisms is consistent with findings from a variety of prior studies (43, 46). For example, Wilkins et al. (43) demonstrated that exogenous VIP causes vasodilation in skin through NO-dependent mechanisms at VIP doses of 100 pmol or greater as well as NO-independent mechanisms. It is well known that physiological concentrations of VIP and/or PACAP38 activate VPAC2/PAC1 receptors to activate adenyl cyclase and increase cAMP generation (4, 5). Our results suggest that this may not be the case in AVD. We found that simultaneous antagonism of VPAC2/PAC1 receptors and NOS attenuated AVD to the same extent as NOS antagonism alone, but more than VPAC2/PAC1 antagonism alone. If cAMP generation by VPAC2/PAC1 receptors was involved in AVD, simultaneous VPAC2/PAC1 and NOS antagonism would have been expected to attenuate AVD to a greater extent than NOS antagonism alone (24). Interestingly, work on AVD in the rabbit ear by Farrell and Bishop (6) suggested that an unknown neurotransmitter from vasodilator nerves stimulates adenyl cyclase production of cAMP, while simultaneous NO production stimulates soluble guanylyl cyclase to produce cyclic GMP. They proposed that cAMP and cGMP act together to stimulate protein kinases leading to AVD. Our results suggest that VPAC2/PAC1 receptors do not play a role in cAMP generation, but rather may participate in NO-induced cGMP generation during AVD, thus leaving unanswered what mechanisms may increase cAMP; indeed, the role for cAMP in human AVD is unclear, as preliminary data from our laboratory indicate that, although interstitial cAMP levels in skin increase during AVD, adenyl cyclase inhibition appears not to attenuate AVD.

As previously alluded, Wilkins at al. (44) showed that NO interacts synergistically with one or more neurotransmitters to increase SkBF during whole body heat stress. This was based on their results that, during NOS inhibition, the increase in CVC in response to exogenous NO administered during concurrent AVD in heat stress was greater than the increase in CVC in response to exogenous NO administered in normothermia. Their related finding that the NO-independent portion of vasodilator response to exogenous VIP was not augmented when administered concomitantly with exogenous NO and NOS antagonism suggested that NO and VIP do not interact synergistically at postsynaptic sites. Our results indicate that such a synergistic NO effect does not occur with VPAC2/PAC1 receptor activation. If VPAC2/PAC1 receptor activation and NO had a synergistic relationship in increasing SkBF during AVD, combined VPAC2/PAC1 receptor and NOS blockade would have produced greater AVD attenuation than either VPAC2/PAC1 receptor or NOS blockade alone. We
PACAP6–38 would not alter our fundamental conclusion that VPAC2/PAC1 receptors, resulting in an underestimation of the consistently and significantly attenuated PACAP38-induced PAC1 receptors. A concentration of 100 μM PACAP6–38 produced an incomplete blockade of VPAC2/PAC1 receptors, resulting in an underestimation of the absolute contribution of VPAC2/PAC1 receptor activation to AVD. Nonetheless, incomplete blockade with 100 μM PACAP6–38 would not alter our fundamental conclusion that VPAC2/PAC1 receptors and NO interact in series to contribute to AVD during whole body heat stress in humans.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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