Journal of Physiology (1991), **442**, *pp.* 391–411
With 11 *figures*
Printed in Great Britain

NEUROEFFECTOR CHARACTERISTICS OF SWEAT GLANDS IN THE HUMAN HAND ACTIVATED BY REGULAR NEURAL STIMULI

BY MASANARI KUNIMOTO*, KLAUS KIRNÖ†, MIKAEL ELAM* AND B. GUNNAR WALLIN*

From the Departments of Clinical Neurophysiology and Anaesthesiology†, Sahlgren's Hospital, University of Göteborg, Sweden*

(*Received 21 February 1991*)

SUMMARY

1. Intraneural electrical stimuli (0·3–1·2 mA, 0·2 ms) were delivered via a tungsten microelectrode inserted into a cutaneous fascicle in the median nerve at the wrist in twenty-eight normal subjects. The effects on sweat glands within the innervation zone were monitored as changes of skin resistance and water vapour partial pressure (WVPP). Regional anaesthesia of the brachial plexus in the axilla eliminated spontaneous sympathetic activity and reflex effects.

2. At stimulation frequencies of 0·1 Hz each stimulus evoked a transient skin resistance reduction, the amplitude of which varied initially but reached a steady state of less than 10 kΩ after, on average, nine responses. If preceded by stimulation-free intervals of 5 min or more, up to fifteen stimuli were required before the first response occurred. With higher frequencies individual responses started to merge, skin resistance levels decreased successively and levelled off around 10 Hz. The total change of resistance (0–10 Hz) was 101 ± 46 ($n = 9$) kΩ and the higher the pre-stimulus level, the larger the reduction ($r = 0{\cdot}68$, $P < 0{\cdot}05$).

3. Stimulus–response latencies to the onset of a skin resistance reduction (single stimuli or trains of six impulses/20 Hz given at 0·1 Hz) shortened initially but reached steady-state values after on average nine to twelve impulses. Average conduction velocity between stimulating electrode and skin resistance recording site was 0·78 m/s and average time for electrical neuroeffector transfer in sweat glands was estimated to be 348 ms.

4. In addition to direct stimulation-induced resistance responses there were also small spontaneous reductions of resistance. They were seen in all subjects and at all frequencies but were more common in some subjects and occurred predominantly at the beginning of stimulation or at changes of frequency. They occurred independently at two skin sites in the same subject and disappeared during stimulation-free periods and after atropine.

5. With train stimulation (six impulses/20 Hz) at 0·1 Hz, each train evoked transient increases of WVPP of 1 mmHg or less in some subjects (latency around 1·6 s). After averaging weak increases were seen also after single stimuli in two subjects. Increases of stimulation current or frequency led to slowly developing sustained increases of WVPP concomitant with decreases in skin resistance.

MS 9170

6. Responses in skin resistance and WVPP to train stimulation at 0·1 Hz were suppressed in a dose-dependent way by I.V. injections of atropine. The cumulative dose necessary for complete inhibition of stimulus-induced responses (0·32 mg or less) had only weak effects on skin resistance responses to arousal stimuli in the opposite, non-anaesthetized hand.

7. We conclude that the intraneural electrical stimulation evoked sweating mainly by direct excitation of cholinergic sudomotor nerve fibres but that a minor addition of sweat was produced by a local mechanism in sweat glands and/or terminal nerve fibres. The delayed development of skin resistance responses after prolonged inactivity and the variability of stimulus–response latencies may indicate either that a certain degree of duct filling is required before skin resistance responses can be measured and/or that the mechanism for sweat production needs potentiation before becoming effective.

INTRODUCTION

Changes of skin resistance are related mainly to sweat formation (McCleary, 1950). As sweat formation is controlled by cholinergic sympathetic sudomotor nerves measurements of skin resistance changes have been used extensively in humans to provide an indirect measure of cutaneous sympathetic nerve traffic in physiological (Carmichael, Honeyman, Kolb & Stewart, 1941; Adams & Vaughan, 1965; Bini, Hagbarth, Hynninen & Wallin, 1980; Lidberg & Wallin, 1981), psychological (Prokasy & Raskin, 1973) and clinical (Öhman, 1981) studies; the method has even been considered in forensic investigations (Podlesny & Raskin, 1977). In spite of this wide usage our understanding of the relationship between sympathetic nerve traffic and resulting skin resistance changes is still incomplete. The main reason is that the strength of sympathetic impulse volleys is difficult to measure. Direct recordings of skin sympathetic nerve activity can be made in humans (Hagbarth, Hallin, Hongell, Torebjörk & Wallin, 1972) but do not provide a solution because sudomotor impulses cannot be separated from other types of impulses in the multiunit neurograms.

In the present study of the neuroeffector function of sweat glands we approached the problem of quantifying sudomotor nerve traffic in another way. Instead of trying to identify sudomotor impulses in naturally occurring sympathetic discharges we used intraneural electrical stimulation (Wallin, Blumberg & Hynninen, 1983; Kirnö, Kunimoto, Lundin, Elam & Wallin, 1991) to evoke a defined number of impulses in sudomotor fibres in the median nerve and then measured the resulting effects on skin resistance and water vapour partial pressure (WVPP) in the innervation zone of the impaled fascicle. Spontaneous sudomotor nerve traffic to the hand was eliminated by regional anaesthesia of the brachial plexus in the axilla.

METHODS

After approval of the local ethics committee and with the written consent of each subject, investigations were made on twenty-eight healthy females aged 24–49 years. Another eleven experiments failed for technical reasons.

Nerve recording and stimulation. Microelectrode recordings and intraneural stimulations were made with tungsten microelectrodes in cutaneous fascicles of the median nerve at the wrist (for technical details see Vallbo, Hagbarth, Torebjörk & Wallin, 1979). The electrode, insulated by

Volta lacquer, had a shaft diameter of 0·2 mm and a tip of a few micrometres. The reference electrode, similar but with a larger uninsulated tip, was inserted subcutaneously 1–2 cm away. For recordings the signal was amplified in two steps with a total gain of 50000. Signal-to-noise ratio was improved by the use of a 700–2000 Hz bandpass filter and an amplitude discriminator. An RC-integrating network with a time constant of 0·1 s was used to obtain a mean voltage display of the multiunit nerve activity. For intraneural stimulation the same electrodes were connected (via a switch on the preamplifier) to the output of a constant-current stimulator (Grass S48 with constant current unit) delivering square-wave pulses (0·3 mA, duration 0·2 ms). Stimulation was delivered as single pulses with frequencies ranging from 0·05 to 20 Hz. When studying the relationship between stimulation frequency and skin resistance the duration of stimulation was around 10 min at 0·05 Hz, 5 min at 0·5 Hz and 1–2 min at higher frequencies. Sometimes trains of six impulses at 20 Hz were given every 10 s. The current strength was changed in some experiments as described in results.

Skin resistance changes. Skin resistance changes (galvanic skin response, GSR) were measured by two modified van Gough GSR modules (type IGSR/7A) using Ag–AgCl electrodes (Medicotest) with a rectangular area of 5·5 × 4 mm. The measuring current was 12 μA and the skin resistance records were displayed with two filter settings, one without low-cut filter (DC display) and the other with a low-cut filter at 0·7 Hz (AC display). The high-cut filter had a cut-off frequency at 4–50 Hz depending on the amplifier gain. When skin resistance measurements were made at two sites filter settings were always identical. The electrode gel contained: hydroxyethylcellulosum, 6 g; NaCl, 0·58 g; ethyl paraoxybenzoas, 0·1 g; propyl paraoxybenzoas, 0·1 g; and purified water to 100 g. In some experiments, both electrodes were placed within the receptive field, in others the positive electrode was within, and the negative outside, the field. Sometimes a common electrode was used as reference for two electrodes within the innervation zone. In such cases the polarity was reversed between the pairs and the current in the common reference electrode was almost zero. In four experiments one pair of electrodes was placed on the palmar surface of the opposite non-anaesthetized hand.

Sweat production. Sweat production was monitored by an evaporimeter (EP1, Servo Med, Stockholm), which contained two pairs of transducers at different distances from the skin surface, one pair measuring humidity, the other temperature. Using the signals from these transducers the instrument computed the partial pressure of water vapour at two distances from the surface, the partial pressure gradient and the evaporation rate (Nilsson, 1977). WVPP at the skin surface was used as indicator of sweat evaporation.

Regional anaesthesia. To eliminate spontaneous and reflex sympathetic activity to the hand and to enable stimulation of C fibres without evoking pain, the brachial plexus was blocked by regional anaesthesia. A Teflon-coated needle was inserted in the axilla and positioned close to the nerve plexus. The needle site was considered appropriate when paresthesias were evoked in the hand by injection of a small amount of cold saline. Complete anaesthesia, i.e. no sensation evoked by skin stimuli or intraneural stimulation and no skin resistance changes induced by arousal stimuli, usually required injection of 35 ml 1% carbocaine but sometimes another 10 ml had to be added later.

Drugs. In some experiments atropine sulphate (Atropine ACO; 1 mg/ml) was injected intravenously in incremental doses (0·01, 0·01, 0·02, 0·04, 0·08, 0·16 mg) every 2 min to a cumulative dose of 0·32 mg.

Analyses. For determination of mean values of skin resistance and WVPP levels during continuous stimulation the analog signals were fed into a PDP 11/70 computer (sampling frequency 96 Hz) which determined average values for the last 30 s at each frequency or current strength. The amplitude of a transient skin resistance change was calculated as the difference between the maximum value after stimulation and the pre-stimulus value. To quantitate the effects of atropine on train-induced skin resistance responses the mean AC response during the last 2 min before the first injection was taken as reference (100%) and compared to the mean amplitude of the last three amplitudes at each dose step. Statistics were made with linear regression analysis and Wilcoxon matched-pair signed-rank test. Values are given as means ± S.E.M. unless stated otherwise.

Experimental procedure. Subjects were supine. When the needle to be used for regional anaesthesia had been positioned in the axilla, the palmar surface of the hand was cleaned with 60% alcohol solution. The arm was put in a supinated position with slight elbow flexion and at an

angle of approximately 30 deg with the body. The microelectrode was inserted in the median nerve at the wrist. The nerve was localized by electrical stimuli as described previously (Vallbo *et al.* 1979). When a suitable intrafascicular site had been obtained, the receptive field was mapped by mechanical skin stimuli, the perception threshold to intraneural electrical stimulation was

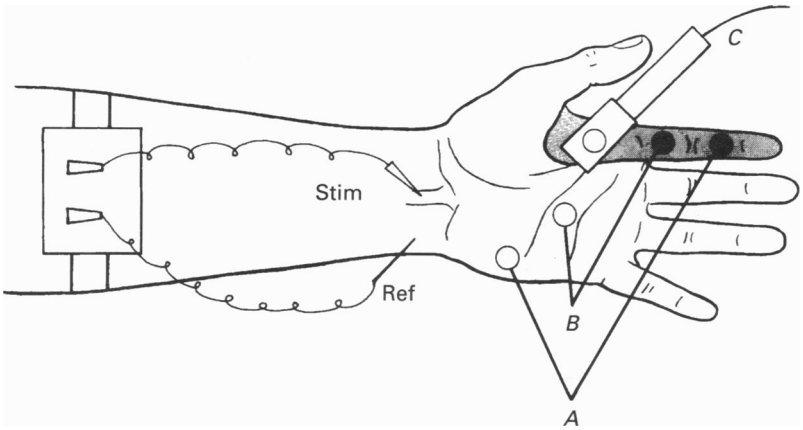


Fig. 1. Experimental set-up. After having determined the afferent receptive field (shaded area) using mechanical skin stimuli, electrodes of skin resistance (*A* and *B*) and evaporimeter probe (*C*) were placed within the field. With all probes in place, regional anaesthesia of the brachial plexus was performed.

determined (always less than 1 V) and it was noted whether or not skin sympathetic activity could be recorded either spontaneously or in response to arousal stimuli. The electrodes for skin resistance measurements and the evaporimeter probe were positioned (Fig. 1). Spontaneous nerve traffic and skin resistance changes were recorded for several minutes and then the axillary block was applied. In most cases development of complete anaesthesia required around 30 min. During this period no intraneural stimulation was given. The stimulation was made irrespective of whether or not sympathetic activity had been recorded prior to the anaesthesia. The room temperature was around 25 °C. The skin temperature varied before anaesthesia (26–34 °C) but increased to a mean level around 35·5 °C after the block and did not change during the experiment.

RESULTS

The change of skin resistance level by regional anaesthesia

After anaesthesia skin resistance in the innervation zone of the impaled fascicle increased in all subjects except two. Quantitative measurements in twenty-one subjects (thirty-seven recording sites) showed that resistance increased from a mean value of 285 (range 60–580) kΩ to 544 (range 180–950) kΩ. After on average 25 (range 5–47) min a new, fairly stable resistance level had been reached, without spontaneous variations or responses to arousal stimuli.

In both exceptional cases skin resistance was recorded at two sites within the innervation zone (with a common reference electrode outside the zone). In the first case skin resistance decreased at both sites (from 375 and 350 kΩ to 290 and 285 kΩ, respectively). In the second case, skin resistance increased from 120 to 295 kΩ at one site but decreased from 510 to 465 kΩ at the other. The reason for the decreases and the discrepancy between the electrodes in the latter case is unclear and the responses to stimulation showed no unusual features in these subjects.

In eleven subjects the skin resistance level was quantified at two sites within the innervation zone of the impaled nerve fascicle with reference electrodes situated outside the field (in five subjects a common reference electrode was used). In two subjects skin resistance before the block was similar at the two sites but after the

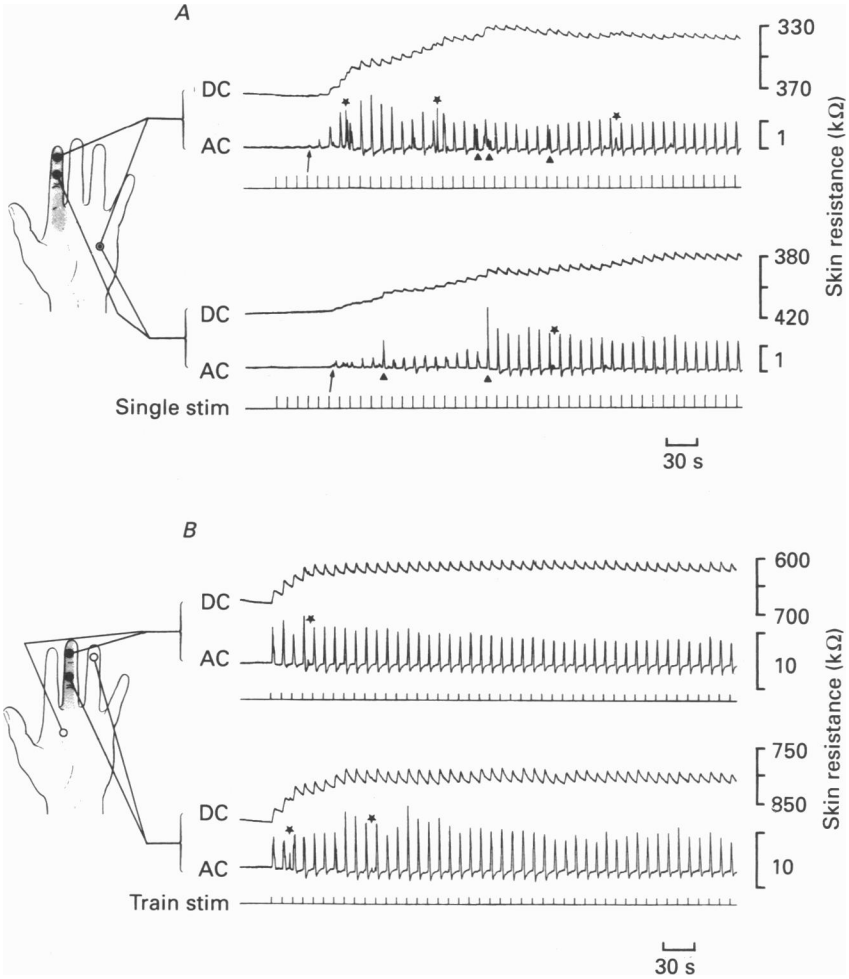


Fig. 2. Skin resistance changes evoked by intraneural stimulation at 0·1 Hz (single stimuli in *A* and trains of six impulses at 20 Hz in *B*) recorded from two sites within the receptive field (common reference electrode outside the field in *A*, separate in *B*). In *A*, arrows indicate that the first responses occurred following the fourth and the sixth stimulus at the distal (upper) and the proximal (lower) site, respectively. In *B*, responses occurred following the first stimulus at both sites. Note the variability of the response amplitudes in the AC displays before steady state was reached. Spontaneous changes of skin resistance indicated by ▲ and ✱. Different subjects in *A* and *B*. Reduction of skin resistance shown as upward deflection in this and all subsequent figures.

block there were stable differences of resistance between the sites of 235 and 375 kΩ, respectively. In the other nine subjects skin resistance differed between the sites by 25–390 (mean 141) kΩ before and 5–300 (mean 135) kΩ after the block. When the

difference of skin resistance level was compared between distal and proximal sites in the fingers and the palm, skin resistance was consistently lower in distal compared to proximal sites both before (111 ± 37 kΩ, $P < 0{\cdot}01$) and after (147 ± 43 kΩ, $P < 0{\cdot}02$) anaesthesia.

Relationship between afferent and efferent innervation zones

In all subjects intraneural electrical stimulation evoked skin resistance changes inside the afferent innervation zone of the impaled nerve fascicle irrespective of whether or not sympathetic activity could be recorded before anaesthesia. In nine subjects skin resistance electrodes were placed also on a neighbouring skin area outside the innervation zone, usually on a different finger. In eight of these cases stimulation-induced responses occurred only within the innervation zone but in one case small responses were seen also in the neighbouring finger (the amplitude in the AC display less than 15% of that in the innervation zone). Furthermore, in one case increases of WVPP were evoked by train stimulation (see below) although the evaporimeter probe was placed slightly outside the afferent field in the palm.

In nineteen experiments effector responses (skin resistance and/or WVPP) were measured at two or three sites within the innervation zone. If possible the recording probes were positioned within skin areas from which dynamic mechanical skin stimuli evoked the strongest afferent discharges. With three probes, however, the whole receptive field often had to be used irrespective of the sensitivity to mechanical stimuli. In three of four subjects with two probes (always one skin resistance and one WVPP) and in ten of fifteen cases with three probes (two pairs of skin resistance and one WVPP) effector responses were obtained at all sites. Changes of WVPP did not occur in the six remaining subjects and in one of them skin resistance changes were observed only in one of two sites. All the non-responsive probes were placed in skin areas from which afferent discharges to touch stimuli were weak.

Effects of stimulation on skin resistance

Single stimuli

In twenty-six subjects the experiment was started by delivering a series of single stimuli at 0·05–0·5 Hz. In all experiments the first stimuli after anaesthesia evoked no skin resistance change, but subsequently small transient resistance reductions started to appear (Fig. 2*A*). In later stimulation sequences, the number of stimuli required to evoke the first response was not related to the frequency of stimulation but did depend on the time elapsed from the end of the previous stimulation to the start of the series. After a pause of less than 5 min the first stimulus usually evoked a response whereas after longer pauses up to fifteen stimuli were needed (Fig. 3). When skin resistance was recorded at two sites within the innervation zone the number of stimuli required to evoke the first response differed at the two sites in ten of twelve subjects. For example, in Fig. 2*A* the fourth stimulus gave a response at the distal site (upper recording), but at the proximal site (lower recording) the first response did not occur until the sixth stimulus.

After the first response had occurred, each stimulus caused a well-defined reduction of skin resistance which started approximately 0·6–0·8 s after each stimulus and had a rise time in the DC curve of 1·5–2·0 s, followed by a slower return

towards the initial level (Fig. 9*A*). The amplitudes of the first responses were small but grew in an irregular way with repeated stimulation at constant strength until an approximate steady state was reached. Sometimes the increase of amplitudes in the AC display occurred gradually, sometimes there was some waxing and waning (Fig.

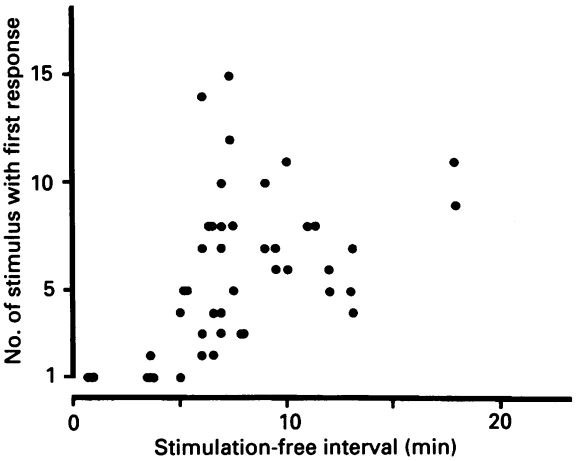


Fig. 3. The relationship between the interval of neural silence preceding the start of a stimulation period (single stimuli at 0·1 Hz) and the number of the stimulus which evoked the first response. Data from forty-five stimulation periods and twenty-five skin sites (fifteen subjects).

2*A* upper recording) and at other times there was more stepwise increase of amplitudes (Fig. 2*A* lower recording). In fifteen sites from ten subjects stimulated at 0·1 Hz the response amplitude reached a steady state after on the average nine stimuli (Fig. 4*A*). The response amplitudes varied widely between skin sites and were influenced also by stimulation frequency but rarely exceeded 10 kΩ in the DC record. Concomitant with the transient changes occurring with each stimulus there were also slowly developing sustained reductions of resistance and in general, the higher the stimulation frequency, the lower the steady-state resistance level (see below).

In addition to reductions of skin resistance directly coupled to (i.e. with approximately constant latency after) a stimulus, transient apparently spontaneous reductions of resistance occurred in all subjects. In fifteen subjects only a few small reductions occurred but in the other thirteen subjects they were more common. As indicated in Fig. 2*A* the number of spontaneous deflections also differed between different skin sites in the same subject. They were never seen during periods without stimulation but when they occurred they had a variable temporal relationship to the stimuli including some which appeared to be superimposed on stimulation-induced responses. Most were small and best seen as short-lasting transients in the AC display but occasionally fairly large and more long-lasting reductions of resistance could occur. The spontaneous events were most common at the beginning of a stimulation period and when strength or frequency of stimulation was changed but could occur also at constant stimulation parameters. They were easy to distinguish at low stimulation frequencies but occurred also above 1 Hz (Fig. 5).

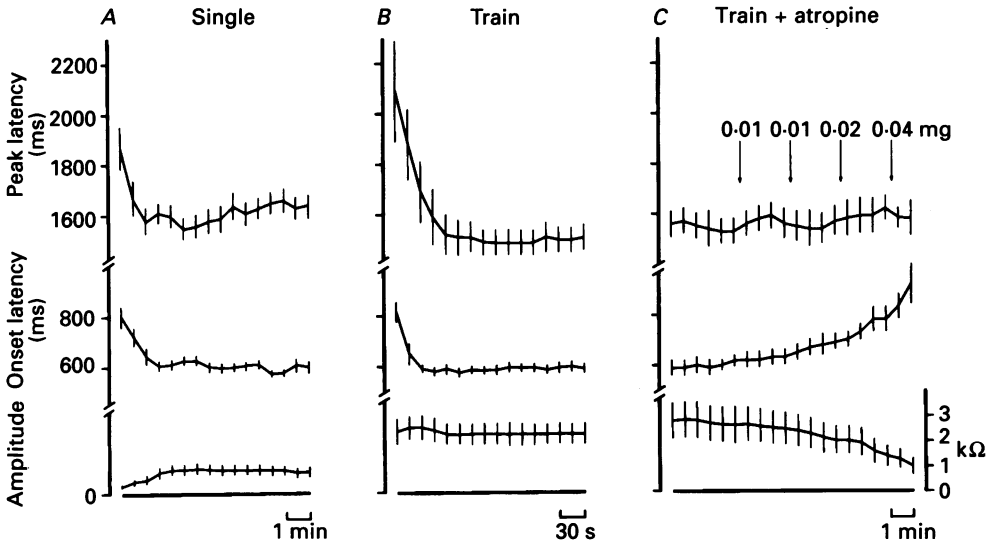


Fig. 4. Changes of amplitudes and latencies to onset and peak of resistance responses in the AC display evoked by single (*A*) and train (*B*) stimulation. *C* summarizes effects of repeated I.V. injections of atropine (arrows) on responses to train stimulation. In *B* each point represents the response to a single train but in *A* and *C* each point is the average of three consecutive responses. Note that onset and peak latencies decreased in the beginning and then reached similar steady states both with single and train stimulation. With atropine injections, onset latency increased with decreasing amplitude but peak latency remained stable. Data (means ± S.E.M.) from fifteen sites (nine subjects) in *A*, nine sites (six subjects) in *B*, and eleven sites (eight subjects) in *C*.

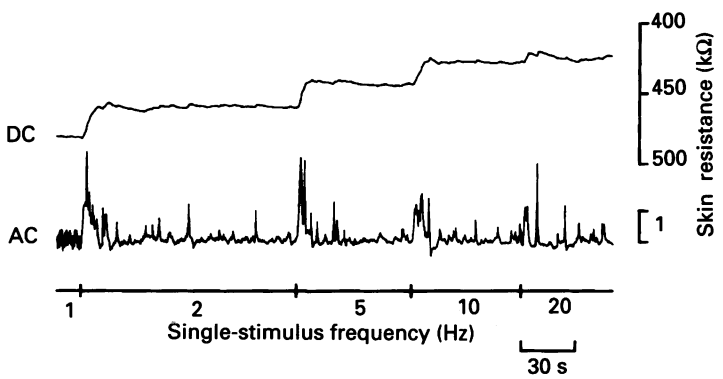


Fig. 5. Spontaneous variability of skin resistance at stimulation frequencies of 2–20 Hz. Note sudden transient reductions of resistance in the AC display with each change of frequency.

Train stimulation

When short trains of impulses (six impulses at 20 Hz) were delivered at 0·1 Hz in twenty subjects, the resulting skin resistance reductions were larger than those evoked by single stimuli and transient changes of 30 kΩ in the DC display did occur. However, also with trains response amplitudes varied widely between skin sites (Fig.

2*B*). The first stimulus usually evoked a response even after 11 min stimulation-free interval and usually steady-state amplitude was reached more rapidly than with single stimuli. When the stimulation-free interval was less than 5 min, the amplitudes of the first responses were often at (and sometimes even above) the steady-state

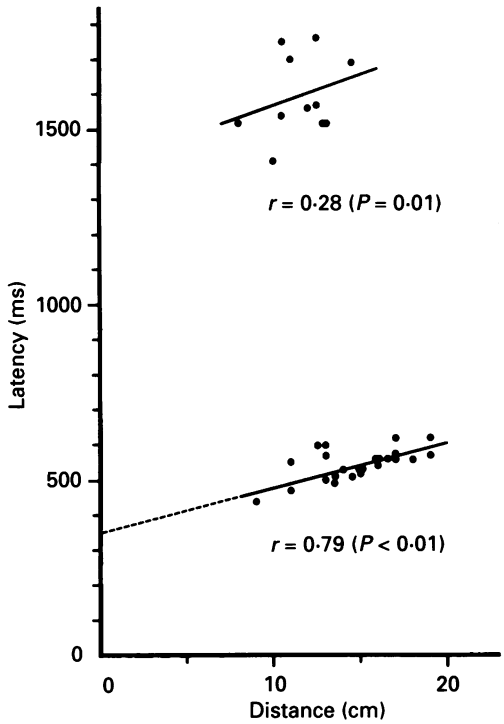


Fig. 6. Latency from start of stimulus (trains of six impulses 20 Hz given every 10 s) to onset of changes of skin resistance (lower part, twenty-four sites in fourteen subjects) and WVPP (upper part, eleven subjects) plotted against distance from stimulating microelectrode to each recording site. Each point represents the averaged latency from twenty (skin resistance) and thirty (WVPP) responses. The slope of the regression line for skin resistance (lower line) represents an average conduction velocity of sudomotor fibres of 0·78 m/s.

amplitude (Fig. 2*B*). This explains why the averaged amplitude in Fig. 4*B* was fairly stable from the beginning. Spontaneous resistance deflections were observed during train stimulation and the character was similar to those occurring with single stimuli.

Stimulus–response latencies

When single stimuli were given every 10 s, latencies from the stimulus to the onset and peak of corresponding skin resistance response in the AC display were around 0·8 and 1·9 s respectively for the first responses. During continuing stimulation both latencies shortened successively to approximate constant values of 0·6 and 1·6 s after six to nine stimuli (Fig. 4*A*). If instead trains of six impulses at 20 Hz were given every 10 s there was a similar reduction of both onset and peak latencies to almost the same steady-state values. Steady-state latency to onset was reached at the third

train (i.e. after twelve stimuli), and to peak at the fifth train (i.e. after twenty-four stimuli) (Fig. 4*B*). Intravenous injection of increasing doses of atropine led to successive reductions of response amplitudes to repeated train stimulation (see below). Concomitant with the amplitude reduction onset latencies increased up to around 0·9 s but peak latencies did not change (Fig. 4*C*).

To determine conduction velocity in the distal part of the sudomotor fibres twenty skin resistance responses to train stimulation were averaged (starting with the fourth response) for twenty-four skin sites in fourteen subjects (see Fig. 9*A*). When average response latency for each site was plotted against the distance between stimulating and recording electrodes the relationship between the variables was linear ($r = 0{\cdot}79$, $P < 0{\cdot}01$) (Fig. 6). A similar plot for the latency to responses in WVPP (see below) showed much larger scatter (1·5–1·7 s) and no significant regression line ($r = 0{\cdot}28$, $P = 0{\cdot}40$). The slope of the regression line for skin resistance was 0·78 m/s which provides a measure of the sudomotor fibre conduction velocity between the stimulation point and the sweat gland. Extrapolating the regression line to the ordinate gave an intercept of 348 ms which represents the average time for electrical neuroeffector transfer. Using the conduction velocity of 0·78 m/s to account for nerve conduction time, the delay between the impulse arrival at the skin and the start of the change in WVPP was 1·3–1·5 s.

Changes of stimulation frequency

In ten subjects (eleven sites, one subject with two sites) the relationship between stimulation frequency and skin resistance level was examined with single stimuli. Skin resistance was reduced with increasing frequency (Fig. 7*A*) and although there were exceptions (see 2 and 5 Hz in Fig. 7*A*) a reasonable steady state was usually achieved at each frequency. The extent to which steady states were reached varied not only between frequencies and experiments, but also between different skin sites in the same subjects.

Figure 7*B* summarizes data from nine recording sites in eight subjects (for technical reasons quantitative calculations could not be made in two subjects) on the relationship between stimulation frequency and resistance levels during the last 30 s at each frequency. All curves were similar in shape and levelled off around 10 Hz. At 20 Hz stimulation a small increase in resistance compared to the value at 10 Hz was observed in three sites in two subjects. The reduction of resistance between the control level before stimulation and the level at 10 Hz was 101 ± 15 (range 14–164, $n = 9$) kΩ. The pre-stimulus resistance level varied considerably between sites (237–915 kΩ) and the higher the level the larger the reduction of resistance with stimulation ($r = 0{\cdot}68$, $P < 0{\cdot}05$).

Changes of current strength

In four experiments (seven skin sites) the strength of the constant current stimulation was changed in a stepwise manner while trains of six impulses at 20 Hz were delivered at 0·1 Hz. The range of current variation differed between experiments, the maximal range in one experiment being from 0·03 to 1·2 mA. With increasing current strength skin resistance sometimes decreased in irregular steps, in other cases it decreased more smoothly until it levelled off at 0·6–1·2 mA (Fig. 8). In

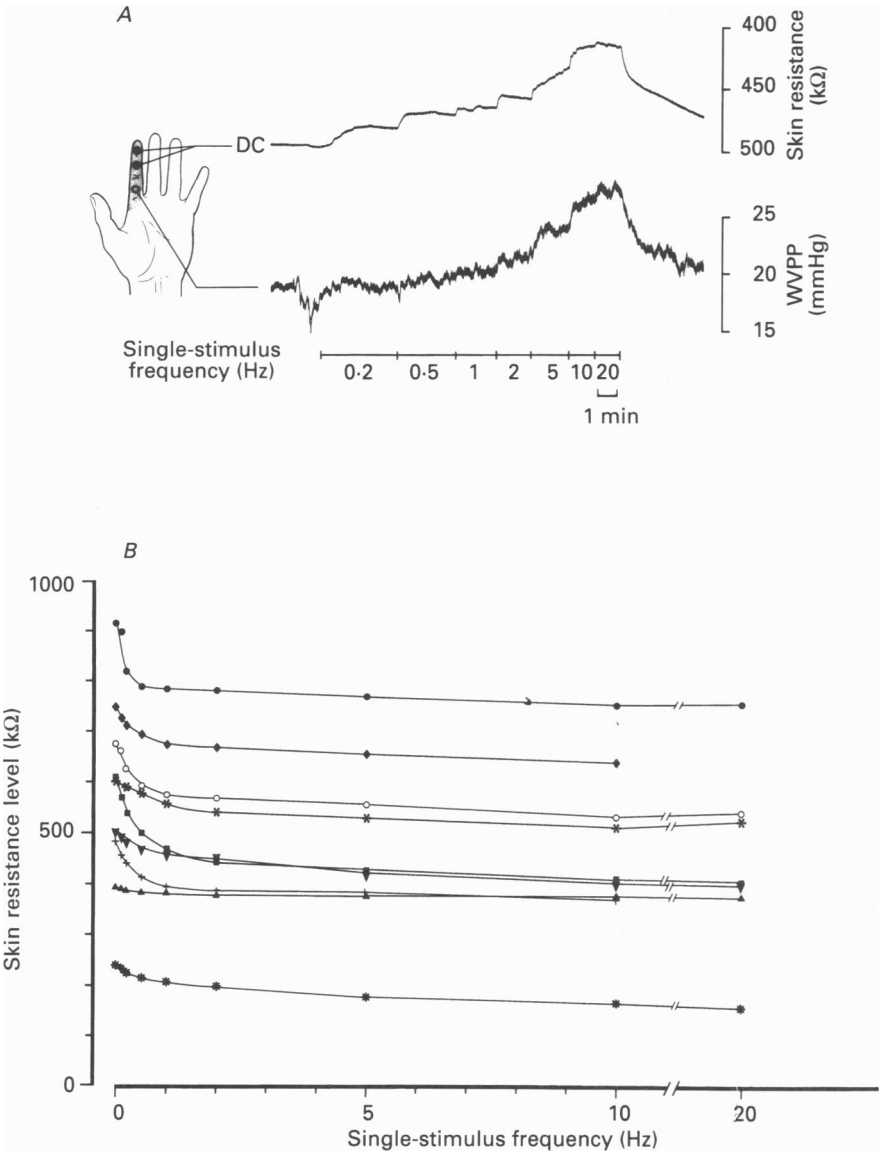


Fig. 7. Effects of stimulation frequency on skin resistance and WVPP. *A*, original record from one subject. Note that skin resistance did not reach steady state at 2 and 5 Hz. The transient reduction in WVPP before stimulation was an artifact induced by air movement. *B*, summarized data from nine sites (eight subjects). Separate reference electrodes in all subjects.

contrast, the relationship between current strength and amplitudes of the transient changes of resistance seen with each stimulus (deflections in the AC display) was much more variable. AC amplitudes in a given recording site sometimes increased and sometimes decreased and if two skin sites in the same subjects were compared even the direction of changes of AC amplitudes could differ (Fig. 8*A*).

Effects of stimulation on water vapour partial pressure

Simultaneous measurements of WVPP and skin resistance were attempted in nineteen experiments and in eleven of these clear changes of WVPP were obtained. Responses of WVPP to single stimuli usually could not be distinguished in the

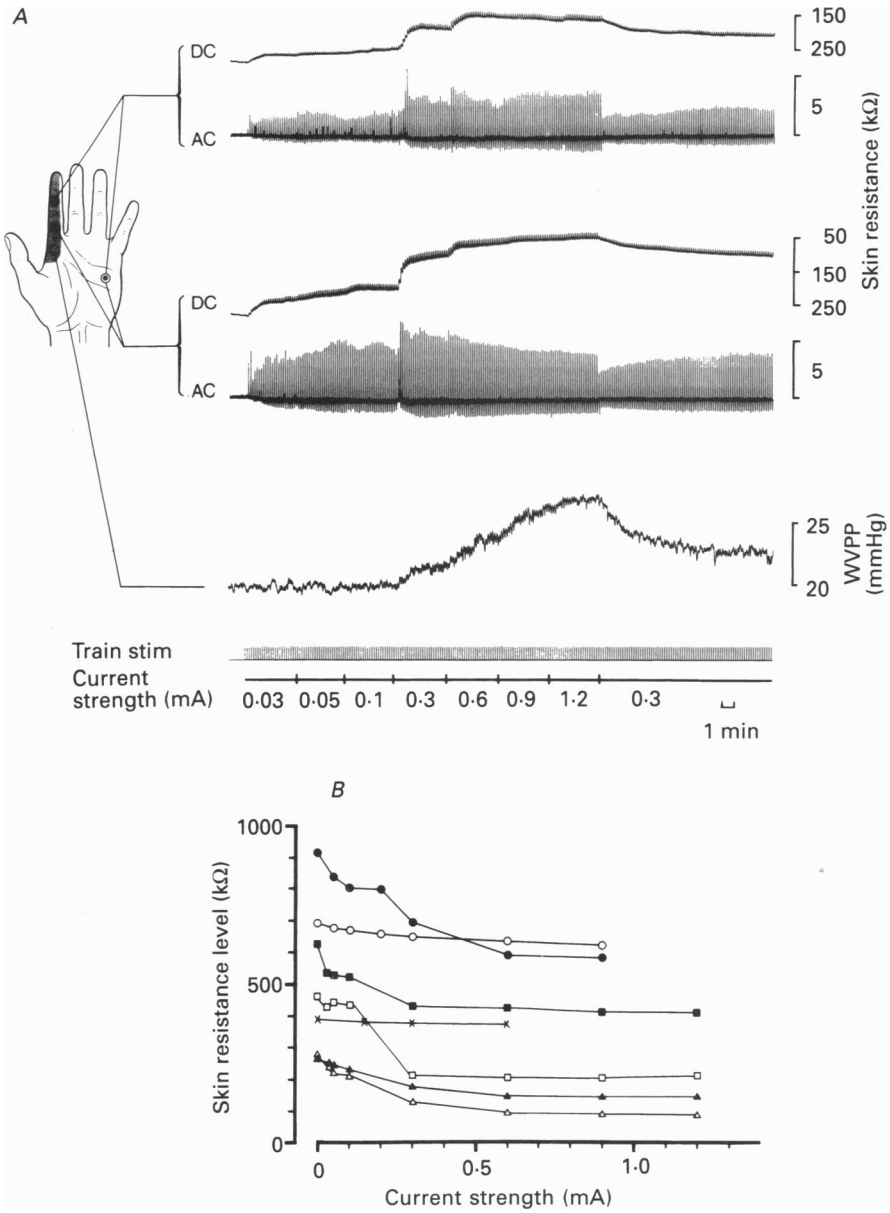
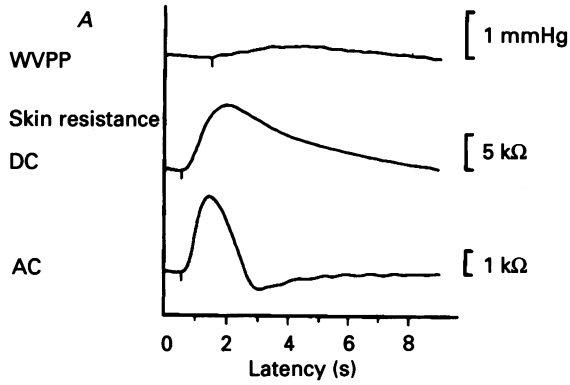


Fig. 8. *A*, effects of stimulation current on responses in skin resistance and WVPP to train stimulation (six impulses 20 Hz given every 10 s). Only minor changes in skin resistance level are seen above 0·6 mA. *B*, summarized data from seven sites (four subjects). Separate reference electrodes were used in two subjects and a common electrode in two.

original records but in two cases a transient increase of WVPP emerged after averaging thirty stimuli delivered at 0·1 Hz (Fig. 9*A*). If instead trains of six impulses at 20 Hz were given at a rate of 0·1 Hz each train was often followed (after approximately 1·6 s) by a well-defined transient increase of WVPP of 1 mmHg or less



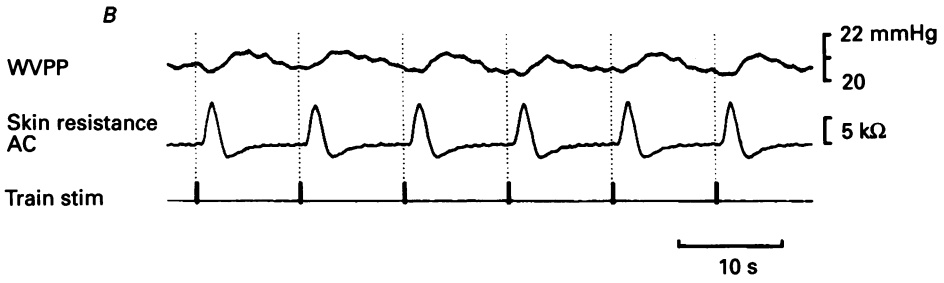


Fig. 9. *A*, average changes of skin resistance and WVPP evoked by single stimulation in one subject ($n = 30$). Stimulus delivered at time zero and onsets of responses indicated by vertical bars. *B*, responses of WVPP and skin resistance (AC display) evoked by train stimulation in the same subject. Each train evokes a clear response in WVPP.

(Fig. 9*B*). Although skin resistance responses to train stimulation usually occurred with the first stimulus WVPP responses were never seen until the second train.

When stimulation frequency was increased from 0 to 20 Hz, WVPP increased more than 5 mmHg in three subjects. In these subjects the relationship between skin resistance (recorded at four sites) and WVPP was more or less non-linear with bigger changes in skin resistance than WVPP at low frequencies. When stimulation current was varied, the relationship was similar in one subject (two sites) and approximately linear in another subject (also two sites) (Fig. 10).

Effects of atropine on skin resistance and WVPP

To study the effects of cholinergic receptor blockade incremental doses of atropine were injected intravenously in ten subjects while trains of six impulses at 20 Hz were delivered at a rate of 0·1 Hz (current strength 0·3 mA in eight subjects and 0·6 mA

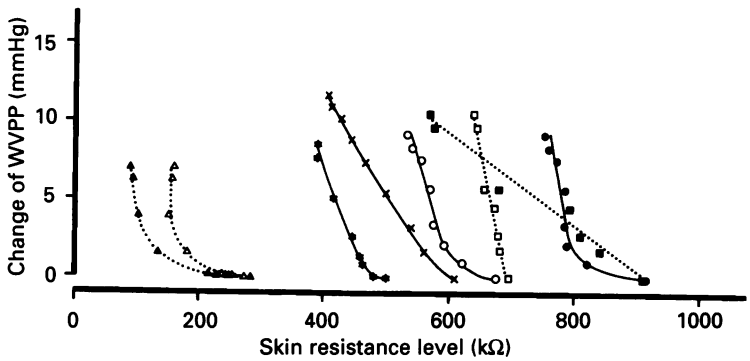


Fig. 10. Relationships between skin resistance level and change of WVPP evoked by changes of stimulation frequency (continuous lines, four sites in three subjects) and current strength (dotted lines, four sites in two subjects). Open (distal) and closed (proximal) symbols of the same type refer to two sites in the same subject. Separate reference electrodes were used in four subjects and a common electrode in one.

in two). WVPP was monitored in six of the ten subjects. An example of experimental records is shown in Fig. 11*A*. Each dose of atropine led to an amplitude reduction of the skin resistance responses (best seen in the AC records) which usually started at the third or fourth stimulus (i.e. 30–40 s) after the injection. Concomitantly there was a slow increase in the skin resistance level and a slow decrease in WVPP. In thirteen sites in nine subjects skin resistance responses were abolished after a cumulative dose of 0·16 mg atropine. In only one of two sites (in a subject stimulated with 0·3 mA) weak responses remained after 0·16 mg but disappeared after 0·32 mg. The dose–response curves were all similar and 50 % amplitude reduction was reached after a total dose of 0·025–0·06 mg (Fig. 11*B*).

After the responses had been abolished by atropine current strength was increased in steps from 0·3 to 0·6, 0·9 and 1·2 mA in two subjects (three sites) and from 0·6 to 0·9 mA in one subject (two sites). In four sites no responses reappeared but in the fifth site small responses reappeared when current was increased from 0·3 to 0·6 mA but did not grow with further increase of current strength. When all stimulation-induced responses had disappeared skin resistance responses to arousal stimuli were still observed in the opposite non-anaesthetized hand ($n = 2$).

DISCUSSION

The present study shows that intraneural electrical stimulation of the median nerve can evoke signs of sweat production (reduced skin resistance and increased WVPP) in the palmar surface of the human hand. Since the responses (*a*) occurred in a hand which was decentralized by regional anaesthesia, (*b*) had latencies

corresponding to a conduction velocity of less than 1 m/s and (*c*) were blocked by atropine, reflex effects can be excluded and the responses must have been evoked by direct stimulation of efferent cholinergic postganglionic sympathetic fibres. The stimulation probably coactivated cutaneous vasoconstrictor fibres but since no

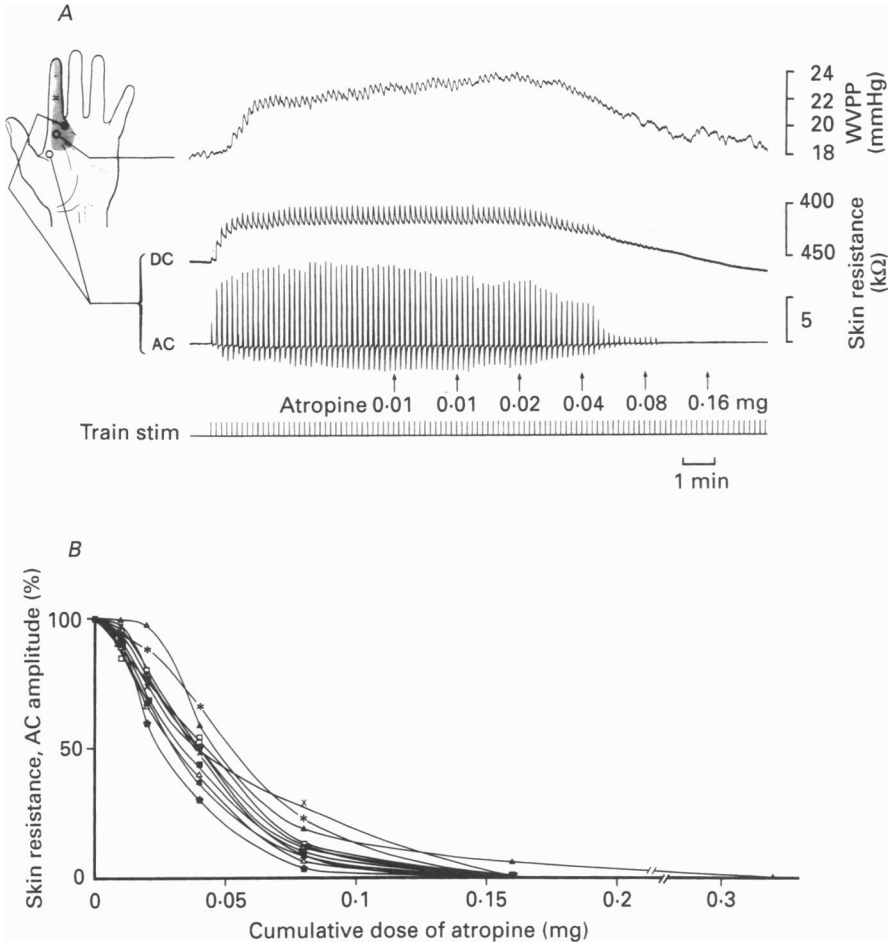


Fig. 11. Effects of successive I.V. injections of atropine on changes of skin resistance and WVPP evoked by train stimulation at 0·1 Hz. *A*, original records from one subject. *B*, dose–response curves from eleven sites (eight subjects) stimulated with current strength 0·3 mA. Each point represents the mean amplitude of the three last responses at each dose.

significant changes of skin temperature occurred, it is unlikely that the vasoconstrictor activation affected the sweat responses. In agreement with previous anatomical data (Guttmann, 1940; Richter & Katz, 1943; Herz, Glaser, Moldover & Hoen, 1946) the correspondance between mechanoreceptor afferent sensory fields and sympathetically innervated fields was essentially good.

Skin resistance levels often differed between two sites within the same innervation zone, before as well as after anaesthesia. This probably depends at least partly on

regional variations in the number of sweat glands. The human palm contains around 260 sweat glands/cm^2 and the finger around 360/cm^2 (Roberts, Salzano & Willson, 1970), i.e. the number of sweat glands covered by our electrodes (0·22 cm^2) may vary between 60 and 80. In agreement with Roberts' finding of increasing numbers of sweat glands distally, skin resistance before local anaesthesia was consistently lower in distal compared to proximal sites in the fingers and the palm. The finding that skin resistance differed between distal and proximal sites after a fully developed axillary block could be due to non-neurally mediated sweating or local skin factors unrelated to sweat gland density.

Initiation of the skin resistance change

When stimulation was initiated with single stimuli, the number of stimuli required before the first skin resistance response occurred increased with the duration of the preceding stimulation-free interval. A possible explanation would be that after prolonged neural inactivity the mechanism for sweat production needs potentiation before becoming effective. Alternatively, the delay may be related to the degree of filling of the sweat ducts. Lloyd (1959) found that the delay to sweat emergence on the skin surface induced by plantar nerve stimulation in the cat correlated positively with the duration of the preceding stimulation-free interval. He suggested that this was due to water being reabsorbed in the gland during neural inactivity and that the delay reflected the number of stimuli needed to refill the ducts. Reabsorption from sweat gland ducts, albeit of electrolytes rather than water, has been confirmed repeatedly (see Sato, 1977). Since skin resistance is a function of sweat content in the duct (i.e. level of duct filling and electrolyte concentration in the sweat) and since the major skin resistance is developed between the germinating layer and the upper corneum (see Fowles, 1974) it is possible that sweat within a duct cannot affect skin resistance significantly before reaching a threshold level around the stratum lucidum. If reabsorption during non-stimulated periods reduces duct filling to levels below this threshold several stimuli may be needed to refill the ducts to the threshold. Since the first skin resistance response occurred after different numbers of stimuli at two recording sites within the same innervation zone, the properties of individual sweat glands may differ, as previously suggested by Sato & Sato (1983). Also the increase in AC amplitude at the beginning of stimulation may indicate successive recruitment of glands with different properties after different numbers of stimuli.

Latency and conduction velocity

The latency from stimulus to corresponding skin resistance response includes the time needed for distal nerve conduction, neuroeffector transmission, sweat gland activation and sweat production resulting in duct filling (see above). The calculated conduction velocity of the distal part of the sudomotor nerve fibres (see. Fig. 6) was 0·78 m/s. This is slower than the velocity of 1·3 m/s previously found for sudomotor fibres in more proximal parts of arms and legs (Fagius & Wallin, 1980). The reason may be distal tapering of sympathetic fibres or an error in the assessment of the length of the nerve between the points of stimulation and registration, due to the branching and irregular course of distal nerve fibres (Miller, Ralston & Kasahara, 1960).

The long latency for electrical neuroeffector transfer to the sweat gland (348 ms) is comparable to the latency in salivary gland cells (Kagayama & Nishiyama, 1974). Since acetylcholine release and diffusion probably occurs in a few milliseconds (see Katz & Miledi, 1965), most of the latency is due to sweat gland activation and sweat production. The relative contribution of these two functions to the latency is not possible to evaluate with our technique. Single-cell recordings from salivary glands (see above) have shown highly variable latencies to glandular cell activation.

Increases of evaporation have been related to spontaneous or reflexly induced bursts of skin sympathetic activity in humans (Iwase, Mano, Sugenoya, Saito & Hakusui, 1988). In that study the delay between a sympathetic discharge at the elbow and the start of evaporation in the palm was 1·8 s. Deducting the time necessary for nerve conduction, this probably agrees well with our values of 1·3–1·5 s.

During the first part of a stimulation sequence after a rest period latencies to the onset of the skin resistance responses decreased during the first nine to twelve impulses (approximately similar for single impulse and train stimulation). In addition, when skin resistance responses were reduced in amplitude by atropine, the latency to onset increased. These results may indicate that the latency to *onset* is related to the number of activated sweat glands, with a low number (i.e. early in a stimulation series or during a partial atropine block) giving longer latencies than a high number. In the beginning of a stimulation series the latency to the *peak* of the skin resistance reduction also decreased but remained unchanged during increasing atropine-induced inhibition of the responses. A possible explanation would be that in the beginning of a stimulation series sweat formation is accelerated by repeated stimulation but once a maximal speed is achieved it remains constant in unblocked synapses.

Spontaneous skin resistance changes

All subjects showed a varying amount of spontaneous transient skin resistance reductions between or superimposed on the stimulation-induced resistance changes. Since the spontaneous events occurred only during periods of stimulation and were independent at two sites, when a common reference electrode was used, they were not electrode artifacts. Similar phenomena have been observed in the salivary gland (Kagayama & Nishiyama, 1974). Whether the spontaneous skin resistance changes were evoked by random spontaneous discharges in individual sudomotor fibres, by spontaneous neurotransmitter 'leakage' or by instability of the sweat glands themselves is unclear. It remains to be investigated whether the relative amount of spontaneous skin resistance changes is correlated to interindividual differences in sweating under physiological conditions. If so, a high level of spontaneous skin resistance changes could be a peripheral mechanism contributing to a relative hyperhidrosis.

Stimulation frequency and skin resistance change

The skin resistance decrease during stimulation with increasing frequencies levelled off at a stimulation rate of 10 Hz. This agrees well with frequency–response curves for vascular sympathetic neuroeffector relationships (Folkow, 1955). In

afferent C fibres activated by electrical skin stimuli frequencies around 2 Hz and higher usually cause intermittent conduction block (Torebjörk & Hallin, 1974). Our finding of fairly stable skin resistance levels at high frequencies suggests that this phenomenon is less prominent in the terminal part of sudomotor fibres.

The steady-state resistance reached at each stimulation frequency probably indicates that the degree of duct filling and electrolyte concentration in the sweat glands is frequency dependent. The possibility that increasing frequencies also recruit more sweat glands within the stimulated field (i.e. that sweat glands have different threshold frequencies for activation) cannot be excluded. The fact that the resistance changes induced both by anaesthesia and by alterations of stimulation frequency differed between two sites within the same innervation zone agrees with the notion that sweat glands may have different characteristics (see above).

Current strength and skin resistance change

When current strength was increased (at constant frequency) skin resistance decreased, suggesting that more sudomotor fibres were recruited within the nerve fascicle. Different nerve fascicles are separated from each other by structures of high impedance which block 'overhearing' between fascicles (Hagbarth, Hongell, Hallin & Torebjörk, 1970) and therefore current spread to neighbouring fascicles is an unlikely explanation. In line with this increasing intraneural stimulation strength in skin fascicles of non-anaesthetized subjects is associated with increasing intensity but unchanged anatomical distribution of the sensation (authors' unpublished observations).

Increasing the current strength above 0·6 mA did not induce significant further decreases of skin resistance, indicating that this current recruited most sudomotor fibres within a fascicle. The stepwise decreases in skin resistance (see Fig. 8) during increases of current strength suggests that at least in some fascicles there is a grouped distribution of sudomotor fibres.

Water vapour partial pressure

During train stimulation WVPP responses were never seen after the first train, suggesting that sweat gland ducts have to be filled to a certain level before evaporation from the ductal orifice can commence. However, once evaporation had started a single impulse was enough to increase WVPP. We found a curved relationship between skin resistance and changes of WVPP. A similar result has been obtained in the non-anaesthetized state (Adams & Vaughan, 1965). The explanation may be that at low stimulation frequencies a greater proportion of the sweat output is reabsorbed before reaching the ductal orifice (Ogawa & Bullard, 1972).

Cholinergic receptor blockade and skin resistance change

The finding that skin resistance changes were inhibited by atropine agrees with previous reports (cf. Edelberg, 1972), confirming the cholinergic innervation of sweat glands. However, this result does not rule out the possibility that other transmitters (including *inter alia* adrenoceptor active substances, vasoactive intestinal polypeptide or calcitonin gene-related peptide) modulate the sudomotoric neuroeffector mechanism (Sato & Sato, 1981, 1987; Tanaka, Uchiyama & Nakano, 1990). The atropine

dose–response curves were similar in all subjects, indicating an interindividual similarity in cholinergic receptor sensitivity on sweat glands. The consistency makes such dose–response curves an attractive model for studying possible modulator systems as well as putative cholinergic receptor affinity and sudomotor side effects of drugs in humans.

The cumulative dose necessary for complete inhibition of skin resistance responses within the receptive field of the impaled fascicle was low, and did not abolish arousal-induced skin resistance changes in the non-anaesthetized contralateral hand. This was not due to submaximal stimulation of the impaled fascicle, since 0·3 mA was found to recruit most sudomotor fibres within the fascicle (see Fig. 8*B*) and an increase in stimulation strength after the atropine injections usually had no effect. Furthermore, two experiments with 0·6 mA stimulation current resulted in similar dose–response curves. A reason for the marked atropine sensitivity of the stimulation-induced response may be that sudomotor fibres from different nerve fascicles converge on the same skin area, as shown for the mouse hindpaw by Kennedy, Sakuta & Quick (1984). If so, electrical stimulation of only one nerve fascicle would lead to a submaximal response which therefore would be more sensitive to atropine.

In conclusion, elimination of spontaneous sympathetic nerve activity in the hand by the use of an axillary blockade offers an experimental model for studying effector responses to a controlled sympathetic sudomotor nerve traffic, evoked by intraneural electrical stimulation. The stimulation-induced reduction of skin resistance and increase of WVPP demonstrates the close relationship between sweat production and skin resistance level. The experimental model also enables pharmacological evaluation of the sensitivity of human eccrine sweat glands under standardized conditions.

We thank Engineer Tomas Karlsson for excellent technical service. This work was supported by the Swedish Medical Research Council Grant No. B91-04X-03546-20A and by travel grants to M.K. from the Japan-Sweden Foundation and the Scandinavia–Japan Sasakawa Foundation.

REFERENCES

ADAMS, T. & VAUGHAN, J. A. (1965). Human eccrine sweat gland activity and palmar electrical skin resistance. *Journal of Applied Physiology* **20**, 980–983.

BINI, G., HAGBARTH, K.-E., HYNNINEN, P. & WALLIN, B. G. (1980). Thermoregulatory and rhythm-generating mechanisms governing the sudomotor and vasoconstrictor outflow in human cutaneous nerves. *Journal of Physiology* **306**, 537–552.

CARMICHAEL, E. A., HONEYMAN, W. M., KOLB, L. C. & STEWART, W. K. (1941). Peripheral conduction rate in the sympathetic nervous system of man. *Journal of Physiology* **99**, 338–343.

EDELBERG, R. (1972). Electrical activity of the skin. In *Handbook of Psychophysiology*, ed. GREENFIELD, N. S. & STERNBACH, R. A., pp. 367–418. Holt, Rinehart and Winston, Inc., New York.

FAGIUS, J. & WALLIN, B. G. (1980). Sympathetic reflex latencies and conduction velocities in normal man. *Journal of the Neurological Sciences* **47**, 433–448.

FOLKOW, B. (1955). Nervous control of the blood vessels. *Physiological Reviews* **35**, 629–663.

FOWLES, D. C. (1974). Mechanisms of electrodermal activity. In *Bioelectric Recording Techniques*, part C, *Receptor and Effector Processes*, ed. THOMPSON, R. F. & PATTERSON, M. M., pp. 231–271. Academic Press, New York.

GUTTMANN, L. (1940). Topographic studies of disturbances of sweat secretion after complete lesions of peripheral nerves. *Journal of Neurology and Psychiatry* **3**, 197–210.

HAGBARTH, K.-E., HALLIN, R. G., HONGELL, A., TOREBJÖRK, H. E. & WALLIN, B. G. (1972). General characteristics of sympathetic activity in human skin nerves. *Acta Physiologica Scandinavica* **84**, 164–176.

HAGBARTH, K.-E., HONGELL, A., HALLIN, R. G. & TOREBJÖRK, H. E. (1970). Afferent impulses in median nerve fascicles evoked by tactile stimuli of the human hand. *Brain Research* **24**, 423–442.

HERZ, E., GLASER, G. H., MOLDOVER, J. & HOEN, T. I. (1946). Electrical skin resistance test in evaluation of peripheral nerve injuries. *Archives of Neurology and Psychiatry* **56**, 365–380.

IWASE, S., MANO, T., SUGENOYA, J., SAITO, M. & HAKUSUI, S. (1988). Relationships among skin sympathetic nerve activity, sweating, and skin blood flow. *Environmental Medicine* **32**, 55–67.

KAGAYAMA, M. & NISHIYAMA, A. (1974). Membrane potential and input resistance in acinar cells from cat and rabbit submaxillary glands *in vivo*: effects of autonomic nerve stimulation. *Journal of Physiology* **242**, 157–172.

KATZ, B. & MILEDI, R. (1965). The measurement of synaptic delay, and the time course of acetylcholine release at the neuromuscular junction. *Proceedings of the Royal Society* B **161**, 483–495.

KENNEDY, W. R., SAKUTA, M. & QUICK, D. C. (1984). Rodent eccrine sweat glands: a case of multiple efferent innervation. *Neuroscience* **11**, 741–749.

KIRNÖ, K., KUNIMOTO, M., LUNDIN, S., ELAM, M. & WALLIN, B. G. (1991). Can galvanic skin response (GSR) be used as a quantitative estimate of sympathetic nerve activity in regional anesthesia? *Anesthesia and Analgesia* (in the Press).

LIDBERG, L. & WALLIN, B. G. (1981). Sympathetic skin nerve discharges in relation to amplitude of skin resistance responses. *Psychophysiology* **18**, 268–270.

LLOYD, D. P. C. (1959). Secretion and reabsorption in sweat glands. *Proceedings of the National Academy of Sciences of the USA* **45**, 405–413.

MCCLEARY, R. A. (1950). The nature of the galvanic skin response. *Psychological Bulletin* **47**, 97–117.

MILLER, M. R., RALSTON, H. J. & KASAHARA, M. (1960). The pattern of cutaneous innervation of the human hand, foot and breast. In *Cutaneous Innervation. Advances in Biology of Skin*, vol. 1, ed. MONTAGNA, W., pp. 1–47. Pergamon Press, Oxford.

NILSSON, G. E. (1977). Measurement of water exchange through skin. *Medical and Biological Engineering and Computing* **15**, 209–218.

OGAWA, T. & BULLARD, R. W. (1972). Characteristics of subthreshold sudomotor neural impulses. *Journal of Applied Physiology* **33**, 300–305.

ÖHMAN, A. (1981). Electrodermal activity and vulnerability to schizophrenia: a review. *Biological Psychology* **12**, 87–145.

PODLESNY, J. A. & RASKIN, D. C. (1977). Physiological measures and the detection of deception. *Psychological Bulletin* **84**, 782–799.

PROKASY, W. F. & RASKIN, D. C. (1973). *Electrodermal Activity in Psychological Research*. Academic Press, New York.

RICHTER, C. P. & KATZ, D. T. (1943). Peripheral nerve injuries determined by the electrical skin resistance method. *Journal of the American Medical Association* **122**, 648–651.

ROBERTS, D. F., SALZANO, F. M. & WILLSON, J. O. C. (1970). Active sweat gland distribution in Caingang Indians. *American Journal of Physical Anthropology* **32**, 395–400.

SATO, K. (1977). The physiology, pharmacology, and biochemistry of the eccrine sweat gland. *Reviews of Physiology, Biochemistry and Pharmacology* **79**, 51–131.

SATO, K. & SATO, F. (1981). Pharmacologic responsiveness of isolated single eccrine sweat glands. *American Journal of Physiology* **240**, R44–51.

SATO, K. & SATO, F. (1983). Individual variations in structure and function of human eccrine sweat gland. *American Journal of Physiology* **245**, R203–208.

SATO, K. & SATO, F. (1987). Effect of VIP on sweat secretion and cAMP accumulation in isolated simian eccrine glands. *American Journal of Physiology* **253**, R935–941.

TANAKA, E., UCHIYAMA, S. & NAKANO, S. (1990). Effects of calcitonin gene-related peptide and vasoactive intestinal peptide on nicotine-induced sweating in man. *Journal of the Autonomic Nervous System* **30**, 265–268.

TOREBJÖRK, H. E. & HALLIN, R. G. (1974). Responses in human A and C fibres to repeated electrical intradermal stimulation. *Journal of Neurology, Neurosurgery and Psychiatry* **37**, 653–664.

VALLBO, Å. B., HAGBARTH, K.-E., TOREBJÖRK, H. E. & WALLIN, B. G. (1979). Somatosensory, proprioceptive and sympathetic activity in human peripheral nerves. *Physiological Reviews* **59**, 919–957.

WALLIN, B. G., BLUMBERG, H. & HYNNINEN, P. (1983). Intraneural stimulation as a method to study sympathetic function in the human skin. *Neuroscience Letters* **36**, 189–194.