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MODULATION OF CUTANEOUS MECHANORECEPTORS BY SYMPATHETIC STIMULATION

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It has long been known that stimulation of the sympathetic nerve supply to skeletal muscle causes relief of fatigue (Orbeli, 1923). In the frog, this has recently been shown to be due to a facilitation of neuromuscular transmission brought about by an increased sensitivity of the motor end-plate to acetylcholine (Hutter & Loewenstein, 1955). The effect is small, the improvement of transmission seldom exceeding 10%. Teleological considerations led to the present work. The smallness of the effect induced speculation on a more general sympathetic facilitation fractioned at various stages of the reflex system. The possibility of a sympathetic influence on cutaneous mechanoreceptors is here tested. It was found that stimulation of the sympathetic nerve supply to an isolated frog's skin or application of adrenaline or noradrenaline alter the response of a tactile receptor to a mechanical stimulus, decreasing adaptation rate and threshold, and, eventually, causing spontaneous activity in the receptor. A preliminary report has appeared elsewhere (Loewenstein, 1955).

Physiological interest in a possible action of the sympathetic system on sense organs dates from Claude Bernard's (1851) observations of changes in cutaneous sensibility following the extirpation of the cervical ganglion in the cat. Since then, a great number of contradictory claims of a sympathetic influence on many kinds of sense modalities have been presented (see v. Brücke, 1932, for a review). Within the technical limitations then existing the conclusions were based either on subjective tests, on clinical observations or on reflex excitability measurements, without eliminating the possibility of the sympathetic system influencing parts of the reflex arc other than the receptor and, generally, of circulatory changes masking the observed effects.

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METHODS

Isolated skin-nerve preparations of the frog's (*Rana pipiens*) dorsum, abdomen and limbs were used. The preparations were set up in Ringer's solution under oil in a lucite vessel (Fig. 1). Ring *B* and the bottom of the vessel functioned like an embroidery hoop. The excised skin was clamped to the bottom by means of ring *B*, its internal surface facing upwards and serving as floor to the bath.

The afferent activity of the corresponding skin nerves was recorded in oil, while Ringer's solutions *R* could be applied or exchanged with a syringe below the interface or while the external skin surface was stimulated mechanically. Alternatively, for applications of drugs or hormones the Ringer's solution bathing the internal surface of the skin (about 0·4 ml.) could be completely sucked away and replaced by another containing the drug or hormone at the desired concentration.

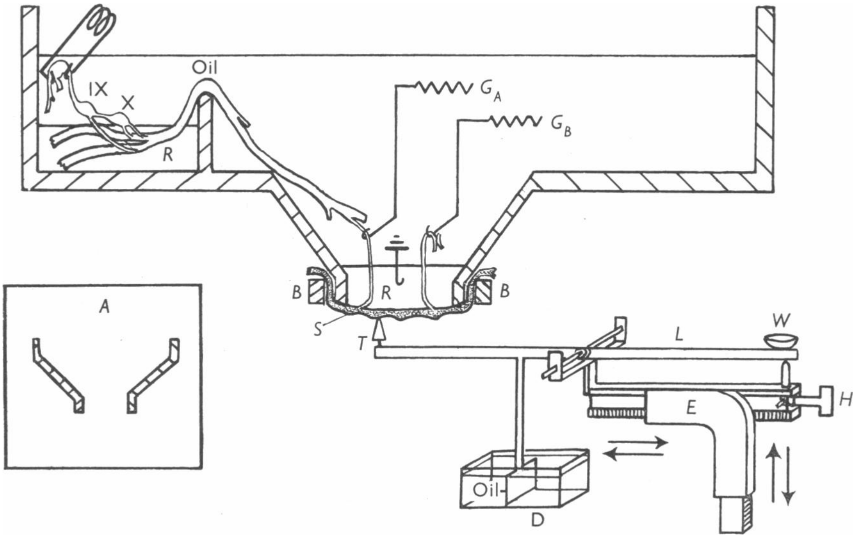

Fig. 1. Electrode arrangements and mechanical stimulator. *R*, Ringer's solution; *S*, skin; *B*, ring serving as skin clamp; *L*, lever for mechanical stimulation of skin; *D*, dashpot; G_A, channel connected with r.c.c.m.; G_B, channel for contiguous overlapping skin nerve; *IX* and *X*, ganglia of the sympathetic chain. Sympathetic chain stimulated with cathode below ganglion VIII.

In experiments in which the cutaneous sympathetic nerve supply was stimulated, a piece of skin from the hind-limb, innervated by ramus cutaneus cruris medialis (r.c.c.m.) was used. The 9th and 10th spinal nerves were cut and dissected from their vertebral emergence and freed up to and together with the offbranching r.c.c.m. The sympathetic chain was prepared as described in an earlier paper (Hutter & Loewenstein, 1955). Thus the isolated preparation consisting of sympathetic chain, sciatic nerve, r.c.c.m. and skin patch was set up in the bath as illustrated in Fig. 1. In a series of experiments a neighbouring fine skin nerve with an overlapping sensory field with r.c.c.m. was also dissected and the afferent activity recorded in both skin nerves, G_A, G_B. The sympathetic chain was stimulated in oil with square voltage pulses of 1 to 2 msec duration, stretches of the sciatic nerve remaining in the Ringer's solution of a small compartment to prevent spread of current to motor or sensory fibres of the sciatic nerve. The stimulating cathode was placed below sympathetic ganglion VIII and the efferent sympathetic volleys recorded at r.c.c.m. Under these conditions mainly preganglionic sympathetic fibres with synapses in ganglia IX and X are stimulated (Hutter & Loewenstein, 1955). Action potentials were fed through condenser-coupled amplifiers into a double-beam oscilloscope.

Mechanical stimulation of the skin was done with lever *L* suspended on clock bearings sensitive to 2–5 mg. An angular lever *H* served as motion trigger. Impact oscillations were damped with an oil dashpot *D*. Mechanical stimuli of 1–3 sec duration were applied to the external skin surface with either of two interchangeable tips *T*, with diameters of 0·25 and 4 mm of contact surface, referred to in the text as fine and broad tip respectively. The diameter of the functionally intact skin was usually 17 mm. In a few experiments, in which a smaller diameter of 8 mm was desired for histological control, a funnel-shaped lucite adapter *A* with ring was pressed to the bottom of the bath. For the study of the effects of stretch a device was constructed which permitted a circular piece of skin to be subjected to circumferential stretching at variable tension, thus, a given point of the skin to which the tactile stimulus was applied would show no detectable displacement at any degree of tension. It is intended to publish a detailed description of the apparatus. When afferent impulses were recorded in single nerve fibres, a functional single fibre was dissected and selected for recording at the entrance of r.c.c.m. into the sciatic trunk.

In view of the results described under the heading of spontaneous tactile activity, all studies on receptor facilitation were carried out on 'slack' preparations giving no spontaneous activity. It should be kept in mind that the present experimental conditions did not allow work at zero stretch. The lowest possible degree of stretch of the preparation, designated by 'slack', is determined by the pressure of the column of fluid of the bath. For the study of spontaneous tactile activity, unless stated otherwise, only well-stretched preparations were used.

Fig. 2 illustrates the arrangement employed for polarization of sensory endings of the skin. Direct currents were passed through the skin by means of two flattened spirals of platinum wire facing the external and internal skin surface respectively. Contact between the external electrode and the skin was generally made with a piece of cotton soaked in Ringer's solution or directly through Ringer's fluid. The internal electrode placed in the Ringer's solution of the bath served usually as the recording ground lead.

Frog's Ringer's solution was used at approx. pH 6·9 of the following ionic composition expressed in m-mole/l.: Na^+, 112·8; K^+, 2·7; Ca^{2+}, 1·8; Cl^-, 117·3; HCO_3, 1·8. Adrenaline chloride (natural, Parke, Davis and Co. or Horton and Converse), adrenaline bitartrate (synthetic, Winthrop-Stearns), noradrenaline bitartrate (Winthrop-Stearns) and acetylcholine chloride (Merck) were used. The experiments were done during the months of October to April at room temperature, varying from 20 to 26° C.

RESULTS

Sympathetic stimulation

Changes in tactile response pattern to an adequate mechanical stimulus. With mechanical stimulation of the skin at constant intensity and at regular intervals, a fairly constant pattern of tactile discharges results. Fig. 3*a* shows such a pattern from a series of mechanical stimulations at 0·5/sec of two active fibres in r.c.c.m. and of two fibres in a neighbouring skin nerve serving an overlapping sensory field with the former. While the mechanical stimulation continues regularly at 0·5/sec at constant strength, the sympathetic nerve supply to the skin is stimulated at 5/sec, causing changes in the pattern (Fig. 3*b*). The duration of the discharge and the total number of tactile spikes increase in both skin nerves under the influence of sympathetic stimulation. The tactile stimulation of the example illustrated in Fig. 3 was done with the fine tip of the lever and a weight of only 250 mg. With a broader stimulated surface and a greater weight, more receptors respond to the mechanical stimulus and sympathetic stimulation brings about a more striking increase in the impulse pattern. For instance, a discharge of 15 tactile impulses lasting 110 msec increased to 25 impulses lasting 260 msec during sympathetic stimulation.

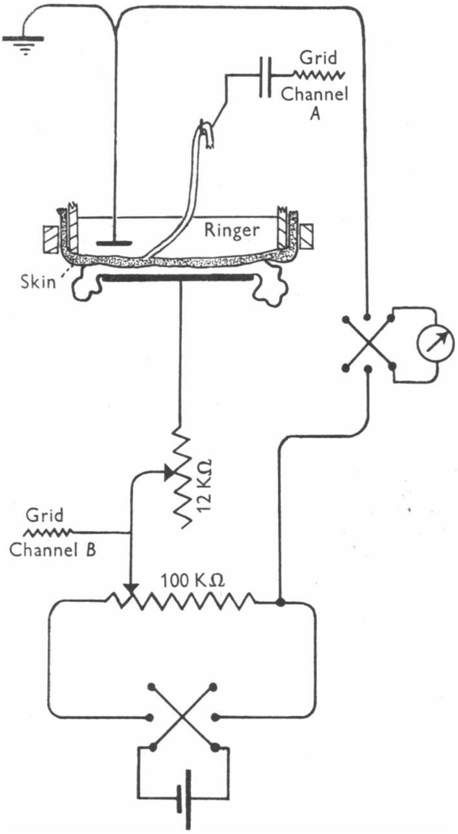

Fig. 2.

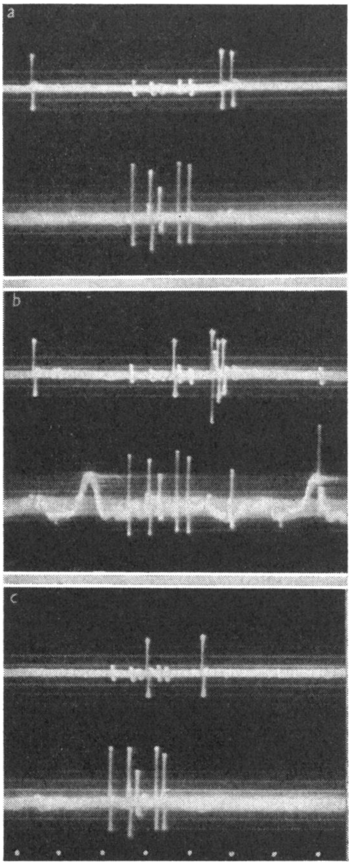

Fig. 3.

Fig. 2. Arrangement for polarization of the skin with direct current. See text for details.

Fig. 3. Changes in threshold and adaptation of tactile receptors due to sympathetic stimulation. Tactile response patterns to a standard mechanical stimulus are recorded simultaneously in r.c.c.m. (lower beam) and in a contiguous overlapping skin nerve (upper beam). Tactile response to 250 mg: *a*, before; *b*, during stimulation of sympathetic chain at 5/sec. Record taken at 8 sec. after start of sympathetic stimulation. Two slow action potentials of the efferent sympathetic volleys are seen in r.c.c.m., together with the fast afferent potentials of tactile fibres. *c*, 12 sec after end of sympathetic stimulation. The small spikes of upper beam are discharges from the tactile fibres of r.c.c.m., which under the recording conditions of this experiment were picked up in the upper beam. Time, 50 msec.

Although this effect is more impressive, a discharge involving only a few receptors, as exemplified in Fig. 3, is more suitable for analysis. Accordingly, most of the experiments under this heading were done with one to three active receptors only. In the skin nerve contiguous to r.c.c.m. a tactile receptor not firing before sympathetic stimulation with the stimulus of 250 mg, is thrown into activity with the same weight under the influence of the incoming sympathetic volleys to the skin, indicating a lowering of threshold (Fig. 3). Another receptor discharging 2 impulses before sympathetic stimulation fires an extra impulse during sympathetic stimulation. Similar effects are seen in r.c.c.m.

Decrease in threshold and increase in the number of discharged impulses of tactile receptors were found in all preparations tested, whenever sympathetic impulses could be detected in the skin nerves. For brevity this effect will henceforth be referred to in the text as 'receptor facilitation'. The effect could, however, not be shown in all tactile receptors of a given preparation. An average of one out of three experiments, several being done on each skin preparation stimulating mechanically different spots, showed an increased amount of discharges and one out of seven lowered threshold. The lowering of threshold seldom amounts to more than 10 %, the change in the number of fired impulses being the more prominent feature. The number of impulses discharged by a single tactile receptor in response to a given mechanical stimulus was found to increase by from 1·25- to 15-fold. In a series of experiments the number of impulses discharged in response to mechanical stimuli of threshold value was found to increase up to 12-fold during sympathetic stimulation. Since the stimulus strength was held at threshold before and during sympathetic stimulation, the enhancement of the discharge can be interpreted as a lowering of adaptation rate of the receptor. The facilitation is preceded by a latent period of 2–4 sec and outlasts sympathetic stimulation by 5–25 sec, the original pattern of tactile discharge being recovered thereafter.

Besides the highly adaptive tactile receptor another mechanoreceptor of much slower adaptation is present in the skin of the frog and toad (Fessard & Segers, 1943*a*, *b*; Maruhashi, Mizuguchi & Tasaki, 1952). This receptor is insensitive to light tactile stimulation but responds readily to stretch applied to the skin, presenting a static discharge and the characteristics of a stretch receptor (Loewenstein, in preparation). Its impulses are conveyed in more slowly conducting axons than the tactile fibres, its action potentials recorded in a compound skin nerve are considerably smaller than the potentials of tactile fibres. The static activity of this stretch receptor, as recorded at various degrees of stretch, remains unaffected by sympathetic stimulation (Figs. 4, 7).

Spontaneous tactile activity. In some extreme cases of facilitation, sympathetic stimulation turns the ordinarily highly adaptive tactile receptor into a slowly adapting one. A given mechanical stimulus, originally producing one single tactile impulse, after sympathetic stimulation may cause a tactile ending to fire up to fifteen times.

Frequently sympathetic stimulation produces spontaneous discharges in tactile endings in the absence of all 'adequate mechanical stimulus'. The 'adequate mechanical stimulus' is here defined as the mechanical stimulus applied to the exterior surface of the skin which ordinarily, without sympathetic activation or other kinds of sensitization, produces firing of touch receptors. Fig. 4 illustrates a typical example. The arrival of four sympathetic volleys to the skin, conveyed by r.c.c.m., causes the heretofore silent tactile receptors of this and of the adjacent skin-nerve to fire in the absence of all mechanical stimulus. For brevity, the effect will henceforth be called 'spontaneous tactile activity'. The spontaneous tactile activity has a latent

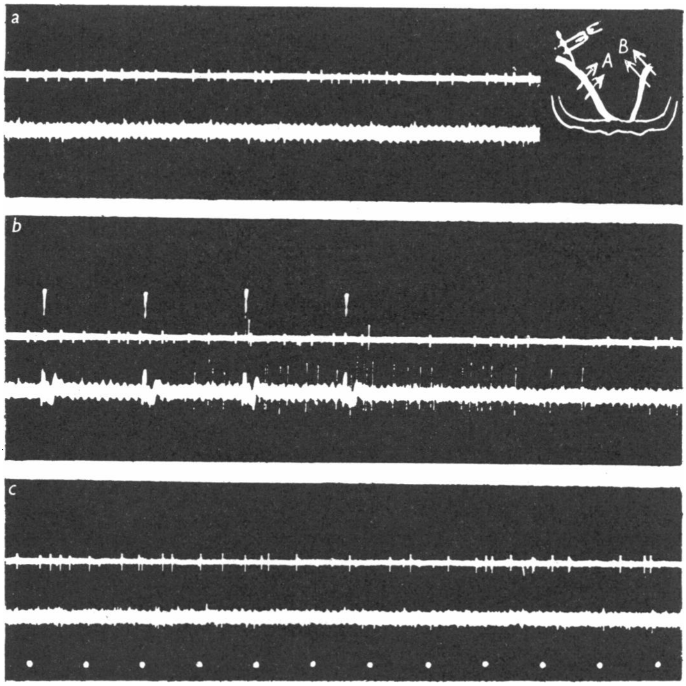

Fig. 4. Spontaneous activity in tactile receptors due to sympathetic stimulation. Records are taken in r.c.c.m. (*A*, lower beam) and adjacent overlapping skin nerve (*B*, upper beam). *a*, tactile silence of the undisturbed preparation; *b*, after the arrival of four sympathetic volleys at 1 per sec to the skin. Note the abundant tactile discharges in r.c.c.m. starting after the arrival of the second sympathetic volley. Two tactile impulses are discharged in neighbouring nerve. The four large deflexions of upper beam are stimulus artifacts. *c*, 5 sec after end of sympathetic stimulation. The small potentials of upper beam in *a*, *b* and *c* are from stretch fibres: note that their activity is not noticeably altered by sympathetic stimulation. Time, 600 msec.

period of 1–4 sec and outlasts sympathetic stimulation by from 0·25 to 15 sec. Reversal of the effect is always obtained.

No regular relationship between the number or frequency of sympathetic volleys and the resulting afferent tactile impulses was found. It is noteworthy that often a few sympathetic impulses were sufficient to trigger the spontaneous activity (see Fig. 4). For instance, a discharge of a single tactile unit containing 12 impulses lasting 3 sec followed the arrival of one single sympathetic impulse to the skin. After prolonged sympathetic stimulation (over 1 min) at 10 per sec, intense spontaneous tactile activity lasting more than 40 sec and containing periodic bursts of impulses was sometimes seen (Fig. 11*a*). Once obtained in a given preparation, the spontaneous activity can repeatedly be produced, but here the above-mentioned fickleness of the effect becomes most apparent: the minimal number and frequency of sympathetic stimuli required to produce the effect, on the one hand, and the number of resulting tactile impulses, on the other, vary greatly in successive sympathetic stimulations at regular intervals. Lability of transmission at the sympathetic ganglionic synapses cannot be blamed for this behaviour, since frequencies of 10 per sec or higher are well carried across the sympathetic synapses of the present preparation.

The degree of initial stretch of the skin is important for the appearance of spontaneous tactile activity. In slack preparations the effect is less frequently found than in well-stretched ones. The relationship between stretch and spontaneous activity is studied in the section on adrenaline.

Sympathetic stimulation has no detectable spontaneous effect on the stretch receptor of the skin.

Adrenaline and noradrenaline

Changes in tactile response pattern to an adequate mechanical stimulus. The described sympathetic effects are readily reproduced with adrenaline (natural or synthetic). All experiments with adrenaline or noradrenaline were done, unless stated otherwise, with concentrations of 1 g in 10^6 ml. Fig. 5 shows an experiment in which application of adrenaline to the inner side of the skin changes the tactile discharge pattern to a standard mechanical stimulus of 200 mg in a sympathomimetic fashion. The total duration of the discharge is prolonged from 39 to 245 msec and the number of contained impulses increased from 4 to 7. Both lowering of threshold, as indicated by recruitment of a new tactile fibre, and slowing of adaptation of two receptors have taken place.

A rough assessment of the increase in tactile excitability produced by adrenaline was gained as follows: a weight was chosen which would cause one to three receptors to discharge, being below threshold by a measurable amount of weight for another receptor. For example 150 mg would fire two receptors. With application of adrenaline a new third receptor joins the discharge pattern. Before the application of the hormone in order to bring this receptor into activity the mechanical stimulus had to be augmented by 15 mg. The lowering of threshold with adrenaline is expressed crudely by the additional weight. The average lowering of threshold was about 10%. Quantitatively more prominent is the change in adaptation rate caused by adrenaline. The number of discharges of a single tactile receptor in response to a given mechanical stimulus increase by from 1·20 to 18 times under the influence of adrenaline. Ten out of fifteen preparations yielded the effect. The latent period of the facilitation caused by adrenaline (4–15 sec) is longer and considerably more variable than the latency of the sympathetic effect. Reversal is usually obtained, but requires prolonged washing of the preparation.

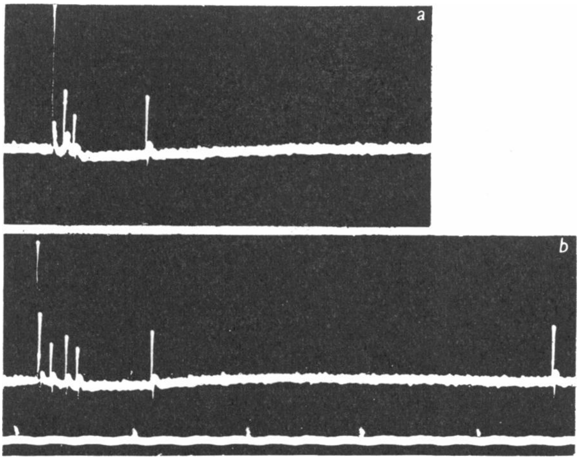

Fig. 5. Changes in threshold and adaptation of tactile receptors caused by adrenaline. Tactile response to 200 mg: *a*, before, *b*, after application of adrenaline, 1 g in 10^6 ml. to the inner skin surface. Time, 50 msec.

Spontaneous tactile activity. In well-stretched preparations application of adrenaline produces a long lasting spontaneous activity of tactile endings in the absence of all adequate mechanical stimulation. The effect was found in 80% of the preparations tested, and generally involves a larger number of tactile fibres than the analogous phenomenon caused by sympathetic stimulation. The time course of a typical spontaneous discharge, recorded in a cutaneous nerve containing multiple active fibres, is shown in Fig. 6. After a latent period of 4 sec a great number of receptors start to fire, the frequency of the total number of impulses reaching a peak at 10–15 sec after application of the hormone.

In fifteen preparations application of adrenaline resulted in peak frequencies varying between 5 and 35 impulses/sec in multifibre preparations. The time course of the discharges often presented two maxima as in Fig. 6. In the same preparation, under equal conditions, successive applications at regular intervals of 20 min often yielded similar time courses, the peak frequencies showing always a declining trend. As with sympathetic stimulation, stretch is a factor

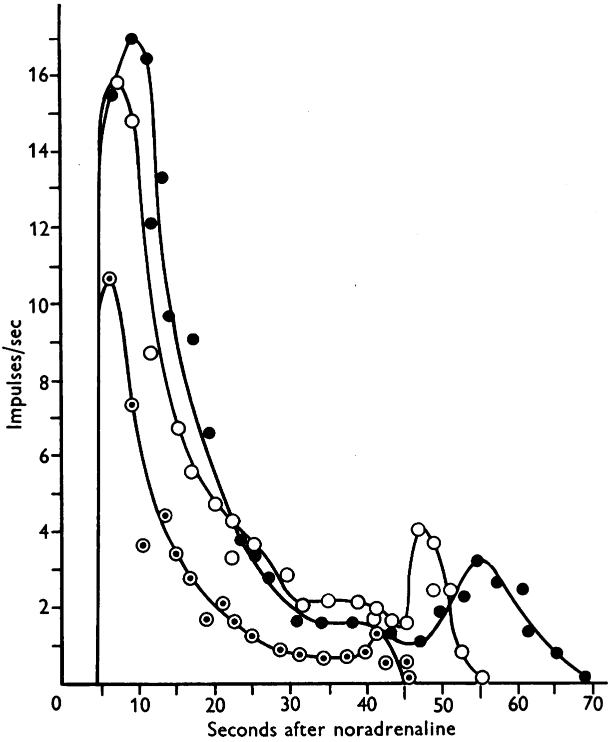

Fig. 6. Spontaneous tactile activity caused by noradrenaline. ●—●, first; ○—○, second; ⊙—⊙, third application of 1 g in 10^6 ml. noradrenaline at 20 min intervals. Ordinates, total frequency of tactile impulses in a multifibre preparation.

favouring the production of spontaneous tactile activity by adrenaline. Fig. 7 gives an example of the influence of stretch. A small nerve bundle is dissected containing two functioning tactile fibres and one stretch fibre with static discharge. Equal doses of adrenaline are applied at intervals of 15 min, while the skin is subjected to a different degree of stretch before each application. The number of tactile impulses augments with increasing stretch in both tactile receptors even against the gradient of time. One of the tactile receptors not activated by adrenaline at lower degrees of stretch is thrown into activity at high stretch. Although stretch is a favouring factor it is not an indispensable condition for all tactile fibres. Spontaneous firing with adrenaline occurs also in 'slack' preparations (see methods) under the experimental conditions of the present work.

Besides the small fibres (Maruhashi *et al.* 1952), the frog's skin presents large fibres sensitive to relatively low concentrations (10^{-3} M) of acetic acid (Diecke, personal communication). The characteristics of their action potentials are apparently the same as those of tactile fibres. Even if their acid specificity remains to be decided, it seemed desirable to ascertain whether the spontaneous receptor activity was actually produced, not by tactile fibres but by acid or other kinds of specific cutaneous receptors. Experiments done with anatomically single tactile fibres yielding spontaneous tactile activity with adrenaline ruled out the former possibility.

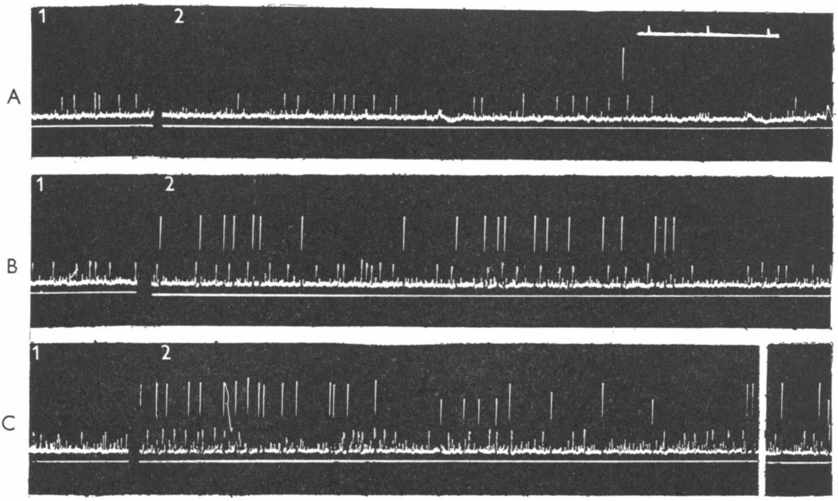

Fig. 7. Effect of skin tension on spontaneous tactile activity caused by adrenaline. Records from a dissected bundle containing two functioning tactile fibres (large spikes) and one stretch fibre (small spikes) at three degrees of initial stretch increasing from A to C. 1, before; 2, after application of 1 g in 10^6 ml. adrenaline. Note that one tactile receptor is activated by adrenaline only at highest stretch. The gap in C means 4·5 sec of tactile silence. Time, 1 sec.

The minimal effective dose of adrenaline causing spontaneous tactile activity was of the order of 1 g in 10^8 ml.

Applications of noradrenaline gave results in all respects similar to those observed with adrenaline. Applications of the amines did not affect the activity of the cutaneous stretch receptor.

Direct current

Changes in tactile response pattern to an adequate mechanical stimulus. The prolonged latent period and duration of the sympathetic receptor facilitation and spontaneous tactile activity strongly suggest the release, at the sympathetic endings of the skin, of a chemical mediator taking some time to reach the tactile endings. It seems desirable to gain histological support for this idea, but, as far as I know, histological studies of the sympathetic innervation of the frog's skin have not been done. The above assumption rests on grounds of analogy with findings in mammals. Boecke (1933) described a Meissner's corpuscle with a sympathetic reticulum coiled around its body. Release of a mediator is also postulated for the sympathetic terminals surrounding the neuromuscular junction. There is little doubt that an adrenaline-like substance is the intermediary of the sympathetic neuromuscular facilitation. In fact, Lehmann (see Tiegs, 1953) and Tiegs (1935) have given direct evidence for it. Let it be assumed here, until further analysis below, that adrenaline or noradrenaline is the mediator of the facilitatory effects on tactile receptors. It is difficult to escape the thought that adrenaline has an electrogenic action. Actually, the response of the tactile receptor to adrenaline bears close resemblance to the behaviour of these receptors under the influence of a direct current. When a direct current is flowing through the frog's skin, action potentials are recorded in tactile fibres (Maruhashi *et al.* 1952; Alanis, 1953). With current intensities below the threshold of firing, modification of the tactile response pattern to an adequate mechanical stimulus is observed. Fig. 8 illustrates this in a representative case. While the skin is stimulated mechanically at regular intervals with the lever device, a subthreshold direct current is passed, flowing from outside to inside through the skin, causing an immediate increase in the number of impulses and duration of the discharge. Lowering of threshold and slowing of adaptation, the latter more frequently than the former, were consistently found. Other cases of interaction in mechanoreceptors between a direct current and a mechanical stimulus have been reported for the labyrinthine end-organs of the ray (O. Lowenstein, 1955) and for the nerve endings of the frog's muscle spindle (Matthews, 1931; Edwards, 1954).

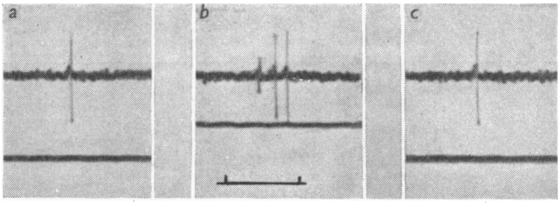

Fig. 8. Facilitation of a tactile response to a mechanical stimulus by a subthreshold current. Tactile response to 100 mg: *a*, before; *b*, during; *c*, after passing direct current of 0·06 mA inwardly across the skin. Time, 50 msec.

Spontaneous tactile activity. With periodical stimulation with stepwise increasing currents flowing inwardly through the skin, the number of tactile impulses and the duration of the discharge augment until finally an intensity is reached causing a relatively slow adapting discharge lasting for about a minute (Fig. 9). It cannot be said with certainty whether the current actually excites the nerve terminal or the thick fibre. However, the interaction of a mechanical stimulus and a subthreshold current can only be understood to have taken place at the receptor level and it becomes likely, therefore, that with threshold and suprathreshold currents the receptor ending is excited too.

Many of the characteristics of nerve fibres under the influence of a direct current, such as proportionality of discharged impulses and stimulating current, cathodal and postanodal changes of excitability (cf. Skoglund, 1942; Granit & Skoglund, 1943; Hodgkin, 1948), were found to be also valid for the receptor.

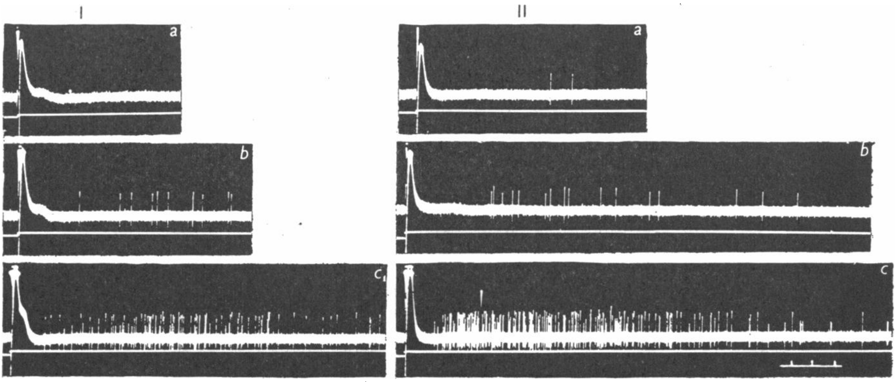

Fig. 9. Interaction of adrenaline and a direct current. Tactile response to *a*, 0·08; *b*, 0·1; *c*. 0·15 mA recorded in a skin nerve, I before and II after addition of subthreshold dose of adrenaline. Time, 600 msec.

Characteristically, excitation of the tactile endings only occurs with a current flowing from outside to inside through the skin. Within certain limits of intensity, currents flowing in an opposite direction not only fail to excite but may depress the receptor's excitability to an inwardly flowing current even for several seconds after reversal of the current.

Interaction of adrenaline and a direct current. The similarities between the effects of a direct current and adrenaline on tactile receptors prompted the search for an interaction between the two agents. I have, in fact, found that subthreshold doses of adrenaline add up to the threshold of firing of tactile units with subthreshold currents and that the response of touch fibres to threshold or suprathreshold direct currents is potentiated with applications of a subthreshold dose of adrenaline (Fig. 9).

The sympathetic innervation of the skin

On stimulation of the sympathetic chain three groups of sympathetic fibres are regularly detected in the sciatic nerve (Fig. 10, II*a*). We find a fast group conducting at velocities between 3 and 6 m/sec at 20–26° C, and a slower group conducting at 0·4–0·6 m/sec. Both components appear simultaneously as the stimulating voltage is increased. With a stimulus strength about ten times that necessary to demonstrate these groups, we detect a third group of fibres conducting at 0·2 m/sec (Erlanger & Gasser, 1930; Bishop & O'Leary, 1938; Hutter & Loewenstein, 1955). In the motor nerves only the slowest-conducting group is present (Fig. 10, II*b*), this group being responsible for a facilitation at the neuromuscular junction (Hutter & Loewenstein, 1955). The skin nerves contain all three components of the sympathetic spectrum, although considerably more dispersed (Fig. 10, II*c*). The relatively small number of sympathetic fibres running in the fine skin nerves contributing little to the integration of the potentials accounts for this dispersion.

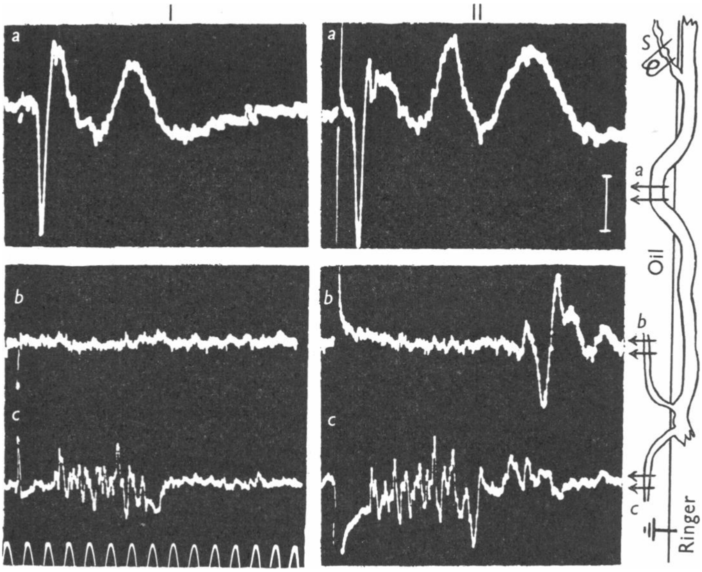

Fig. 10. Sympathetic fibre spectrum of cutaneous and motor nerves. Sympathetic chain *S* is stimulated with cathode below ganglion VIII, I with maximal intensity for the two faster fibre groups only; II with maximal intensity for all groups. Records taken at: *a*, sciatic trunk; *b*, motor nerve to gastrocnemius; *c*, skin nerve r.c.c.m.: *b* and *c* taken simultaneously. Stimulation and recording is done in oil while portions of the sciatic not in contact with electrodes are submerged in Ringer's solution. Calibration of abscissa 50 cycles; ordinates 50 mV.

Which are the sympathetic fibres responsible for the facilitation and spontaneous activity observed in tactile receptors? The large difference in threshold between the slowest-conducting group, on one hand, and the two 'faster' groups, on the other, made it easy to establish that the sympathetic tactile effects are concomitant with the appearance of the two 'faster' components, but do not depend on the 'slowest' group. It was not possible, owing to their practically equal thresholds, further to distinguish between the 'faster' fibre groups for decision on the origin of the effect.

The mediator

In view of the long latency and the analogous results with adrenaline, it was held implicit throughout the present work that the sympathetic fibres involved were adrenergic and that the effect was mediated by a substance. Fortunately the high sensitivity to adrenaline of well-stretched preparations, yielding spontaneous tactile activity, made it possible to give direct evidence for the release of a substance and its nature. Two skin preparations were set up in these experiments (Fig. 11). The sympathetic supply to preparation S_1 was stimulated for 1 min at 10 per sec, spontaneous tactile activity being detected in the skin nerve of S_1. The Ringer's solution bathing preparation S_1 was now drained to the second preparation S_2. After a latent period of 10 sec, tactile impulses were recorded in the cutaneous nerve of S_2. Previous controls with the superfusate of S_1 gave negative results. After another period of 1 min of sympathetic stimulation at 10 per sec in S_1, with its consecutive tactile discharge, the Ringer's solution of S_1 was now used to perfuse a sensitive, isolated, spontaneously beating frog's heart (*Rana pipiens*). A positive chrono- and inotropic cardiac effect could be detected. The results show that an adrenaline-like substance is released during sympathetic stimulation in one preparation which is able to reproduce the sympathetic tactile effects in another one.

Although the experiments were not specially designed for quantitative purposes, the high sensitivity of the tactile receptors to the released adrenaline-like substance was revealed by the following observation: while in about half of nine pairs of skin preparations tested, S_2 would respond with tactile activity to the content of S_1, a proportion of only approximately one out of ten frog's hearts was found sensitive enough to show a chrono- or inotropic effect with the content of S_1. These hearts gave a clear response to adrenaline at a concentration of 10^{-8} g/ml., comparable to the sensitivity of those in Loewi's (1921, 1936) classical experiments. The tactile ending in the frog thus compares in sensitivity with pharmacological test organs for adrenaline.

Acetylcholine. Before the results described in the previous section were obtained, and in the hope of gaining some differential evidence on whether the effect is mediated by cholinergic or adrenergic cutaneous sympathetic fibres, a few experiments with acetylcholine were done. It appeared of general interest to study the action of acetylcholine on nerve endings in view of the well-known depolarizing action at junctions. While adrenaline and sympathetic stimulation affect selectively the tactile endings, acetylcholine was found to act mainly on other types of receptors, the impulses of which travel in smaller fibres. With concentrations of 7 g in 10^6 ml. the spontaneous small fibre activity of the skin nerves increased two- to threefold its original frequency and, at the same time, very slowly conducted potentials appeared (Fig. 12). It is not safe to state anything else for the present about the functional individuality of these fibres than that they are not tactile ones. At concentrations of 1 g in 10^5 ml. and higher generally some tactile fibres were also activated, discharging at average frequencies of 0·1–5/sec; but this effect looks meagre indeed if compared with the tactile activity produced by adrenaline. The results with acetylcholine on tactile fibres are in accord with Jarrett's (1955) recent report on excitation of tactile fibres of the frog's skin with acetylcholine. After prolonged washing reversal is obtained.

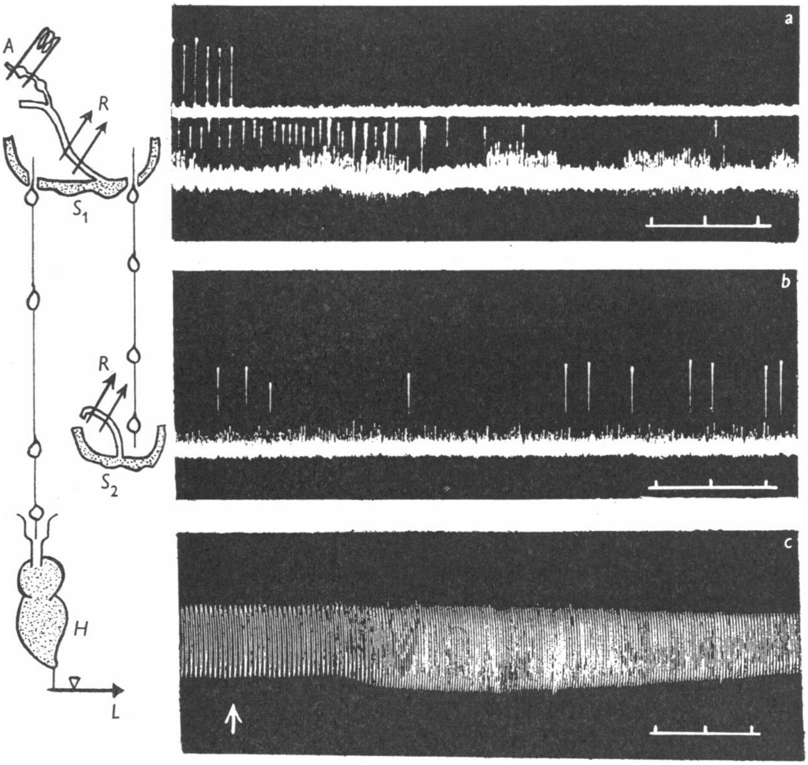

Fig. 11. The adrenaline-like mediator. *a*, stimulation of sympathetic supply *A* to skin preparation S_1 results in tactile activity in the hitherto silent skin nerve *R* (lower beam). Note burst-like discharge of touch receptors. Upper beam shows last artifacts of 1 min stimulation at 10 per sec. Time, 500 msec. *b*, spontaneous tactile activity resulting in nerve *R* of skin preparation S_2 starting 10 sec after bathing S_2 with Ringer's solution drained from S_1 after end of sympathetic stimulation in S_1. Time, 500 msec. *c*, effects on isolated frog's heart *H* perfused with Ringer's solution content of S_1 after a new sympathetic stimulation in *a*. Arrow marks start of perfusion with content of S_1. Kymographic record. Time, 10 sec.

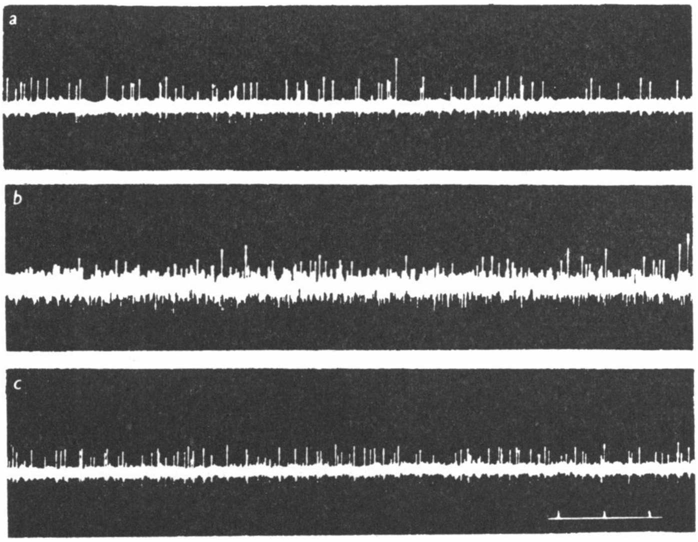

Fig. 12. Effect of acetylcholine on small fibre activity. Spontaneous activity in cutaneous small fibres: *a*, before; *b*, 10 sec after application of acetylcholine 8 g in 10^6 ml.; *c*, after washing. Time 500 msec.

DISCUSSION

Chronologically, the spontaneous tactile activity in dorsal skin, where smooth musculature is well known to exist, was my first observation. This motivated a series of experiments with preparations of reduced diameter (8 mm) which, once a spontaneous effect with adrenaline was obtained in them, were serially sectioned and searched for cutaneous smooth muscle. Skin pieces of the abdomen were used for this purpose, since smooth musculature is very rare in this region (Ecker & Wiedersheim, 1896). At least in three preparations which had given good spontaneous activity no smooth muscle was found. These histological controls, although eliminating one possibility, are not conclusive, since movements of blood vessels as sources of tactile excitation cannot be entirely excluded even in a non-circulated preparation. The later results on receptor facilitation, considered in conjunction with the mentioned histological controls, were more decisive. The interaction of a mechanical adequate stimulus and adrenaline, as revealed by the changes in threshold and adaptation rate and, furthermore, the interaction between a direct current and adrenaline, point toward a direct effect of the hormone on the receptor. Of special significance in this regard are the results with subthreshold doses of adrenaline potentiated to threshold of firing of tactile fibres with subthreshold currents. It seems justifiable to infer that the facilitation and the spontaneous activity of tactile units caused by sympathetic stimulation or application of the hormone are true receptor phenomena.

Spontaneous activity and receptor facilitation have been treated as separate events throughout this paper only in order to render description easier. Concerning the underlying mechanisms, and in view of the following considerations, the spontaneous tactile activity and the receptor facilitation should be considered as fundamentally the same phenomenon. The present experiments demonstrate that the frequency of a receptor discharge may be modulated by sympathetic stimulation or by adrenaline. They do not, however, show how this increase is brought about, since the cutaneous tactile receptor of the frog is not suitable for recording of the generator potential. It seems that two mechanisms can account for the observed effects. Either adrenaline is truly a depolarizing agent of tactile endings or it modifies the generator potential of the receptor. The first possibility implies that adrenaline by itself is excitatory and needs no further explanation; the second, that for the excitation of the receptor, i.e. the setting up of a generator potential, a mechanical or other stimulus is required. Increase in steepness of the rising phase of the receptor's generator potential, such as is observed in the heart fibre with adrenaline (Hutter & Trautwein, personal communication) or an increase of its time constant of decay may account for the phenomenon of receptor facilitation, an extra tactile impulse fired during the slowing of adaptation being thus comparable to an extra systole of the heart caused by the same hormone. In the light of this hypothesis, the spontaneous activity in tactile units becomes a special case of receptor facilitation in which, instead of the adequate tactile stimulus applied to the external face of the skin, stretch is interacting with adrenaline. Stretch alone applied to the skin is able to excite tactile endings in a way similar to an adequate mechanical stimulus acting on the external surface of the skin. At low degrees of stretch fast-adapting discharges occur, but with stepwise increasing stretch, the tactile receptors become less and less adaptive until at high tension they behave like non-adaptive stretch receptors or as in a spontaneous discharge with adrenaline, firing for a minute or so. It is conceivable, therefore, that, as with an adequate mechanical stimulus, a change in generator potential caused by adrenaline may facilitate the response to stretch, the latter acting as excitatory factor for the ending. The dependency found between the degree of skin stretch and the number of impulses discharged during spontaneous tactile activity caused by adrenaline is consistent with this view. The occurrence of spontaneous tactile activity in apparently slack preparations does not necessarily contradict the hypothesis, since the experimental conditions of the present work did not permit work with preparations at zero tension (see Methods).

Unless one is willing to put up with the idea of sympathetic tactile 'hallucinations' occurring under physiological conditions, the effect of spontaneous tactile activity appears teleologically disturbing. But in view of the above considerations, it cannot be said whether spontaneous firing of tactile fibres caused by sympathetic activity appears at all under physiological conditions of cutaneous stretch and discussion of its physiological meaning is premature.

The rate of adaptation in mechanoreceptors appears to be linked with the time constant of decay of the generator potential. With a continuously sustained mechanical stimulus the generator potential of the fast adapting stretch receptor in the crayfish's dorsal musculature (Eyzaguirre & Kuffler, 1955) and in the Pacinian corpuscle (Alvarez-Buylla & de Arellano, 1953; Gray & Sato, 1953) rapidly decays to subthreshold level, while the generator potentials of the slowly adapting frog's muscle spindle (Katz, 1950) and of the crayfish's slow adapting RM_1 stretch receptor (Eyzaguirre & Kuffler, 1955) are maintained for a relatively long time. Assuming a similar behaviour for the tactile and stretch receptors respectively of the frog's skin, the specificity of the sympathetic effect, exerting influence on the fast adapting tactile but not on the slowly adapting stretch receptor, fits into the picture of adrenaline producing changes in the generator potential, provided the hormone affects the time constants of rise or decay but not the size of the generator potential.

It is not intended to pursue these speculations seriously until direct evidence is available to support them, but it is tempting to compare the actions of adrenaline on structures as diverse as the tactile ending and the motor end-plate. At the latter, adrenaline increases the end-plate potential without apparent change of its time course, suggesting a facilitation of the reaction between the end-plate receptor and acetylcholine rather than a direct action on the membrane permeability (Hutter & Loewenstein, 1955). If, similarly, the increase in magnitude is the only change in generator potential of the tactile ending induced by adrenaline, some kind of selective mechanism of the hormone on tactile receptors must be invoked in order to explain the different behaviour on the fast- and slow-adapting mechanoreceptors of the frog's skin.

The fact that under equal experimental conditions adrenaline causes a greater number of tactile receptors of a given skin area to fire spontaneously than with sympathetic stimulation, suggests that the receptors of a given skin patch receive sympathetic innervation via various skin nerves. That fields of innervation of sympathetic fibres conveyed by some skin nerves overlap with fields of sensory fibres conveyed by other skin nerves, is revealed by the observation that impulses conducted in sympathetic fibres of ramus cruris cutaneus medialis commonly aroused spontaneous activity in tactile fibres of a neighbouring skin nerve. In a few experiments, the sympathetic fibres were stimulated with electrodes placed on the sciatic nerve. This led to spontaneous tactile activity similar to that resulting from stimulation of the sympathetic chain. It would appear therefore that the 'antidromic' effect of Habgood (1950), attributed to antidromic stimulation of sensory fibres, can be explained as resulting from orthodromic stimulation of sympathetic fibres included in the sciatic nerve.

It would appear that the receptor facilitation is part of a more general facilitatory function of the sympathetic system. The facilitation at the receptor level and at the neuromuscular junction places at least two stages of the reflex system under the modulatory influence of the sympathetic system. A sensitization to acetylcholine caused by adrenaline at the spinal synapse has also been reported (Bülbring & Burn, 1941). The possibility therefore exists that a further stage of the reflex system may be facilitated by the sympathetic system. The great sensitivity of the receptor to adrenaline and noradrenaline allows us to think of the sympathetic hormone released at the cutaneous sympathetic terminals, as well as that circulating in the blood stream, serving as a modulator of tactile receptor activity. Teleologically the sympathetic receptor facilitation, as part of a more general facilitatory action of the sympathetic system, is well in accord with Cannon's (1928) picture of a role for readiness in alarm situations of the sympatho-adrenal system.

The physiological principle involved in the present work namely, the interaction of two axonic processes, is exceptional in vertebrates. In the invertebrates (Bullock, 1952) axon to axon relations are common, and one speaks, in view of the fact that transmission of impulses normally occurs, of a synapse. Since between the sympathetic terminals and tactile endings true transmission of impulses is apparently non-existent, the denomination of synapse is not applicable. The term 'modulapse', defined as the interplay of two processes in which the function of one is modulated by the other, is here tentatively proposed.

The results of the present work provide direct evidence for a facilitatory control of the sensory input, located at the receptor level. That modulation of activity occurs at higher levels of the central nervous system, i.e. in cell bodies, is established (Hagbarth & Kerr, 1954; Hernández-Peón & Scherrer, 1955). But apparently the only case in which a direct neural effect upon receptor elements has heretofore been established is that of inhibition occurring in the dendritic processes of a crustacean stretch receptor nerve cell (Kuffler & Eyzaguirre, 1955). Although inhibition of dendrites may not be immediately comparable with modulation of axon terminals, the analogy with the present results underlines the probable similarity between dendrites and sensory axon endings.

SUMMARY

1. Stimulation of the sympathetic nerve supply to an isolated frog's skin preparation causes lowering of threshold and slowing of adaptation in touch receptors.

2. In well-stretched preparations the arrival of sympathetic volleys to the skin may cause spontaneous activity in tactile receptors in absence of all adequate mechanical stimulation.

3. The sympathetic effects on tactile endings are mediated by fibres of the 'faster' groups of the sympathetic spectrum conducting at 0·4–6 m/sec at 20–26° C.

4. Sympathetic stimulation liberates an adrenaline-like substance in the skin able to reproduce the sympathetic effects on tactile receptors of another skin preparation.

5. The facilitatory effects of sympathetic stimulation can be reproduced with adrenaline or noradrenaline. Application of the amines to the inner skin surface causes lowering of threshold, slowing of adaptation rate and, eventually, spontaneous firing in tactile receptors.

6. The degree of tension of the skin is a favouring factor for the appearance and magnitude of the spontaneous tactile activity produced by sympathetic stimulation or addition of sympathetic hormones.

7. Neither sympathetic stimulation nor application of adrenaline or noradrenaline produces detectable changes in the activity of the cutaneous stretch receptor.

8. Subthreshold direct currents flowing from outside to inside through the skin cause decrease of threshold and adaptation rate in touch receptors.

9. Interaction of a direct current and adrenaline at the tactile receptor is shown. It is found that subthreshold currents add up to threshold of tactile firing with a subthreshold dose of adrenaline and that the tactile response to suprathreshold currents is potentiated by subthreshold concentrations of adrenaline.

10. Evidence for modulation of the sensory input at the receptor level is given, and its possible role in a more general facilitatory function of the sympathetic system is discussed.

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