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Effect of local acetylcholinesterase inhibition on sweat rate in humans

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Shibasaki, Manabu, and Craig G. Crandall. Effect of local acetylcholinesterase inhibition on sweat rate in humans. *J Appl Physiol* 90: 757–762, 2001.— ACh is the neurotransmitter responsible for increasing sweat rate (SR) in humans. Because ACh is rapidly hydrolyzed by acetylcholinesterase (AChE), it is possible that AChE contributes to the modulation of SR. Thus the primary purpose of this project was to identify whether AChE around human sweat glands is capable of modulating SR during local application of various concentrations of ACh in vivo, as well as during a heat stress. In seven subjects, two microdialysis probes were placed in the intradermal space of the forearm. One probe was perfused with the AChE inhibitor neostigmine (10 μM); the adjacent membrane was perfused with the vehicle (Ringer solution). SR over both membranes was monitored via capacitance hygrometry during microdialysis administration of various concentrations of ACh (1×10^{-7}–2 M) and during whole body heating. SR was significantly greater at the neostigmine-treated site than at the control site during administration of lower concentrations of ACh (1×10^{-7}–1×10^{-3} M, $P < 0.05$), but not during administration of higher concentrations of ACh (1×10^{-2}–2 M, $P > 0.05$). Moreover, the core temperature threshold for the onset of sweating at the neostigmine-treated site was significantly reduced relative to that at the control site. However, no differences in SR were observed between sites after 35 min of whole body heating. These results suggest that AChE is capable of modulating SR when ACh concentrations are low to moderate (i.e., when sudomotor activity is low) but is less effective in governing SR after SR has increased substantially.

hyperthermia; temperature regulation; microdialysis; neostigmine; acetylcholine

INCREASING INTERNAL TEMPERATURE in humans leads to an increase in sweating rate (SR). This event occurs after the release of ACh from sudomotor nerves innervating muscarinic receptors on sweat glands (13). ACh released from sudomotor nerves is rapidly inactivated via hydrolysis into choline and acetate by acetylcholinesterase (AChE). Given this characteristic of ACh, coupled with the dependence of sweating on adequate concentrations of ACh, it is likely that AChE is capable of modulating SR. Prior work investigating the effects of AChE inhibition on sweating responses has been equivocal. Longmore et al. (10) reported an increase in the number of activated sweat glands induced by intradermal injection of a single dose of ACh (1×10^{-4} M) when AChE was inhibited relative to control sites in normothermic humans. In contrast, MacIntyre et al. (12) observed only a tendency (16 of 25 observations) for an increase in SR at AChE-inhibited sites during local heating in subjects exposed to a 40°C room compared with non-AChE-inhibited sites. Taken together, the effectiveness of AChE in modulating SR remains unclear. These apparently contradictory findings may be related to the level of background sudomotor activity and, thus, the local concentration of ACh in the area of SR measurement.

Investigators studying the effects of AChE on sweating responses during exercise in the heat administered an inhibitor of AChE systemically while monitoring SR (3, 4, 8, 17, 18). This method of drug delivery precludes identifying whether altered sweating responses, if present, were due to factors associated with systemic administration of the drug (9). Thus, whether AChE modulates sweat gland function during conditions in which SR increases, such as heat stress or exercise, remains unclear. Given this concern, coupled with the uncertainty of the aforementioned studies in which the effects of local AChE inhibition were investigated (10, 12), a primary purpose of this project was to test the hypothesis that AChE around sudomotor endings is capable of modulating SR during local administration of ACh and during whole body heating in humans.

To assess the effects of AChE on sweat gland function, it would be beneficial if a method was employed to continually deliver drugs into the skin while simultaneously monitoring the response of those drugs at the same location. Previously, methods such as intradermal injection or iontophoretic application of sudorific drugs have been used to assess human sweat gland function in vivo (7, 9, 11, 15). However, these techniques are limited, in that they preclude the administration of multiple doses of a drug at the same location while simultaneously measuring a response, such as

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SR or the number of activated sweat glands, at that site. Thus a dose-response curve cannot be constructed using the aforementioned methods at a given site. Recently, we (1, 6) used intradermal microdialysis to deliver pharmacological agents into the skin while simultaneously assessing the effects of these agents over the microdialysis membrane. Given the success of this technique to locally deliver drugs (1, 5, 6), it seemed reasonable that the microdialysis method could be used to assess the effects of AChE on sweat gland function. Accordingly, a secondary goal of this project was to identify whether intradermal microdialysis could be coupled with capacitance hygrometry to assess sweat gland function in vivo.

METHODS

Seven healthy subjects (4 men and 3 women) participated in *protocol 1*, and six healthy subjects (5 men and 1 woman) participated in *protocols 2* and *3*. Their ages ranged from 21 to 38 yr, and all were of normal weight (72 $\pm$ 4 kg) and height (175 $\pm$ 3 cm). Each subject was informed of the purpose and risks of this institutionally approved study before providing their written consent.

Protocols 1 and 2. After subjects entered the laboratory, two microdialysis probes were placed in the dermal space of the dorsal aspect of a forearm of each subject. The probes were constructed in our laboratory from a semipermeable cellulose membrane (18,000 mol wt cutoff; Spectrum) glued between two polyimide tubes (Polymicro Technologies) and reinforced by a 51-μm-diameter stainless steel wire placed in the lumen of the membrane and tubes (1, 6). The membrane window for each probe was 10 mm. The probes were placed by piercing a 25-gauge needle in the dermal space and then having the needle exit 20–25 mm from the point of entry. The microdialysis probe was inserted through the lumen of the needle. The needle was then withdrawn, leaving the probe in place. After placement, the probes were perfused with lactated Ringer solution at a rate of 2 μl/min. Chambers having a small window (10 $\times$ 5 mm) were positioned over each membrane to measure SR by the ventilated capsule method with compressed nitrogen used as the perfusion gas (2, 16). Location of capsule placement was aided through the use of markings on the polyimide tubing that indicated the center of the membrane portion of the microdialysis probe. Local skin temperature around the SR chambers was maintained at 40°C through the use of a lamp and thermocouple in all protocols. This temperature was chosen, inasmuch as pilot data revealed that local stimulation of SR was maximized at this temperature. Data collection did not begin until $\geq$60 min after probe placement.

The purpose of *protocol 1* was to identify the reproducibility of sweating responses during intradermal delivery of various concentrations of ACh. This goal was accomplished by comparing SR between two sites on the same forearm during administration of identical concentrations of ACh. After 5 min of baseline data collection, 1×10^{-6}–1×10^{-1} M ACh (6 doses) dissolved in Ringer solution was perfused through both membranes. Each dose was administered in 5-min increments. Absolute humidity over the microdialysis probes was continuously monitored via capacitance hygrometry (Vaisala, Woburn, WA) and immediately converted to SR via the data acquisition program (Biopac, Santa Barbara, CA).

In *protocol 2*, 10 μM neostigmine (an AChE inhibitor) was perfused through one microdialysis probe; the adjacent probe received the vehicle (Ringer solution). Ten minutes after the onset of neostigmine administration, 1×10^{-7} to 2 M ACh (9 doses) dissolved in Ringer solution was perfused through one probe; the same concentrations of ACh dissolved in a 10 μM neostigmine solution were administered through the adjacent probe. As in *protocol 1*, each dose of ACh was administered for 5 min and SR was continuously measured over both sites.

Protocol 3. Each subject was instrumented for the measurement of esophageal (5 subjects) or sublingual (1 subject) temperature [core temperature (T_c)] with a thermistor. Mean skin temperature (T_{sk}) was monitored from the electrical average of six thermocouples placed on skin. The subject was then placed in a tube-lined suit that permitted the control of T_{sk}. The suit covered the entire body surface except for the head, feet, and right forearm. Each subject rested in the supine position while two microdialysis probes were placed in the dermal space of the right forearm. The aforementioned SR capsules were placed over each microdialysis membrane. A minimum of 60 min elapsed before the onset of data collection, during which both sites were perfused with Ringer solution. Then one membrane was perfused with a 10 μM neostigmine solution, while the adjacent membrane continued to be perfused with Ringer solution. Ten minutes after the onset of neostigmine administration, the heat stress began by an increase in the temperature of the water perfusing the tube-lined suit to 46°C. The goal of this heat stress was to increase internal temperature ~0.7–1.0°C. Throughout the heat stress, neostigmine was perfused through one membrane while the adjacent membrane was continually perfused with Ringer solution.

Data collection and analysis. SR, local T_{sk}, and T_c were recorded at 20 Hz (Biopac). Data from *protocols 1* and *2* were averaged at 10-s intervals, with the maximum value from each stage being selected for each ACh dose. Differences in SR between sites at each dose of ACh were statistically analyzed via a paired *t*-test. Moreover, for *protocol 1*, the reproducibility of detecting sweating across different concentrations of ACh was analyzed using simple linear regression. For *protocol 3*, the onset of sweating (time to sweating and the T_c threshold at the onset of sweating) was statistically compared using a paired *t*-test. SR was also statistically compared between sites after 35 min of heating (longest duration that all subjects completed) using a paired *t*-test. Regression analysis of the SR-T_c relationship beyond the previously identified threshold for sweating was performed to identify whether AChE inhibition altered the sensitivity (i.e., slope) of the sweating response. Slopes derived from the regression analysis were statistically compared between sites using a paired *t*-test. Values are means $\pm$ SE. The level of statistical significance was set at $P = 0.05$.

RESULTS

Protocol 1. This procedure was performed to verify that intradermal microdialysis coupled with capacitance hygrometry was a viable and repeatable method of assessing sweat gland function in vivo. As illustrated in Fig. 1, SR increased progressively with increasing concentrations of ACh in the perfusate. There were no statistical differences in SR between sites for any dose of ACh ($P > 0.05$). Moreover, a strong linear relationship existed between sites across all subjects (mean slope = 1.07 ± 0.13, $R = 0.99 \pm 0.001$), demonstrating the repeatability of the method.

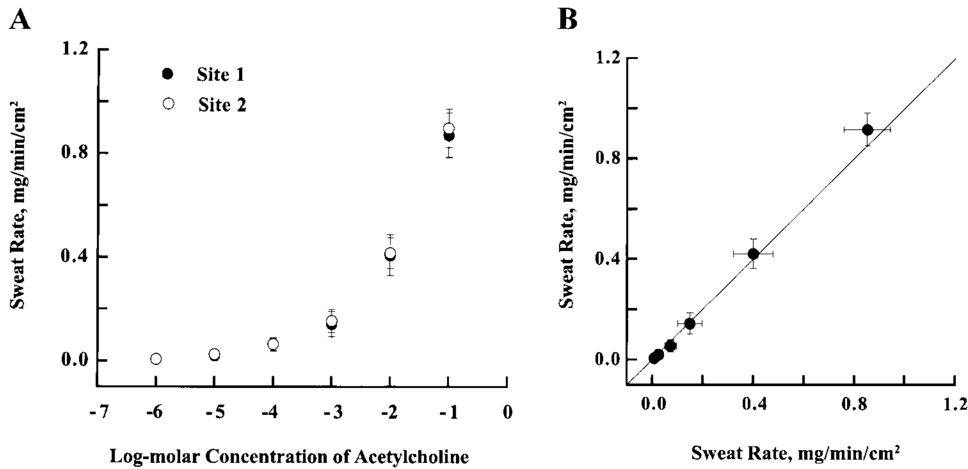

Fig. 1. Sweat rate at 2 sites during local administration of 10^{-6} to 10^{-1} M ACh. Data demonstrate that a dose-response curve can be obtained during local administration of ACh via microdialysis. *A*: ACh concentration in the perfusate at threshold for sweating ranged from 10^{-5} to 10^{-3} M. *B*: linear regression of sweat rate between sites. Data show the strong linear relationship (slope = 1.07 ± 0.13, R = 0.99 ± 0.001) between sites, verifying the repeatability of the measurement.

Protocol 2. Administration of ACh increased SR at both neostigmine-treated and untreated sites. However, from the lowest dose of ACh (1×10^{-7} to 1×10^{-3} M), SR was significantly greater at the neostigmine-treated site than at the untreated site ($P < 0.05$ for each dose). This response resulted in a leftward shift of the neostigmine dose-response curve (Fig. 2) relative to the curve at the untreated site, as identified by a significant difference in the dose of ACh required to elicit 50% of the maximal response (i.e., EC_{50}; −3.1 ± 0.2 and −1.9 ± 0.3 log-molar ACh concentration at the neostigmine-treated and untreated sites, respectively, $P = 0.006$). In contrast, SR was not different between sites during administration of $>1 \times 10^{-3}$ M ACh. Although sweating increased at the neostigmine-treated site with the application of the smallest concentration of ACh (1×10^{-7} M), sweating at the untreated site started at $\sim 1 \times 10^{-5}$ M (range 1×10^{-6} to 1×10^{-4} M).

To further illustrate the effects AChE inhibition on SR, the difference in SR between sites relative to SR at the neostigmine site was plotted across various concentrations of ACh in the perfusate (Fig. 3). This was accomplished using the following formula

$$[(SR_{Neo} - SR_{Con})/SR_{Neo}] \times 100$$

where SR_{Con} represents SR at the control (Ringer) site and SR_{Neo} represents SR at the neostigmine-treated site. A value close to 100% suggests that ACh delivered

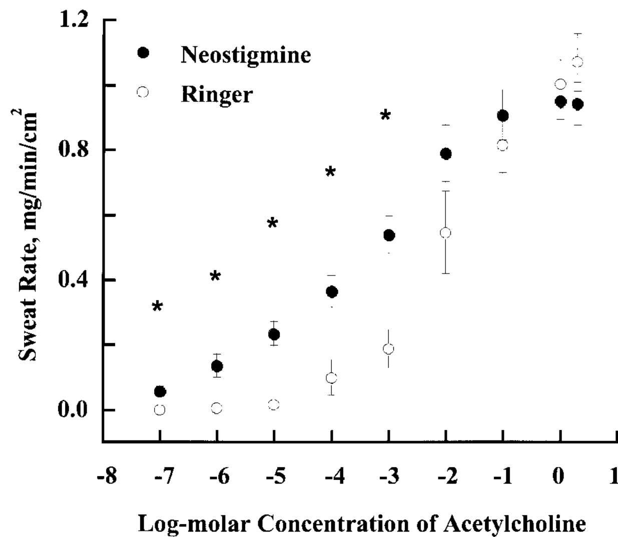

Fig. 2. Effect of inhibition of acetylcholinesterase on ACh-stimulated sweating responses. Various concentrations of ACh were administered via microdialysis at a control site (ACh dissolved in Ringer solution) and at a site at which acetylcholinesterase was inhibited via coadministration of 10 μM neostigmine. Inhibition of acetylcholinesterase shifted the dose-response curve to the left (●) compared with the control site (○). *Significant difference between the neostigmine and control site at that dose, $P < 0.05$.

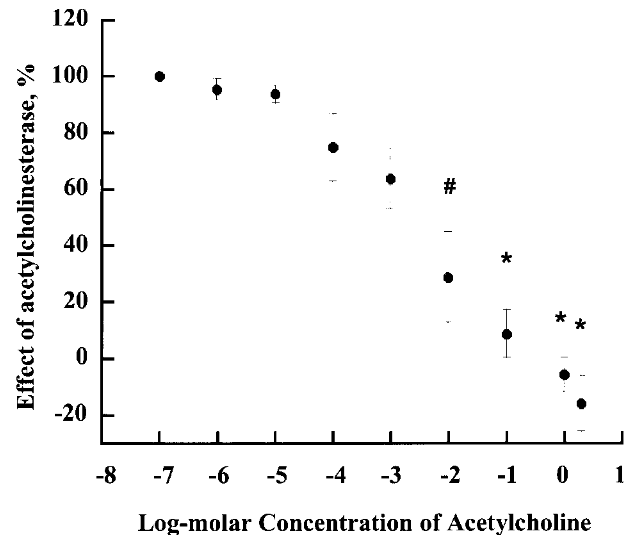

Fig. 3. Effectiveness of acetylcholinesterase on sweat rate at each dose of ACh administered via microdialysis. Each value was calculated using the following formula: $[(SR_{Neo} - SR_{Con})/SR_{Neo}] \times 100$, where SR_{Con} represents sweat rate at the Ringer site and SR_{Neo} represents sweat rate at the neostigmine-treated site. A value close to 100% suggests that the dose of ACh delivered through the microdialysis membrane was degraded sufficiently by acetylcholinesterase to abolish a sweating response. Conversely, a value close to 0% suggests that there was little difference in sweat rate between the control and neostigmine-treated sites, and thus AChE inhibition had little effect on the response at that dose of ACh. *Significantly different from 10^{-7} to 10^{-4}; #significantly different from 10^{-7} to 10^{-5}, $P < 0.05$ (1-way repeated-measures ANOVA).

through the microdialysis membrane was degraded sufficiently by AChE to abolish a sweating response. Conversely, a value close to 0% suggests that there was little difference in SR between the control and neostigmine-treated sites. These values were statistically compared using a one-way repeated-measures ANOVA. It is clear from this analysis, as illustrated in Fig. 3, that AChE exerts its greatest influence when ACh concentrations are low and becomes less important in modulating SR at higher concentrations of ACh. Thus AChE may influence the threshold for sweating and early sweating responses but is less likely to modulate sweating responses after SR has increased substantially.

Protocol 3. Whole body heating significantly increased T_{sk} (from 34.8 ± 0.2 to 38.9 ± 0.4°C) and T_c (from 36.56 ± 0.16 to 37.44 ± 0.20°C, $P < 0.05$ in each case). The increase of T_c from rest to the end of whole body heating averaged 0.88 ± 0.09°C. The onset of sweating occurred significantly earlier at the neostigmine-treated than at the Ringer site (Fig. 4; 397 ± 127 vs. 636 ± 132 s, $P < 0.05$) and at a significantly lower T_c (36.44 ± 0.10 vs. 36.60 ± 0.08°C, $P < 0.05$). In contrast, the slope of the relationship between SR and T_c was not affected by AChE inhibition (2.35 ± 0.22 and 2.21 ± 0.23 $mg \cdot cm^{-2} \cdot min^{-1} \cdot °C^{-1}$ at the neostigmine-treated and Ringer sites, respectively). After 35 min of heating, SR tended to be greater at the neostigmine-treated site than at the untreated site: 1.60 ± 0.29 and 1.38 ± 0.26 $mg \cdot min^{-1} \cdot cm^{-2}$, respectively ($P = 0.15$).

DISCUSSION

The primary findings of this study were twofold: *1*) AChE is capable of modulating sweat gland function, and *2*) the impact of AChE on governing SR is greatest when ACh concentrations are low to moderate. These findings are supported by the observation that the onset of sweating induced by local ACh administration or whole body heating occurs earlier at the site in which AChE is inhibited, whereas no significant differences in SR were observed between sites with higher concentrations of ACh or after 35 min of whole body heating.

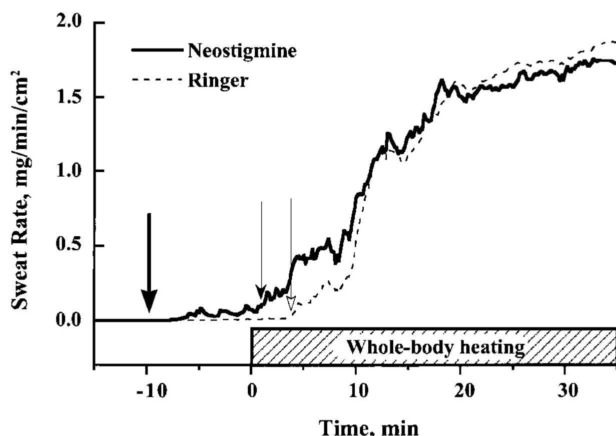

Fig. 4. Data from 1 subject demonstrating differences in sweat rate at the neostigmine-treated site and at the control site during whole body heating. Onset of sweating occurred earlier at the neostigmine-treated site than at the control site. In contrast, after 35 min of heating, there was little difference in sweat rate between sites. Thick arrow, beginning of neostigmine administration through 1 membrane; thin filled arrow, onset of sweating at the neostigmine-treated site; thin open arrow, onset of sweating at the control site.

A secondary goal of this study was to demonstrate the utility and reproducibility of using microdialysis with capacitance hygrometry to assess sweat gland function in vivo. The results from *protocol 1* support this contention. The delivery of drugs via microdialysis may be influenced by a variety of factors, including flow rate, local temperature, membrane characteristics, and concentration differences of the substance across the membrane (1, 5). However, in the present experiment, SR measured at both sites were similar at each ACh concentration (via paired *t*-test), and a strong linear relationship existed between sites. These findings confirm that differences in SR between the neostigmine and the untreated sites represented the effect of AChE.

Previous studies investigating the effects of AChE inhibition on SR have shown that AChE inhibition increases the number of activated sweat glands, whereas there was a slight tendency for an increase in SR (10, 12). However, these studies did not identify whether the effectiveness of AChE in altering SR is predicated on the level of sudomotor activity and, thus, the local concentration of ACh. To clarify this issue, in *protocols 2* and *3*, the effects of AChE inhibition on SR were evaluated during local administration of multiple doses of ACh via intradermal microdialysis, as well as during progressive whole body heating. It is clear from Fig. 2 and the results of the EC_{50} analysis that sweating responses were shifted to the left at the sites where AChE was inhibited by neostigmine. Moreover, an index of inhibition of AChE (Fig. 3) demonstrated that AChE effectively diminished ACh activity when ACh concentrations were low to moderate, but not during administration of higher concentrations of ACh. These data suggest that AChE modulates SR when sudomotor activity and, thus, local concentrations of ACh are low to moderate. These findings were confirmed during the whole body heating protocol, in which the T_c threshold for the onset of sweating was lower, and SR early in the heat stress was higher at the neostigmine-treated site than at the control site. However, when sudomotor activity was further elevated during the later stages of whole body heating, inhibition of AChE had little effect on SR. Taken together, these results suggest that AChE modulates SR when sudomotor activity is low to moderate but has less influence on SR when sudomotor activity is relatively high.

In an analogous study using in vitro techniques, methacholine, a substance more resistant to hydrolysis than ACh, was used to assess sweat gland function (14, 15). It was found that sweating started at ~1×10^{-8} M methacholine and was saturated at ~1×10^{-5} M (14). These values are in contrast to the present in vivo study in which the onset of sweating at the untreated

sites occurred at $\sim 1 \times 10^{-5}$ M ACh. The difference in the threshold for sweating between the cited study and the present study is likely due to the drug administered, the route of drug delivery, and the methodology employed (i.e., in vitro vs. in vivo). However, at the neostigmine-treated site, the onset of sweating occurred at the lowest dose of ACh (1×10^{-7} M), which is similar to the dose reported in the aforementioned in vitro study (14). In contrast, regardless of the site, tremendously high concentrations of ACh were required to saturate the sweating response in the present study. Differences in the dose of drug required to saturate the sweating response between in vivo and in vitro studies are not clear but may be due to differences in the concentration of the drug reaching the receptors on the sweat glands when delivered via microdialysis compared with in vitro procedures.

During the heat stress, the onset of sweating and the T_c threshold for the onset of sweating were significantly different between sites. In contrast, the slope of the relationship between T_c and SR after the onset of sweating and SR after 35 min of heating was not different between sites. These observations are consistent with the findings from *protocol 2*. Previous studies investigating the effects of AChE inhibition on thermoregulatory responses report that SR is not changed or is slightly elevated during exercise in the heat after oral administration of an AChE inhibitor (3, 4, 8, 17, 18). However, caution must be used when these studies are compared with the present study, because in the cited studies AChE inhibitor was administered systemically, which likely affects other tissues, including autonomic ganglia, as evidenced by lower heart rates (3, 17, 18). In contrast, in the present study, microdialysis was used to administer relatively minute amounts of the drug to the skin. For example, in *protocol 3,* the maximum quantity of neostigmine delivered, even if 100% of the drug was delivered through the microdialysis membrane, was <0.4 µg. Thus we are confident that because of the extremely small amounts of neostigmine administered, the responses observed were due solely to the effects of the drug in the region surrounding the sweat gland.

Limitation of the study. Only one dose of neostigmine (10 µM) was administered through the microdialysis membrane. The efficacy of this dose is demonstrated by the difference in SR during the initial doses of ACh in *protocol 2* and the difference in the threshold for sweating in *protocol 3*. However, we cannot conclusively state that this dose totally abolished all AChE activity. For this reason, we do not know whether the differences in SR at the lower doses of ACh or during the initial periods of whole body heating would have been exaggerated had larger doses of neostigmine been administered. Similarly unknown is the effect of larger doses of neostigmine on SR during administration of higher concentrations of ACh or after pronounced whole body heating. Nevertheless, we are confident that the dose of neostigmine administered caused some degree of inhibition of AChE, and this degree of inhibition was significant to affect sweating responses when ACh concentrations were low to moderate, but not when ACh concentrations were substantially elevated.

In the present experiment the concentration of ACh in the dialysate draining the membrane was not measured, and thus the amount of ACh delivered through the membrane was not calculated. Rather, it was presumed that the aforementioned factors affecting drug delivery through the microdialysis membrane would be comparable between sites, and thus the same amount of ACh crossed the membrane into the interstitium at both sites. Similarities in SR between sites at each dose of ACh support this contention (Fig. 1).

In conclusion, these findings demonstrate that intradermal microdialysis coupled with capacitance hygrometry is a viable method for assessing sweat gland function in vivo. Furthermore, the data suggest that AChE in the vicinity of cholinergic synapses regulates SR when sudomotor activity is low but likely has less effect in modulating SR after SR is substantially elevated.

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