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SYMPATHETIC MODULATION OF MECHANORECEPTOR SENSITIVITY IN FROG SKIN

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SUMMARY

1. Sympathetic effects on the mechanical sensitivity of frog cutaneous mechanoreceptors were examined *in vivo*.

2. Functionally identified units were tested with repetitive mechanical stimuli of threshold intensity during electrical stimulation of the sympathetic trunk.

3. Sympathetic activity resulted in increased sensitivity for three classes of afferents; slowly adapting compression receptors, slowly adapting stroke receptors, and rapidly adapting stroke receptors. Decreased sensitivity was produced in the fourth class, rapidly adapting compression receptors.

4. Preliminary tests of several possible modes of sympathetic influence indicated that blood flow changes, changes in probe–skin coupling and changes in tissue compliance could not account for the observed changes in receptor sensitivity. Na^+ and Cl^- ions, secreted by cutaneous mucous glands were found to be possible contributors to the decreased sensitivity of rapidly adapting compression receptors. Direct neurotransmitter action on the receptors, a likely mechanism of sympathetic action, was not tested.

5. The data indicate that systematic changes in cutaneous sensibility occur with modest changes in sympathetic efferent activity. Possible mechanisms of these sympathetic effects are discussed.

INTRODUCTION

Efferent modulation of sensory input has long been known to occur in vertebrate vision and audition, but it is not widely known to have a role in somatosensation. Evidence for sympathetic modulation of cutaneous sensibility is found in physiological, behavioural and clinical studies. Frog tactile and cold receptors were shown to receive an excitatory sympathetic influence (Loewenstein, 1956; Chernetski, 1964; Spray, 1974). The threshold for detection of vibratory cutaneous stimuli was shown

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to decrease with increasing sympathetic activity in human subjects (Edelberg, 1961). Lumbar sympathectomy in cats was reported to result in hyperaesthesia and hyperalgesia (Bernard, 1851; Dusser de Barenne, 1931; Tourney, 1932). A similar hyperalgesia was reported to occur in some humans after sympathectomy (Tracy & Cockett, 1957). Sympathetic activity has also been found in many studies to exacerbate the pain of causalgia or the sympathetic reflex dystrophies while sympathetic block relieves such pains (reviewed by Bonica, 1979). These several lines of evidence indicate that the sympathetic system does influence cutaneous sensibility through a peripheral mechanism, and that sympathetic activity is most commonly associated with decreased thresholds.

In the present study we re-examined electrophysiologically the influence of sympathetic stimulation on four classes of tactile receptors in intact frogs. The mechanical thresholds of all four classes were found to be modulated by low-frequency sympathetic stimulation, and the direction of change was consistent within each class. The sympathetic effect was found to be qualitatively different between the classes of receptors and, with one exception, appeared not to be mediated by glandular or mechanical responses. These data support the view that cutaneous receptors are subject to efferent modulation and that this effect is not uniform for all receptor classes.

METHODS

Experimental animals

Data were obtained from 110 adult leopard frogs of both sexes acquired throughout the year from two dealers. The frogs originated from Northwestern or Northeastern Mexico and were not identified as to their species but were likely to be *Rana berlandieri forreri*, *R. magnaocularis*, or *R. berlandieri berlandieri* (Bagnara & Frost, 1977). These species do not normally hibernate.

The frogs were held from 1 to 100 days at 21–24 °C with a 12 hr light/dark cycle. Tetracycline hydrochloride (Squibb; 8 mg) was administered daily to most frogs for 5 days following their arrival. Animals with visible skin lesions were not used for the study.

Preparation

Frogs were anaesthetized with tricaine methanesulphonate 0·16 mg/g (Sigma), which produced areflexic preparations with vigorous blood circulation. They were ventilated by positive pressure respiration, and their skin was moistened periodically with deionized water to help maintain gas exchange. Experiments were performed at room temperature (21–24 °C).

A bipolar stimulating electrode was implanted around the sympathetic trunk between the connectives to the sixth and seventh spinal nerves for activation of pre-ganglionic efferent fibres innervating the lower leg (Pick, 1957). In most animals the sympathetic trunk was cut central to its connexion with the sixth spinal nerve.

The ipsilateral peroneal nerve was exposed in the thigh and its muscular branch was cut, as was the posterior tibial nerve. The intact cutaneous branch of the peroneal nerve was desheathed and covered with a pool of mineral oil. Activity in single afferent fibres was recorded from small filaments which had been dissected from the main body of the nerve and placed over a Ag-AgCl electrode.

Stimulation and recording techniques

Supramaximal stimuli were delivered to the sympathetic trunk through a bipolar Ag-AgCl electrode using monophasic 300 μsec pulses at rates of 5 or 20/sec. These stimulus frequencies are within the range of post-ganglionic firing frequencies which can be reflexively evoked in mammals (Douglas & Ritchie, 1957; Koizumi & Brooks, 1972). Two populations of efferent fibres were identified, having conduction velocities of 2–4 m/sec and 0·2–0·4 m/sec (cf. Loewenstein, 1956). The velocity of the faster population suggests that these are myelinated, post-ganglionic fibres, as shown anatomically (Pick, 1957).

An electrodermal potential (e.d.r.) was recorded from the skin of the ipsilateral calf, outside with respect to inside, with a Ag-AgCl electrode placed on the skin near the mechanical stimulus probe. The e.d.r. provided a measure of the activation of cutaneous mucous glands, and thus served as a monitor of the efficacy of electrical stimulation of the sympathetic trunk. The e.d.r. recording amplifier had a 1 MΩ input impedance and a band width of DC to 1 kHz. The Ag-AgCl reference was placed on a thigh muscle.

Mechanical stimuli to the skin of the calf were applied with a cylindrical Teflon probe, 3·0 mm in diameter. Semiconductor strain gauges mounted on the probe linkage allowed measurement of the stimulus forces. A soleonoid drive system was used to deliver repeated, identical step displacements to the probe. The mechanical compliance of this system was less than one tenth that of the stimulated tissue.

Testing procedures

Characterization of receptors. After isolation of a mechanoreceptive afferent unit, tests were made to determine its preferred stimulus characteristics and receptive field size using mechanical stimulation delivered both by hand with cotton-tipped swabs and by the solenoid. Test stimuli included *compressive* stimuli, one or more cotton fibres moved perpendicular to the skin surface, and *stroke* stimuli, one or more cotton fibres dragged across the skin surface. The rate of adaptation of compression receptors was tested with suprathreshold stimuli delivered by the solenoid. For this, the *threshold* stimulus force was first determined (22 msec rise time, 300 msec duration, 0·5 probability of response). Steps of 6 × threshold force and 1 sec in duration then were delivered every 10 sec. Units which responded repetitively throughout such a stimulus were classified as slowly adapting compression (s.a.c.) receptors, whereas rapidly adapting compression (r.a.c.) receptors gave no more than three spikes at onset and offset of the stimulus.

Testing with sympathetic stimulation. A base line response pattern was first established by centring the solenoid-driven probe in the receptive field with the solenoid axis oriented perpendicular to the skin for compression receptors or at an angle of 60–70° to the skin for stroke receptors. When testing compression receptors, mechanical stimulus pulses were applied from a *base line force* of 40 mg wt.; this force was selected as being sufficiently large to maintain stimulus probe contact and sufficiently small to avoid local ischaemia during prolonged testing. Repetitive test stimuli (see above) were delivered with sufficient amplitude to give a probability of response between 0 and 1. When a stable base line response was attained, sympathetic stimulation was imposed for 2–60 sec. Between successive tests of sympathetic influence the probe was lifted from the skin and the skin rinsed with deionized water in order to remove the secretory products of cutaneous mucous glands. This rinsing procedure was essential to maintain electrodermal responses of consistent magnitude and configuration throughout many hours of testing.

Statistical analysis. The data from all acceptable tests of units within each receptor class were time indexed using the onset of sympathetic stimulation as the marker. The mean number of responses to each block of five mechanical stimuli then was calculated for each receptor class. Changes in the mean as a function of time were tested for significance using Student's *t* test (Dixon & Massey, 1957).

RESULTS

Rapidly adapting compression (r.a.c.) receptors. These receptors were exquisitely sensitive to compressive stimuli, most responding to the touch of a single cotton fibre. They responded with three or fewer spikes at the onset of a 6 × threshold step (Fig. 1*B*), and were the most numerous in our sample (88 of 199 or 45%). Mechanical step stimuli of near-threshold intensity elicited responses with relatively invariant latencies, as can be seen in the superimposed traces of Fig. 1*A*. R.a.c. units had the lowest thresholds of the four receptor types tested (Fig. 1*E*), and their thresholds were the same for steps having either 10 or 22 msec rise times in the few units tested with both. They showed little fatigue to repetitive mechanical stimulation (1 pulse every 2 sec).

The effects of *brief, high-frequency* sympathetic stimulation (20/sec for 2 sec) on r.a.c. units varied somewhat among individual afferent units. The probability of response of the r.a.c. unit shown in Fig. 2*A* was reduced by brief sympathetic stimulation, indicating a desensitization of the unit. This was the most common response to brief sympathetic stimulation observed in this class of receptors (see

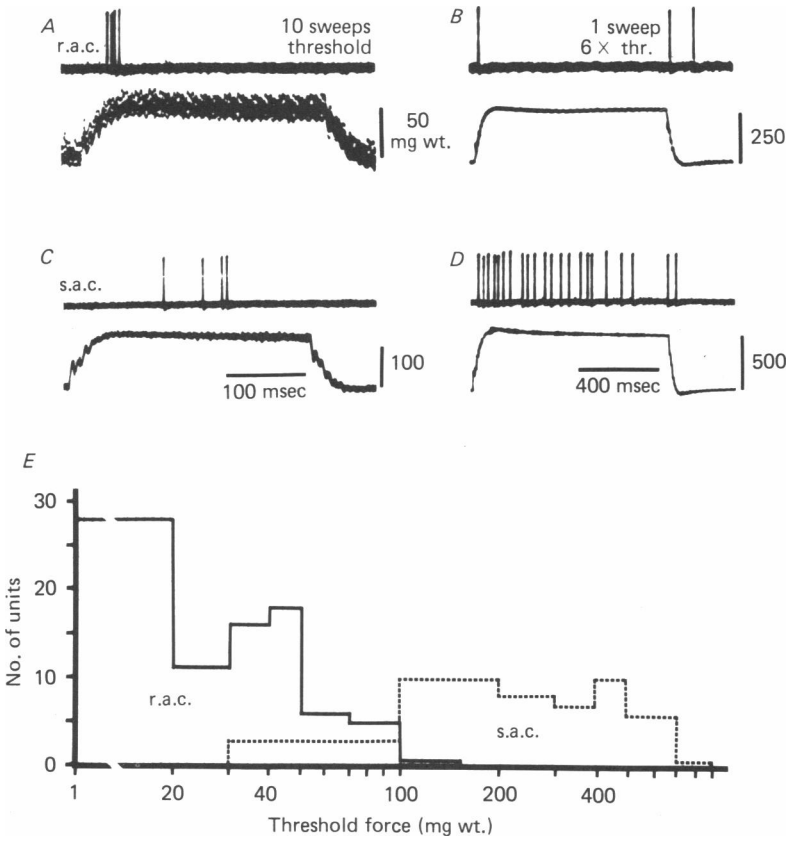

Fig. 1. Response characteristics of compression receptors. The records in *A* and *C* show ten superimposed traces of spike activity and stimulus force during ten consecutive stimuli of threshold intensity ($P \simeq 0{\cdot}5$). Note that the spike latencies are longer and more variable for the slowly adapting compression receptor (*C*) than for the rapidly adapting compression receptor (*A*). The records in *B* and *D* show the responses of the same units to single mechanical stimuli of about 6 × threshold, illustrating the characteristic difference in adaptation rates. The graph in *E* shows the distribution of mechanical thresholds for the two populations of compression receptors.

below). The negative-going e.d.r. that paralleled this reduction in sensitivity can be seen in the upper trace of the oscilloscope record.

More consistent sympathetic effects on r.a.c. receptor sensitivity were obtained with *maintained, low-frequency* sympathetic stimulation (60 sec of 5/sec). A maintained reduction in sensitivity similar to that of the r.a.c. unit shown in Fig. 2*B* was the typical effect.

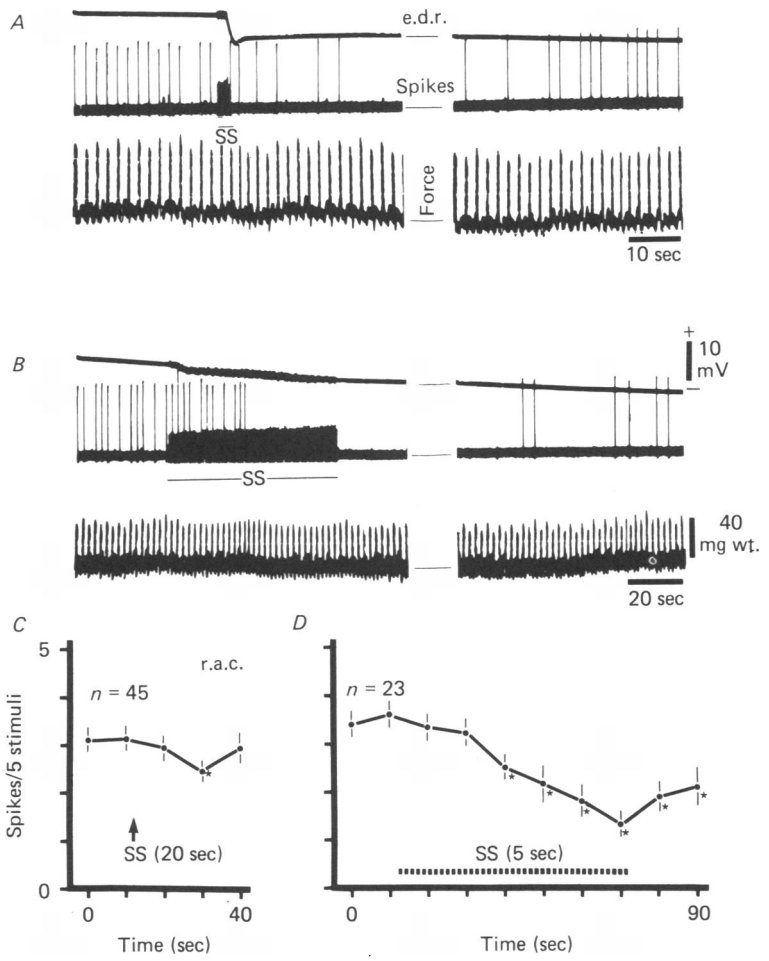

Fig. 2. Sympathetic effects on rapidly adapting compression receptors. The oscilloscope records in *A* show the electrodermal response (e.d.r.) and spike activity for one unit in response to repetitive mechanical stimuli (lower trace) before and after sympathetic stimulation (SS) for 2 sec at 20/sec. Note the decreased probability of response after sympathetic stimulation. *B* shows the same unit during 5/sec sympathetic stimulation for 60 sec. The graphs in *C* and *D* show the mean number of spikes per 5 mechanical stimuli for all r.a.c. receptors tested as functions of time, the data from each unit being time indexed to the onset of sympathetic stimulation. Bars show the standard error of the mean, and the * denotes those data which are significantly different from the pre-sympathetic stimulation activity ($P < 0{\cdot}025$).

Not all r.a.c. receptors responded to sympathetic stimulation in the manner illustrated in Fig. 2. However, the mean number of responses calculated from all units of this type does show a significant decrease during or following sympathetic stimulation. The graph in Fig. 2*C*, compiled from tests of 45 units, shows a small but significant reduction in r.a.c. afferent activity about 20 sec after 20/sec sympathetic stimulation. The reduction in evoked activity with maintained 5/sec sympathetic stimulation shown in Fig. 2*D* is much more pronounced.

The magnitude of the decrease in r.a.c. receptor sensitivity was measured in only a few cases. In these tests, receptors showing a marked decrease in activity during maintained 5/sec sympathetic stimulation (probability of response reduced from 1 to 0) required stimulus force increases of about 30 % over pre-sympathetic stimulation levels to maintain their pre-sympathetic stimulation probabilities of response. Such testing was made difficult by the very sharp thresholds common to r.a.c. units, resulting in increases in their response probability from 0 to 1 with step force increases on the order of 20 mg wt. Thus, precise adjustment of stimulus amplitudes was required in order to allow changes in sensitivity to be measured.

'Spontaneous' unitary activity (spikes occurring before or more than 50 msec after mechanical stimulation) was observed subsequent to sympathetic stimulation in 5 of 48 (about 10 %) r.a.c. receptors studied. This activity generally lasted no more than 10 sec after sympathetic stimulation onset and occurred more commonly in tests with 20/sec than in those with 5/sec.

Slowly adapting compression (s.a.c.) receptors. These receptors were responsive to gentle compressive stimulation with most having thresholds of 100–500 mg wt. (Fig. 1*E*). Response latencies of s.a.c. receptors stimulated at threshold were quite long and variable (Fig. 1*C*) relative to those of r.a.c. receptors, and with mechanical stimulation at 6 × threshold the s.a.c. receptors fired repeatedly throughout a 1 sec stimulus (Fig. 1*D*). Thus s.a.c. receptors appeared to be a class of compression receptors distinguishable from r.a.c. receptors on the basis of three response characteristics: latency variability at threshold, mechanical threshold, and response to 6 × threshold stimulation. S.a.c. units were less numerous than r.a.c. in our sample, accounting for 52 of the 199 units studied (26 %). These receptors showed considerable fatigue to mechanical stimuli delivered once every 2 sec.

In contrast to r.a.c. units, s.a.c. units were commonly sensitized by sympathetic stimulation. One example of this sensitization is shown in the records of Fig. 3*A* and *B*. Note that the probability of response increased following brief, high-frequency sympathetic stimulation (*A*) and during maintained, low-frequency sympathetic stimulation (*B*).

A compilation of the results from all s.a.c. receptors tested with 20/sec sympathetic stimulation is shown in Fig. 3*C*. It can be seen that these receptors responded more readily to mechanical stimulation during the first 10 sec period following sympathetic stimulation. During maintained 5/sec sympathetic stimulation, the only significant changes in the number of evoked responses occurred at 10 and 20 sec after sympathetic stimulation onset (Fig. 3*D*); this facilitation was not maintained.

As a class, s.a.c. receptors showed less pronounced and more variable effects during maintained sympathetic stimulation than any of the other receptor classes. S.a.c. receptors also showed the highest incidence of spontaneous activity subsequent to sympathetic stimulation of any receptor class. Such activity was observed in 54 % of 30 s.a.c. receptors tested with 20/sec sympathetic stimulation, and occurred even when the stimulus probe was not in contact with the skin. Spontaneous activity began as early as 1 sec after the onset of sympathetic stimulation and generally persisted for less than 6 sec, even during maintained sympathetic stimulation.

Slowly adapting stroke (s.a.s.) receptors. These receptors were extremely sensitive to forces oriented tangential to the skin surface, responding vigorously when a single

cotton fibre was dragged slowly across the skin. They did not respond to moderate skin stretch and responded only weakly to compressive stimuli. Thus the observed range of s.a.s. threshold forces (300–4000 mg wt.) obtained with *compressive* stimuli is somewhat misleading, helping to distinguish this type of receptor from compression receptors only when combined with information on preferred stimulus orientation.

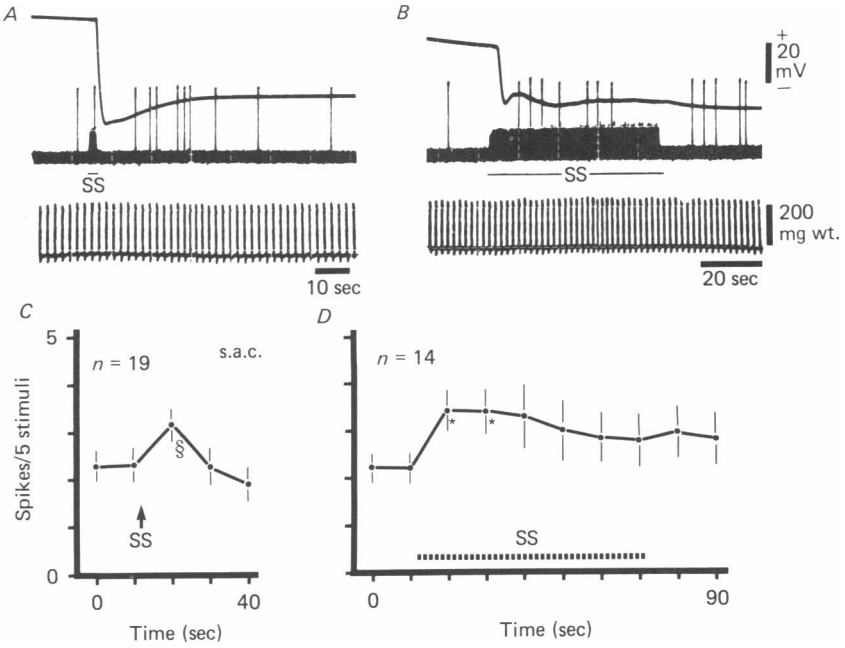

Fig. 3. Sympathetic effects on slowly adapting compression receptors. Records in *A* show the electrodermal response (e.d.r.) and spike activity of one unit during repetitive mechanical stimulation. After sympathetic stimulation (20/sec, 2 sec) the probability of response is seen to increase. For the same unit in *B*, sympathetic stimulation (5/sec, 60 sec) also produced an increased probability of response. The graphs in *C* and *D* show the mean number of spikes per five mechanical stimuli as functions of time for all s.a.c. Note the transient increases in mean response probability for both 20/sec and 5/sec sympathetic stimulation (§ denotes $P < 0{\cdot}05$; * denotes $P < 0{\cdot}025$).

S.a.s. units constituted 18% of all units tested. They showed little fatigue with interstimulus intervals of 2 sec, and only two units (6%) showed spontaneous activity subsequent to sympathetic stimulation.

Examples of the most common effect of sympathetic stimulation on the sensitivity of s.a.s. receptors are shown in the records of Fig. 4. This unit showed a marked transient increase in the number of responses to mechanical stimulation subsequent to a brief 20/sec sympathetic stimulation train (*A*) and a maintained increase during 5/sec sympathetic stimulation (*B*). At both frequencies the facilitatory effect of sympathetic stimulation was remarkably similar for most units in this class.

Changes in the mean response probability of 15 s.a.s. receptors tested with 20/sec sympathetic stimulation are shown in Fig. 4*C*. The marked increase in mechanically evoked activity lasted for more than 30 sec after sympathetic activation. An increased probability of response was also evident in the s.a.s. population tested with

5/sec sympathetic stimulation (Fig. 4*D*). Sensitization at that frequency developed rather slowly, and outlasted the 60 sec period of low-frequency sympathetic stimulation.

Rapidly adapting stroke (r.a.s.) receptors. R.a.s. receptors were the least sensitive of the four receptor types; during hand testing, firm, rapid stroking of the skin was required for their activation. They were relatively unresponsive to skin stretch and

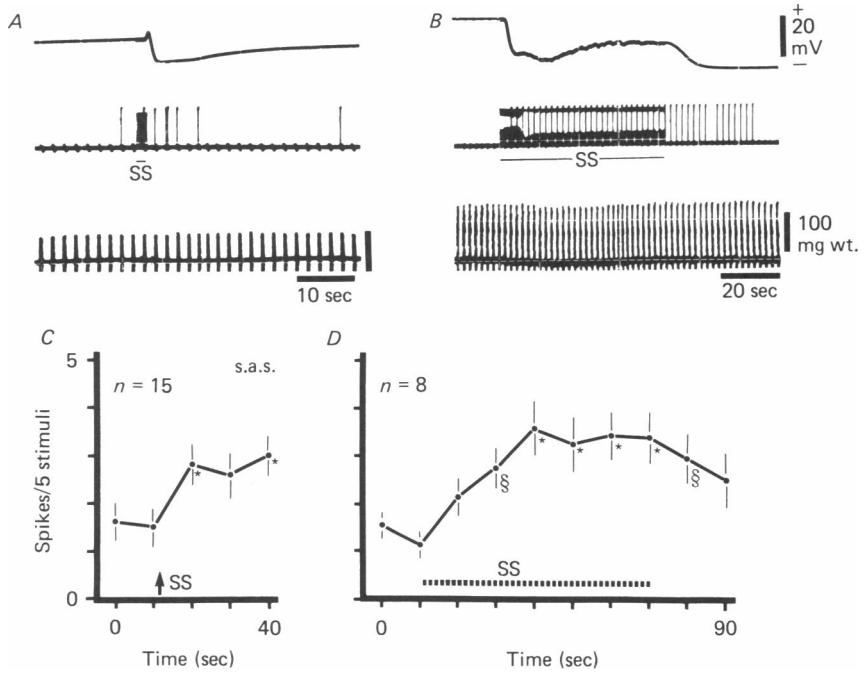

Fig. 4. Sympathetic effects on slowly adapting stroke receptors. The records and graphs are similar to those in Figs. 2 and 3. Characteristic increases in the probability of response are shown for one unit following sympathetic stimulation at 20/sec (*A*) and 5/sec (*B*). Graphs of the mean responses from s.a.s. units (*C* and *D*) show significant increases in response probability at both stimulus frequencies.

to pin prick. In order to elicit responses from r.a.s. receptors with the solenoid-driven probe, it was generally necessary to begin probe movement off the skin and to allow the probe to strike the skin at an angle of $\leqslant 70°$ during a fast step (10 msec rise time). Such a stimulus produced visible dimpling of the skin but was non-noxious when applied to human skin. The response latencies of r.a.s. receptors at threshold showed very little variability. These units showed an unusual type of sensitization during repetitive mechanical stimulation: they did not show increased sensitivity during repeated *subthreshold* stimulation, but once they began to respond they tended to fire with a probability of 1, even when the stimulus intensity was reduced subsequently by as much as 20%.

Twenty r.a.s. afferents (10%) were identified in our total sample of 199 mechanoreceptors. This number represents a minimum estimate because such units have not been described previously in studies of frog cutaneous receptors, and we failed to use appropriate search stimuli during our earlier experiments. In the last seven animals,

3 of 17 (18%) units were identified as r.a.s. receptors. None of the 12 r.a.s. units adequately tested showed spontaneous activity subsequent to sympathetic stimulation.

Conditioning sympathetic stimulation at 20/sec for 2 sec resulted in an increase in mechanically evoked responses from all r.a.s. afferents tested, as shown in the records from one unit in Fig. 5*A*. Similarly, maintained sympathetic stimulation

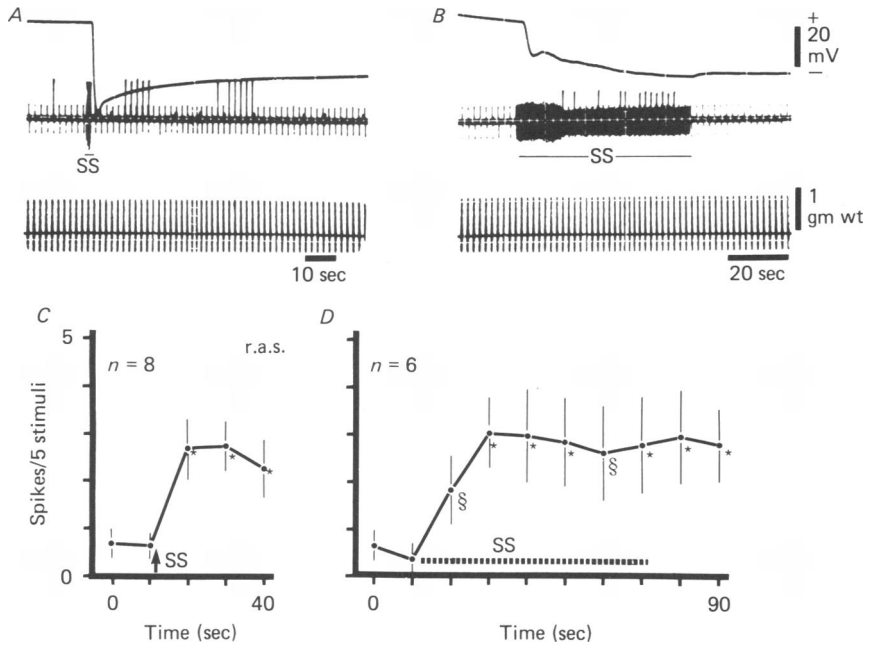

Fig. 5. Sympathetic effects on rapidly adapting stroke receptors. The records and graphs are similar to those in Figs. 2–4. Characteristic increases in response probability are shown for one unit following sympathetic stimulation at 20/sec (*A*) and 5/sec (*B*). Graphs of the mean responses for all r.a.s. units (*C* and *D*) show significant increases in response probability at both stimulus frequencies.

always resulted in a greater probability of response of these units (Fig. 5*B*). The sensitization of r.a.s. receptors with both frequencies of sympathetic stimulation is evident in graphs of the mean responses from all such receptors tested (Fig. 5*C*, *D*).

Tests of glandular and mechanical influences. The changes in receptor sensitivity during sympathetic stimulation might be secondary to changes in blood flow which occur with vasoconstriction. This possibility was tested by simulating the flow reduction with aneurysm clips applied to the sciatic and lateral femoral cutaneous arteries. Eight receptors, including at least one from each class, were tested for sympathetic effects before, during, and after arterial occlusion. No qualitative changes in sympathetic effects on receptor activity were observed during seven to 33 min of occlusion, suggesting that blood flow changes are not responsible for the observed changes in receptor activity which accompany sympathetic activation. However, slow decreases in the sensitivity of some units were noted during arterial occlusion.

The amount of fluid at the probe–skin interface was found to influence the mechanical threshold of some receptors, most commonly r.a.c. receptors. One example of this influence is seen in the records of Fig. 6*A*, in which wetting the interface resulted in a 60% reduction in threshold. Because sympathetic stimulation is known to result in fluid secretion from skin glands (Lindley, 1969; Lang, Sjöberg

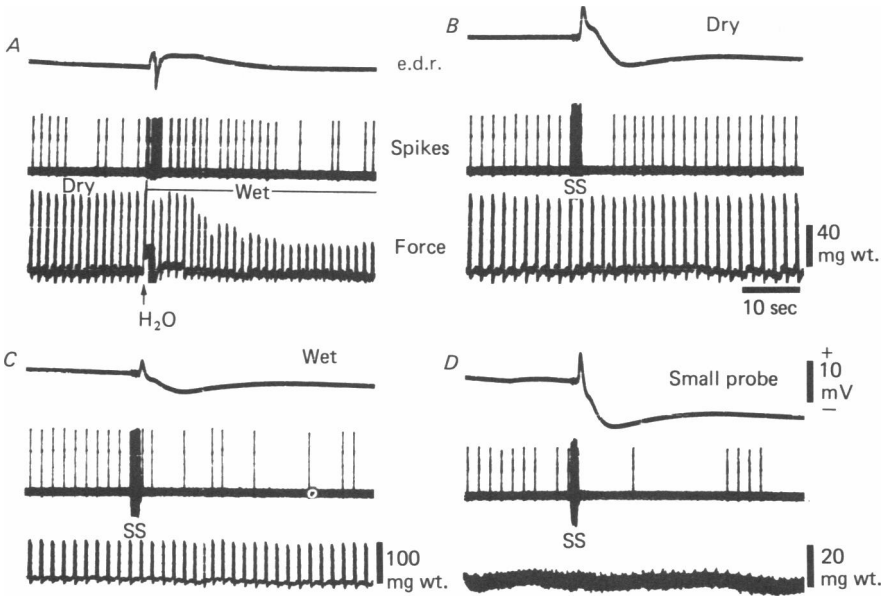

Fig. 6. Similarity of sympathetic effects obtained with both wet and dry probe–skin interface. For the first part of the test shown in *A* the stimulus intensity was adjusted to give a probability of response of about 0·5 for this r.a.c. unit. At the arrow, water was applied topically, and the stimulus intensity was subsequently reduced to again give a probability of 0·5. Note the decrease in threshold force. In *B*, 20/sec sympathetic stimulation for 2 sec resulted in a decreased response probability when the skin was dried before testing. In *C* a qualitatively similar decrease in probability was observed with a 'wet' interface. The test in *D* was conducted with a 0·25 mm diam. probe with 'dry' skin. All tests of this unit showed a decreased response probability following sympathetic stimulation.

& Skoglund, 1975), individual receptors were tested with both a 'dry' and a 'wet' probe–skin interface. The 'dry' condition was produced by wiping the skin with cotton after a water rinse; the 'wet' condition was established by topical application of deionized water while the probe was in contact with the skin. The most common finding, illustrated with records in Fig. 6*B* and *C*, was that the sympathetic effect was qualitatively similar under both conditions. Testing with a small probe (0·25 mm diameter) gave similar results (Fig. 6*D*). To summarize these results: wetting of the probe–skin interface generally reduced the force required for activation of r.a.c. receptors, had little or no effect on the threshold of other types, and for all four classes of receptors tested, the influence of sympathetic activation on their responses to mechanical stimuli is qualitatively independent of the wetness of this interface.

Sympathetic stimulation was observed to alter tissue compliance in some tests. This

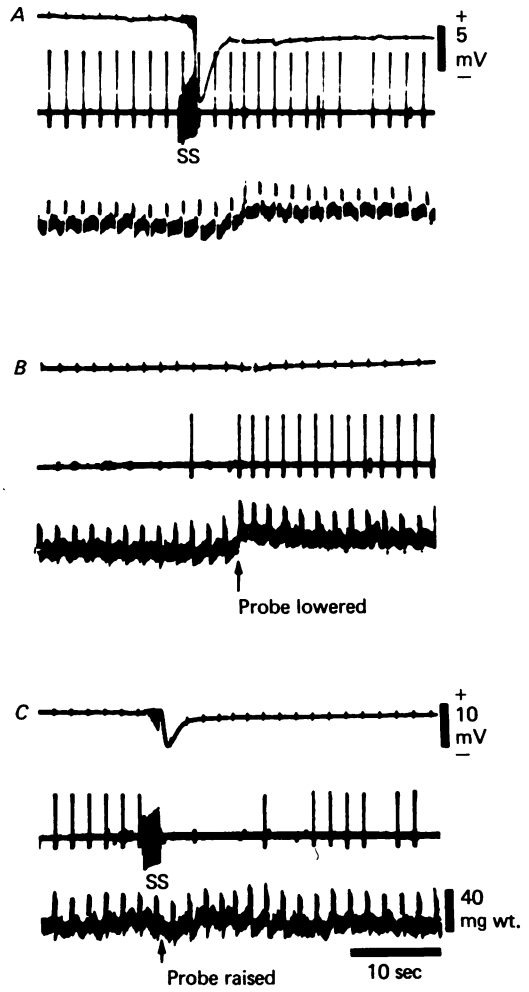

Fig. 7. Influence of sympathetically induced changes in tissue compliance on the apparent sensitivity of a r.a.c. receptor. During the test shown in *A*, 20/sec sympathetic stimulation produced a small increase in the base line stimulus force (lower trace) but little change in response probability. For the test shown in *B* the stimulus probe was simply lowered by about 15 μm to produce a force increase similar to that observed in *A*. Note the increased probability of response in *B*. During the sympathetic test in *C* the experimenter raised the probe slightly after sympathetic stimulation to maintain the initial base line force (40 mg wt.). Note the decrease in probability of response when constant stimulus conditions were maintained.

response appeared either as a change in base line force during a constant skin indentation, or as a change in the observed step force during repeated step displacements of equal amplitude. One example of the former is seen in the records of Fig. 7. In *A* the r.a.c. unit continues to respond to all but one stimulus following brief sympathetic stimulation, however the recorded force increased from its initial 40 mg wt. In the test shown in *B* no sympathetic stimulation was delivered, but

midway through the trace the solenoid was lowered by about 15 μm to stimulate the force increase observed in *A*, resulting in an increase in evoked activity. During test *C* the solenoid position was adjusted by the experimenter during sympathetic stimulation to maintain a constant base line force. This adjustment allowed the sympathetic-induced desensitization of the receptor to appear as a reduction in its response probability.

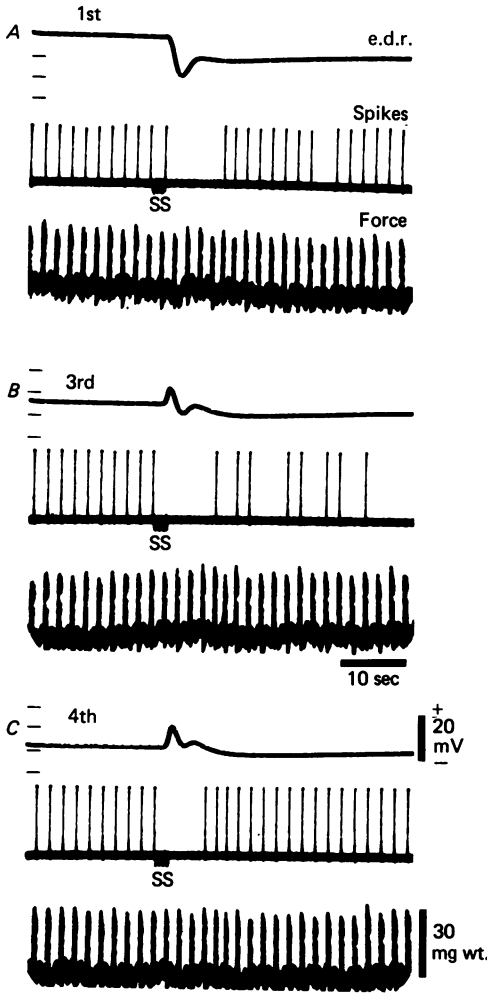

Fig. 8. Similarity of sympathetic effects on r.a.c. receptor during repeated testing without rinsing. The records in *A*, *B* and *C* were taken during the first, third and fourth of successive tests of sympathetic effect. Note that the recorded skin potential (upper trace) became progressively more negative and that the transient in that potential changed, but that the sympathetic effect on the response probability remained qualitatively unchanged.

Such changes in the base line stimulus force were observed in 52 % of all tests of compression receptors. The changes were commonly 10–20 mg wt. in amplitude from a base line of 40 mg wt. and were decreases as often as increases; sometimes changes in opposite direction were observed in consecutive trials at the same probe location. Compliance changes occurred at both rates of sympathetic stimulation, with both wet

and dry probe–skin interfaces, with both large- and small-diameter probes, and during occlusion of the arterial blood supply. The observed changes in receptor response probability were found not to be related consistently to the amplitude, direction, or time course of sympathetic stimulation-evoked changes in the base line force.

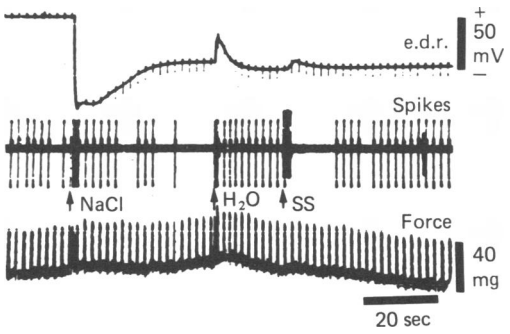

Fig. 9. Similarity of effects between topical NaCl and sympathetic stimulation on r.a.c. receptor. The initial high probability of response of this r.a.c. unit, obtained with a wet probe–skin interface, was reduced by topical application of a 0·2 % solution of NaCl, then restored by rinsing with water, and finally, reduced by sympathetic stimulation at 20/sec for 2 sec.

Two types of tests were done to study the possible influence of cutaneous glandular secretions on receptor sensitivity. In the first, brief periods of sympathetic stimulation were repeated at 3 min intervals without rinsing the skin to remove the accumulated secretory products. This procedure resulted in a progressive reversal of the evoked electrodermal response (e.d.r.) as seen in the records of Fig. 8. Note, however, that the response probability decreased similarly in all three tests. The increasing negativity of the base line e.d.r. reflects increased chloride concentration at the epidermal electrode. In all such tests we saw no consistent correlation between the time course, direction, and amplitude of the e.d.r. and changes in receptor activity.

In another series of tests we applied weak aqueous solutions of NaCl to the skin surface during repeated mechanical stimulation in order to simulate the glandular secretions (McAfee, 1970; Lang, *et al.* 1975). Deionized water was first applied while the probe was in contact with the skin to eliminate effects due solely to wetting of the probe–skin interface. Subsequent application of solutions with sodium chloride concentrations ranging from 0·02 to 0·2 % by weight (cf. House, 1971) produced measured potentials from the skin which were similar in time course and polarity to those resulting from sympathetic stimulation, but were larger in amplitude (Fig. 9, upper trace, compare with Fig. 8*A*).

Such tests were conducted on four to six units in each of three receptor classes (s.a.c., s.a.s., r.a.s.) with no evidence for any effect of the solutions on the activity of the units. However, seven of 15 rapidly adapting compression (r.a.c.) receptors tested showed a reduced probability of response subsequent to NaCl application as illustrated by the records from one unit in Fig. 9. These tests, although not conclusive, suggest that glandular secretion of sodium and chloride ions subsequent to sympathetic stimulation may be related to the reduced sensitivity seen in r.a.c. receptors.

DISCUSSION

In recordings from cutaneous nerves of frogs, we have distinguished four classes of mechanoreceptive myelinated afferents on the basis of their preferred mechanical stimulus, threshold force magnitude, and adaptation rate: rapidly adapting compression receptors, slowly adapting compression receptors, rapidly adapting stroke receptors, and slowly adapting stroke receptors. The sensitivity of these receptors to mechanical stimuli is altered by electrical stimulation of the sympathetic trunk, and the general characteristics of the sympathetic-evoked sensitivity changes are relatively consistent for receptors within each of these four classes. Our results indicate that probe–skin coupling effects, changes in blood flow, and changes in tissue compliance cannot explain the observed sympathetic effects; however, local changes in ion concentrations may be responsible for the desensitization of one class of mechanoreceptors. These results leave open the possibility that the influence of the sympathetic system on the sensitivity of cutaneous mechanoreceptors is mediated by a direct action of efferent neurotransmitter on the receptor membrane.

Receptor classification. The system of receptor classification used in the present study is not identical to that reported by any previous investigator, as no existing system seemed adequate to describe the receptor types encountered. The characteristics of our rapidly adapting compression (r.a.c.) receptors that were noted by other investigators include exquisite sensitivity to compressive stimuli and phasic 'on' and 'off' responses to sustained suprathreshold compression (Hogg, 1935; Fessard & Segers, 1943; Maruhashi, Mizuguchi & Tasaki, 1952; Catton, 1958, 1976; Loewenstein, 1956; Höglund & Lindblom, 1961; Lindblom, 1962, 1963). However, Hogg and Lindblom reported pressure thresholds for rapidly adapting receptors which ranged from 2 to 50 times greater than the least sensitive r.a.c. receptor observed in the present study (50–1000 mg wt./mm^2 compared to $\leqslant$ 22 mg wt./mm^2). Several differences in experimental conditions may have contributed to this discrepancy: differences in stimulus rise times (not reported in those papers), differences in the condition of the skin (earlier studies were done using isolated skin), and differences in probe sizes.

Although quantitative tests of adaptation rate were generally not conducted in earlier studies, the response characteristics of s.a.c. receptors are most similar to the type A_2 receptors of Fessard & Segers (1943), the 'pressure' fibres of Maruhashi *et al.* (1952), and the 'type *b*' discharges of Catton (1958). They may also be the same as part of Loewenstein's (1956) 'highly adaptive tactile receptors' since both types give only a few responses to near-threshold stimuli and show increased sensitivity after sympathetic stimulation. The 6 times threshold test for adaptation rate, based on the observation by Gray & Malcolm (1951) that stimuli of 2–6 times threshold evoke repetitive firing in 'touch' receptors, proved to be a very simple and discriminating method for distinguishing between rapidly and slowly adapting compression receptors.

We found no evidence that the thresholds of compression receptors varied systematically with the location of their receptive fields, as has been reported for toad skin mechanoreceptors (Lindblom, 1958). This difference most likely results from our use of force rather than displacement as the independent variable because force minimizes the variability due to differences in subcutaneous tissues (see below).

In none of the studies cited above were *stroke* sensitive tactile units reported. The units we describe as stroke units could be considered simply as tactile or touch units with high thresholds and thereby made to conform to previous classification schemes; however, this would obscure their very clear preference for stimuli oriented tangential to the skin surface.

Methodology. Force rather than displacement was recorded as the independent variable in the present study in order to eliminate the differences in mechanical effect on the skin related to differences in the compliance of the subcutaneous tissue (cf. Lindblom, 1958). A given change in force will produce the same distortion of the skin independently of the underlying tissue if one neglects stretch, as is appropriate for small indentations. Measurement of the stimulus force had the additional advantage of revealing active changes in the compliance of the skin subsequent to sympathetic stimulation, and allowed observation of fluctuations in tissue volume due to blood pressure pulses, which could sometimes interact with the imposed stimuli to alter receptor firing.

Sympathetic effects on receptors. Two previous studies of sympathetic actions on frog cutaneous mechanoreceptors have shown only facilitatory effects (Chernetski, 1964; Loewenstein, 1956) these effects being similar to those observed in the present study with s.a.c., r.a.s. and s.a.s. mechanoreceptors. Their failure to observe receptor desensitization, which was commonly observed with rapidly adapting compression receptors in the present study, may be attributable to a number of factors. Tissue viability was more compromised in the isolated preparation of Loewenstein and the pithed frogs of Chernetski; although short periods of occlusion in our study failed to block desensitization. The frog species tested were different. Methodological differences may also have contributed to the discrepancy. Desensitization was most apparent when the pre-sympathetic stimulation probability of response was slightly less than unity (cf. Fig. 2), and neither Loewenstein nor Chernetski reported the use of this test condition. In two previous reports of sympathetic-induced receptor desensitization in cat spindle afferents and tooth pulp receptors (Hunt, 1960; Edwall & Scott, 1971) the desensitization followed an initial sensitization. The present report is the first to describe a sympathetic-induced, monophasic desensitization of a cutaneous mechanoreceptor.

Both Chernetski and Loewenstein reported that sympathetic stimulation can evoke bursts of 'spontaneous' afferent activity in the absence of overt mechanical stimulation. Our results are consistent with this finding and indicate in addition that such activity occurs predominantly in slowly adapting compression receptors. We did not find that spontaneous activity persisted for more than 15 sec with maintained sympathetic stimulation, as Chernetski reported. It is possible that the maintained spontaneous activity he observed occurred in cold receptors rather than mechanoreceptors (Spray, 1974).

Loewenstein (1956) suggested that sympathetic stimulation-induced spontaneous activity and receptor facilitation may be different manifestations of the same underlying phenomenon. Our data are not consistent with that view in so far as most receptors which showed increased sensitivity during sympathetic stimulation did not show spontaneous activity. Furthermore, most r.a.c. units were desensitized, even those which showed transient spontaneous activity. One possible origin of the spontaneous activity is considered in the next section.

Nature of sympathetic influence. Sympathetic modulation of blood flow does not appear to be responsible for the changes in receptor sensitivity we observed, since sympathetic-evoked sensitivity changes were the same in the presence and absence of arterial circulation for receptors of all classes. Similary, Loewenstein (1956) observed sympathetic-induced sensitization of receptors in isolated skin-nerve preparations. Hunt (1960) found that in cats, sympathetic modulation of muscle spindle afferent activity occurred in the absence of blood flow; however, Edwall & Scott (1971) reported that blood flow and tooth pulp afferent activity were correlated in cats.

Our results clearly show that the amount of fluid between probe and skin had a major influence on the measured sensitivity of the very sensitive, rapidly adapting compression receptors. However, the effect of sympathetic stimulation on the evoked activity of these receptors was qualitatively independent of the amount of fluid at the probe–skin interface, suggesting that fluid secretion and sympathetic-induced changes in receptor sensitivity are separate phenomena.

The sympathetic-induced changes in tissue compliance shown earlier are likely to originate within the skin because the underlying muscles were denervated (except for possible perivascular efferents) and because the compliance changes were unaltered by temporary occlusion of the arterial blood supply. Within the skin, the most likely source of a mechanical response to sympathetic activation is contraction or relaxation of the scattered population of smooth muscle bundles oriented perpendicular to the skin surface (Whitear, 1974*a*).

Just how mechanical responses within the skin relate to force changes at the probe and to the status of individual receptors is not known, so no definite conclusions can be drawn regarding the sensory role of mechanical responses within the skin. However, our finding that receptor sensitivity was often changed when there was no detectable mechanical response, and that there was no consistent relationship between the direction, amplitude or time course of compliance changes and sensitivity changes in receptors, makes it unlikely that mechanical responses to sympathetic stimulation are responsible for the observed changes in receptor sensitivity.

The sympathetic-evoked spontaneous activity observed primarily in slowly adapting compression receptors may result from mechanical responses within the skin. The latency of onset is similar for both and the relatively slow rate of force change (generally 2–5 sec to plateau) should be more effective on the s.a.c. receptors than on the other, more rapidly adapting classes. Against to this speculation, however, is the report by Loewenstein (1956) that sympathetic-evoked spontaneous activity occurs in abdominal skin devoid of smooth muscles.

Sympathetic-evoked glandular secretions were considered to be of potential importance in mediating sympathetic effects on receptors for several reasons: (*a*) sympathetic stimulation has been demonstrated to result in the secretion of Na^+ and Cl^- ions from frog skin glands (Lindley, 1969; Lang *et al.* 1975); (*b*) there is evidence that Na^+ and Cl^- concentrations influence the potential difference across frog skin (House, 1971); and (*c*) Loewenstein (1956) has shown that potential changes imposed across frog skin can summate with the action of monoamines in modulating mechanoreceptor sensitivity. Our experimental data are not consistent with such a mechanism of action for three of the four receptor classes studied (s.a.c., r.a.s. and

s.a.s.). However, seven of the 15 r.a.c. receptors showed reductions in response probability to both sympathetic stimulation and topical application of NaCl, suggesting that their desensitization may be related to glandular secretions. This view is not supported by our findings that repeated periods of sympathetic stimulation without rinsing failed to produce a progressive change in r.a.c. receptor thresholds and that similar sympathetic desensitizations were observed with both 'wet' and 'dry' skin. The dilution of glandular secretions would presumably be quite different under those two conditions. Further study is needed to resolve these conflicting results.

Studies from other laboratories are consistent with a *direct* action of sympathetic efferent neurotransmitter on the receptors themselves. Whitear (1974*b*) presented morphological evidence for the presence in frog skin of nerve fibres with numerous varicosities containing dense-cored vesicles. These fibres, which are located as superficially as the epidermal layers of the skin, might mediate sympathetic actions on nearby sensory neurones. Monoaminergic innervation of frog skin has also been demonstrated by fluorescent microscopy (Fuxe & Nilsson, 1965; Sjöberg & Flock, 1976; Sjöberg, 1977). In addition, Loewenstein & Altamirano-Orrego (1956) applied adrenaline and noradrenaline to isolated Pacinian corpuscles from cat mesentary and observed reversible lowering of threshold to mechanical stimulation. Such evidence, coupled with our inability to find indirect effects of sympathetic system activation that might account for the observed changes in mechanoreceptor sensitivity, make a direct action of efferent neurotransmitter on the receptor membrane seem the most likely mode of sympathetic influence on receptor sensitivity.

Possible behavioural consequences of sympathetic effects on receptors. The sympathetic-induced desensitization consistently observed in rapidly adapting compression receptors is not sufficiently great to render the frog insensible to rapid compressive stimuli; however, the desensitization would likely reduce the number of r.a.c. receptors responding to a supraliminal stimulus as the stimulus propagates rapidly from the site of initiation (cf. Johnson, 1974). Thus the frog would be less capable of perceiving a very weak compressive stimulus but might be better able to localize a supraliminal stimulus because the number of adjacent responding units would be reduced. Sympathetic-induced sensitization of slowly adapting compression receptors appears to be a transient response, having little or no lasting influence; however, the sympathetic stimulation-induced 'spontaneous' activity which occurred in half of this population would likely produce a paraesthesia as noted earlier by Chernetski (1964).

The sympathetic-induced sensitization of both rapidly and slowly adapting stroke receptors appeared to be both persistent (> 1 min) and pronounced. Thus the aroused frog should be more sensitive to stimuli which are moving across the skin (in contrast to compressive stimuli). This increased sensitivity might have some survival value during flight.

Conclusions. Our results indicate that sympathetic activity influences mechano-receptor sensitivity in intact frogs. Furthermore, this change in sensitivity is quite consistent for most receptors within any one class, and includes both a desensitization of some receptors (rapidly adapting compression receptors) and a sensitization of others (slowly adapting compression receptors and rapidly and slowly adapting stroke

receptors). Direct action of efferent neurotransmitter on the receptor membrane is the most likely mode of sympathetic influence on receptor sensitivity.

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