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## Cutaneous blood flow and sweat rate responses to exogenous administration of acetylcholine and methacholine

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**Kimura K, Low DA, Keller DM, Davis SL, Crandall CG.** Cutaneous blood flow and sweat rate responses to exogenous administration of acetylcholine and methacholine. *J Appl Physiol* 102: 1856–1861, 2007. First published January 18, 2007; doi:10.1152/jappphysiol.01069.2006.—The aim of this study was to evaluate cutaneous vasodilation and sweating responses to exogenous administration of acetylcholine (ACh) and methacholine (MCh), which have different sensitivities to endogenous cholinesterase. Four intradermal microdialysis probes were placed in dorsal forearm skin: two sites were perfused with ACh ( $1 \times 10^{-7}$ – $1$  M) and the other two with the same molar concentrations of MCh. Sweat rate (SR) and cutaneous blood flow were simultaneously assessed directly over each microdialysis membrane. Dose-response curves were constructed, and the effective concentration of the drug resulting in 50% of the maximal response ( $EC_{50}$ ) was identified. For SR and cutaneous vascular conductance (CVC), there were no significant differences in  $EC_{50}$  between sites receiving the same drug:  $-1.52 \pm 0.18$  and  $-1.19 \pm 0.09$  log-molar concentration of ACh at distal and proximal sites, respectively, and  $-2.35 \pm 0.24$  and  $-2.42 \pm 0.23$  log-molar concentration of MCh at distal and proximal sites, respectively, for SR ( $P > 0.05$ ) and  $-3.87 \pm 0.32$  and  $-3.97 \pm 0.27$  log-molar concentration of ACh at distal and proximal sites, respectively, and  $-4.78 \pm 0.17$  and  $-4.46 \pm 0.16$  log-molar concentration of MCh at distal and proximal sites, respectively, for CVC ( $P > 0.05$ ). However, the  $EC_{50}$  for CVC and SR was significantly lower at the MCh than at the ACh sites. A second procedure was performed to confirm that differences in responses between ACh and MCh could be attributed to different cholinesterase sensitivities. Similarly, four microdialysis membranes were placed in dorsal forearm skin: two sites were perfused with ACh and other two with MCh. However, one of each of the ACh and MCh sites was also perfused with  $10 \mu\text{M}$  neostigmine (an acetylcholinesterase inhibitor). Neostigmine at the ACh site induced a leftward shift (i.e., lower  $EC_{50}$ ) of the SR and CVC dose-response curves compared with the site treated with ACh alone, resulting in no difference in the  $EC_{50}$  for SR and CVC between the ACh + neostigmine and the MCh site. These results suggest that elevations in SR and CVC occur earlier with MCh than with ACh treatment because of differences in cholinesterase susceptibility between these drugs.

thermoregulation; sweating; microdialysis

ALTERATIONS IN SWEATING and skin blood flow are vital for body core temperature regulation. Elevations in internal temperature increase sweat rate (SR) and skin blood flow to promote the transfer of heat from the body core to the skin. Sweating occurs on the release of acetylcholine (ACh) from cholinergic nerves and subsequent binding to muscarinic receptors on the eccrine sweat gland (28, 29, 37). In addition, ACh activates muscarinic receptors on endothelial cells of cutaneous blood vessels and

induces cutaneous vasodilation via nitric oxide, prostaglandin, and possibly endothelium-derived hyperpolarizing factor mechanisms (14, 17, 18, 26, 27, 32, 33). After release into the synaptic cleft, ACh is rapidly degraded by hydrolysis to acetate and choline by acetylcholinesterase (AChE).

A number of studies have sought to identify cutaneous vascular and sudomotor responsiveness to exogenous administration of ACh in healthy and diseased populations (1, 3, 4, 11, 16, 18, 32, 34, 36). Some studies propose that cutaneous vasomotor responses to ACh may provide insight into pathological conditions that alter endothelium-mediated vasodilation (1, 11, 19, 21, 30). However, varying responses between subjects and medical conditions may also be related to differences in the effectiveness of AChE in ACh hydrolysis. Consistent with this thought, Shibasaki and Crandall (34) showed that AChE is capable of modulating SR at low-to-moderate ACh concentrations. Methacholine (MCh), an analog of ACh, is resistant to hydrolysis by AChE (9, 22). Thus MCh may be preferred over ACh for assessment of postsynaptic responsiveness to exogenous administration of cholinergic drugs. Previous studies investigating differences between ACh- and MCh-induced sweating and cutaneous vascular responsiveness have used repeated intradermal injections or iontophoresis (10, 13, 25), both of which have the potential to confound the results because of hyperemia associated with drug administration. Furthermore, these methods are limited, in that they preclude the administration of various concentrations of a drug at the same location during measurement of SR and/or skin blood flow at that location. Thus the aforementioned methods cannot be used to construct dose-response curves at the same location. This methodological limitation is particularly concerning given the large degree of heterogeneity of blood flow in human forearm skin (6, 7, 38). Therefore, the primary aim of this research was to compare local SR and skin blood flow responses at the same location to different doses of MCh and ACh administered via intradermal microdialysis to test the hypothesis that MCh-mediated responses will be different from ACh-mediated responses. A second objective was to evaluate intersite variability, with respect to SR and CVC responses, between multiples sites receiving ACh or MCh.

### METHODS

Twelve healthy subjects (6 men and 6 women) participated in *protocol 1*; their age, height, and weight (mean  $\pm$  SE) were as follows:  $36 \pm 2$  yr,  $176 \pm 4$  cm, and  $79 \pm 6$  kg. Seven healthy subjects (5 men and 2 women) participated in *protocol 2*; their age,

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height, and weight were as follows:  $34 \pm 2$  yr,  $171 \pm 4$  cm, and  $61 \pm 3$  kg. Each subject was informed of the purpose and risks of the study before providing their written consent. The Institutional Review Boards of the University of Texas Southwestern Medical Center at Dallas and Presbyterian Hospital of Dallas approved all protocols and informed consents.

### Instrumentation and Measurements

On entering the laboratory, each subject rested in the supine position during placement of four intradermal microdialysis probes in the dermal space of the dorsal forearm. Each probe was separated by  $\geq 2.5$  cm. The membrane window for each probe was 10 mm. For placement of the probes, a 25-gauge needle pierced the dermal space without local anesthetic and exited 20–25 mm from the point of entry. The microdialysis probe was threaded through the lumen of the needle. The needle was then removed, leaving the probe in place. The depth of probe placement was not identified, although a depth of 0.3–1.0 mm was reported in a prior study using similar procedures (17). After membrane placement, the probes were perfused with lactated Ringer solution at 2  $\mu$ l/min via an infusion pump (Pump 11, Harvard Apparatus, Natick, MA). Chambers with a small ( $10 \times 5$  mm, i.e., 0.5-cm<sup>2</sup> surface area) window were positioned over each membrane to measure SR via capacitance hygrometry (Viasala, Woburn, WA) using compressed nitrogen gas at a flow of 300 ml/min. Absolute humidity over each probe was converted to SR from gas flow and the measured surface area. Capsule placement was aided by markings on the probe that indicated the center of the membrane portion of the microdialysis probe. Local skin blood flow was measured via laser-Doppler flowmetry (System 4000, Perimed) using integrating probes (model PF 413) that were housed within the SR chambers. Thus skin blood flow and SR were simultaneously assessed from the same location. Ambient temperature in the laboratory was controlled at 24°C. Arterial blood pressure was obtained from the opposite arm, relative to microdialysis probe placement, via auscultation of the brachial artery (SunTech Medical Instruments, Raleigh, NC). Mean arterial blood pressure was calculated as one-third pulse pressure + diastolic blood pressure. Cutaneous vascular conductance (CVC) was calculated as the ratio of laser-Doppler flux to mean arterial blood pressure.

### Protocol 1

The primary purpose of *protocol 1* was to compare local SR and CVC responses to exogenous ACh and MCh administration. A secondary goal was to examine intersite variations in SR and CVC for each drug. After microdialysis membrane placement and  $\geq 90$  min for the hyperemic response associated with membrane placement to subside, baseline SR and CVC data were collected for 5 min. Subsequently, two membranes were perfused with increasing doses of ACh ( $1 \times 10^{-7}$ –1 M; 8 doses at 10-fold increments) dissolved in Ringer solution, while the other two membranes were perfused with the same concentrations of MCh, with each dose being administered for 7 min at a perfusion rate of 2  $\mu$ l/min. ACh and MCh were administered to randomly assigned sites. After the last dose of the drug, 50 mM sodium nitroprusside (SNP) was administered for 10 min at all sites. This duration of SNP administration is adequate to maximally dilate the skin when sites previously received high doses of vasodilating drugs such as ACh or MCh. CVC was normalized relative to each site's maximal CVC.

### Protocol 2

*Protocol 2* was performed on a subset of subjects to investigate the effects of AChE on local SR and CVC responsiveness to ACh and MCh administration. Four microdialysis membranes were placed in forearm skin as described for *protocol 1*. One site was perfused with increasing concentrations of ACh ( $1 \times 10^{-7}$ –1 M; 8 doses at 10-fold

increments), a second site was perfused with the same concentrations of MCh, a third site was perfused with the same concentrations of ACh + 10  $\mu$ M neostigmine (an AChE inhibitor), and a fourth site was perfused with the same concentrations of MCh + 10  $\mu$ M neostigmine. The dose of neostigmine was based on prior studies in which 10  $\mu$ M was demonstrated to effectively inhibit AChE (34–36). Similar to *protocol 1*, each dose of drug was administered for 7 min at a perfusion rate of 2  $\mu$ l/min. After the last dose, 50 mM SNP was administered for 10 min to maximally vasodilate the assessed areas. Sites were randomly selected to receive ACh, MCh, ACh + neostigmine, and MCh + neostigmine.

### Data Collection and Statistical Analysis

Data were continuously obtained and digitized at a sampling rate of 50 Hz (Biopac System, Santa Barbara, CA). For each dose, SR and skin blood flow were averaged at 10-s intervals, and the 60-s average around the maximum 10-s value from each stage was selected for analysis. This 60-s period typically occurred during the last 1–2 min of the perfused dose. Dose-response curves for CVC and SR at each of the four sites in both protocols were constructed using a nonlinear fitting technique, with a Hill slope of 1, from which the effective concentration causing 50% of the maximal response ( $EC_{50}$ ) was identified (Prism, Graphpad Software). For the intersite analysis for *protocol 1* (i.e., evaluation of responses between ACh sites and between MCh sites), SR and CVC between the proximal and distal sites receiving ACh, as well as between the proximal and distal sites receiving MCh, were statistically analyzed across doses of drug via a repeated-measures two-way ANOVA. In addition, the  $EC_{50}$  values of the proximal and distal sites for each drug were compared using paired *t*-tests. For the between-drug analysis (i.e., ACh responses compared with MCh responses), SR and CVC for the sites receiving the same drug were first averaged for each dose. These averaged responses were then modeled via the nonlinear fitting technique, and differences in responses between drugs across the dose range were compared via a repeated-measures two-way ANOVA. Moreover, differences in the  $EC_{50}$  between ACh and MCh dose-response curves were compared using paired *t*-tests. Because of a technical error, SR was not measured in two subjects for *protocol 1*. Thus, for *protocol 1*, SR is reported from 10 subjects and CVC is reported from 12 subjects. For *protocol 2*, the  $EC_{50}$  values for SR and CVC to ACh and MCh at neostigmine-treated and untreated sites were compared using a repeated-measures one-way ANOVA. Differences in responses between ACh and MCh at the neostigmine-treated and untreated sites across the dose range were compared via a repeated-measures two-way ANOVA. Values are means  $\pm$  SE. The level of statistical significance was set at  $P < 0.05$ .

## RESULTS

### Protocol 1

**Within-drug comparisons.** The  $EC_{50}$  for SR and CVC dose-response curves showed no significant difference between proximal and distal sites for ACh or MCh ( $P > 0.05$ ; Figs. 1 and 2). A linear relation for SR existed between the two ACh sites (slope =  $1.29 \pm 0.14$ ,  $R = 0.96 \pm 0.01$ ) and the two MCh sites (slope =  $0.68 \pm 0.09$ ,  $R = 0.96 \pm 0.01$ ). Consistent with this observation, a linear relation for CVC existed between the two ACh sites (slope =  $0.96 \pm 0.05$ ,  $R = 0.92 \pm 0.02$ ) and the two MCh sites (slope =  $0.84 \pm 0.05$ ,  $R = 0.94 \pm 0.01$ ). There was no significant difference in CVC between sites receiving ACh or MCh (i.e., main effect of site  $P > 0.05$ ; Fig. 2). However, for SR, there was a significant interaction between site and dose for ACh and MCh trials ( $P < 0.05$ ; Fig. 1).

**Between-drug comparisons.** SR was significantly greater at the MCh than at the ACh sites during administration of moderate

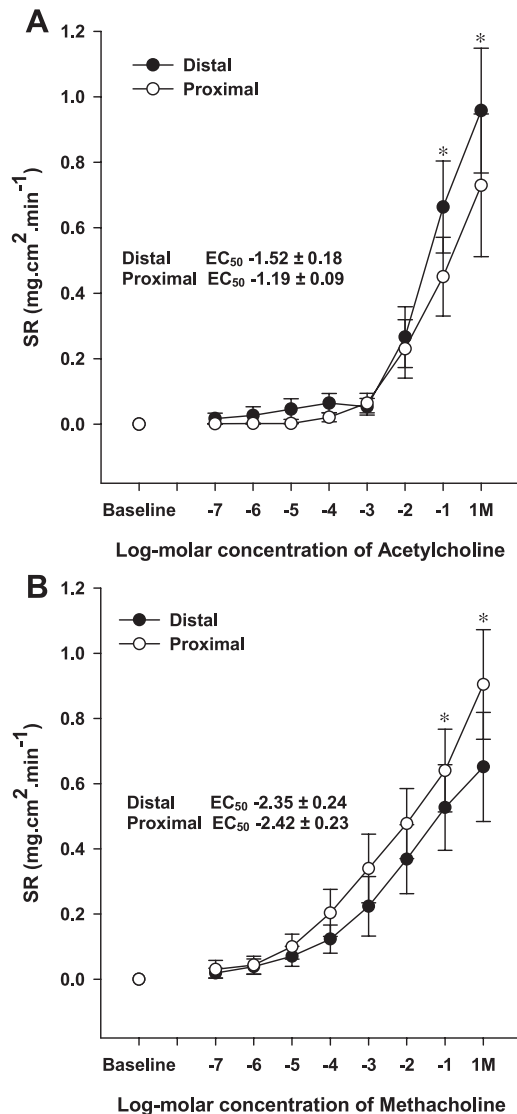


Fig. 1. Sweat rate (SR) dose-response curves to exogenous administration of  $10^{-7}$ – $1$  M ACh (A) and methacholine (MCh, B) at proximal and distal sites.  $EC_{50}$  values were not significantly different between proximal and distal sites treated with the same drug. \*Significant difference between sites at indicated dose ( $P < 0.05$ ).

doses of these drugs ( $1 \times 10^{-4}$ – $1 \times 10^{-2}$  M; Fig. 3). The  $EC_{50}$  for SR was significantly higher at the ACh sites ( $-1.50 \pm 0.16$  log-molar concentration of ACh) than at the MCh sites ( $-2.46 \pm 0.21$  log-molar concentration of MCh,  $P = 0.007$ ). Moreover, the concentration of the drug at the onset of sweating was significantly lower for MCh ( $-4.86 \pm 0.29$  log-molar concentration of MCh) than for ACh ( $-2.38 \pm 0.25$  log-molar concentration of ACh,  $P < 0.001$ ; Fig. 3). CVC was significantly greater at the MCh than at the ACh sites during administration of moderate doses of these drugs ( $1 \times 10^{-5}$ – $1 \times 10^{-3}$  M; Fig. 4). The  $EC_{50}$  was significantly higher for the ACh sites ( $-3.68 \pm 0.17$  log-molar concentration of ACh,  $P = 0.002$ ) than for the MCh sites ( $-4.56 \pm 0.12$  log-molar concentrations of MCh).

#### Protocol 2

Administration of ACh and MCh increased SR at neostigmine-treated and untreated sites. Neostigmine at the ACh site

induced a leftward shift of the SR dose-response curve compared with the non-neostigmine-treated site, as evidenced by a significant difference in the  $EC_{50}$  between sites ( $-2.18 \pm 0.39$  and  $-0.87 \pm 0.22$  log-molar ACh concentration for neostigmine-treated and untreated sites, respectively,  $P = 0.002$ ). The  $EC_{50}$  for SR to various doses of MCh was unaffected by neostigmine in the perfusion solution ( $-2.48 \pm 0.26$  and  $-2.36 \pm 0.16$  log-molar concentration of MCh for neostigmine-treated and untreated sites, respectively,  $P > 0.05$ ). There was no significant difference in the  $EC_{50}$  between the MCh ( $-2.36 \pm 0.16$  log-molar concentration) and ACh sites when neostigmine was included at the ACh site ( $-2.18 \pm 0.39$  log-molar concentration,  $P > 0.05$ ). At the neostigmine-treated sites, ACh and MCh induced sweating at the lowest concentrations of drug ( $1 \times 10^{-7}$  M), while sweating did not occur at the non-neostigmine-treated sites until a higher concentration of ACh was administered.

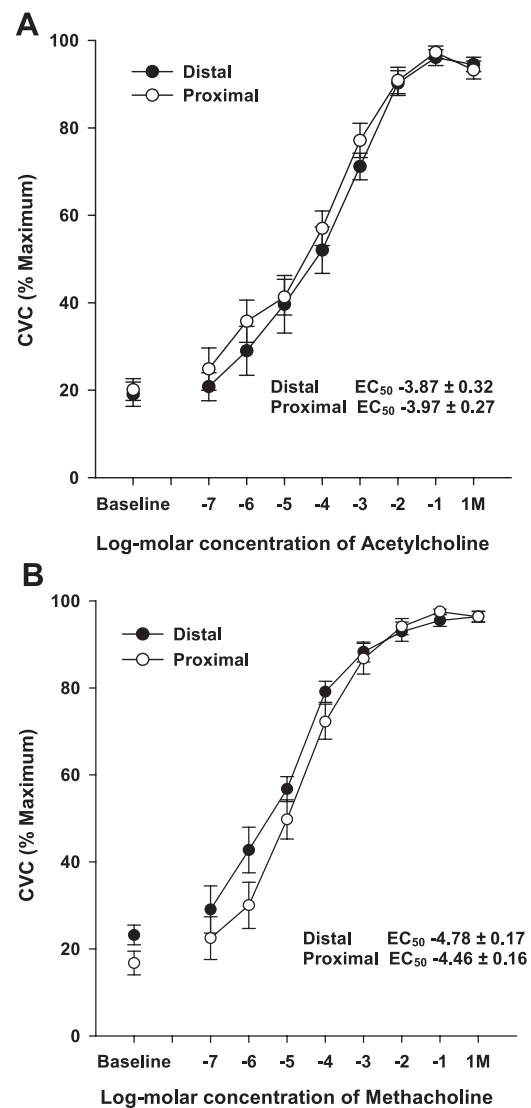


Fig. 2. Cutaneous vascular conductance (CVC) dose-response curves to exogenous administration of  $10^{-7}$ – $1$  M ACh (A) and MCh (B) at proximal and distal sites.  $EC_{50}$  values were not significantly different between proximal and distal sites treated with the same drug.

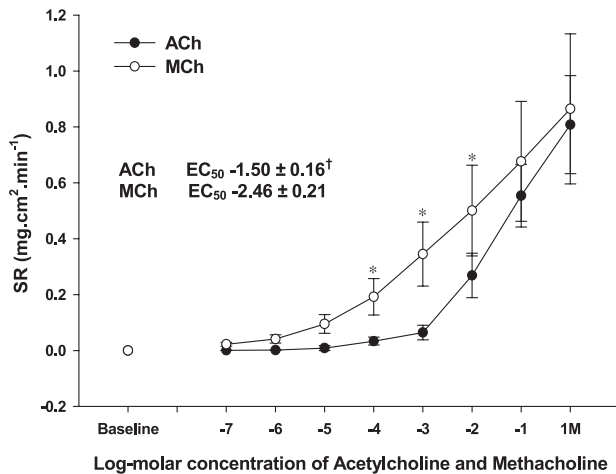


Fig. 3. SR dose-response curves to exogenous administration of  $10^{-7}$ – $1$  M ACh and MCh. Before analysis, data from both sites treated with the same drug (for each subject) were averaged, resulting in a single value for each dose.  $EC_{50}$  was significantly greater for the ACh than for the MCh dose-response curve. \*Significant difference between sites at the indicated dose ( $P < 0.05$ ). †Significant difference between  $EC_{50}$  values ( $P < 0.05$ ).

CVC at all sites increased during ACh and MCh administration. When neostigmine was included in the ACh perfusion solution, the CVC dose-response curve was shifted leftward (from  $-3.23 \pm 0.38$  to  $-4.74 \pm 0.39$  log-molar ACh concentration at the untreated and neostigmine-treated sites, respectively,  $P = 0.01$ ). The  $EC_{50}$  for CVC at the MCh site was unaffected by neostigmine ( $-4.61 \pm 0.15$  and  $-4.59 \pm 0.22$  log-molar concentration of MCh for MCh and MCh + neostigmine, respectively,  $P > 0.05$ ). The  $EC_{50}$  for the CVC dose-response curve at the MCh site was not different from that at the ACh site treated with neostigmine ( $-4.74 \pm 0.39$  and  $-4.61 \pm 0.15$  log-molar concentration of ACh and MCh for ACh + neostigmine and MCh, respectively,  $P > 0.05$ ).

## DISCUSSION

The primary aim of this study was to compare local SR and CVC responses to exogenous ACh and MCh administration when these agents were administered via intradermal microdialysis. The main finding of the study is that SR and CVC responses to ACh were significantly less sensitive, as indexed by the  $EC_{50}$  of the dose-response curve, than SR and CVC responses to MCh. Furthermore, the onset of sweating occurred at a lower dose of MCh than ACh. On the basis of these findings, *protocol 2* was included to evaluate whether the observed differences between ACh and MCh responses could be attributed to different degrees of sensitivity of ACh and MCh to AChE. Coadministration of an AChE inhibitor caused a significant leftward shift of the ACh dose-response curve for CVC and SR, demonstrating that the lower  $EC_{50}$  for SR and CVC to MCh than to ACh is likely due to the susceptibility of ACh to hydrolysis by AChE. Finally, the intersite differences (i.e., within-drug comparison) for CVC were similar when the  $EC_{50}$  to various doses of ACh, as well as MCh, was evaluated. However, SR between sites receiving the same drug were significantly different at higher doses of ACh and MCh.

## Sweating Responses

As internal and skin temperatures increase, sweating occurs on the release of ACh from cholinergic nerves and subsequent binding to muscarinic receptors on the eccrine sweat gland (28, 29, 37). ACh is rapidly hydrolyzed to choline and acetate by AChE. Previous studies investigating the effects of AChE inhibition on SR have shown that AChE inhibition increases the number of activated sweat glands and that AChE tends to increase SR (23, 24). Consistent with previous findings, our laboratory has shown that inhibition of AChE with coadministration of neostigmine caused a leftward shift of the dose-response curve to exogenous ACh (34). Together, these findings suggest that SR responsiveness to ACh can be affected by the activity of AChE. A key objective of *protocol 1* was to evaluate differences in responses between ACh and an analog of ACh, MCh, which is not as rapidly degraded by AChE (9, 22). The  $EC_{50}$  of the sweating response was significantly lower with administration of MCh than with administration of ACh (Fig. 3). In addition, the onset of sweating occurred at a lower concentration of MCh than ACh. At higher concentrations of these drugs (i.e.,  $1 \times 10^{-1}$ – $1$  M), sweating responses between ACh and MCh became similar. This observation suggests that the effect of hydrolysis of ACh on sweating responses can be overcome as the exogenous dose of ACh approaches  $1 \times 10^{-1}$  M. When AChE activity was inhibited with coadministration of neostigmine, sweating responsiveness to ACh ( $EC_{50}$  and threshold for the onset of sweating) was similar to that observed during MCh administration (Fig. 5). At higher concentrations of ACh (i.e., approaching 1 M), sweating responses were unaffected by AChE inhibition, consistent with the aforementioned observation of similar sweating responses at higher doses of ACh and MCh in the absence of neostigmine. Coadministration of neostigmine with MCh did not significantly alter the dose-response curve relative to administration of MCh alone. Together, these observations strongly suggest that differences in sweating responses between exogenous ACh and MCh are due to the effect of AChE on hydrolysis of ACh.

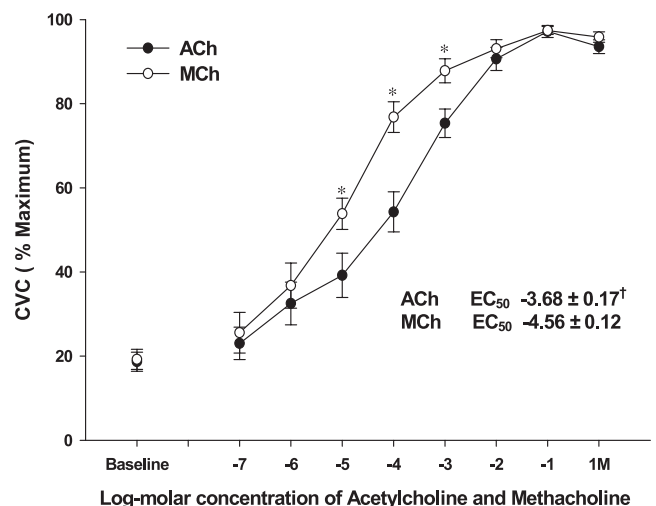


Fig. 4. CVC dose-response curves to exogenous administration of  $10^{-7}$ – $1$  M ACh and MCh. Before analysis, data from both sites treated with the same drug (for each subject) were averaged, resulting in a single value for each dose.  $EC_{50}$  was significantly greater for ACh than for MCh dose-response curve. \*Significant difference between sites at the indicated dose ( $P < 0.05$ ). †Significant difference between  $EC_{50}$  values ( $P < 0.05$ ).

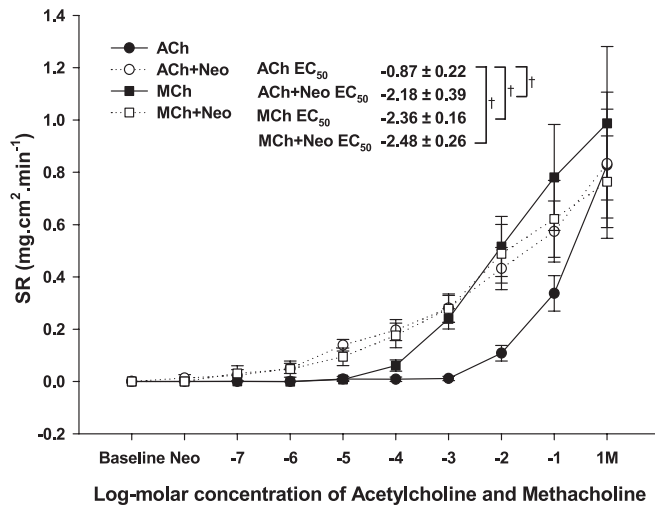


Fig. 5. Effect of inhibition of acetylcholinesterase (AChE) on SR dose-response curves to exogenous administration of ACh and MCh. Inhibition of AChE shifted ACh dose-response curve to the left compared with control site, such that EC<sub>50</sub> was significantly greater for ACh than for ACh + neostigmine (Neo), MCh, or MCh + neostigmine. †Significant difference between EC<sub>50</sub> values ( $P < 0.05$ ).

### Cutaneous Vasodilator Responses

Increasing concentrations of exogenous ACh and MCh resulted in progressive increases in CVC. The mechanism by which ACh induces cutaneous vasodilation has recently been investigated. It is generally agreed that ACh mediates increases in CVC through a combination of nitric oxide, prostaglandins, and possibly endothelium-derived hyperpolarizing factor mechanisms (5, 12, 14, 16–18, 26, 27, 31, 33). In the present study, it is assumed that one or all of these mechanisms were responsible for the elevation in CVC in response to ACh and MCh. At moderate doses of MCh ( $1 \times 10^{-5}$ – $1 \times 10^{-3}$  M), the increase in CVC was significantly greater than that observed in response to ACh administration (Fig. 4), resulting in a significant reduction in the EC<sub>50</sub> of MCh relative to ACh. As outlined above, these differences are proposed to be due to differences in AChE susceptibility between these agents. In support of this hypothesis, when neostigmine was added to the ACh solution, the EC<sub>50</sub> values of CVC dose-response curves to ACh were not different from those to MCh, whereas coadministration of neostigmine with MCh did not significantly alter the EC<sub>50</sub> of the MCh site (Fig. 6). Given these observations, differences in CVC between ACh and MCh in the absence of neostigmine were not due to differences in the ability of these drugs to evoke nitric oxide-, prostaglandin-, and endothelium-derived hyperpolarizing factor-stimulated vasodilation. Rather, different CVC responses of these drugs were likely due to greater sensitivity of ACh to AChE. Similar to its effect on sweating, CVC responses were unaffected by AChE inhibition at higher concentrations of ACh, suggesting that the dose of ACh can overwhelm the capacity of AChE to hydrolyze ACh.

### Intersite Variability

Despite the widespread use of exogenous ACh and MCh administration in evaluating cutaneous vascular and sudomotor responses, little is known regarding the intersite variability of this procedure, particularly during microdialysis administration

of these drugs. A number of variables could result in different responses to the same drug at different sites, including heterogeneity of skin blood flow, sweat gland density, and the potential for differences in characteristics of each microdialysis membrane (6–8, 15). Thus a secondary goal of this study was to examine intersite variability of ACh- and MCh-induced increases in SR and CVC. This objective was accomplished by comparing dose responses to identical drug administration at proximal and distal sites. Although CVC was not significantly different between sites receiving the same drug, SR was significantly different between sites at higher doses for ACh and MCh (Fig. 1). These data were further analyzed by evaluation of the linear regression between the two sites receiving the same drug. Although the  $R$  values for these analyses were very high (i.e., averaging  $>0.92$ ), the slope of the relation between the proximal and distal sites for SR averaged  $0.68 \pm 0.09$  when MCh was administered and  $1.29 \pm 0.14$  when ACh was administered, whereas the slope of the relation between sites for CVC averaged  $0.84 \pm 0.06$  when MCh was administered and  $0.96 \pm 0.05$  when ACh was administered. These observations raise a concern regarding the intersite variability when cutaneous vascular and sweating responses to exogenous ACh and MCh administration are evaluated. Unfortunately, we do not have a clear explanation for these varying responses, despite what may appear to be otherwise reproducible findings. Nevertheless, these observations raise concern about the appropriateness of comparing CVC and SR responses between sites, unless the expected difference is greater than the observed variability between sites.

In conclusion, the present data demonstrate attenuation of sudomotor and cutaneous vasodilator responses to exogenous ACh compared with MCh administration. These different responses are likely due to the responsiveness of AChE in hydrolysis of ACh, whereas MCh is more resistant to AChE hydrolysis. Perhaps because of its accessibility, a number of investigators are using the skin to evaluate the effects of various pathologies on vascular and sudomotor responsiveness (1–4, 11, 19–21, 30). Commonly, endothelium-dependent vasodilation and sudomotor responses are assessed through

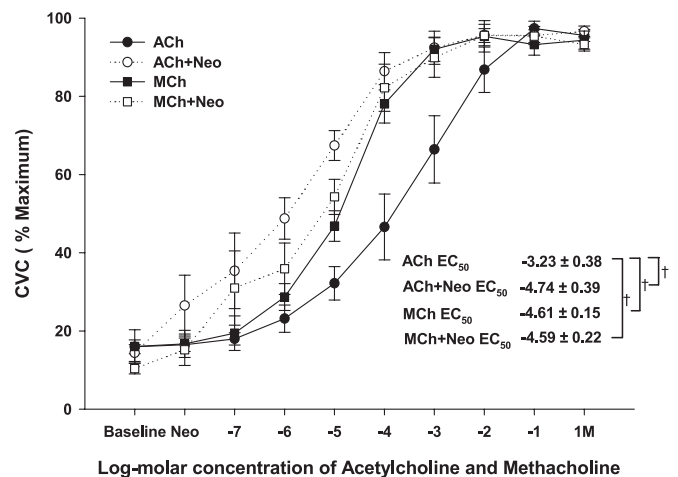


Fig. 6. Effect of inhibition of AChE on CVC dose-response curves to exogenous administration of ACh and MCh. Inhibition of AChE shifted ACh dose-response curve to the left compared with the control site, such that EC<sub>50</sub> was significantly greater for ACh than for ACh + neostigmine, MCh, or MCh + neostigmine. †Significant difference between EC<sub>50</sub> values ( $P < 0.05$ ).

exogenous administration of ACh via intradermal injection, iontophoresis, and intradermal microdialysis. However, given the effects of AChE in inactivation of ACh, findings from these studies may be clouded by the possibility that different responses could be attributed to the effect of that pathology in alteration of AChE-mediated hydrolysis of ACh, as opposed to a direct effect of that pathology on endothelium-dependent vasodilation and sudomotor responsiveness. To overcome this potential limitation, data from the present investigation suggest that MCh may be preferred over ACh for evaluation of sudomotor and endothelium-dependent cutaneous vasodilator responses.

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#### GRANTS

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