Cyclooxygenase inhibition does not alter methacholine-induced sweating

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Fujii N, McGinn R, Paull G, Stapleton JM, Meade RD, Kenny GP. Cyclooxygenase inhibition does not alter methacholine-induced sweating. J Appl Physiol 117: 1055–1062, 2014. First published September 11, 2014; doi:10.1152/japplphysiol.00644.2014.—Cholinergic agents (e.g., methacholine) induce cutaneous vasodilation and sweating. Reports indicate that either nitric oxide (NO), cyclooxygenase (COX), or both can contribute to cholinergic cutaneous vasodilation. Also, NO is reportedly involved in cholinergic sweating; however, whether COX contributes to cholinergic sweating is unclear. Forearm sweat rate (ventilated capsule) and cutaneous vascular conductance (CVC, laser-Doppler perfusion units/mean arterial pressure) were evaluated in 10 healthy young (24 ± 4 yr) adults (7 men, 3 women) at four skin sites that were continuously perfused via intradermal microdialysis with 1 lactated Ringer (control), 2) 10 mM ketorolac (a nonselective COX inhibitor), 3) 10 mM Nω-nitro-L-arginine methyl ester (i-NAME, a nonselective NO synthase inhibitor), or 4) a combination of 10 mM ketorolac + 10 mM i-NAME. At the four skin sites, methacholine was simultaneously infused in a dose-dependent manner (1, 10, 100, 1,000, 2,000 mM). Relative to the control site, forearm CVC was not influenced by ketorolac throughout the protocol (all P > 0.05), whereas i-NAME and ketorolac + i-NAME reduced forearm CVC at and above 10 mM methacholine (all P < 0.05). Conversely, there was no main effect of treatment site (P = 0.488) and no interaction of methacholine dose and treatment site (P = 0.711) on forearm sweating. Thus forearm sweating (in mg·min⁻¹·cm⁻²) from baseline up to the maximal dose of methacholine (2,000 mM) was not different between the four sites (at 2,000 mM, control 0.50 ± 0.23, ketorolac 0.44 ± 0.23, i-NAME 0.51 ± 0.22, and ketorolac + i-NAME 0.51 ± 0.23). We show that both NO synthase and COX inhibition do not influence cholinergic sweating induced by 1–2,000 mM methacholine.

sudomotor activity; prostanoids; muscarinic receptor; thermoregulation; nitric oxide

THERMOREGULATORY CONTROL OF sweating and cutaneous active vasodilation is crucial to the maintenance of a stable core body temperature during heat stress in humans. Numerous reports have shown that acetylcholine (ACh) released from cholinergic nerves is a key neurotransmitter involved in the control of sweating (24, 31, 48, and, to a lesser extent, cutaneous active vasodilation during heat stress (24, 49). Indeed, during passive heating at rest, the administration of a muscarinic receptor antagonist (atropine) abolished sweating (24, 31) and partly inhibited cutaneous active vasodilation (24). Furthermore, acetycholinesterase inhibition, which increases ACh bioavailability, enhances both sweating and cutaneous active vasodilation in the early stages during passive heating with <0.4°C increase in core body temperature (49).

Previous human studies in vivo have evaluated whether nitric oxide (NO) and cyclooxygenase (COX) are involved in cholinergic cutaneous vasodilation induced by administration of ACh via iontophoresis (13, 40) or intradermal microdialysis (6, 16, 22, 25, 34). Although some studies demonstrated a contribution of NO (6, 16, 25, 34) and COX (16, 25, 34, 40) to ACh-induced cutaneous vasodilation, others reported no effect of NO (22, 40) or COX (13). Thus whereas current evidence remains inconclusive, it is plausible that either NO or COX, or both may be involved in cholinergic cutaneous vasodilation. On the other hand, the mechanisms of cholinergic sweating have been examined extensively in sweat glands in vitro (43, 45, 47). More recently, however, studies using microdialysis have also expanded on the mechanisms of human cholinergic sweating in vivo (28, 35, 36, 46). Specifically, Lee and Mack (28) showed that the sweating response to methacholine, a cholinergic agent, was lower with simultaneous NO synthase (NOS) inhibition relative to methacholine alone. Therefore, NO is believed to be involved in cholinergic sweating. In addition, some observations in vitro infer the possibility that COX contributes to cholinergic sweating. For example, the presence of COX-1 and, to a lesser extent, COX-2, have been observed in the epithelial cells of the basal coil in the eccrine sweat gland and secretory ductal tubules in normal human skin (38). Accordingly, local administration of prostaglandin E1 and E2, which are products of COX, caused increased sweat secretion in human eccrine sweat glands in vitro (45). The role of prostaglandins in sweat production may arise through increasing cAMP, which is known to be synthesized by prostaglandins E1 and E2 (23), and has been shown to augment sweating in human sweat glands in vitro (47).

To date, the influence of local COX on cholinergic sweating in vivo has not been directly assessed in humans. Given that sweat evaporation is the major avenue for heat loss when ambient temperature is high (e.g., 35°C) (17), and that a COX inhibitor such as aspirin is commonly used to relieve pain, headache, and fever, and to minimize cardiovascular risk (39), it is important to elucidate the influence of local COX on cholinergic sweating. Moreover, an interaction of NO and COX was first introduced by Salvemini et al. (44) who demonstrated that NO augmented the production of prostaglandins by activating COX in the mouse macrophage cell line. In addition, this interaction has also been observed in the salivary gland of rats (29). Given that the exocrine nature of the salivary gland is similar to that of the sweat gland, it is plausible that NO and COX may also interact in human sweat glands such that any effects of NO and COX on cholinergic sweating may not be independent. Thus the purpose of the present study was to evaluate the separate and combined influences of NO and COX on cholinergic sweating by administering incremental doses of methacholine via microdialysis. We hypothesized that NO and COX would contribute to cholinergic sweating, and that the combined influence of NO and COX would not be additive. In this study, we also evaluated the roles of NO and COX. We also assessed the role of NO synthase inhibition in the presence of COX-1 and, to a lesser extent, COX-2, by administering incremental doses of methacholine via microdialysis.
COX in cholinergic cutaneous vasodilation to affirm the results reported in the previous studies.

MATERIALS AND METHODS

Ethical approval. This study was approved by the University of Ottawa Health Sciences and Science Research Ethics Board and conformed to the guidelines set forth by the Declaration of Helsinki. Verbal and written informed consent was obtained from all volunteers prior to their participation in the study.

Subjects. Ten healthy, habitually active (2–4 days per wk, ≥30 min of exercise per day) young adults (seven men, three women) participated in this study. Subjects were excluded if they had a history of cystic fibrosis transmembrane conductance regulator mutations, skin disorders, hypertension, heart disease, diabetes, autonomic disorders, and cigarette smoking. All subjects were not currently taking prescription medications with the exception of contraceptives. Two of the three women were using contraceptives (one used orally administered pills and one used an intrauterine device). All women completed their experimental session during the early follicular phase (within 6 days of the start of menstruation) or during the placebo phase if they used contraceptives. This was necessary to minimize the reported effects of female sex hormones on sweating (27). Subjects’ body mass, height, surface area, and age were (mean ± SD) 76.0 ± 16.9 kg, 1.69 ± 0.05 m, 1.86 ± 0.20 m², and 24 ± 4 yr, respectively.

Experimental session. All subjects abstained from taking over-the-counter medications (including nonsteroidal anti-inflammatory agents and vitamins) for at least 48 h before the study, as well as alcohol and caffeine at least 12 h before the study. They also refrained from heavy exercise the day prior to the study. On the study day, subjects did not consume any food 2 h before and throughout the study. Upon arrival at the laboratory, subjects provided a urine sample and voided their bladder, after which a measurement of body mass was taken using a digital weight scale platform (model CBU150X; Mettler Toledo, IND560; Mettler Toledo). Thereafter, the subject’s body height was measured using an eye-level physician stadiometer (model 2391; Detecto Scale, Webb City, MO). Body surface area was subsequently estimated from the measurements of body mass and height (14). Subjects were then seated in a semirecumbent position in a thermoneutral room (~23°C) and instrumented with four microdialysis fibers (model MD2000; Bioanalytical Systems, West Lafayette, IN) (30 kDa cutoff, 10 mm membrane) on the dorsal side of the left forearm in the dermal layer of the skin. A 25-gauge needle was first inserted into the unanesthetized skin [i.e., ice or local anesthetic cream used previously (19) was not employed] using aseptic technique. The entry and exit points were ~2.5 cm apart. The microdialysis fiber was then threaded through the lumen of the needle, after which the needle was withdrawn leaving the fiber in place. Microdialysis fibers were secured with surgical tape. Each fiber was separated by at least 4.0 cm.

Approximately 20 min after the placement of the microdialysis fibers, perfusion of pharmacological agents began via microdialysis. Fibers were randomly assigned to receive 1) lactated Ringer (control); 2) 10 mM ketorolac (Sigma-Aldrich, St. Louis, MO), a nonselective COX inhibitor; 3) 10 mM L-NAME (Sigma-Aldrich) to nonspecifically inhibit NOS and thus NO production; or 4) a combination of 10 mM ketorolac and 10 mM L-NAME. These concentrations were determined on the basis of previous studies in which intradermal microdialysis was employed in human skin (16, 21, 22, 25, 32–34, 50). It has been suggested that L-NAME has antimuscarnic effects in the rabbit coronary artery and canine colonic smooth muscle (9); however, a more recent study in humans reported minimal antimuscarnic effects on methacholine-induced sweating using 10 mM L-NAME (28). Each drug was continuously perfused at a rate of 2.0 µl/min using a microinfusion pump (model 400; CMA Microdialysis, Solna, Sweden) for at least 75 min to ensure the establishment of each blockade

After 10 min of baseline data collection, each microdialysis fiber was perfused with methacholine in a dose-dependent manner (5 doses: 1, 10, 100, 1,000, and 2,000 mM) in combination with the site-specific pharmacological agents. Methacholine has been perfused at concentrations of 1, 10, 100, and 1,000 mM in previous studies wherein cholinergic sweating and/or cutaneous vasodilation were evaluated (26, 51). We also infused 2,000 mM methacholine to induce a more pronounced increase in sweating given that NO-dependent sweating during exercise is evident only at higher local sweat rates (50, 53). Conversely, we did not employ <1 mM methacholine because this is associated with minimal increases in sweating (26, 51). Each dose of methacholine was continuously administered until a clear plateau of sweating and cutaneous vasodilation was observed (i.e., for ~10 to 25 min). The plateau was defined as a stable value for at least 2 min. The actual drug administration time for 1, 10, 100, 1,000, and 2,000 mM methacholine was (mean ± SD) 14 ± 2, 16 ± 5, 19 ± 3, 23 ± 3, and 22 ± 2 min, respectively. Importantly, we did not observe any marked differences in the time to reach a plateau across the four skin sites.

After observing a plateau at 2,000 mM methacholine, administration of 50 mM sodium nitroprusside (SNP, Sigma-Aldrich) at a rate of 3.0 µl/min was initiated. Our pilot work indicated that 50 mM SNP induces maximal cutaneous vasodilation to a similar extent as would be observed during local heating to 44°C. SNP administration lasted for approximately 25–30 min, until a stable plateau for at least 2 min was observed. At this point, blood pressure was measured to quantify maximal cutaneous vascular conductance (CVC).

Additional session. We conducted an additional session to examine the effect of different concentrations of ketorolac in modulating the sweating response to methacholine administration in four habitually active (2–4 days per wk, ≥30 min of exercise per day), young men. Subjects’ body mass, height, surface area, and age were (mean) 86.1 kg, 1.70 m, 1.97 m², and 24 yr, respectively. The protocol was equivalent to the experimental session described above with the exception that pharmacological agents were delivered before and during methacholine dose response. Four forearm skin sites continuously received either J) lactated Ringer (control), 2) 5 mM ketorolac, 3) 10 mM ketorolac, or 4) 15 mM ketorolac.

Measurements. Cutaneous blood flow (expressed in perfusion units), which is an index of cutaneous blood flow, was locally measured at a sampling rate of 32 Hz with laser-Doppler flowmetry (PeriFlux System 5000; Perimed, Stockholm, Sweden). Integrated laser-Doppler flowmetry probes with a seven-laser array (model 413; Perimed) were housed in the center of each sweat capsule over each microdialysis fiber, allowing for simultaneous measurement of both local forearm sweat rate and cutaneous red blood cell flux at the four skin sites. Beat-by-beat mean arterial pressure was monitored from the right middle finger using finger photoplethysmography (Finometer; Finapres Medical System, Amsterdam, The Netherlands). The right arm was placed on a foam pad to keep the middle finger at heart level. The Finometer was calibrated using upper arm return-to-flow systolic pressure detection (3) and physical criteria (54) following brachial artery pressure reconstruction (18). CVC was evaluated as perfusion units divided by mean arterial pressure. CVC data were expressed as a percentage of maximum as evaluated during the maximal cutaneous vasodilation procedure to minimize the effect of site-to-site heterogeneous in the level of cutaneous blood flow (37).

Sweat capsules, each of which had an area of 3.8 cm², were placed directly over the center of each microdialysis membrane. The sweat capsules were attached to the skin with adhesive rings and topical skin glue (Collodion HV; Maidron Medical Products, Lake Worth, FL). Dry compressed air in the gas tank located in the thermoneutral room was supplied to each capsule at a rate of 0.5 liter/min, while water content of the effluent air from the sweat capsule was measured with high-precision dew point mirrors (model 473; RH Systems, Albuquerque, NM). Long vinyl tubes were used for...
connections between the gas tank and the sweat capsule, and between the sweat capsule and the dew point mirror so that internal gas temperature was equilibrated to near room temperature (≈23°C) before reaching the sweat capsule (inlet) and the dew point mirror (outlet). Local forearm sweat rate was calculated every 5 s from the difference in water content between influent and effluent air multiplied by the flow rate and normalized for the skin surface area under the capsule (mg·min⁻¹·cm⁻²). Due to technical difficulties, one subject in the additional session did not have a local forearm sweat rate at the control site at 10, 100, 1,000, or 2,000 mM methacholine.

Urinary specific gravity was assessed in 9 of 10 subjects from the urine samples obtained before the start of the experimental protocol using a handheld total solids refractometer (model TS400; Reichert, Depew, NY).

Data analyses. Baseline values were obtained by averaging measurements made over ~10 min. Local forearm sweat rate and CVC data from the final minute at each dose of methacholine was used for data analysis. Of all CVC values obtained during administration of methacholine, the highest CVC measured over 1 min was considered as the peak at each site. CVC values during administration of SNP at the end of the experimental protocol were determined from averaging CVC data over at least 2 min. Of note, maximal CVC at the control and ketorolac sites was often observed during methacholine administration, whereas at the L-NAME site, only one subject exhibited maximal CVC during methacholine administration. In contrast, maximal CVC was observed during SNP administration in all subjects at the ketorolac + L-NAME site. The higher CVC measured during methacholine administration compared with that response observed during SNP administration was also reported in a previous study (28). The half maximal effective concentration (i.e., the concentration required to elicit half of the maximal response, EC₅₀) for sweating was evaluated as reported previously (51) using commercially available software (GraphPad Prism 6.0; GraphPad Software, La Jolla, CA). However, given that the lowest dose of methacholine (1 mM) caused large increases in forearm CVC, we did not evaluate EC₅₀ for forearm CVC.

Statistical analyses. Using 80% power and a significance level of 0.05, we calculated a minimal sample size on the basis of data in our previous study (51) with an effect size of 1.21 for sweating EC₅₀, and 1.33 for local forearm sweat rate. Subsequently, the calculated minimal sample size for sweating EC₅₀ and local forearm sweat rate was n = 8 and 7, respectively. Thus our sample size of n = 10 should have been sufficient. All data used for parametric statistical analyses in the experimental session was normally distributed, as verified by D’Agostino’s K-squared test. Local forearm sweat rate and CVC were analyzed using a two-way repeated measures ANOVA with the factor of methacholine dose (six levels: baseline, 1, 10, 100, 1,000, and 2,000 mM) and of treatment site (four levels: control, ketorolac, L-NAME, and ketorolac + L-NAME). Forearm absolute maximal CVC (expressed in perfusion units mmHg⁻¹) was analyzed with a one-way repeated-measures ANOVA with the factor of treatment site (four levels: control, ketorolac, L-NAME, and ketorolac + L-NAME). Mean arterial pressure was analyzed using a two-way repeated measures ANOVA with the factor of dose (seven levels: baseline, 1, 10, 100, 1,000, and 2,000 mM methacholine; and 50 mM SNP). When a significant main effect was observed, post hoc comparisons were carried out using Student’s paired t-tests corrected for multiple comparisons using the Holm-Bonferroni procedure. In addition, two tailed Student’s paired t-tests were used to evaluate differences in CVC from the peak value to that observed during perfusion of 2,000 mM methacholine at each skin site. For this analysis, we did not include individuals who showed peak values in CVC at 2,000 mM methacholine (n = 1, 2, and 1 for the control site, ketorolac site, and ketorolac + L-NAME site, respectively). Furthermore, due to the small sample size in the additional substudy, we did not perform statistical analyses to differentiate mean values. The level of significance for all analyses was set at P ≤ 0.05. All values are reported as mean ± SD.

RESULTS

Local forearm sweat rate. We found a main effect of methacholine dose (P < 0.001) for local forearm sweat rate. However, there was no main effect of treatment site (P = 0.488) and no interaction of methacholine dose and treatment site (P = 0.711) for local forearm sweat rate. Therefore, local forearm sweat rate did not differ between the four forearm skin sites at baseline or at any dose of methacholine (Fig. 1). Furthermore, there was no main effect of treatment site on EC₅₀ for local forearm sweating (P = 0.162), such that EC₅₀ for local forearm sweating (in mM) was similar between the four sites (control 288 ± 59; ketorolac 268 ± 134; L-NAME 237 ± 136, and ketorolac + L-NAME 272 ± 118).

It is also important to note that in the additional experiment wherein we assessed the influence of differing concentrations of ketorolac (5, 10, and 15 mM) on local forearm sweat rate, there were no clear differences in local forearm sweat rate across the four sites during baseline or any concentration of methacholine (Table 1).

Local forearm cutaneous vascular response. There was an interaction of methacholine dose and treatment site (P = 0.011) for local forearm CVC. No differences in local forearm CVC across treatment sites were observed at baseline or at 1 mM methacholine (all P > 0.05, Fig. 2). However, at or above 10 mM methacholine, L-NAME and/or ketorolac + L-NAME reduced local forearm CVC relative to the control group, albeit no effect of ketorolac was detected (Fig. 2). Local forearm CVC at 2,000 mM methacholine was lower than local forearm peak CVC observed during methacholine administration irrespective of treatment site (Fig. 3). In addition, there was no main effect of treatment site on local forearm absolute maximal CVC (P = 0.296). Hence, local forearm absolute maximal
various concentrations site and during simultaneous perfusion of ketorolac at during incremental methacholine administration at a control site and during simultaneous perfusion of ketorolac at various concentrations.

Local forearm sweat response at baseline and during incremental methacholine administration from 1 to 2,000 mM (five levels) at four skin sites receiving 1) lactated Ringer (control, open circles), 2) 10 mM ketorolac (squares), 3) 10 mM L-NAME (triangles), or 4) a combination of 10 mM ketorolac + 10 mM L-NAME (diamonds). Values are mean ± SD (n = 10). *Significantly different vs. control (P < 0.05).

Fig. 2. Local forearm cutaneous vascular conductance at baseline and during methacholine administration from 1 to 2,000 mM (five levels) at four skin sites receiving 1) lactated Ringer (control, open circles), 2) 10 mM ketorolac (squares), 3) 10 mM L-NAME (triangles), or 4) a combination of 10 mM ketorolac + 10 mM L-NAME (diamonds). Values are mean ± SD (n = 10). *Significantly different vs. control (P < 0.05).

CVC was comparable between the four sites (control 1.89 ± 0.54, ketorolac 1.79 ± 0.45, L-NAME 1.79 ± 0.27, and ketorolac + L-NAME 1.57 + 0.38 perfusion units mmHg⁻¹).

Urinary specific gravity. Urinary specific gravity before the experiment session was 1.019 ± 0.008, indicating the participants were not dehydrated before the experimental session.

Mean arterial pressure. No main effect of dose was detected for mean arterial pressure (P = 0.753), indicating that mean arterial pressure did not change from baseline levels (88 ± 5 mmHg) throughout the incremental doses of methacholine (1 mM 91 ± 8, 10 mM 89 ± 9, 100 mM 91 ± 7, 1,000 mM 91 ± 8, and 2,000 mM 90 ± 10 mmHg) or during the perfusion of 50 mM SNP (90 ± 9 mmHg).

DISCUSSION

A key finding of the present study was our observation that ketorolac (a nonselective COX inhibitor), L-NAME (a nonselective NOS inhibitor), and the combination of ketorolac + L-NAME did not influence cholinergic sweating as assessed using incremental doses of methacholine (a muscarinic receptor agonist), which is in contrast to our original hypothesis. We also demonstrated that L-NAME but not ketorolac attenuated cholinergic-mediated cutaneous vasodilation. In addition, cholinergic cutaneous vasodilation became greater with increasing doses of methacholine at or below ~500 mM, but thereafter the response gradually decreased despite increases in methacholine concentration up to 2,000 mM. Therefore, we show that both NOS and COX inhibition do not modulate cholinergic sweating achieved by administration of 1–2,000 mM methacholine.

NO and COX in methacholine-induced sweating. We did not observe any differences in EC50 (i.e., the concentration of methacholine required to achieve half of the maximal response) in local forearm sweating between the control and L-NAME sites. This is inconsistent with the previous study by Lee and Mack (28) who reported that EC50 for sweating was higher (indicating the sweating response was attenuated) with both NOS and COX inhibition compared with that of the control site. This discrepancy between studies may be due to differences in the doses employed during the experimental session [i.e., Lee and Mack (28) reported 0.033–243 mM in nine steps vs. the present study using 1, 10, 100, 1,000, and 2,000 mM doses]. It is likely that had we used a lower dose (<1 mM) of methacholine, we may have observed differences in EC50 for local forearm sweating between the control and L-NAME sites. In addition, it is important to note that Lee and Mack (28) reported baseline sweat rates (~0.17 mg-min⁻¹·cm⁻²) and CVC (~35%max) that were greater than those observed in the present study (sweat rate ~0.06 mg-min⁻¹·cm⁻², CVC ~13%max). This
may be due to higher ambient temperatures (28°C), and therefore higher mean skin temperatures, in the study by Lee and Mack (28) compared with the present study (~23°C). It is possible that differences in mean or local skin temperature may in part explain the disparity observed in the NO-dependent cholinergic sweating between the present study and that by Lee and Mack (28). This notion is indirectly supported by the fact that higher local skin temperature itself induces NO-dependent cutaneous vasodilation (11).

It was also reported by Lee and Mack (28) that peak local forearm sweat rate observed with 243 mM methacholine administration was not influenced by NOS inhibition. Our findings paralleled these observations such that we found no differences in local forearm sweat rate between the control and i.-NAME sites even at high doses of methacholine (i.e., 2.000 mM, Fig. 1). It is therefore possible that at higher levels of cholinergic stimulation (or high doses of methacholine as in the present study of ≥1 mM), the effect of NO would not be a limiting factor to the volume of sweat produced. Along these lines, a recent human study in vivo reported that although Ca2⁺ plays a role in sweating at low doses of ACh (as evaluated by EC50), it does not contribute to sweating induced by a higher dose of ACh (i.e., 1,000 mM) (35). These findings indicate that greater muscarinic receptor activation with a higher concentration of cholinergic agents may induce sweating without marked increases in Ca2⁺. Given that NOS is activated by increases in Ca2⁺ (8), this may explain the mechanism underlying the conflicting findings regarding the role of NO in modulating cholinergic sweating.

A robust suppression of cutaneous blood flow can attenuate sweating as demonstrated by Wingo et al. (55), in that a reduction in cutaneous blood flow by ~80% was associated with a decrease in sweating during resting whole-body heating (i.e., an increase in core temperature of ~1°C). However, sweating was maintained at the i.-NAME site compared with the control site in the present study (Fig. 1) despite the fact that i.-NAME decreased CVC by ~20% (Fig. 2). Thus our results may indicate that a partial decrease in cutaneous blood flow (~20%) associated with NOS inhibition does not substantially alter low-to-moderate levels of sweating (maximal sweat rate of ~0.5 mg·min⁻¹·cm⁻² in our study).

Local forearm sweat rate at any dose of methacholine (Fig. 1) and the EC50 for local forearm sweating were not different between the control and ketorolac sites. Moreover, the additional study demonstrated that different doses of ketorolac (i.e., 5, 10, and 15 mM) did not modify local forearm sweat rate relative to the control site (Table 1). These findings indicate that COX is not involved in methacholine-induced cholinergic sweating. One plausible interpretation of our results is that COX enzyme activation, and thus increases in local prostanoid levels, do not subsequently increase sweat production. In fact, although administration of prostaglandins reportedly increases sweat production in vitro (45), it remains to be seen whether this is true for human eccrine sweat gland in vivo. On the other hand, inhibiting COX can increase the amount of bioavailable arachidonic acid to be metabolized by cytochrome P-450, thereby producing more epoxyeicosatrienoic acids (EETs). Potassium channels are believed to be involved in sweat production (43), and EETs reportedly activate calcium-activated big-conductance potassium channels in the cortical collecting duct in rats or rabbits (52). Thus in the present study, local COX inhibition may have enhanced EET-mediated sweat production, which in turn offsets the local COX inhibition-induced decrease in prostaglandin-dependent sweating (45).

The role of COX inhibition on cholinergic sweating may have also been confounded by the possibility of a concomitant alteration or modulation of other signaling pathways. Specifically, COX inhibition may have influenced multiple cells such as dark secretory, clear secretory, and myoepithelial cells as well as endothelial cells in the microvascular and sensory nerve cells surrounding the sweat glands. Although it is not elucidated which prostaglandin receptors are located in what type of cells, it may be possible that some cells specifically contain inhibitory prostaglandin E4 receptors, which are known to decrease bioavailability of cAMP (23). In contrast, other cells may particularly contain stimulatory prostaglandin E4 receptors and/or prostacyclin receptors, all of which are known to increase levels of cAMP (23). Taken together, COX inhibition with ketorolac may have influenced both inhibitory and stimulatory prostaglandin receptors simultaneously, which are located in different cells, thereby resulting in no influence on sweat production.

Interaction between NO and COX on methacholine-induced sweating. COX produces not only prostanoids, but also superoxide (15), which can reduce NO bioavailability because superoxide rapidly binds with NO to produce peroxynitrite. If this is true for sweating, inhibition of COX may enhance NO-dependent sweating. On the other hand, peroxynitrite irreversibly blocks biosynthesis of prostacyclin (56), which is a product of COX. Therefore, inhibiting NO may decrease peroxynitrite, which in turn enhances COX-dependent sweating. Although neither NOS nor COX inhibition affected cholinergic sweating (Fig. 1), one might speculate that this may be due to one enzyme inhibition activating the other, thereby balancing total sweat rate. However, inhibiting both enzymes simultaneously also did not alter cholinergic sweating, suggesting that NOS and COX pathways are not complementary to each other for methacholine-induced cholinergic sweating.

Insight into sweating mechanisms during heat stress. Because muscarinic receptor activation is necessary to induce sweating during heat stress (24, 31), the mechanisms of cholinergic sweating at first glance seem to account almost entirely for the increase in sweat production during heat stress. However, it is important to mention that although we could not detect NO-dependent cholinergic sweating in the present study, NO-dependent sweating is consistently observed during exercise in the heat in young adults in our (50) and other laboratories (53). Cholinergic nerves innervating the eccrine sweat gland release not only ACh, but also cholinergic cotransmitters, such as vasoactive intestinal peptide. Importantly, the cholinergic cotransmitters may modulate cholinergic sweating during heat stress in such a manner that cannot be reproduced with a dose-response protocol (i.e., in the absence of hyperthermia). As such, there exists a possibility that COX modulates sweating during heat stress, although further work is needed to evaluate this hypothesis during passive and/or exercise-induced heat stress.

NO and COX in methacholine-induced cutaneous vasodilation. We show that NO is involved in methacholine-dependent cholinergic cutaneous vasodilation, which is in accordance with previous studies using methacholine (28) and ACh (16, 25, 34) delivered via microdialysis. In contrast, no role of COX...
in cholinergic cutaneous vasodilation is observed in the present study (Fig. 2). This is in contrast to previous studies in which the same drug (10 mM ketorolac) was shown to attenuate ACh-induced cutaneous vasodilation using intradermal microdialysis in the forearm (16, 22, 25) and calf (34) skin. These disparate findings are likely caused by the differing characteristics between ACh and methacholine. For example, ACh can activate both nicotinic and muscarinic receptors, which are involved in the axon reflex-dependent vasodilation (2, 42) and endothelium-dependent vasodilation (2), respectively. Conversely, methacholine activates only muscarinic receptors (i.e., endothelium-dependent vasodilation) (41). Hence, we speculate that the axon reflex, but not the endothelium, is the primary mechanism by which COX-dependent cutaneous vasodilation occurs.

Die-away of methacholine-induced cutaneous vasodilation.

We found that above ~500 mM methacholine, the level of vasodilation gradually subsided despite further increases in methacholine concentration (Fig. 3). The die-away effect was originally reported during local heating (1), and the current study extends this observation to cholinergic stimulation via methacholine. This die-away potentially reflects impaired endothelium-dependent vasodilation, the components of which include NO, COX, and/or endothelium-derived hyperpolarizing factors (EDHFs). Because this die-away effect was observed to a similar extent at the control, L-NAME, and ketorolac sites, we excluded the possibility that NO- and/or COX-dependent mechanisms are involved. Hence, impaired EDHF-dependent vasodilation may account for the die-away effect observed in the present study. EDHFs have previously been shown to play a role in the cutaneous vasodilator response (7, 12, 30). On the other hand, other mechanisms may be also involved, including activation of muscarinic receptors located on the smooth muscle cells, which can cause vasoconstriction (41) and increased sympathetic adrenergic nerve activity, which have previously been postulated to be major factors in the die-away effect during cutaneous thermal hyperemia to local heating (20).

Limitations. Although 10 mM ketorolac is known to effectively inhibit cutaneous vasodilation in response to the administration of cholinergic agents (16, 22, 25), we do not have any direct evidence supporting the effectiveness of 10 mM ketorolac in inhibiting COX at the level of sweat gland. Nevertheless, we believe 10 mM to be the optimal concentration of ketorolac to inhibit COX for sweating, because concentrations greater than 10 mM ketorolac can cause a consistent increase in cutaneous blood flow (22, 25), which may influence sweating independent of COX inhibition. Indeed, it has been suggested that the level of cutaneous blood flow can influence sweat rate (55).

In the present study, methacholine administration at each dose continued until a clear plateau was observed over a 2-min period to ensure a stable and complete diffusion to the tissue. We believe this approach is an effective way of ensuring a complete diffusion of methacholine, and that monitoring this response on the basis of the time at which a stable and consistent sweat output occurs would ensure a more reliable evaluation of the dose to sweating relationship. In contrast, employing a standard time for drug delivery could lead to variable responses in sweat output and therefore alter the dose to sweating relationship. Future studies are required to examine the extent to which differences in infusion time may alter the pattern of response.

Clinical perspectives. COX inhibitors are commonly prescribed to relieve pain, fever, and headache. Also, aspirin, a COX inhibitor, is often prescribed to reduce the risk of cardiovascular diseases (39). On the basis of our results, we speculate that an acute intake of COX inhibitors may not have a detrimental effect on whole-body sweating and thus core body temperature regulation during a heat stress challenge (e.g., passive and/or exercise-induced heat stress). Indeed, Charkoudian and Johnson (10) showed that acute oral administration of 800 mg of ibuprofen (a nonselective COX inhibitor) did not affect sweating during passive heating at rest in young women. In addition, Bradford et al. (4) demonstrated that oral administration of rofecoxib (a COX-2 inhibitor) for 6 days (50 mg/day) did not affect sweating during exercise in young men. On the other hand, a recent study by Bruning et al. (5) demonstrated that taking low-dose aspirin orally for 7 days increased core body temperature during heat exposure at rest and exercise in the heat in middle-aged adults. Thus the effect of COX inhibitors on sweating and core body temperature during passive- or exercise-induced heat stress may be dependent on the type of COX inhibitor, method of administration (e.g., oral ingestion, transdermal patch, eye drops), duration, frequency, dose of administration, and population group (e.g., women, older adults, individuals with chronic disease), all of which need to be elucidated in future studies.

Conclusion. We show that neither NOS nor COX inhibition influences cholinergic sweating induced by 1–2,000 mM methacholine. We also observed that inhibiting NOS but not COX attenuates methacholine-induced cholinergic cutaneous vasodilation, and that cutaneous vasodilation during methacholine administration exhibits gradual decay despite an increase in methacholine concentration above ~500 mM (i.e., die-away).

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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