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BRADYKININ FORMATION IN HUMAN SKIN AS A FACTOR IN HEAT VASODILATATION

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The vasodilatation in the skin of the human forearm produced by indirect heating is not due to the release of vasoconstrictor tone but is the result of an active vasodilator mechanism (Grant & Holling, 1938; Edholm, Fox & Macpherson, 1956*a*; Roddie, Shepherd & Whelan, 1956). This active vasodilatation might be caused through the excitation of specific vasodilator nerve fibres or by the action of vasodilator substances released from the activated sweat glands. In a discussion with Dr O. G. Edholm, concerning the mechanism involved, the question was raised whether the polypeptide bradykinin might be produced by the sweat glands and play a similar role in the causation of the cutaneous vasodilatation to that found with the vasodilatation of the activated salivary gland (Hilton & Lewis, 1955*a*, *b*). The present experiments were initiated as a result of this discussion. They show that the vasodilatation which occurs in the human forearm on indirect heating has several features in common with the vasodilatation in the activated salivary gland, and further that sweat gland activity leads to the appearance of the bradykinin-forming enzyme in the sweat and to the formation of bradykinin itself in the skin of the human forearm.

METHODS

Collection of sweat during indirect heating

The subject sat in a bath with the legs and lower part of the trunk immersed in water. The temperature of the bath water could be regulated as desired. An inflated immersion suit covering the trunk, head and upper arms reduced the heat loss from the non-immersed parts of the body and polythene bags were fixed over both forearms. The subject was heated, by raising the bath temperature, until his oral temperature was about 38° C. The bags were drained as completely as possible at intervals over a period of 1½ hr. The samples of sweat so obtained were cooled immediately after collection to about 0° C and kept at −4° C until tested for bradykinin-forming activity.

Occlusion of sweat gland ducts

In some experiments the effects of occlusion of the sweat gland ducts on the vasodilator and sweating responses to body heating were examined. The commercial preparation 'Newskin'

(Harwoods Laboratories) which was used for this purpose was painted on one forearm in longitudinal strips, leaving unpainted skin between in order to permit the forearm to swell during heating. The subject was seated in a bath, as in the experiments for collection of sweat, and the blood flow before and after heating was measured simultaneously in the two arms, using the experimental procedure described by Edholm, Fox & Macpherson (1956*b*).

Perfusion of subcutaneous tissue space

To study the formation of bradykinin in human forearm skin, the following procedure was adopted. Holes were drilled along the shaft of two hypodermic needles (20 s.w.g. × 1·25 cm) to enable fluid to pass in or out along their whole length. One of the needles was pushed carefully into the subcutaneous tissue space on the anterior aspect of the forearm; the second was inserted, from the opposite direction, at a distance of 1·5–2 cm from the first and parallel to it. A small subcutaneous depot of fluid was then produced by injecting 1 ml. of sterile saline solution through each needle. Subsequently, sterile saline was infused at a constant rate (0·4 ml./min) through

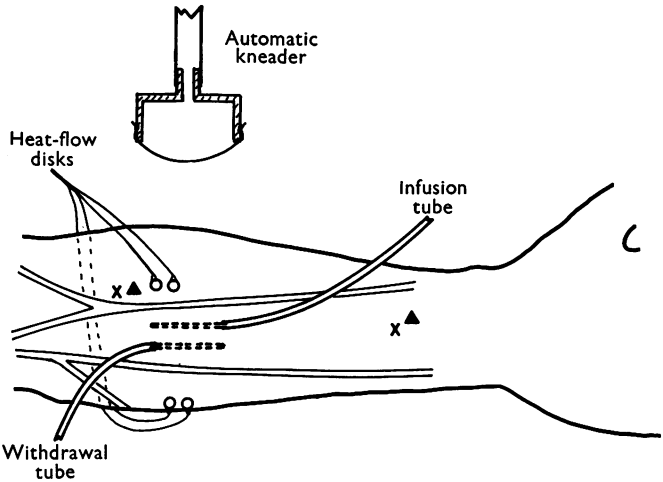

Fig. 1. Diagram showing sites of perfusion of subcutaneous space and measurement of heat flow, skin temperature (×) and skin resistance (▲). Automatic kneader shown in cross-section.

one needle, and fluid was continuously withdrawn into the barrel of a graduated 1 ml. syringe through the other needle under a small negative pressure (20–40 mm Hg). The fluid withdrawn was clear of blood in nearly every experiment. The arrangement is shown diagrammatically in Fig. 1. To facilitate the passage of fluid from one needle to the other, intermittent pressure was applied to the raised area of skin by an automatic kneader, also shown in Fig. 1, and consisting of a polythene bell with a rubber diaphragm stretched across its open end. The rubber diaphragm was inflated about 30 times/min by a Dale-Schuster pump. The samples of fluid which were not immediately tested for bradykinin-like activity were kept on ice until the test was made. All needles, tubing and glassware in contact with the fluid were previously siliconed.

In these perfusion experiments, the onset and development of the cutaneous vasodilatation in the forearm skin was determined using copper-constantan thermocouples with a potentiometer and galvanometer. Skin temperature was measured at two sites, one above and the other below the area of the infusion. In addition, the rate of heat elimination through the skin was measured using Hatfield's heat-flow disks. The onset of sweat secretion was detected by measuring skin resistance at two or more sites on the forearm.

In these experiments it was necessary to control body temperature by some method other than immersing the subject in a water-bath, and for this a body temperature-regulating suit was

devised. The suit is shown in schematic cross-section in Fig. 2. During the control period the subject was kept cool by circulating cold water through the tubes inside the insulating suit. To heat the subject and produce a peripheral vasodilatation, hot water was circulated through the suit and the heating pads were turned on; the polythene lining prevented the subject being cooled by evaporation of his own sweat.

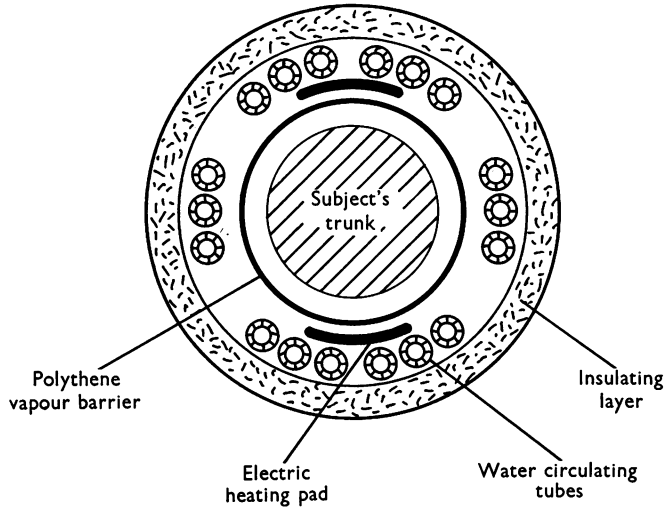

Fig. 2. Diagrammatic cross-section of temperature-regulating suit.

Assay of sweat and perfusate

Sweat and perfusate were assayed for bradykinin-forming enzyme activity and for bradykinin-like activity, using the isolated non-pregnant rat uterus. Each horn was mounted in a 15 ml. organ bath containing de Jalon's solution and maintained at a temperature of 29–30° C. When assaying for bradykinin-like activity the samples of sweat or perfusion fluid were transferred directly to the organ bath. When testing for bradykinin-forming enzyme, the samples were first incubated with a solution of dog pseudo-globulin. This solution contained the plasma protein fraction precipitated by $(NH_4)_2SO_4$, 33–46% saturation. The precipitate was redissolved in distilled water, dialysed for 48 hr against running tap water and finally made up to one third of the original volume with distilled water. The incubation and assay were carried out by the method of Hilton & Lewis (1956). The samples of sweat (or perfusate) were diluted, when necessary, before mixing, in order to ensure that the pseudo-globulin substrate was in excess. In each test between 0·1 and 0·3 ml. pseudo-globulin solution was added to two volumes of solution of sweat (or perfusate). The mixtures were incubated for 1 min at 30° C. All assays were performed against a standard preparation of bradykinin, kindly supplied by Dr Rocha e Silva.

Estimation of protein and peptide

In one experiment samples of perfusate from the forearm skin were examined by the method of Lowry, Rosebrough, Farr & Randall (1951) which gives a measure of free and peptide tyrosine. Heparinized plasma from the same subject was diluted and examined in the same way.

Preparation of cat's submandibular salivary gland

To compare the heat vasodilatation in forearm skin with the functional vasodilatation in the salivary gland, cats were anaesthetized with pentobarbitone (Nembutal, Abbott Laboratories, 40 mg/kg), and the submandibular gland was prepared for isolation of its arterial inflow and venous outflow (via the external jugular vein), for cannulation of its duct, and for stimulation of the

chordolingual nerve as previously described (Hilton & Lewis, 1955*a*). The lingual artery was cannulated for injection. The venous outflow was measured using a photo-electric drop recorder (Hilton & Lywood, 1954), drop formation being registered with a Gaddum drop-timer.

Drops of saliva were registered by a Thorp impulse counter, so giving a profile of the rate and amount of secretion. The chordolingual nerve was stimulated with square waves of 0·5 msec duration of varying frequency and voltage.

One femoral vein was cannulated for intravenous injections, and the femoral artery of the other leg for the blood pressure record. Heparin (10 mg/kg) was injected by the venous cannula before the femoral and lingual arteries and the external jugular vein were opened.

RESULTS

Experiments on the human subject

The presence of bradykinin-forming enzyme in sweat

Sweat collected in the polythene bag from the forearm of a human subject heated to 38° C caused no contraction of the isolated rat uterus, a preparation which is very sensitive to bradykinin, even when as much as 1 ml. was added to the organ bath: however, 0·5ml. of a 1:10 dilution of sweat in normal saline solution, when incubated with pseudo-globulin solution, caused a strong contraction. Like bradykinin, this smooth muscle-stimulating activity disappeared when incubation was prolonged, and it was destroyed by a few minutes incubation with chymotrypsin. Sweat collected from the hand under the same conditions possessed similar bradykinin-forming activity.

In one experiment the sweat was collected from the forearm bag in successive samples, the bag being drained as much as possible whenever a few millilitres of sweat had collected. The volumes of these samples were measured and their bradykinin-forming activity was then assayed on the rat uterus. The rate of sweat production and output of bradykinin-forming enzyme are shown as histograms in Fig. 3. Although the body temperature was raised quickly, the peak of sweat production was not reached for 10–15 min. Thereafter it gradually declined, even though body temperature was maintained at a high level.

The bradykinin-forming activity reached a maximum 20–30 min after sweating began and then declined, but was still detectable in the sample of sweat collected up to the 90th minute.

The presence of bradykinin in perfusate from the subcutaneous tissue space

Direct evidence of bradykinin-formation in the skin was provided by experiments in which sterile saline was infused subcutaneously. When the subject was cool, the fluid withdrawn from the subcutaneous space and tested on the isolated rat uterus was found to contain small amounts of bradykinin-like activity. This activity, assayed against a standard preparation of bradykinin, corresponded to between 1 and 5 μg bradykinin/ml. Like bradykinin, this

activity disappeared if the fluid was kept for 15–20 min at room temperature or after a few minutes incubation with chymotrypsin. As shown in Fig. 4, the activity of successive samples withdrawn during more than an hour of perfusion remained relatively steady. The fluid did not contain any bradykinin-forming enzyme, since incubation with pseudo-globulin solution did not increase the amount of smooth muscle-stimulating activity.

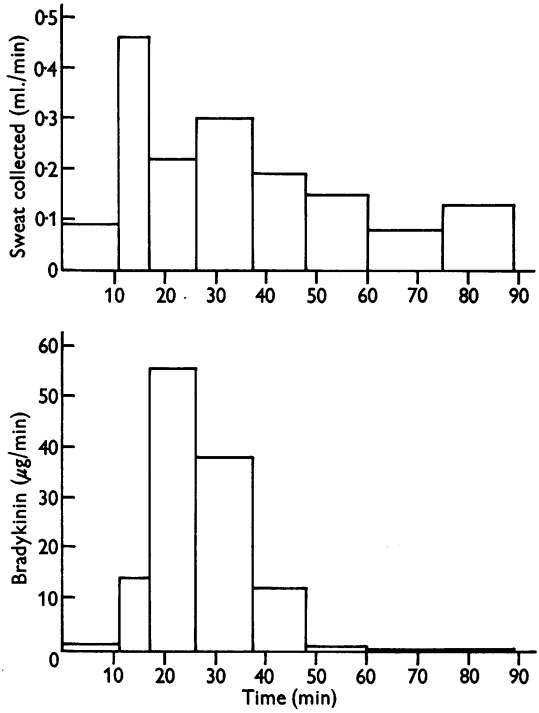

Fig. 3. Histograms of the amounts of sweat collected from a human forearm during a period of body heating and of the corresponding bradykinin-forming activity of each sample.

Human plasma proteins readily develop bradykinin-like activity when diluted with saline *in vitro* (Schachter, 1956). The activity of the fluid from the subject when cool could likewise be accounted for by the dilution of the tissue space proteins by the saline infusion. When the peptide and free tyrosine content of the fluid withdrawn was examined, it was found to correspond to that of a 1–2% dilution of plasma from the same subject. Accordingly, a 1–2% dilution of plasma in saline was tested for bradykinin-like activity and the activity developed corresponded to 3μg bradykinin/ml.

When the subject was heated, the bradykinin-like activity of the perfusion fluid increased up to five times the previous level. As shown in the experiment illustrated in Fig. 5, this increase in activity corresponded with the onset of vasodilatation. In this experiment the subject's temperature was raised slowly

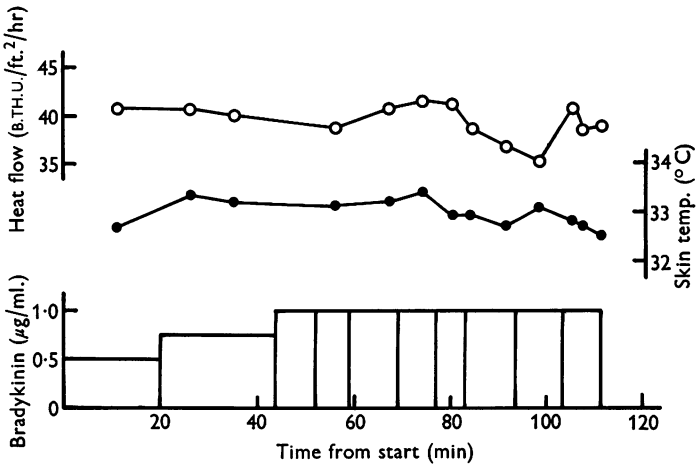

Fig. 4. Results of an experiment in which the subject was kept cool throughout a 2 hr period, showing measurements of forearm skin temperature (●), heat flow (○) and equivalent bradykinin content of successive samples of perfusate from the subcutaneous space.

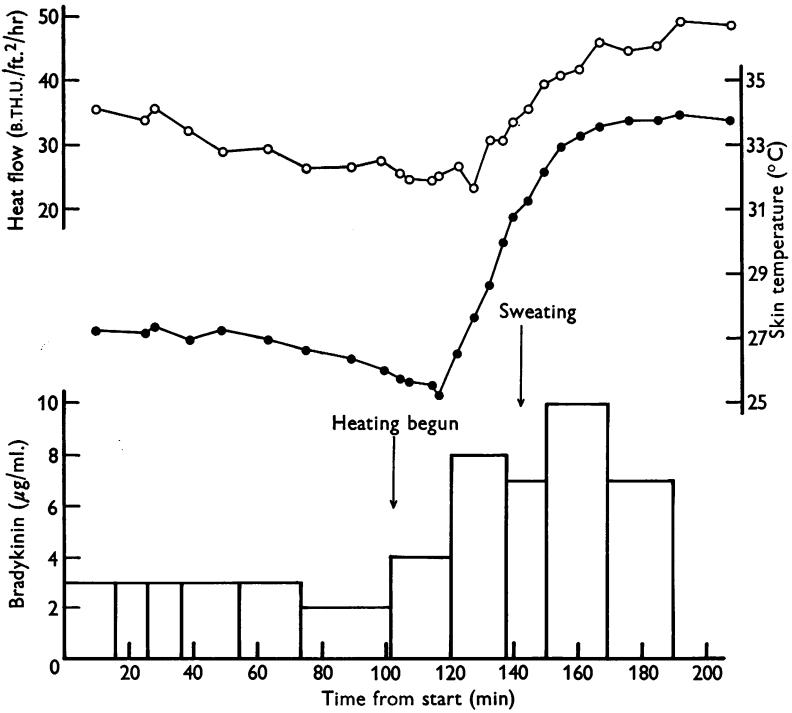

Fig. 5. Results of an experiment in which the subject was heated after a control period of cooling, showing measurements of forearm skin temperature (●), heat flow (○) and equivalent bradykinin content of successive samples of perfusate from the subcutaneous space.

so as to produce vasodilatation some time before sweating commenced, and the bradykinin-like activity had almost reached its maximum when sweating first occurred. In another experiment, in which heating produced a threefold increase in bradykinin-like activity, the protein content of the fluid withdrawn did not change as the forearm vasodilatation developed. The temperature of the forearm skin increased to a maximum of 34° C during the body heating, and diluted plasma was therefore incubated *in vitro* at this temperature. The bradykinin-like activity of the diluted plasma was not increased by this treatment. The increase in bradykinin-like activity cannot, therefore, be attributed either to the raised skin temperature or to an increase in passage of protein across the capillary walls secondary to arteriolar dilatation and increased rate of blood flow through the skin.

The effect of occluding sweat gland ducts

There is evidence that reabsorption of the constituents of sweat occurs through the sweat gland ducts (Lobitz & Mason, 1948). Experiments were therefore carried out in an attempt to increase this reabsorption by obstructing the outflow of sweat by painting layers of 'Newskin' on the forearm. It was thought that this might potentiate the vasodilator response; but no evidence of increased vasodilatation was found. The resting level of flow was not obviously affected in the treated arm. Further, when the subject was rapidly heated the flow increased equally in the two arms.

On removing the plethysmographs, inspection of the treated arm revealed only occasional blebs of sweat, like sago grains, under the film of 'Newskin'. These were so small and sparse that it was clear that either sweat secretion had been largely suppressed, or the sweat secreted during the period of heating had been re-absorbed.

The effect of atropine on vasodilatation and sweating

The salivary glands and the eccrine sweat glands both have a cholinergic secretomotor innervation. In the salivary gland stimulation of these nerves produces secretion together with an accompanying vasodilatation brought about by the local formation of bradykinin (Hilton & Lewis, 1955*a*, *b*). It is known that atropine suppresses salivary secretion more readily than the accompanying vasodilatation. This drug has a similar effect on the secretory and vasodilator responses in the human forearm during body heating. After an injection of 0·3 mg of atropine into the brachial artery, the subject was heated in a bath. The onset of vasodilatation in the treated arm was slightly delayed compared with that in the normal arm (Fig. 6). Thereafter the vasodilatation in the two arms was almost identical. The onset of sweating in the treated arm was, however, considerably delayed and sweat secretion was markedly reduced when compared with that of the normal arm.

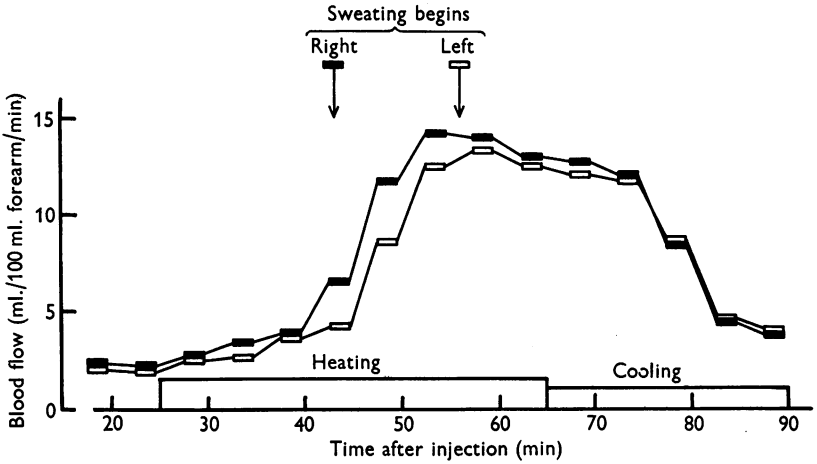

Fig. 6. Results of an experiment in which the blood flow in both forearms was measured by venous occlusion plethysmography after arterial injection of atropine (0·3 mg) into the left brachial artery. The differential effect of body heating on the blood flow of the two forearms is shown.

Experiments on the cat's salivary gland

The present experiments suggest that there is a mechanism contributing to heat vasodilatation in human skin similar to that of the functional vasodilatation in the cat's salivary gland. In human skin the vasodilatation on heating usually develops before sweating begins. In such experiments it is likely that the sweat glands are not being maximally stimulated; experiments were therefore performed to determine whether submaximal stimulation of the cat's chorda tympani can produce vasodilatation in the submandibular salivary gland before secretion starts.

It was found that when the nerve was stimulated with square waves of submaximal frequency and voltage, the vasodilatation in the gland began promptly, and always before salivary secretion. In one experiment stimulation just adequate to produce a vascular effect caused vasodilatation without any secretion, even though stimulation was maintained for 5 min. In two other experiments an interval as long as 75 sec passed after the onset of vasodilatation before secretion commenced. This confirms and extends previous findings that submaximal stimulation of the chorda tympani can lead to vasodilatation alone (von Beznák, 1934).

Another feature of the vasodilatation in the salivary gland is that it rapidly reaches a peak and then begins to fall off, even though stimulation is being continued. It then settles down to a steady value which depends on the intensity of stimulation used. According to the intensity of stimulation, the blood flow may be steady, or falling, or even increasing at the time that secretion commences.

Effect of atropine. Small doses of atropine injected via the artery into the salivary gland, which scarcely affect the vasodilatation resulting from supramaximal chorda stimulation, greatly reduce the vasodilatation produced by submaximal stimulation. The experiment illustrated in Fig. 7 shows the vasodilator and secretory effects of stimulation with a submaximal voltage at 4 pulses/sec before (at *a*) and after (at *c*) an arterial injection of atropine sulphate (5 μg/kg). After atropine the secretory effect was abolished while the vasodilator effect had almost completely disappeared. By comparison, the vasodilator effect of supramaximal chorda stimulation was hardly affected by the atropine (compare *b* with *d*), though secretion was abolished.

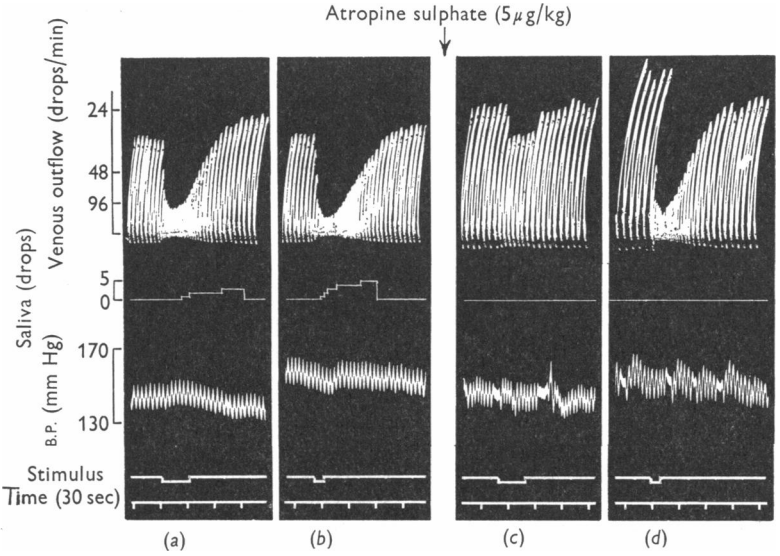

Fig. 7. Cat, submandibular salivary gland. Records of venous outflow (Gaddum drop-timer), secretion of saliva and arterial blood pressure. Effect of stimulation of chordolingual nerve at 4 pulses/sec with submaximal voltage for 30 sec (*a* and *c*) and supramaximal voltage for 10 sec (*b* and *d*). Atropine sulphate (5 μg/kg) infused arterially between *b* and *c*.

DISCUSSION

Skin vasodilatation and secretion of sweat are two mechanisms controlling heat exchange in man which have generally been thought to be functionally independent, each having its separate system of peripheral neural control. The present experiments suggest that they are interrelated responses activated by the secretomotor nerves to the eccrine sweat glands, and that the functional relationship is similar to that found in the cat submandibular salivary gland, where the vasodilatation accompanying secretion is mediated by a bradykinin-like polypeptide formed in the tissue spaces (Hilton & Lewis, 1955*a*, *b*).

The bradykinin-forming enzyme is found in sweat as it is in saliva. The increase in the bradykinin-like activity of the perfusate from the subcutaneous

tissue space of the forearm during body heating is analogous to the increase in bradykinin-forming enzyme in the venous effluent of the perfused salivary gland on chorda stimulation. Following an intra-arterial injection of atropine into the human forearm, the sweating produced by body heating is markedly delayed and reduced, whereas the vasodilatation is slightly delayed only. In the salivary gland, an intra-arterial dose of atropine sufficient to suppress secretion has hardly any effect on the vasodilatation and does not prevent the release of increased amounts of bradykinin-forming enzyme (Hilton & Lewis, 1956). When the human subject is heated relatively slowly, the vasodilatation in the atropinized forearm is reduced and only appears 20 min after its onset in the normal forearm (Roddie, Shepherd & Whelan, 1957). Similarly, we found that atropine has a marked inhibitory action on the vasodilatation produced by submaximal stimulation of the nerve supplying the salivary gland.

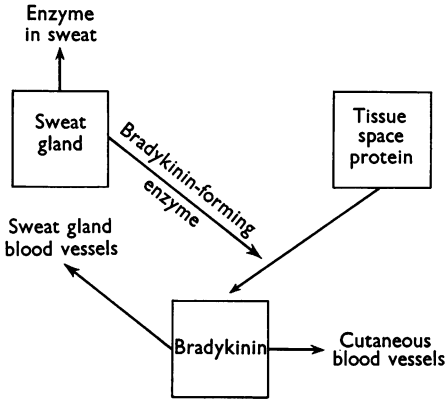

Fig. 8. A schematic diagram to illustrate the bradykinin mechanism in human skin.

The possibility that the vasodilatation in the human forearm might be caused by the action of vasodilator substances released from the activated sweat glands was considered by Grant & Holling (1938) but was questioned by Doupe, Cullen & Macaulay (1943) because atropine can prevent visible sweating without abolishing the vasodilatation. The hypothesis that the cutaneous vasodilatation results from the formation of the bradykinin, as in the salivary gland, removes this objection. A simple schematic diagram showing the probable mode of action of the bradykinin mechanism in human skin is given in Fig. 8.

It has been suggested that the time relationship between the onset of visible sweating and the vasodilatation in the human forearm exhibits such a marked variability as to make it unlikely that the two phenomena are produced by the same nerve supply (Hellon & Lind, 1956). In some of our experiments the onset of sweating and vasodilatation were nearly simultaneous and in others sweating did not appear until the vasodilatation was

almost complete. These variations, however, can be readily explained by differences of intensity in the activation of the glands. In our experiments on the cat's salivary gland, in which the chorda tympani was stimulated at low intensities and frequencies, a small vasodilatation could be produced without any secretion of saliva. With increasing intensity of stimulation the latent period between vasodilatation and secretion shortened until the two responses became virtually simultaneous. Further, with submaximal stimulation, the blood flow first increased, then decreased a little to reach a plateau which was maintained until stimulation was stopped. According to the time of onset of secretion, the blood flow might at this particular moment be increasing, decreasing or even steady.

The degree of thermal stress giving rise to a particular discharge rate along the secretomotor fibres to the sweat gland will almost certainly be an important factor determining the time interval between the onset of vasodilatation and the onset of sweating. In situations imposing only a low thermal stress the threshold for stimulating sweat secretion would not be reached, and only a vasodilatation would occur which, by raising skin temperature, would re-establish thermal equilibrium. Furthermore, the magnitude of the vasodilator and sweating responses can be greatly influenced by the local skin temperatures prevailing at any one time at different parts of the body surface. With so many factors involved, a marked individual variability in response is not surprising, despite the relationship between vasodilatation and glandular activity.

One point at which there is a difference between the findings with the salivary and sweat glands is found in the experiments in which the outflow of secretion was prevented during glandular activation. In the salivary gland, the vasodilatation during nerve stimulation under these conditions was smaller than normal, but when the obstruction was relieved the blood flow was rapid for many minutes, even though the original stimulation had only been maintained for 20 sec (Hilton & Lewis, 1955*b*). In the human forearm, however, when the outflow of sweat was prevented by application of a film of 'Newskin', the vasodilatation was the same as in the control arm, both during and after the period of heating. It is possible that this difference is due to the fact that the salivary glands are encapsulated while the sweat glands are not. A firm capsule would limit blood flow when the pressure in the ducts is raised and it would further increase the rise of pressure in the ducts themselves. It might also act by restricting the diffusion of enzyme or bradykinin away from the blood vessels. It is likely that sweat can pass through the duct walls quite readily; this was suggested by the absence of sweat under pressure in the sweat ducts when the film of 'Newskin' was removed from the arm following a period of body heating and, indeed, dyes have been seen to diffuse horizontally from the ducts into the surrounding tissue (Kuno, 1956).

The anatomy of the skin is such that the concept of bradykinin diffusing from the sweat glands to affect the skin blood vessels seems reasonable. Both the bodies and ducts of the sweat glands are enveloped in a profuse network of blood vessels, and bradykinin formed in the immediate vicinity of the gland would readily affect all these vessels; the resulting vasodilatation probably plays an essential role in enabling the sweat glands to produce their copious watery secretion. The bodies of the sweat glands, which are not encapsulated, are located mainly below or in the lower part of the subdermal layer of the skin, close to the deep or cutaneous vascular plexus.

In view of the evidence for a relationship between sweat gland activity and the active vasodilatation in human skin, it is significant that in the skin of the limbs of the cat and dog, which lack eccrine sweat glands, there is no indication of an active vasodilator mechanism, and heat vasodilatation is the result of the release of vasoconstrictor tone (Folkow, Frost, Haegar & Uvnäs, 1949; Green, Howard & Kenan, 1956).

If sweat secretion and cutaneous vasodilatation are interdependent functions as is suggested, then sweat gland activity should be accompanied by vasodilatation when initiated in other ways than by indirect heating. For instance, eating sharp and pungent foods, such as chillies, produces sweating associated with vasodilatation, usually restricted to the head and neck but sometimes extending over the trunk. Herxheimer (1956) observed that the skin reaction of cholinogenic urticaria is always associated with both sweating and vasodilatation, and therefore suggested that it was secondary to some product of sweat gland activity.

On the other hand, the sweating found in the clinical condition of shock is accompanied by a general cutaneous vasoconstriction, but here the local vasodilator mechanism has probably been overcome by a more powerful vasoconstrictor influence.

So far the discussion has dealt with the active vasodilatation in forearm skin. The finding of bradykinin-forming activity in sweat collected from the human hand, however, raises a problem in so far as the vasodilatation occurring here on body heating has been explained on the basis of a release of vasoconstrictor tone alone (Arnott & MacFie, 1948; Gaskell, 1956). The skin of the hand differs from that of the forearm in having a large number of arteriovenous anastomoses, and these anastomoses may well exert a predominant influence on the control of skin blood flow in the hand, masking the vasodilatation resulting from the activity of the eccrine sweat glands known to be present. Furthermore, the eccrine sweat glands of the palmar skin differ from those of the forearm in responding to emotional rather than thermal stimuli. As Lewis & Pickering (1931) have pointed out, it may only be in pathological states in which arterial vessels are partially blocked that the active vasodilator mechanism becomes apparent.

SUMMARY

1. The mechanisms controlling the active vasodilatation and eccrine sweat gland activity occurring in human forearm skin on body heating have been studied and compared with those in the cat submandibular salivary gland.

2. The bradykinin-forming enzyme was shown to be present in human eccrine sweat as in saliva.

3. The subcutaneous space of the human forearm was perfused with saline. With body heating the bradykinin content of the perfusate increased up to five times the level observed when the subject was kept cool, and this increase is attributed to sweat gland activity.

4. The onset of skin vasodilatation may precede the onset of sweating, particularly when the subject is exposed to low thermal stress. Experiments on secretion and venous outflow from the cat's submandibular gland showed that whereas with maximal stimulation of the chorda tympani, vasodilatation and extrusion of saliva were observed simultaneously, with submaximal stimulation, vasodilatation could appear before, or even without, extrusion of saliva.

5. Using forearm venous occlusion plethysmography it was shown that during rapid body heating atropine suppressed overt sweat secretion more readily than the vasodilatation. In the atropinized salivary gland maximal stimulation elicited an almost normal vasodilatation without extrusion of saliva, whereas with submaximal stimulation the vasodilatation was greatly reduced.

6. It is concluded that bradykinin formation in human eccrine sweat glands, by producing a periglandular vasodilatation, plays an important role in sweat secretion closely analogous to its role in the salivary gland. It is further concluded that the active vasodilatation in human forearm skin accompanying body heating is also produced in the main by bradykinin resulting from sweat gland activity.

We wish to thank Dr J. L. Simkin for performing the free and bound peptide estimations.

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