Sweat induction from an isolated eccrine sweat gland

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SATO, Kenzo. Sweat induction from an isolated eccrine sweat gland. Am. J. Physiol. 225(5): 1147-1152. 1973.—A method has been developed for in vitro induction of sweating from an isolated monkey-palm eccrine sweat gland. The validity of this methodology is shown in the following results. Mecholyl-induced sweating was completely inhibited by atropine and by cooling the incubation medium to 4 °C. Ouabain inhibited both sweat secretion and sodium reabsorption. Sweat samples collected from the end of the proximal duct had a sodium concentration from 20 to 90 mm and a potassium concentration from 5 to 18 mm, whereas samples collected directly from the isolated secretory coil contained both sodium and potassium in concentrations isotonic to the incubation medium; the results are consistent with the previous studies of others with micropuncture techniques. Epinephrine was shown to induce sweat secretion comparable in both secretory rate and sodium and potassium concentrations to those of Mecholyl-induced sweat. An attempt was made to estimate the transport rate of the secretory coil expressed in terms of tubular length. Volume flux from 3 to 4.7 × 10⁻⁷ cm/s/cm²/sec thus calculated was approximately half as much as that of the rat kidney proximal tubule.

PREVIOUS STUDIES on the function and reactivity of the eccrine sweat gland in vivo have generally been performed by collecting and analyzing skin surface sweat induced by the intradermal injection of sudorific drugs, exposure to thermal stimuli, or by the electrical stimulation of nerves supplying the sweat glands. In many studies, however, interpretation of the data has been complicated by uncertainty as to the periglandular conditions such as blood circulation, the composition of intersitial fluid, temperature, the possible effect of some endogenous hormonal factors, and the influence of sweat center. Furthermore, the inability to handle and study specific segments of the sweat gland has prevented us from thoroughly understanding the mechanism of sweat formation at the cellular level. In the present study methods have been developed to induce sweating directly from an isolated single eccrine sweat gland in vitro. The preliminary results indicate that the isolated eccrine sweat glands remain viable in vitro and secrete sweat in response to both cholinergic and adrenergic drugs.

METHODS

Isolation of sweat gland. The detailed description of the methods have been published previously (4-6). Skin biopsy specimens were obtained from the palms of rhesus monkeys tranquilized with phenytoin-HCl (Parke, Davis & Company). The specimens were quickly sliced freehand with a razor blade, rinsed in several changes of ice-cold Ringer solution (Krebs-Ringer bicarbonate containing 11 mM glucose gassed with 95% O₂ + 5% CO₂) and placed in a dissection chamber (Fig. 1). The Ringer solution in the dissection chamber was kept cold by introducing ice-cold water into the water jacket of the chamber, which was stirred constantly and oxygenated by gas lift with a mixture of 95% O₂ + 5% CO₂. Fresh, unstained eccrine sweat glands were isolated under a stereoscopic microscope (×40-80) using Dumont no. 5 stainless tweezers and sharp steel needles. In some glands the secretory portion was further isolated from the sweat gland coils (secretory portion plus duct).

Transfer of sweat glands. To avoid any damage to the tissue, the isolated sweat glands were transferred to the incubation chamber (the same model as the dissection chamber) in a small volume of medium in a Pasteur pipet.

In vitro induction of sweating. All the procedures were performed under a stereoscopic microscope (×40-80) with appropriate illumination. The Ringer solution in the incubation chamber was stirred and oxygenated as described for the dissection chamber and was kept cold (4°C) until the sweat induction was initiated. Glass pipets were mounted in a pipet holder (Fig. 2) modified from the collection pipet holder of Burg and co-workers (1) used for the in vitro perfusion of isolated rabbit kidney tubules. The pipet holder was constructed in the Instrument Section, University of Nijmegen School of Medicine and mounted on a microinmanipulator (Narishige, Tokyo). The outer pipet (o.d. 1.2 mm) was pulled using a horizontal pipet puller (Narishige, Tokyo). A fine constriction (i.d. 30-50 μm) was made on the tip of the pulled pipets by means of a heating ring and a small motor for rotating the pipets (also constructed in the Instrument Section). The outer pipet was inserted into the incubation chamber and end of the sweat duct or the secretory coil was drawn by suction into the tip of the pipet. The tissue-glass junction was sealed with Sylgard-184 (Dow Corning Corporation, Midland, Mich.) as described by Helman and Grantham (2). The pipet was then filled with mineral oil up to the water level to offset any hydrostatic pressure difference between inside and outside the pipet, to detect the leakage of the medium into the pipet, if any, and to prevent the evaporation of secreted sweat. The temperature of the incubation medium was raised to 38°C by circulating warm water into the water jacket of the chamber, and the sweat gland was preincubated for 10 min, during which time completeness of the seal between the glass capillary and the tissue was checked. At this point Mecholyl...
(acetyl-β-methylcholine, Sigma Chemical Company) was added to the incubation medium. The inner (sampling) pipets (o.d. 0.8 mm), one end of which had been drawn out to a long thin tip (o.d. 60 μm), was mounted in the inner pipet holder and the sweat was collected at 5- or 10-min intervals. After each sweat collection the sampling pipet was sealed with mineral oil to minimize evaporation. At the end of the experiments, the tissue-glass scaling was again checked by either cooling the medium to 4°C or adding atropine to the medium. The complete termination of sweating was taken as an indication of the completeness of sealing. The evaporation of the incubation media in the chamber was corrected by periodically adding distilled water.

**Determination of sample volume and sodium and potassium concentrations in sweat.** The sweat sample in the sampling pipet was blown out into the cavity of an oil-filled, siliconized glass slide and the sample size was measured with a calibrated standard-volume pipet, a procedure routinely employed in many laboratories of renal physiology (10). Sample size ranged from 0.5 to 35 nl (10⁻⁹ liter). Sodium and potassium were determined by a picomolar helium-glow spectrophotometer (Clifton Technical Physics, New York); the range of error was ±5%.

**RESULTS**

Figure 3 is a photograph of sweat induction from an isolated sweat gland coil (secretory portion + proximal duct) and Fig. 4 shows that of an isolated segment of the secretory portion. In two sweat gland coils the dose response to Mecholyl was studied (Fig. 5). Sweat glands responded to the lowest concentration of Mecholyl, 5 X 10⁻⁸ M, although the secretory rate was less than one-third the maximal value which was reached at 5 X 10⁻⁷ to 5 X 10⁻⁶ M Mecholyl. The highest dose of Mecholyl, 10⁻² M, however, seemed to suppress the secretory rate. Sweat secretion was completely terminated by quickly cooling the incubation medium to 4°C. In half the cases sweat secretion continued for more than 1 hr before the secretion was terminated artificially. Figure 6 illustrates such a typical experiment. Although there were small variations in the secretory rate, the starting secretory rate was maintained for the 2-hr incubation period, at which point sweat secretion was stopped by the addition of atropine (4 X 10⁻⁸ M). Although sweat posat-

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**FIG. 1.** Diagrammatic illustration of glass chamber used for dissection and incubation. Size, ca. 10 cm x 10 cm x 5 cm. A, compartment for dissection and incubation; B and G, inlet and outlet, respectively, of water jacket connected to pump of a thermostat bath; C, glass tubing for circulating Ringer solution; D, water jacket of chamber; E, stone filter; F, inlet of 95% O₂ + 5% CO₂ for oxygenation and circulation of Ringer solution.

**FIG. 2.** Schematic drawing of pipet holder. A, outer pipet holder; B, Teflon screw; C, inner pipet holder; E, Lucite ring for tightening rubber gaskets (D); F, pinion to advance inner pipet holder; G, rack; H, connector for mounting on a micromanipulator of Narishige type; I, brake to immobilize A.

**FIG. 3.** Photograph of sweat induction from an isolated, whole sweat gland. Cut end of proximal tubule is drawn into outer pipet by suction and sweat is induced by addition of Mecholyl. S, secretory coil; D, proximal duct.
SWEAT INDUCTION IN VITRO

Figure 4. Photograph of sweat induction from an isolated secretory coil. S, secretory coil.

Figure 5. Dose response of sweat rate to Mecholyl. Sweat samples were collected at 10-min intervals from whole sweat gland coils. Sweat rate expressed as nanoliters per hour per gland. In this paper, terms sweat rate and secretory rate were used interchangeably.

Sodium concentration was relatively constant during the entire induction period (7–8 mM), that of sodium increased with time (47 mM initially to ca. 75 mM finally) and showed no secretory-rate dependency. The effect of ouabain was studied on four whole sweat glands. At higher concentrations of ouabain, from $10^{-3}$ to $10^{-4}$ M, sweating stopped almost instantaneously; thus the small sample size of sweat collected after the addition of ouabain did not permit us to measure its electrolyte content (data not shown). However, this was not the case when a lower dose of ouabain was added to the medium. Figure 7 shows the effect of $10^{-6}$ M ouabain on secretory rate and sodium and potassium concentrations in a typical experiment with a whole sweat gland coil. In contrast to the addition of atropine or higher doses of ouabain, nearly 10 min were required after the administration of ouabain for the termination of sweat secretion. The most remarkable change occurred in sweat sodium concentration, which rose from 80 mM before the addition of ouabain to 140 mM in the final sample. Potassium concentration was increased by only 1–2 mM in the presence of ouabain. Figure 8 illustrates the sodium and potassium concentrations in sweat induced directly from an isolated secretory coil (see also Fig. 4). Sweat secretion was initiated first by $5 \times 10^{-7}$ M Mecholyl. At 30 min, atropine $5 \times 10^{-6}$ M was added; this stopped sweat secretion almost completely. Five minutes later, 0.01 mg epinephrine ($2.7 \times 10^{-5}$ M) was added to the incubation medium. Surprisingly, the secretory coil began to secrete sweat at approximately the same rate as before;
FIG. 8. Sodium and potassium concentrations in sweat induced directly from an isolated secretory coil. MCH, Mecholyl; AT, atropine; AD, epinephrine; RG, Regitine. Doses of epinephrine and Regitine represent absolute amount of drugs added to incubation medium of 2 ml. Sweat samples were collected at 5-min intervals.

however, the secretion was spontaneously terminated within 5 min. At the same time the incubation medium was observed to acquire a salmon-pink color due probably to the oxidized epinephrine since the medium was continuously bubbled with 95% O₂ + 5% CO₂. At 45 min, the secretory coil resumed secretion in response to the subsequently added Mecholyl at a final concentration of 2 × 10⁻³ M. After the inhibition of Mecholyl-induced sweating, again by atropine, the higher dose of epinephrine (0.05 mg) was added 3 times at 5-min intervals during the next 15 min, to which the secretory coil responded by secreting sweat at a constant rate although the sweat rates during this period of time were only 50–70% of the initial samples. Although Regitine (phentolamine, Ciba) added at 75 min stopped sweating in 5 min, the spontaneous termination of sweating cannot be ruled out in this experiment since Mecholyl added at 5 × 10⁻⁴ M at 90 min failed to reestablish sweat secretion. Both sodium and potassium concentrations were rather constant during the entire period, despite the difference in the pharmacological stimulants. Similar patterns of drug response and sweat electrolyte concentration were observed in two other secretory coils studied (data not shown). Figure 9 compares sodium and potassium concentrations in sweat obtained from three secretory coils and the end of the proximal ducts of more than 10 whole glands. Sweat samples obtained from the end of the proximal tubules contained considerably lower sodium but higher potassium concentrations than those obtained from the secretory coils. Both sodium and potassium concentrations in the secretory coil sweat were approximately isotonic to the incubation media.
seems especially the case with the isolated secretory coils where only three of seven coils showed a secretory response to the sudoriferous agents. The use of collagenase, however, was found to be of no use in removing the still-adherent collagen fibers or in isolating the secretory portion since without intact collagen fibers the handling of the glands with tweezers proved almost impossible. With regard to the second group of glands which showed a spontaneous termination of secretion, great caution must be exercised especially when studying the effects of inhibitors. It would be more desirable, therefore, to experiment on more than two sweat glands simultaneously or to use stimulators, if possible, to reverse the initial inhibitory effect. As shown in Fig. 6, sweat sodium concentration increased with time in the absence of the change in secretory rate after 1 hr of incubation, which indicates a decrease in ductal sodium reabsorption. It seems essential, therefore, in future experiments, to select a more ideal incubation medium and maintain the activity of the gland constant for a longer period of time.

Electrolyte components of sweat. The present data on sweat electrolytes are consistent with the earlier results obtained by the micropuncture (8) or cryoscopic (9) technique that nearly isotonic precursor fluid is secreted by the secretory coil and sodium in excess of water is reabsorbed by the duct thus producing hypotonic sweat. The present study also shows that the potassium concentration tends to be higher in sweat collected from the end of the proximal tubule than in secretory coil sweat, which offers indirect evidence that potassium is secreted into sweat by the duct. However, this assumption may not be valid if considerable reabsorption of water occurs in the proximal duct.

Pharmacology of eccrine sweat secretion. The eccrine sweat gland has been regarded as predominantly cholinergic; this is based on the observation that the sudorific response of the sweat glands to epinephrine is extremely low, being less than 1/10 of the cholinergic sweating in both human and rhesus monkey (unpublished observation). In isolated monkey and human eccrine sweat glands both lactate and $^{14}CO_2$ production are markedly stimulated by $10^{-6}$ to $10^{-5}$ M epinephrine, although to a lesser extent than by Methocholyl (5, 6). Since the increased energy metabolism in the presence of stimulants is, most likely, due secondarily to the increased membrane transport (6), this discrepancy between in vivo secretory and in vitro metabolic function has posed a difficulty in the interpretation of adrenergically stimulated energy metabolism in vitro. The present evidence that the secretory response of the sweat gland is similar in magnitude to both Methocholyl and epinephrine agrees well with our previous observations on in vitro energy metabolism (5, 6). The present study has also pointed out that epinephrine is extremely unstable in the incubation medium in the presence of oxygen, which might at least partly explain the lower metabolic response of the incubated sweat glands to epinephrine than to Methocholyl. The fact that both Methocholyl and epinephrine produced precursor sweat of comparable flow rate and ionic content suggests that both drugs stimulated the same transport system, probably by increasing the influx of sodium from the interstitial space to the secretory cell interior and secondarily activating the sodium pump (6); however, it remains to be shown whether both drugs share the same receptor site, if any, at the basal side of the secretory cell membrane, or whether each drug acts on different receptor sites. The low in vivo secretory response of the sweat gland to epinephrine can probably be explained by a strong vasoconstrictor effect of the drug on the periglandular blood vessels.

Estimation of transport rate in sweat gland secretory coils. Both the sweat gland secretory coil and the kidney proximal tubule are lined by single-layered, transporting, epithelial cells (although, in addition, the secretory coil contains myoepithelial and dark cells). Since both epithelia perform isotonic transport and possess more or less the same tubular diameter (see Figs. 3 and 4), the transport parameters can be compared with each other if the volume flux ($J_V$) is related to the tubular length. The mean sweat rate per gland per hour was calculated from 10 whole glands which showed reasonably constant secretory rates for 1 hr and whose sweating of which was terminated by the addition of atropine or by cooling the media. Assuming negligible water reabsorption by the sweat duct, the mean secretory rate per secretory coil equals the mean secretory rate per gland, $218 \pm 37$ (SE) nl/gland per hr. This estimate is considerably higher than our previously calculated in vivo secretory rate (secretory rate), $136$ nl/gland per hr (7). The discrepancy between the two might have been caused by the selection of the sweat glands in the present study and the differences between in vivo and in vitro periglandular conditions. By analyzing the photographs of the monkey sweat glands previously published (4, 7), we estimated the tubular length of the secretory coil to be approximately 1.3 mm. Thus $218$ and $136$ nl/gland per hr are equivalent to $4.7$ and $3.0 \times 10^{-7}$ cm$^2$/hr sec, respectively. These values are approximately half as much as that of the rat proximal tubule, $J_V = 6.7 \times 10^{-7}$ cm$^2$/hr sec (3).

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