

Local Sweat Stimulation with the Skin Prick Technique

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Intracutaneous injections of cholinergic agonists are usually employed for induction of local sweating. The possibility to apply a less painful skin prick technique as a sweat test was examined. Pricks were made on the forearm skin of 25 young men by puncturing the skin through drops of methacholine (MCH) and control saline solutions with lancets having a point length of 1 mm. The drops were kept on skin for 10 min and were then wiped off. Using an evaporimeter the measurements of water loss (WL) were started 5 min later and continued at 5 min intervals. Sweat loss (SL) was calculated from the difference of rates of evaporative WL recorded at the MCH and saline pricks. Ten, 1, 0.1, and 0.01% solutions of MCH elicited the following average peak evaporative sweat losses: 107.1, 77.5, 32.4 and 5.4 g m⁻²h⁻¹. Both the degree and duration of the sweat responses were dependent on concentration. The prick technique is well tolerated and may serve for comparative studies of local sweating. *Key words: Evaporative water loss; Eccrine sweat glands.* (Received December 18, 1983.)

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Experimental local sweating has been most usually elicited by intradermal injections of cholinergic agonists. Since intradermally administered fluids are injected with force the expanded tissues often tend to give rise to trickling for some time (1). Such a leak-back of fluid onto the test area is unacceptable for sweat measurements. To avoid such a contamination of test area by non-sweat fluids the needle tip has to traverse a long intracutaneous course to reach the midpoint of the test area (2, 3). Multiple intradermal testing produces discomfort or pain (4) and may induce unwanted emotional sweating. One way to circumvent this problem is the use of indwelling needles or catheters (2). The latter technique is time-consuming and cannot be used routinely for the study of clinical patients.

In allergy skin tests the relatively cumbersome intradermal tests have been largely replaced by simple prick tests. The use of sterile disposable lancets permitting a standard prick depth has increased the ease and reproducibility of the prick technique (5, 6). In our experience disposable lancets with a short point length are suitable for producing a channel into the skin for penetration of cholinergic agents in sufficient amounts to stimulate sweat responses on forearm skin. The objective of this report is to demonstrate the applicability of the prick technique for the study of sweat gland function following local stimulation. The sweat gland activation was recorded with a sensitive evaporimeter, designed for the measurement of evaporative water losses on skin (7).

MATERIAL AND METHODS

Subjects. The tests were performed on the forearm skin of 25 conscripts (mean age 20, range 17-27 yrs) with minor surgical, venereological or skin complaints. No peroral or topical medication on arm

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skin was allowed during three days before testing. Informed consent was obtained from all participants.

Cholinergic drug. Methacholine (MCH) chloride (Mecholyl, Sigma) dissolved in saline was used for induction of sweating and physiologic saline served as the control. Prior screening tests suggested that only fairly high concentrations would give a measurable sweat response in prick tests. Hence, four concentrations of MCH, 10, 1, 0.1 and 0.01% (5.1×10^{-1} – 5.1×10^{-4} mol/l) were used for a more detailed analysis. The specificity of the sweat responses was proved by finding that they could be inhibited by intradermal injections of atropine sulphate (10^{-3} mg/0.1 ml).

The sweat test procedure. The sweat tests were performed by a specially trained nurse in a laboratory room during the winter season at mean ambient temperature of 23.2°C (range 22.4–24.1°C) and at mean relative humidity of 29.2% (range 18–35%).

The test sites located on noneczematous volar forearm skin in one straight row approximately 4 cm below the antecubital flexure to 4 cm above the wrist and at intervals of about 4 cm. Before testing the subjects rested supine for 10 to 15 minutes with the upper part of the body uncovered. The baseline water loss (BWL) was then recorded (see below).

One drop (40 µl) of various MCH concentrations and one drop of control saline were placed topically in a random order along the forearm with an automatic pipette (Finnpipette, Labsystems, Helsinki). Usually three different MCH concentrations were tested on each person. The skin was immediately punctured through the drops with disposable lancets having a standard point length of 1.0 mm (Dome/Hollister-Stier) (5, 6).

The drops were maintained on the punctured skin for a period of 10 min and were then wiped off gently with absorbent paper tissue. This period was chosen for practical reasons, since in each test person the prick tests could be performed and the subsequent responses assessed consecutively. An other reason was that preliminary tests ($n=8$) had indicated a weaker reactivity if the drops were removed immediately after puncturing as contrasted to the drop kept on the skin for 5 or 10 min.

For subsequent measurements of water loss the removal of the test drop from the skin was designated as zero time. Measurements were started after a latency period of 5 min, because the skin was still moist at zero time. This was evidenced by measurements showing that within one minute of saline drop removal relatively high water losses were observed (mean \pm SD, 50 ± 21 g m⁻²h⁻¹, $n=15$). However, during the latency of 5 min approximately 90% of this residual free water was found to evaporate, and did not markedly interfere with the onset of sweat measurements.

Measurement of water loss. Rates of water loss (WL) were measured with an evaporimeter (7) (Evaporimeter EP 1, Servomed, Stockholm) from every test site at 5 min intervals until the pretest baseline level (BWL) was obtained, or for 60 min.

The measured water loss rates were the baseline water loss (BWL) recorded from the undisturbed skin before prick testing, and the (total) water loss (WL), recorded from any prick test site after removal of the drop. The residual free water content on skin surface remaining after drop removal was considered as the difference of saline site WL and BWL. Rates of sweat loss (SL) were calculated by subtracting the saline site WL from the corresponding MCH site WL, or in case the saline site WL had decreased to a level equal to BWL, the latter was subtracted to obtain SL. Thus, SL was considered as "pure" sweat. All data are expressed as water evaporation rates (g m⁻²h⁻¹).

RESULTS

The average WL rates are given as time response curves in Figs. 1a and 1b for different MCH-concentrations and for saline control. With all MCH-concentrations the peak rates occurred in the first reading at 5 min and were dependent on concentration (mean \pm SD): 117 ± 23.7 ($n=22$), 90.3 ± 18.4 ($n=24$), 44 ± 10.9 ($n=21$), 16.5 ± 4.3 g m⁻²h⁻¹ ($n=11$). The corresponding WL for saline was 12.8 ± 3.9 g m⁻²h⁻¹ ($n=25$) and the BWL was 8.2 ± 2.0 g m⁻²h⁻¹ ($n=25$). Thus, at 5 min the residual free water content remaining on the skin surface after drop removal corresponded to WL about 4.6 g m⁻²h⁻¹ and contributed by 35.9% to the saline site WL. Based on similar calculations the free water contributed to MCH site WL by 3.9, 5.1, 10.2 and 27.9%, in a decreasing order of methacholine concentration.

In 10 min readings all curves showed definite declines with the exception of that for the highest MCH-concentration. Compared with the measurement 5 min earlier the free water content at saline sites had decreased as much as 57%. Consequently, the free water

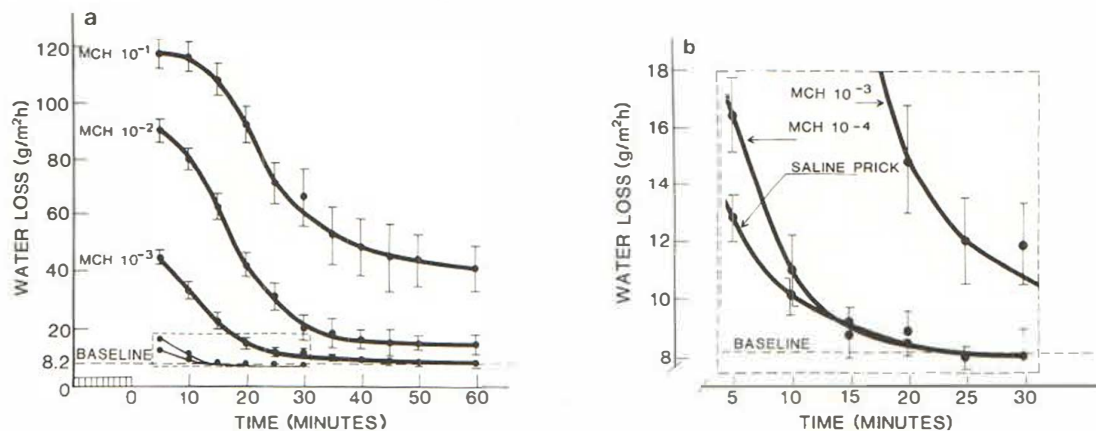


Fig. 1a. Rates of total water loss ($\text{g m}^{-2}\text{h}^{-1}$, mean \pm SE) in response to skin pricks with MCH concentrations 5.1×10^{-1} to 5.1×10^{-4} mol/l in forearm skin of 25 young males. Interrupted line denotes baseline level (BWL); hatched area denotes time of drop maintenance on the skin after pricking. Inset see Fig. 1b.

Fig. 1b. Inset of Fig. 1a. Rates of water loss in skin pricks induced by MCH 5.1×10^{-3} and 10^{-4} mol/l, and by physiologic saline.

contributed to WL at two higher MCH-concentrations by 1.7 and 2.4% and at two lower concentrations by 6.0 and 17.8%.

As shown in Figs. 1a and 1b the saline site WL reached the baseline level within 25–30 min, i.e. the zero level of the residual free water. The lowest MCH-concentration was near the threshold, as it gave rise to a response clearly distinguishable from that of saline control only in the recording at 5 min ($p < 0.05$, paired *t*-test). After 10 min the response faded and the curve coincided with that of the control.

Fig. 2 illustrates the sweat response (SL) curves based on the above data. The elicited average peak evaporative sweat losses with the tested four MCH-concentrations were: 107.1, 77.5, 32.4 and 5.4 $\text{g m}^{-2}\text{h}^{-1}$, respectively. Both maximum sweat production and the duration of the responses were clearly dependent on dose. There was a 19.8-fold difference in the peak sweat responses between the highest and lowest agonist concentra-

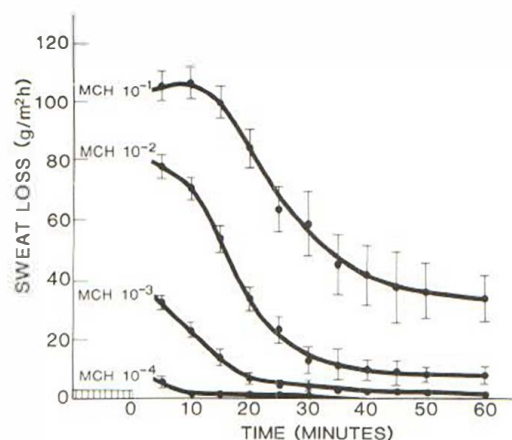


Fig. 2. Rates of pure sweat loss (mean \pm SE) induced with pricks of different MCH concentrations.

tions. Also the difference in the duration of the responses was over four-fold. Actually, for the two higher MCH concentrations the response persisted beyond the observation period of 60 min.

DISCUSSION

There are several prerequisites for using the prick technique as a sweat test. First, the minute volumes of the agonist solution which can be carried by the prick needle into the skin must affect the deep portions of the sweat glands to activate their secretion. Second, the instrumentation should be sensitive enough to record the induced activity of the glands, confined to a relatively small area around the actual prick test site. Third, the obligatory non-sweat water present on skin moistened by the test drop should be set under control, and other accidental and less well controllable non-sweat fluids eliminated. Otherwise this water will be falsely superimposed on evaporative sweat rates.

Skin pricks were made with a precision lancet having a point length of only 1 mm. This needle has been shown to elicit a minuscule prick hole and reproducible reactions in allergological tests (5). Lancets with a longer needle point penetrate deeper into the skin and might therefore increase sensitivity of the sweat test. However, they were considered unsatisfactory for the present purpose owing to their tendency to produce greater discomfort and a higher frequency of bleeding. Both factors could lead to falsely high WL levels. Indeed, bleeding on the sweat measuring site is rather unacceptable because of its haphazard occurrence. In agreement with previous observations (6) skin pricks using the lancets having a point length of 1 mm had to be repeated due to bleeding in only about 5% of the tests.

Prolonged oozing of extravascular tissue fluid or leak-back of test solution through the prick lesion might provide other potential sources for contamination of the skin surface with non-sweat water. However, in the absence of frank capillary lesion, and provided that WL measurements are started after a latency period of 5 min, our experience suggests that mere pricking of normal skin or of skin moistened by saline drop does not give rise to higher WL levels than does the corresponding skin without pricking. Bulk water is therefore unlikely to trickle from the prick, the surface cut of the minuscule slit being less than 1 mm. Also the amount of water vapor diffusing through this puncture is obviously too small to contribute to WL measured with the evaporimeter from a circular area 1.2 cm in diameter.

The present technique is regularly associated with free water deposit remaining on skin after drop removal. Because skin drying with absorbent tissue may vary in effectiveness, a latency period of 5 min was found appropriate before starting sweat measurements. Indeed, approximately 90% of the residual free water had evaporated during this time interval. Thus, during sweat measurements the free water content was of minor importance and, in addition, was constantly distinguishable from other sources of water loss. At low sweat rates, however, parallel measurements on the test site and on the nearby control saline site were required. Sweat stimulation using iontophoresis of cholinergic solutions is another technique based on application of liquids directly on the test area and requires drying of the skin followed by sweat collections (8). It is clear that in both techniques the proportion of non-sweat water relative to the amount of sweat is in practise unimportant when submaximal concentrations of a potent sweat stimulant are used.

In this study the highest sweat rates were recorded with a 10% MCH solution (5.1×10^{-1} mol/l) probably representing a sub-maximal stimulation. In some trials still higher MCH concentrations appeared to give slightly higher and more prolonged stimulation, but were not subjected to further analysis, as the evaporimeter tends to underestimate high WL rates (9, 10).

A 0.1% MCH concentration was found to be near the threshold. This concentration is approximately 1000-fold higher than is required to elicit a threshold sweat response using intradermal sweat tests (2) including evaporimetry (11). This difference in threshold concentrations between the prick and intradermal sweat tests is similar to endpoint titrations in allergological practice; a prick test requires about a one thousand-fold higher concentration than an intradermal test for a positive reaction of the same size (12). The difference is at least partly related to the administered volumes. In intradermal sweat tests the injected volumes are usually in the range of 0.05 to 0.1 ml whereas the volumes deposited by a prick needle tip have been shown to be less than 2 mm³ (13).

In conclusion, the present results demonstrate that the skin prick technique can be adapted for the study of local sweat gland responsiveness in human skin. Employing MCH as the agonist it was shown that both the peak and duration of sweat response were dependent on concentration. The technique is simple and produces less inconvenience than the traditional intradermal sweat test and should be suitable for comparative purpose particularly in multiple screening tests.

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