Human sweating response to electrophoresed acetylcholine: a test of postganglionic sympathetic function

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In recent years it has become possible to measure a number of activities of the autonomic nervous system in man, and in abnormal man attempts are made to localize the site of the lesion. When an abnormality of sympathetic activity has been shown to be due to a lesion in the efferent pathway, it is desirable to show whether the lesion is in the postganglionic fibre or more proximal. Existing tests based on the effect of injected acetylcholine have proved difficult to interpret. The present communication describes observations on the electrophoresis of acetylcholine and its effect on local sweating in normal and abnormal man. It presents evidence that electrophoresis of acetylcholine provides a reliable means of examining the function of postganglionic nerves supplying sweat glands. It is simple to perform and interpret, semi-quantitative, objective, and can localize the skin areas involved.

METHODS AND MATERIAL

ELECTROPHORESIS Acetylcholine was applied to the sweat glands by electrophoresis. The anode was a silver electroencephalography electrode enclosed in lint to give an area of 1 cm² in contact with the skin. The cathode was much larger, a sheet of malleable metal wrapped in lint. The current, provided by three nine-volt batteries connected in series, was regulated with a 100,000Ω variable resistance and measured on a 2mA meter. The anode was moistened with a freshly prepared 1% solution of acetylcholine perchlorate, the cathode with tape. To apply the drug a current of 0.125, 0.25, 0.50, 1.0, or 2.0 mA was passed between the electrodes for a timed period of 0.25, 0.50, 1.0, or 2.0 minutes.

Thermal sweating The subject's central temperature was raised either in a hot bath (40° to 44°C), or with a radiant heat cradle over the trunk or by placing one forearm and hand in a stirred waterbath at 40° to 44°C. This was continued until there was profuse generalized sweating or the oral temperature had risen 1°C.

DETECTION OF SWEATING Iodine paper (Dole and Thaysen, 1953) was used. Paper containing starch was suspended over iodine in a closed vessel in a warm place; after a few hours sublimation of iodine colored the paper pale brown; it was then ready for use, for when wet it turned dark blue. After electrophoresis the anode was removed and the skin dried, which took about 10 seconds. The paper was then taped on to the skin with transparent adhesive tape (Sellotape). Active sweat glands showed as dots on the paper. Electrophoresis of a near threshold amount of acetylcholine produced sweating which was greatest two minutes after removal of the anode. The sweating response to acetylcholine was described as negative when iodine paper which had been in place for three minutes after removal of the anode showed three or less active sweat glands. Using these criteria sweating thresholds are reproducible. Quinizarin powder (Guttmann, 1940) was used to demonstrate that thermal sweating was present over substantial parts of the body and areas which appeared not to sweat when tested in this way were further examined using iodine paper.

STATISTICAL METHODS The population distribution of acetylcholine thresholds is far from normal (see Fig. 2). Non-parametric statistics have therefore been used (Siegel, 1956).

CONTROL SUBJECTS The acetylcholine threshold for sweating was measured in 24 subjects—11 women aged 18 to 79 years and 13 men aged 25 to 59 years. Twelve were healthy volunteers and 12 patients whose final diagnoses were pyloroplasty, phantom limb pain, cervical spondylosis, post-traumatic epilepsy, cervical neurofibroma, accidental hypothermia of the elderly, brain-stem thrombosis, and (five patients) no neurological abnormality. The skin areas examined most often were the anterior surface of the forearms and the calves. Thresholds were also measured on the proximal limbs, trunk, and forehead, giving a total of 12 sites.

SUBJECTS WITH THERMAL ANHIDROSIS Eight subjects were examined. The clinical diagnoses and extent of complete anhidrosis are shown in Table I.

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TABLE I
SUBJECTS SHOWING THERMAL ANHIDROSIS

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Neurological diagnosis</th>
<th>Extent of thermal anhidrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46</td>
<td>M</td>
<td>Leprosy with neuropathy</td>
<td>Over left scapula</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>M</td>
<td>Leprosy with neuropathy</td>
<td>Over dorsum of hands</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>M</td>
<td>Leprosy with neuropathy</td>
<td>Over left lateral calf</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>M</td>
<td>Traumatic paraplegia C7</td>
<td>Below neck</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>F</td>
<td>Traumatic paraplegia T1</td>
<td>Below umbilicus</td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>M</td>
<td>Traumatic paraplegia C6-7</td>
<td>Total except for narrow midline strip over anterior trunk</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>F</td>
<td>Anterior spinal artery thrombosis C6</td>
<td>Total except on left forehead</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>M</td>
<td>Brain-stem encephalopathy</td>
<td>Below umbilicus</td>
</tr>
</tbody>
</table>

RESULTS

EXAMINATION OF METHOD  Dose of electrophoresed acetylcholine  The dose is expressed as the quantity of electricity passed through unit area of skin (mA min/cm²). To establish validity of this mode of expression of dose, the threshold duration of electrophoresis at different current was measured. For a threshold dose at one skin site on one subject the duration of electrophoresis varied inversely with the current used, that is the quantity of electricity remained constant (Fig. 1).

Acetylcholine solution  In two control subjects the threshold dose of acetylcholine required to provoke sweating was measured using 0·1%, 1·0% and 10% solutions of acetylcholine perchlorate. The threshold consistently decreased as the concentration of acetylcholine was increased (Table II). Normally freshly prepared 1% acetylcholine perchlorate solution was used in the determination of acetylcholine thresholds, but on two occasions the threshold was compared with that found with using 1% acetylcholine perchlorate solution which had been stored for one month at room temperature. There was no change in the potency of the stored solution.

TABLE II
ELECTROPHORESED ACETYLCHOLINE THRESHOLD IN mA MIN/CM²

<table>
<thead>
<tr>
<th>Concentration of acetylcholine perchlorate solution (g/100 ml)</th>
<th>Subject 1</th>
<th>Subject 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0·1</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>1·0</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>10·0</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Body temperature  The effect of varying the oral and skin temperatures on the acetylcholine threshold was investigated. A scantily clad normal subject was seated in a room while the air temperature was raised and then lowered using electric heaters and fans. The oral temperature rose from 36·4°C to 37·2°C and then fell to 36·6°C. At times when thermal sweating was absent, the acetylcholine threshold was determined on the posterior chest. This threshold varied between two neighbouring doses 1 and 2 mA min/cm² and these variations did not correlate with the changes in oral or skin temperatures.

Acetylcholine threshold in control subjects  The threshold dose of acetylcholine for local sweating was determined at up to 12 skin sites on 24 control subjects on 35 occasions, giving a total of 197
threshold determinations. On every occasion local sweating was produced by electrophoresis of acetylcholine. The commonest threshold was 1 mA min/cm² with a range from 1/3 to 4 mA min/cm². The distribution of the results is shown in Fig. 2, and, as the horizontal scale is logarithmic, the distribution is far from normal.

The results were examined for bilateral symmetry of the threshold using 87 paired observations each representing the thresholds at the same skin area on the right and left in a normal subject. The right-left pair differences were expressed in rank units as shown on the horizontal scale of Figure 2. While bilateral asymmetry of one rank unit was not uncommon, asymmetry of two rank units occurred in only 3% of observations (Fig. 3). Thus a bilateral asymmetry of more than one rank unit was rare.

Variation of the threshold dose of acetylcholine between different skin sites was examined. Results from eight normal subjects in whom all skin sites were examined were analysed by Friedman's two-way analysis of variance (Siegel, 1956) and showed that differences in acetylcholine threshold exist between sites (P < 0.01). The sites arranged in descending order of magnitude of the threshold dose are trunk, arm, thigh, forearm, forehead, and calf. There is a large overlap in the threshold dose required at each site between individuals (Fig. 4). The threshold on the trunk is significantly or highly significantly higher than at other sites and that on the calf is lower.

The results were examined for an effect of age and sex. Figure 5 suggests the possibility that the threshold decreases with age, but this effect is not significant (Spearman's rank correlation coefficient). The threshold in women is not significantly different from that in men (Mann-Whitney U test).

**Acetylcholine threshold in subjects with thermal anhidrosis** Local sweating response to acetylcholine was examined in subjects (Table I) who had thermal anhidrosis. Three patients (Nos. 1 to 3) showed regional thermal anhidrosis due to neuropathic leprosy. This disorder produces a multiple mononeuropathy affecting the peripheral nerves, and the sudomotor lesion is postganglionic. The areas of thermal anhidrosis were on the calf, dorsum of the hand, and trunk. Large doses of acetylcholine by electrophoresis failed to produce sweating at these sites, whereas moderate or small doses produced sweating on contralateral or neighbouring skin areas which showed thermal sweating. Thus postganglionic sudomotor lesions abolished the local sweating response to acetylcholine.

Five patients (Nos. 4 to 8) with central sudomotor lesions were examined. Three of these (Nos. 4 to 6) had a clinically complete high transverse lesion of

![FIG. 2. Threshold dose for local sweating of electrophoresed acetylcholine; 197 observations on 24 control subjects at 12 skin sites.](image)

![FIG. 3. Bilateral symmetry of threshold dose for local sweating of electrophoresed acetylcholine; difference in threshold in rank units of dose (see abscissa Fig. 2) between equivalent sites on right and left of control subjects, 87 paired observations.](image)
the spinal cord due to trauma occurring four months
to six years previously. They showed complete
thermal anhidrosis on the skin which was examined
by electrophoresis. On the arms and legs they
showed sweating following electrophoreses of \( \frac{1}{2} \) to
\( \frac{1}{4} \) mA min/cm\(^2\) acetylcholine, a response which falls
within the normal range (Fig. 2). One patient
(No. 7) had thermal anhidrosis which spared
only the face after an anterior spinal artery occlusion
one month previously. She showed local sweating in
response to \( \frac{1}{2} \) to \( \frac{1}{4} \) mA min/cm\(^2\) acetylcholine on the
arms and legs, a response which falls in the normal
range. A patient (No. 8) with a brain-stem lesion
showed thermal anhidrosis below the umbilicus. He
showed local sweating in response to \( \frac{1}{2} \) to \( \frac{1}{4} \) mA
min/cm\(^2\) acetylcholine in the legs and to \( \frac{1}{2} \) to \( \frac{1}{4} \) mA
min/cm\(^2\) in the arms, responses which are in the
normal range. Thus subjects with thermal anhidrosis
due to preganglionic or more central sudomotor
lesions showed a local sweating response to acetyl-
choline with thresholds similar to those in normal
subjects.

**DISCUSSION**

Sweating may occur in response to drugs when the
drugs are given either systemically or locally.
Subcutaneous injection of pilocarpine 10 to 16 mg
or mecholyl 12 to 25 mg, the maximum practical
doses, can produce widespread sweating on head,
neck, and upper trunk, but usually not legs (List and
Peet, 1938a, b). It occurs even in areas which show
thermal anhidrosis due to spinal cord lesion (List
and Peet, 1938b) or preganglionic sympathectomy
(Hyndman and Wolkin, 1941: Netsky, 1948).
Procaine block or recent section of a peripheral
nerve does not abolish this type of sweating (List
and Peet, 1938b, c), but one to eight weeks after
peripheral nerve section the sweating response is
absent or very slight (Adson, Craig, and Brown,
1935; List and Peet, 1938b; Hyndman and Wolkin,
1941). The local sweating response to deep in-
dermal injection of parasympathomimetic drugs
changes similarly. The concentration of acetyl-
choline or mecholyl required to provoke local
sweating is little changed by preganglionic sympa-
thectomy or procaine nerve block (Gurney and
Bunnell, 1942; Chalmers, 1950) but is absent or very
slight in two to 62 days after postganglionic
sympathectomy (Kahn and Rothman, 1942;
Janowitz and Grossman, 1950) and after ulnar
nerve section (Chalmers and Keele, 1952; Silver,
Versuci, and Montagna, 1963). The denervated
sweat glands are histologically normal (Adson
et al., 1935; Lofgren, 1950), except that there is
disappearance of nearby acetylcholine-esterase
(Silver et al., 1963).

Disadvantages of injecting acetylcholine or
metholyl subcutaneously or intradermally as a test
of postganglionic function are that sweating does
not always occur in normal subjects (List and Peet,
1938b, c; Chalmers and Keele, 1951, 1952) and that
it is unpleasant for the subject. The technique
described in this paper is semi-quantitative, and no
false negative responses have been found in over
200 determinations of the threshold dose for sweat-
ing. It is simple, convenient, and does not cause the
subject discomfort, a current of 2 mA/cm\(^2\) being just
perceptible. The results support the view that the
sweating response is prevented by a lesion of the
postganglionic pathway but not by more proximal lesions.

In normal subjects there is wide variation in the sweating threshold dose of acetylcholine by intradermal injection. On the forearm the variation is from $10^{-3}$ to $10^{-7}$ g/ml., a 10,000-fold variation (Chalmers and Keele, 1952). When the acetylcholine is applied by electrophoresis, however, the variation is much less—from $1/8$ to 4 mA min/cm$^2$, a 64-fold variation. Whatever the threshold, it is reproducible and bilaterally symmetrical. It is possible that the variation between subjects might be related to sex and age. Janowitz and Grossman (1950) found the threshold to intradermal acetylcholine on the forearm was higher in five women than in five men. Chalmers and Keele (1952) repeated this on 25 women and 25 men and found no sex difference for the threshold. Using electrophoresis of acetylcholine, our results (Fig. 5) might suggest a lower threshold in women; this difference is not statistically significant. The sex difference in the sweating response to intradermal cholinergic drugs (Kahn and Rothman, 1942; Gibson and Shelley, 1948; Gordon and Maibach, 1966) is due to changes in the sweat rate of active glands (Silver, Montagna, and Karacan, 1964) and not to the sweat gland thresholds. In the present work no correlation was found between acetylcholine threshold and age, although Silver et al. (1964) found that the volume of sweat secreted by each active sweat gland decreased with increasing age.

In addition to the variation between subjects, there is considerable variation in the threshold dose of electrophoresed acetylcholine for sweating between different skin sites. The two extremes are the trunk, which requires a large dose, and the calf, where sweating is provoked by small doses. This usually produces a four-to-eight-fold variation in the acetylcholine threshold.

While there is wide variation in the threshold dose of electrophoresed acetylcholine required for sweating by sweat glands with intact postganglionic axons, in all instances examined they have responded to 4 mA min/cm$^2$ or less, whereas sweat glands without intact postganglionic innervation have not responded.
A procedure is described for electrophoresis of acetylcholine through the skin and observation of the sweating with paper impregnated with iodine.

In normal subjects sweating is produced by electrophoresis of a 1% solution of acetylcholine for 1/8 to 4 mA min/cm². The threshold dose varies with skin site and between individuals, but not with age or sex.

In subjects with postganglionic lesions of sudomotor nerves, but not in those with more proximal lesions, such sweating does not occur.

This procedure is valuable in distinguishing a postganglionic lesion of the sympathetic nervous system from a more proximal lesion.

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