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CHARACTERISTICS, SPECIFICITY, AND EFFERENT CONTROL OF FROG CUTANEOUS COLD RECEPTORS

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SUMMARY

1. Thermal stimulation of frog skin produces a discharge in afferents in the dorsocutaneous nerve. The characteristics of this response have been examined with regard to static and dynamic sensitivity to thermal stimuli and to mechanical sensitivity. Frog cutaneous receptors respond only to cooling, with no response to warming through the same thermal range.

2. The static temperature at which these receptors are maximally active is about 24° C for *Rana pipiens* and about 27° C for *R. catesbiana*.

3. The dynamic sensitivity of frog cutaneous receptors is linearly related to both stimulus slope and magnitude. Maximum dynamic sensitivity was between -90 and -120 impulses/° C.sec.

4. Antidromic occlusion experiments demonstrate relative insensitivity of these receptors to tonic mechanical stimulation. At high stimulus intensities, however, larger fibres are recruited into the response; this recruitment of action potentials of larger amplitude is a linear function of both stimulus slope and magnitude.

5. Spike heights are linearly related to conduction velocities in the dorsocutaneous nerve; tonic mechanoreceptors have a mean spike height of $28{\cdot}4 \pm 0{\cdot}6\ \mu$V and conduction velocities about 6–8 m/sec, whereas these temperature sensitive receptors have spike heights $15{\cdot}8 \pm 0{\cdot}4\ \mu$V and conduction velocities about 3–4 m/sec.

6. Maximum dynamic sensitivity skin is increased following stimulation of the first or second sympathetic ganglion. This increase is both marked and progressive, reaching a maximal enhancement of about 150–160% control at a stimulus rate of 5 stimuli/train, each train delivered once every 5 sec.

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7. Static sensitivity of the cold receptors is also increased following sympathetic stimulation. This increased sensitivity is shown by both increased discharge rate within the same thermal range and by decreased temperature of maximum static sensitivity.

8. Sympathetic modulation of dynamic thermal sensitivity is mimicked by epinephrine and norepinephrine in doses of 10^{-6}–10^{-7} g/ml. Ephedrine, another adrenergic agonist, also mimics the enhancement of cold receptors by sympathetic stimulation.

9. Larger fibres are recruited to account for the increased sensitivity of thermoreceptors following sympathetic stimulation and epinephrine application.

10. Propranolol and phentolamine both block the enhancement of the response by sympathetic stimulation, but propranolol blocks the response of the receptor to thermal stimulation as well. Reserpine pre-treatment blocks the effect of sympathetic stimulation on the cold response.

INTRODUCTION

Frog cutaneous receptors that fully meet the strict electrophysiological criteria applied to thermoreceptors have not been reported previously. These criteria include both static and dynamic temperature sensitivity, little or no response to mechanical stimulation, and temperature sensitivity (both threshold and firing rate) comparable to those receptors mediating the thermal sense in humans (Hensel, Iggo & Witt, 1960). Certainly, cutaneous receptors that respond to thermal stimuli are found in amphibians (Maruhashi, Mitzuguchi & Tasaki, 1952; Adrian, 1932; Hogg, 1935), but these receptors are either additionally responsive to other sorts of stimuli or respond only to temperatures that may cause tissue damage. Clearly, these receptors do not fully satisfy Hensel's criteria.

The first part of the present study was undertaken to investigate the characteristics of the responses of dorsal cutaneous afferents to thermal stimuli; during these investigations receptors were found that fulfilled Hensel's criteria for thermoreceptors. This finding suggested that the simple nerve-skin preparation could be used in the study of several aspects of thermoreceptor function that have been largely ignored in the past. These problems include the degree of sensory specificity of the thermoreceptors, whether coding by size is a feature of thermoreceptor afferents, and the extent of efferent and adrenergic modulation of thermoreceptor sensitivity.

The small number of afferent fibres contained within the dorsal cutaneous nerves (Spray & Chronister, submitted for publication) allows one to record single action potentials from a population of fibres. This advantage

permits access to problems of coding in a group of axons supplying a discrete cutaneous area. Efferent modulation of thermoreceptor response can also be studied in this preparation with less ambiguity than in others because the preparation does not require an intact blood supply for its maintenance. One difficulty in demonstrating the phenomenon of efferent modulation in preparations from homoeotherms is that sympathetic stimulation alters vasomotor tone, increasing peripheral vascular resistance and decreasing both blood flow and skin temperature.

The purpose of the present paper is to describe the thermal responsiveness of dorsal cutaneous afferents, the specificity of this response, the recruitment of larger fibres as stimulus strength is increased, and the enhancement of frog cutaneous cold receptor activity by sympathetic stimulation and graded doses of adrenergic agonists. The recruitment of fibres of larger size includes the recruitment of a larger proportion of mechanoreceptors into the total response. Other authors have speculated on the importance of thermoceptive mechanoreceptors in the discrimination of temperature at both cutaneous (Burton, Terashima & Clark, 1972) and thalamic levels (Poulos & Benjamin, 1968). The data presented in this paper support their speculations.

METHODS

Data were obtained from more than 800 nerves of about 500 healthy *Rana pipiens* and *R. catesbiana* that had been held at room temperature for from 1 to 3 weeks prior to use. All experiments were performed at an ambient temperature of 21–23° C between the months of March and October. A traditional nerve-skin preparation was employed in most of the experiments (Adrian, 1928); for the experiments involving sympathetic stimulation the preparation consisted of a decapitated, eviscerated frog with fore and hind limbs removed. In all experiments the skin was placed dorsum down atop a thermo-electric or water-cooled thermode with a thermistor at the interface between skin and thermode.

The inner skin surface was rinsed with air-equilibrated amphibian saline (Forster 1948). One to three dorsal cutaneous nerves and the first or second sympathetic ganglion were isolated, placed upon bright platinum wire electrodes and covered with mineral or silicon oil. In some experiments, one or more dorsal cutaneous nerves were dissected into finer fibre bundles using collagenase and chymotrypsin enzyme treatment (Perl, 1968).

Nervous activity and temperature of the stimulating surface were monitored and recorded. Data were anlysed either by counting the impulses from photographed records or from the integrated output of a trigger with discriminator. Base line activity averaged about 5 μV; action potentials of various amplitudes above this level could be integrated selectively. Maximum dynamic sensitivity (impulses/°C.sec) was determined for each preparation at various stimulus slopes and magnitudes; peak static sensitivity (impulses/sec at an optimal temperature) was determined for many fibres.

Impulse amplitude and conduction velocity were correlated in twelve whole dorsal cutaneous nerves by recording either monopolarly or bipolarly from two sections of the same nerve at a separation of 3·2–12 mm. The recorded action potentials were

then played back and the temporal separation of impulses was measured and correlated with the measured action potential amplitude.

Using seventy nerves from twenty animals, the antidromic occlusion technique (Douglas & Ritchie, 1957) was used to verify the results obtained with other methods and to determine the spectrum of conduction velocities of fibres within the frog dorsal cutaneous nerve. The whole nerve was placed upon a pair of recording electrodes proximal to the skin and upon a pair of stimulating electrodes 10–30 mm distal to the recording electrodes. An electronic filter was used to filter below 60 Hz and above 2 kHz, thus eliminating much of the stimulus artifact. After these records were taken, the signal was differentiated through an RC circuit (1000 Ω, 0·001 μF; Coombs, Curtis & Eccles, 1957) and the experiment repeated. Amplitudes of each of the biphasic, positive-negative deflexions were measured and histograms were constructed for each stimulus condition.

Statistical procedures throughout this study were derived from Steele & Torrie (1960). Significance of data between groups was assessed using the Student's *t* test, and correlation coefficients were calculated as warranted.

Sympathetic stimulation consisted of 200 msec trains of 1, 5, 10, 15 and 20/sec, 0·2 V (8–10 μA), 5 msec pulses presented at 5 sec intervals. Drugs used in this study were as follows: ephedrine sulphate (Cheplin), L-norepinephrine (L-Arterenol: Sigma), reserpine (Serpasil: CIBA), phentolamine mesylate (Regitine: CIBA), propranolol hydrochloride (Inderal: Ayerst), epinephrine chloride (Adrenalin: Parke, Davis & Company). Drugs were freshly diluted in amphibian salt solution and doses cited are in g/ml. The procedure for sympathetic stimulation and drug administration was as follows: between three and eight dynamic responses to identical cooling ramps were recorded. Then the skin temperature was held as 23° C and a selected drug was applied to the inner skin surface at a concentration between 10^{-10} and 10^{-3} g/ml. The volume of each applied drug was about 1 ml., resulting in a concentration at the skin surface of 0·1 ml. drug solution/cm^2. These solutions were maintained at 23° C so that no difference in temperature developed between inner and outer skin surfaces as a consequence of the drug. Each solution remained on the skin for 2 min, after which it was removed and the nerve again covered with mineral or silicon oil. At this time, another sample of three to eight dynamic responses to temperature change were measured. The skin was then washed with air-equilibrated salt solution and the next drug dose applied.

The maximum dynamic sensitivity of each nerve was determined as a percentage of control response for each drug dose. Mean and variability were calculated, and this response was plotted as a function of drug dose. Each dose level was compared to control using the Wilcoxon matched-pairs signed-rank test, taking into account both direction and magnitude of the effect in assignment of significance (Siegel, 1956).

RESULTS

Static sensitivity

Impulses were counted from photographed record; an impulse frequency that depended upon temperature of the skin was recorded from the dorsal cutaneous nerves (Fig. 1). Each point in this Figure represents the means of several 1 sec responses from five animals measured after the skin had been held at that temperature from 30 to 90 sec. Standard errors are smaller than the symbols in each case. In *Rana pipiens*, the temperature at which the skin was maximally responsive was $24{\cdot}8 \pm 0{\cdot}8°$ C; in *R.*

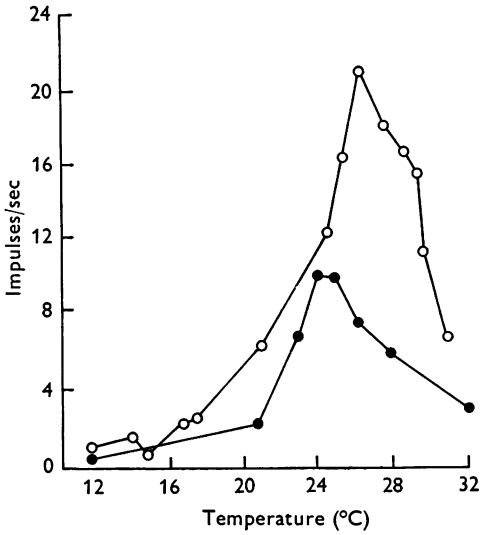

Fig. 1. Impulse frequency recorded from dorsocutaneous nerves of *Rana pipiens* (twelve nerves) and *R. catesbiana* (four nerves) after temperature was held constant for 60–90 sec. Symbols (○, *R. catesbiana*; ●, *R. pipiens*) represent means of 3–5 sec at each temperature; standard errors are smaller than symbols in each case. Both temperature at which the maximum firing rate occurred and the absolute value of the maximum rate of firing are significantly different between species ($P < 0{\cdot}05$; $P < 0{\cdot}01$, respectively).

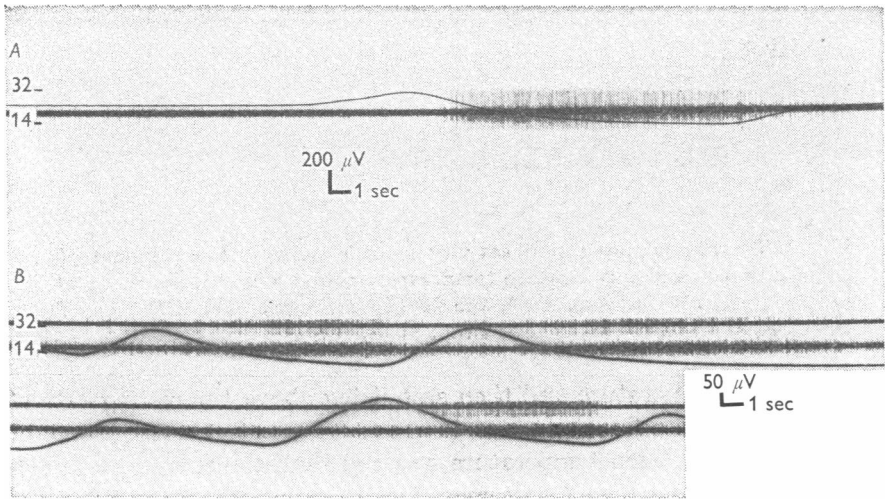

Fig. 2. Nervous activity recorded from a twig of the dorsocutaneous nerve (*A*) and two entire nerves innervating the same receptive fields (*B*) in response to cooling. Voltage and time calibrations are given.

catesbiana, 27·5 ± 0·7° C. These two species showed similar response ranges, from about 12 to 32° C. The curve from the bullfrog was shifted to the right when compared to that of the grass-frog, with a significantly higher peak temperature ($P < 0{\cdot}05$).

Dynamic sensitivity

In twigs of any dorsal cutaneous nerve, action potentials were initiated by a change in temperature (Fig. 2*a*). This characteristic discharge was also observed in simultaneous recordings from two or more entire nerves innervating the same stimulated field (Fig. 2*b*). These fibres were always stimulated by a decrease in temperature, the response increasing during

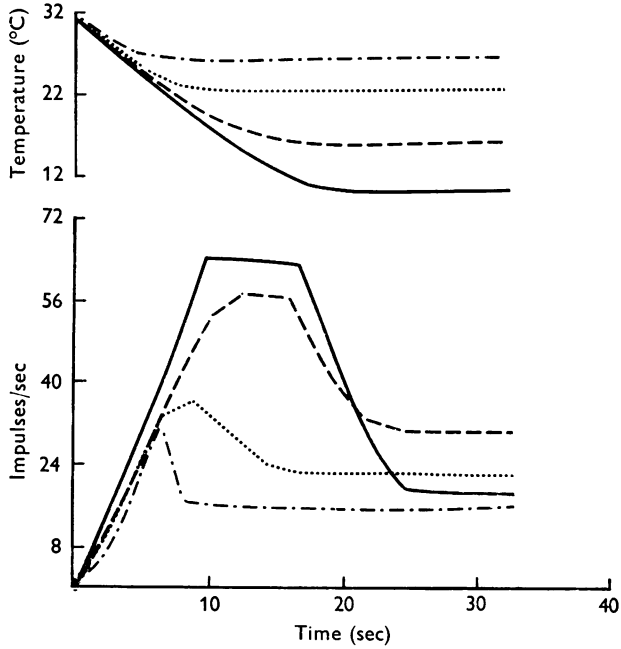

Fig. 3. Tracings of integrated activity records showing the response of a single dorsocutaneous nerve to temperature changes of 5° C (· – ·), 10° C (dotted line), 15° C (dashed line), and 20° C (continuous line). The slope of the thermal stimulus was held constant at 0·7° C/sec.

the change in temperature and then stabilizing above the resting rate of discharge unless the final stimulus temperature was below the range of static sensitivity. When temperature was elevated, the frequency of impulses recorded from the skin decreased, unless the rate of warming was slow. With very slow rates of warming (below 0·1° C/sec) an increased discharge was seen as the skin passed through its temperature of maximum static sensitivity.

The maximum dynamic sensitivity of these cool-responsive receptors depended upon the magnitude of the cooling (Fig. 3), being directly proportional to the height of the cooling ramp. At the higher magnitude of stimulus change, the maximum dynamic sensitivity approached −90 impulses/° C.sec. The maximum dynamic sensitivity also depended upon the rate of temperature change (Fig. 4*a*), the sensitivity varying as a linear function of the slope (Fig. 4*b*). At higher rates of temperature change, the maximum dynamic sensitivity approached −90 to −120 impulses/° C.sec.

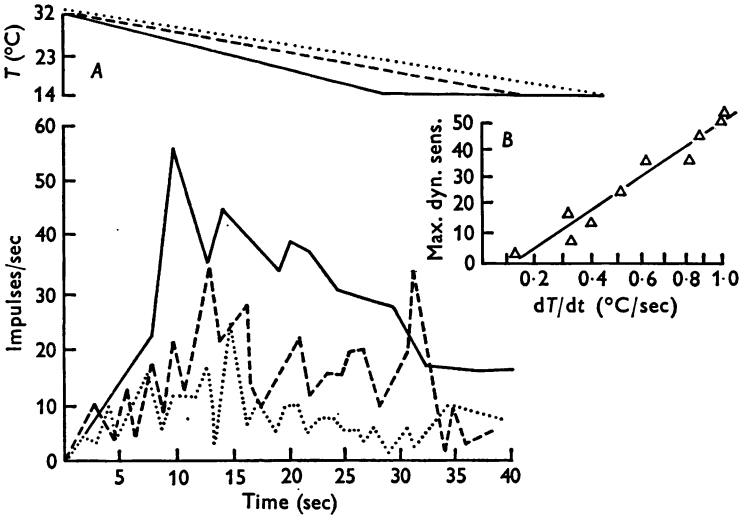

Fig. 4. Responses of the dorsocutaneous nerve to different stimulus slopes of the same magnitude (20° C). Slopes used in *A* were 0·67° C/sec (continuous line), 0·5° C/sec (dashed line), 0·25° C/sec (stippled line). In *B*, the maximum dynamic sensitivity (impulses/° C.sec) is plotted as a function of slope for three nerves. This relationship is linear (r = 0·98).

In Figs. 3 and 4, the peak dynamic response occurred at a temperature corresponding to that producing a peak static response. Thus, as stimulus slope increased, the latency to peak dynamic response decreased.

Except for the temperature at which the peak dynamic response occurred, no difference was noted between the dynamic responses of *R. pipiens* and *R. catesbiana.*

Impulse size as a function of stimulus intensity

The magnitude of the thermal stimulus was varied from 1 to 24° C at a constant stimulus slope (dT/dt = 1·0° C/sec). The mean and standard errors were calculated for the amplitudes of the action potentials associated with each temperature change (Fig. 5*a*). This relationship is linear, with a correlation coefficient of 0·93. The slope of the thermal stimulus was

varied from 0·1 to 1·1 °C/sec over a constant fall in temperature (ΔT = 20° C), and the means and standard errors for the action potentials recorded with each temperature change were calculated (Fig. 5*b*). This relationship between slope and action potential amplitude was also linear (r = 0·94). It follows that the mean amplitude of impulses recorded from the nerve is directly related to stimulus strength (both slope and magnitude).

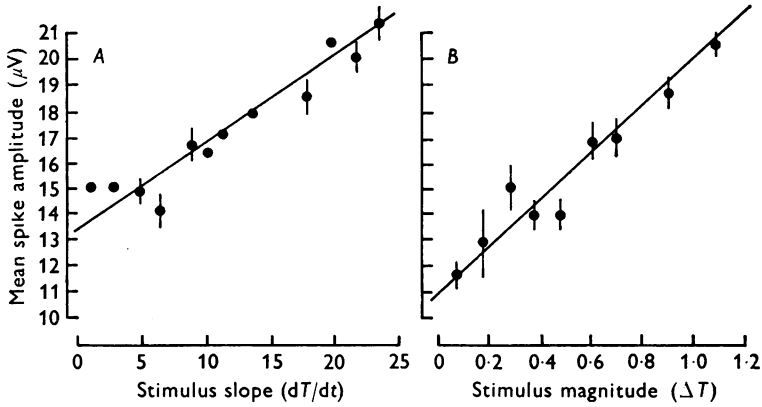

Fig. 5. Relationship between amplitudes of action potentials (μV) recorded from dorsocutaneous nerve as a function of magnitude of temperature change (ΔT) with a constant slope (*A*) and slope (dT/dt) at a constant magnitude (*B*). These relationships are linear with correlation coefficients of 0·93 and 0·94, respectively.

Correlation of action potential amplitude with conduction velocity

Amplitudes of many action potentials were either measured by hand from photographed records or determined by integration. The mean amplitude of these action potentials increased as a linear function of stimulus intensity (Fig. 5). In order to show that this increase in amplitude represented an increase in conduction velocity of the fibres, a correlation was performed on 153 impulses obtained from several temperature changes or with constant mechanical stimulation (3·5 g, 1·5 cm diameter circular stimulating surface). This correlation was linear with a correlation coefficient of 0·98 (Fig. 6).

Using a larger sample of 563 impulses, the separation of sizes of fibres responsive to thermal and mechanical stimuli can be seen (Fig. 7). Spike heights averaged $15{\cdot}77 \pm 0{\cdot}44$ μV for receptors responding to thermal stimuli, $28{\cdot}39 \pm 0{\cdot}62$ μV for receptors responding to light, steady mechanical stimuli and $30{\cdot}81 \pm 1{\cdot}22$ μV for receptors responding to more intense, sudden mechanical stimuli (pressing on the mechanical stimulus in place). The difference between amplitudes of thermally sensitive units and those

that responded to light tactile stimuli was clearly significant ($P < 0{\cdot}001$). Interestingly, there appeared to be a secondary peak in those thermally responsive fibres with a mode of about 30–40 μV, which might well represent a population of temperature-sensitive mechanoreceptors. Action potentials above 30 μV were very rarely encountered, except when stimulus slope exceeded 0·5° C/sec.

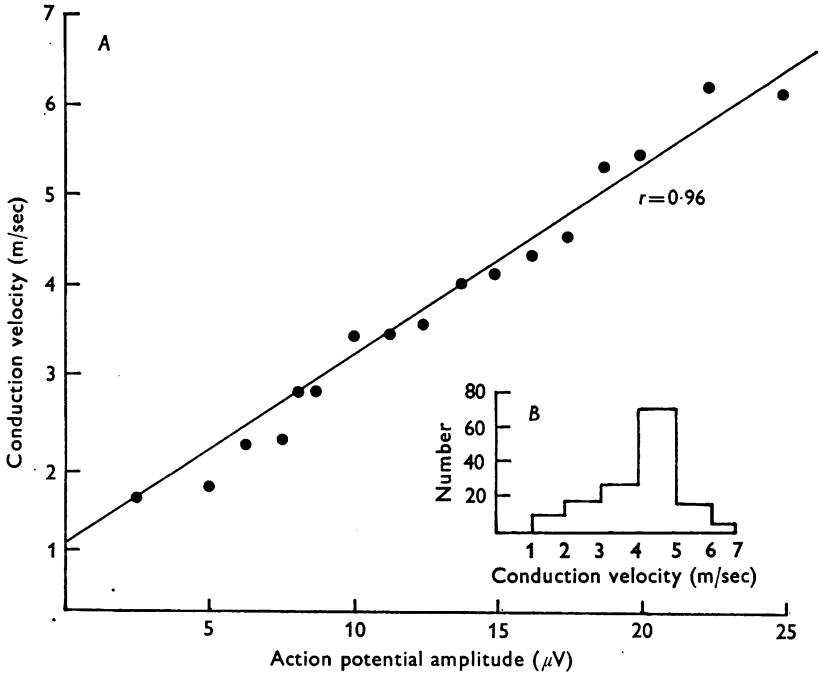

Fig. 6. Relationship between conduction velocity (m/sec) and action potential amplitude (μV) in the frog dorsocutaneous nerve (*A*). This relationship is linear ($r = 0{\cdot}96$) and is derived from 153 action potentials distributed according to *B*.

Antidromic occlusion

Stimulation parameters for maximal excitation of all fibres were typically between 0·01 and 0·1 msec, 10–29 V. Twenty to fifty control antidromic responses were photographed, then photographs of the antidromic response were made with thermal stimulation, tonic mechanical stimulation, or the two stimuli presented simultaneously. Three clearly recognizable peaks were seen reliably in the response of the dorsal cutaneous nerves to antidromic stimulation (Fig. 8*a*). These peaks corresponded to fibres with conduction velocities of 15, 7, and 4 m/sec. When the skin was cooled, the third of these peaks (labelled *B* in Fig. 8*a*, *b*) decreased in amplitude while

the second peak (labelled *A* in Fig. 8*a*, *b*) remained relatively constant in height. When a constant mechanical stimulus was applied to the skin, peak *A* was depressed (Fig. 8*a*, *b*) while peak *B* remained relatively constant in height. When the skin was cooled with the weight still in place, both peaks *A* and *B* were decreased in amplitude.

The slight decrease in the first peak that was seen when comparing the tracings in Fig. 8*a* was seen only during the first 10–30 sec after application of the mechanical stimulus. The three discrete peaks, which were seen in nearly every antidromic experiment, were reduced in response to phasic

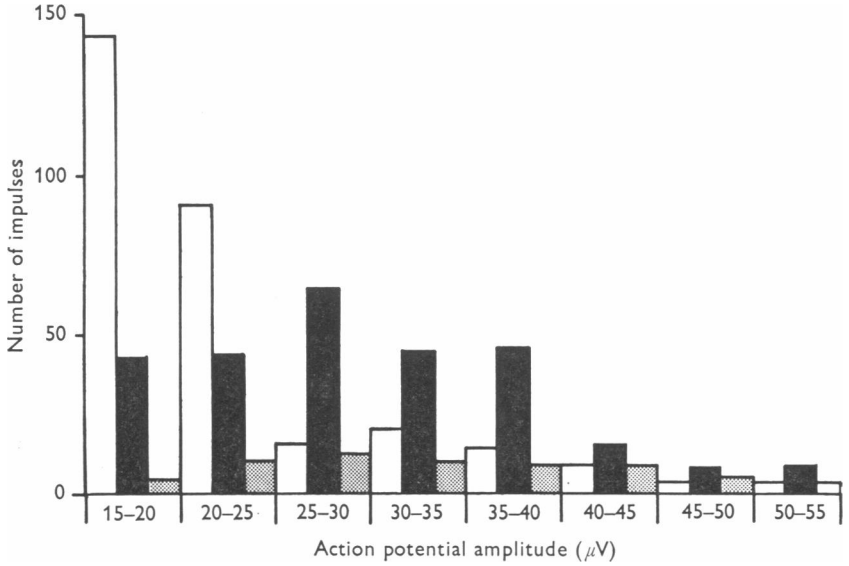

Fig. 7. Distribution of 625 action potential amplitudes according to responses to thermal (open figure), light mechanical (dark figure), and strong mechanical (stippled figure) stimulation.

mechanical, constant mechanical, and thermal stimulation, respectively. That there was some overlap in responses of mechanical and thermal receptors was consistent with the overlap of conduction velocities of fibres responding to different stimulus modalities (Fig. 7).

Sympathetic stimulation

The first or second sympathetic ganglion was stimulated supramaximally (200 msec trains of 1–5 stimuli; 0·2 V, 5 msec). Sympathetic stimulation at 1–5/train markedly and progressively increased the sensitivity of cold receptors (Fig. 9, 10*a*). This enhancement was characterized by increased range of thermal sensitivity (Fig. 9), decreased latency to maximum

dynamic sensitivity, as well as increased dynamic sensitivity at every point in time during the cooling slope (Fig. 9). Maximum dynamic sensitivity was increased to 140 % control at 5 stimuli/train (Fig. 10*a*), a significant increase ($P < 0{\cdot}05$). There was also a slight shift in static sensitivity of the cold-responsive receptors, both to a higher static frequency (impulses/

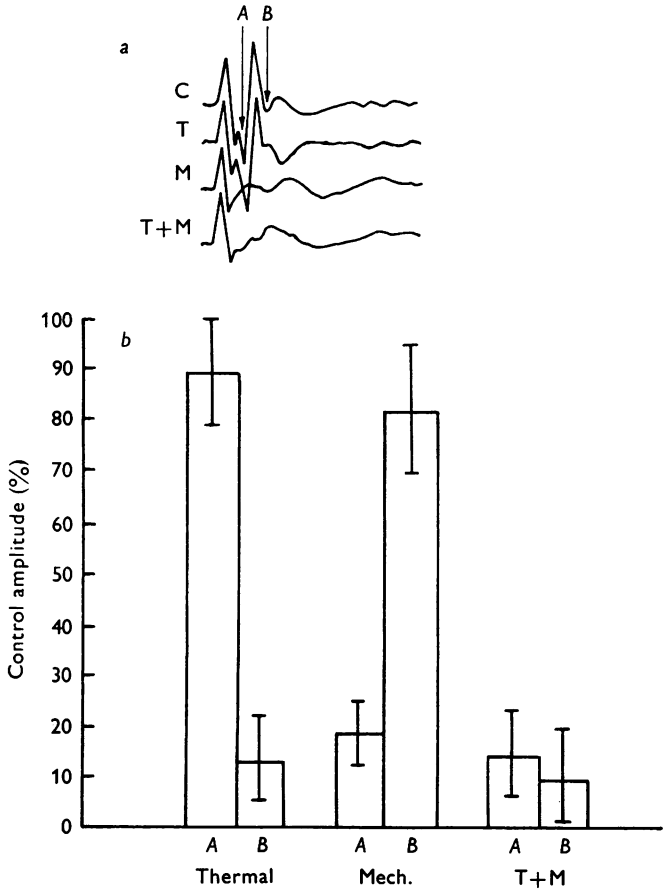

Fig. 8. Different antidromic recordings from dorsocutaneous nerves (*a*). Two prominent peaks (*A* and *B*) are indicated by arrows in the control record (*a*). Thermal stimulation causes a prominent reduction in the amplitude of peak *B* (T), mechanical stimulation causes a reduction in the amplitude of peak A (M), and thermal and mechanical stimulation together cause reductions in both peaks (T and M). The histogram is derived from the responses of ten nerves.

sec) and also to a lower temperature range. Usually, the shift in temperature of peak response was significant ($P < 0{\cdot}05$ for six experiments), while the shift in amplitude was not.

The recruitment of larger fibres (characterized by larger amplitudes of impulses) to account for this increased sensitivity is seen in Figs. 9 and 11. The amplitude of each action potential occurring during the cooling ramp was measured for control conditions and then with stimulation of the sympathetic chain at 1–3/train (Fig. 11). The mean size of the fibres responding to cooling is reflected in action potential amplitudes: At 1/sec, this mean amplitude was $25{\cdot}8 \pm 0{\cdot}6\,\mu$V; 2/sec, $24{\cdot}1 \pm 0{\cdot}3\,\mu$V; 3/sec,

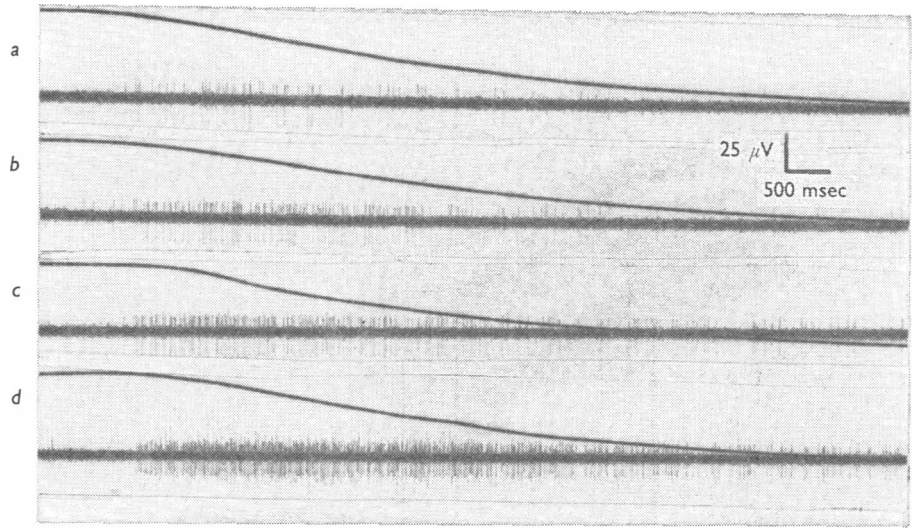

Fig. 9. Nervous activity recorded from the dorsal cutaneous nerves in response to cooling the skin before (*a*) and after stimulation of the first sympathetic ganglion at 1 (*b*), 2 (*c*), and 3 (*d*) stimuli per train. Cooling ramps were very similar, the temperature changing from 32 to 14° C with a slope of about 1·4° C/sec. Calibration mark represents a horizontal calibration of 1 sec and vertical calibration of 25 μV.

$25{\cdot}2 \pm 0{\cdot}3\,\mu$V. Compared to control amplitudes ($20{\cdot}3 \pm 0{\cdot}4\,\mu$V), the amplitudes obtained with sympathetic stimulation are significantly higher ($P < 0{\cdot}001$ for each). This shift in action potential amplitude represents a shift in conduction velocity (see Fig. 6).

Epinephrine added to the inner skin surface in concentrations graded from 10^{-8} to 10^{-5} g/ml. increased the maximum dynamic sensitivity to as much as 200% control (Fig. 10*b*). Doses as low as 10^{-8} g/ml. evoked a significant ($P < 0{\cdot}05$) increase in the maximum dynamic sensitivity. The same range of concentrations of norepinephrine and ephedrine produced maximal enhancements of 160–180%, respectively (Fig. 10*c*, *d*). Again, concentrations of 10^{-8} g/ml. were sufficient to produce significant increases in activity of the temperature receptors. These three adrenergic agonists

showed different dose–response curves: norepinephrine caused an increase in the response that approached an asymptote at about 160% control; ephedrine produced an enhancement that was maximal at 10^{-6}–10^{-5} g/ml., after which the enhancement was reduced; epinephrine produced a sigmoidal response. These three different dose–response curves may be due to the range of concentrations employed. The order of effectiveness of these agonists at concentrations of 10^{-5} g/ml. was epinephrine > norepinephrine > ephedrine.

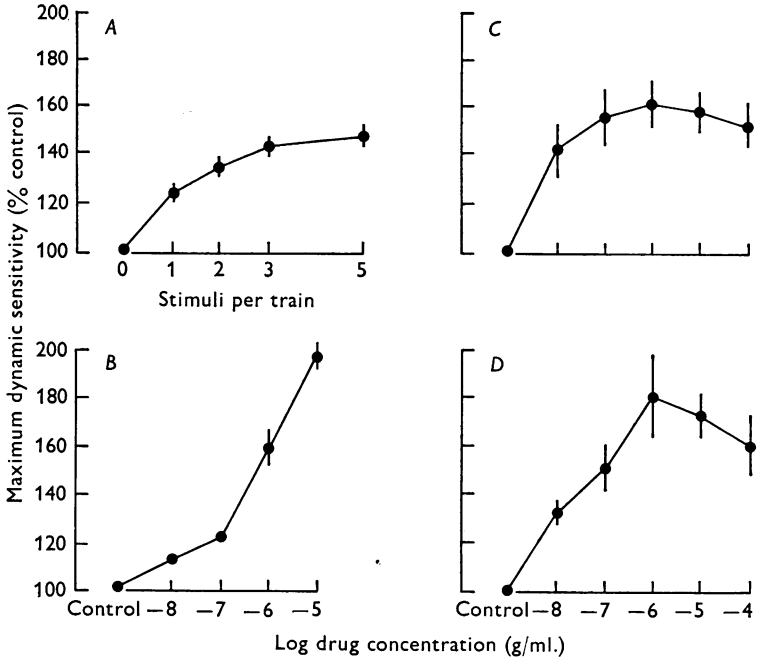

Fig. 10. Effects of graded sympathetic stimulation (*a*), epinephrine (*b*) norepinephrine (*c*), and ephedrine (*d*) on maximum dynamic sensitivity (% control). Means and standard errors are given for each stimulation frequency and drug dose (n = 19, 12, 12, 8, respectively). At all stimulation rates, sympathetic stimulation produced a significant increase in maximum dynamic sensitivity (*a*); maximum dynamic sensitivity was elevated above control at all doses of both epinephrine and norepinephrine (*b*, *c*); ephedrine produced enhancement at all doses below 10^{-3} g/ml.

The increase in responsivity of the cold receptors following epinephrine administration was due in large measure to the proportional recruitment of fibres with large action potentials and the decreased contribution of smaller fibres (Fig. 12). As the concentration of epinephrine was increased, the proportion of the response that could be accounted for by the larger fibres

also increased (Fig. 12). From both this Figure and Fig. 11, it can be argued that the increase in mean fibre size consists of the recruitment of new fibres into the firing population, rather than a simple decrease in firing rate of smaller fibres.

Reserpine (6 mg/kg) was given to six frogs three times a week for 4 weeks before testing (Miller & Mizell, 1972). In the ten nerves tested from six frogs, none exhibited any enhancement of cold receptor response following sympathetic stimulation.

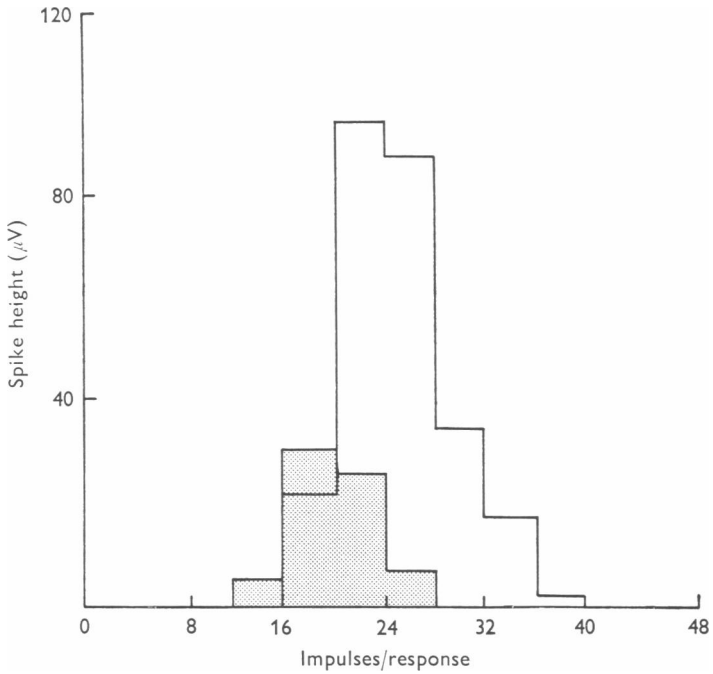

Fig. 11. Effects of sympathetic stimulation at 2/train on the response spectrum of action potentials recorded from the dorsal cutaneous nerve. Number of action potentials in each size class is indicated by shaded area; clear area represents spectrum following stimulation. Both number of impulses evoked by thermal stimulation and the amplitude of these impulses are increased after sympathetic stimulation.

Both phentolamine (an α-adrenergic blocking agent) and propanolol (a β-blocking agent) decreased the enhancement by sympathetic stimulation at very low doses (5 and 1×10^{-9} g/ml., respectively: Fig. 13). Although the control response to thermal stimulation was unaltered by the highest phentolamine doses, responses from skin both before and after sympathetic stimulation were depressed at higher (above 10^{-9} g/ml.) doses of propranolol.

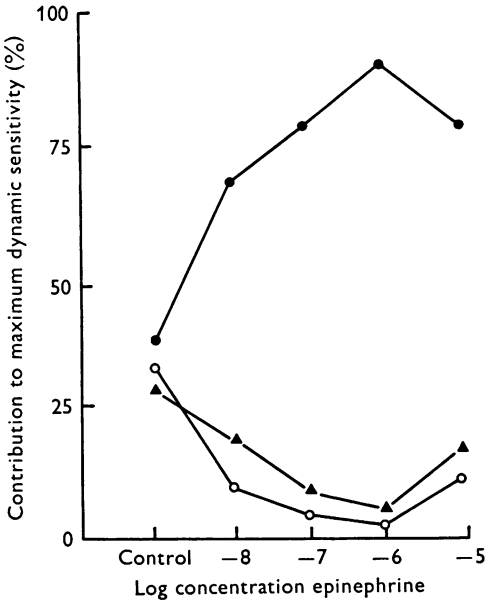

Fig. 12. Contribution of large fibres (more than 32 μV; ●), medium-sized fibres (15–32 μV; ▲), and small fibres (less than 15 μV; ○) to the total maximum dynamic sensitivity of the skin to thermal stimulation.

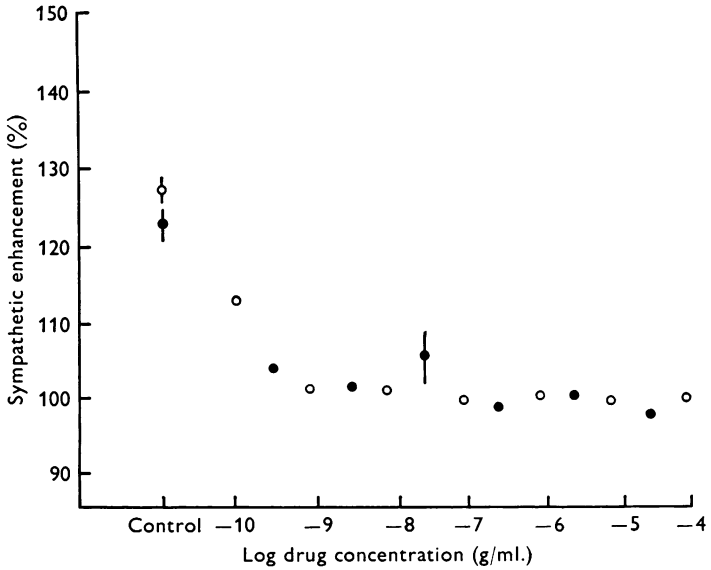

Fig. 13. Decrease in sympathetic enhancement of the maximum dynamic sensitivity following administration of propanolol (○; n = 9) and phentolamine (●; n = 12). At all doses above 5×10^{-10} g/ml., no difference existed between control response and effect of sympathetic stimulation.

DISCUSSION

Characteristics of frog cutaneous cold receptors

Afferents within the dorsal cutaneous nerve displayed firing rates that were related to the static temperature of the skin. Receptors of *R. pipiens* and *R. catesbiana* were maximally active at temperatures of 24–25° C and 27–28° C, respectively (Fig. 1); these temperatures correspond to temperatures chosen by these two species in the field (Brattstrom, 1963; Lillywhite, 1970). Most mammalian temperature receptors have similar peaks in activity (cf. Boman, 1958).

Cooling the skin of the frog elicited a discharge that could be recorded either from twigs (Fig. 2*a*) or from the whole dorsal cutaneous nerve (Fig. 2*b*). The maximum dynamic sensitivity of these receptors was typically from -60 to -90 impulses/° C.sec (Fig. 4*b*). This maximum dynamic sensitivity is within the range of values reported from unimodal temperature receptors in mammalian skin (cf. Iggo, 1968). With some exceptions, the phasic increase in activity of non-specific receptors is ordinarily much lower than that of specific thermoreceptors (Hensel & Boman, 1960). But since more pronounced temperature dependence is observed in some other nerves (Hensel, 1955; Dorai Raj & Murray, 1962), this high sensitivity cannot be used as an argument for specificity (Iggo, 1969).

Dependence of maximum dynamic sensitivity on stimulus strength (Figs. 3, 4) has been demonstrated for mammalian thermoreceptors (Hensel, 1953), and decreased latency to discharge seen in frog receptors as stimulus strength increases (Figs. 3, 4) also has its mammalian counterpart (Hensel, 1953; Boman, 1958). The point of maximum dynamic sensitivity always occurred in the range of static sensitivity, again in agreement with work on receptors of mammals (Hensel, 1953; Boman, 1958; Iggo, 1969; Kenshalo, Holmes & Wood, 1968).

Several early authors (Adrian, 1932; Hogg, 1935; Dodt, 1954) reported the discovery of temperature-sensitive receptors in anurans, but these receptors were all stimulated by extreme heating. Maruhashi *et al.* (1952) found that very small fibres (conduction velocity 0·1–1·0 m/sec) responded to the application of ice to the skin, but these receptors were also clearly different from those reported above.

An excellent survey of dorsal cutaneous afferents was provided by Catton (1958). Catton classified these receptors into four groups (*a–d*) on the basis of conduction velocity, spike height, and response to mechanical stimulation. Fast adapting touch receptors were found with conduction velocity 20–30 m/sec and spike heights 350–450 μV (type '*a*'), more slowly adapting receptors with conduction velocity 10–15 m/sec and spike height 300 μV (type '*b*'), vibration-sensitive endings with conduction

velocity 5–10 m/sec and spike height 100–150 μV (type '*c*'), and tonic pressure receptors with conduction velocity 0·1–0·3 m/sec and spike height less than 100 μV (type '*d*'). Catton suggested that type '*c*' fibres might mediate thermal sensation (corresponding to very slow *A* delta fibres of mammals).

The four peaks in the histogram of axonal diameters comprising the dorsal cutaneous nerve are linearly related to conduction velocities of Catton's fibre groups (Spray & Chronister, 1973). The present study is concerned, therefore, with axons having conduction velocities 4–8 m/sec and axonal diameters of 2·5–3·5 μm.

Specificity of frog temperature receptors and recruitment by size

Kenshalo's use (1970) of Beidler's (1953) technique of integrating multiple unit activity from cutaneous nerve strands has been criticized (Iggo, 1969, 1970) and has led to the alternative technique of analysing separate fibres, then artificially summing responses to yield an 'integration' of activity restricted to the single fibres analysed (Benzing, Hensel & Wurster, 1969). This method is not entirely satisfactory, being restricted to only a few fibres. Utilizing a pulse-generating trigger that could be adjusted to pick off action potentials of various amplitudes, it has been possible to distinguish among stimulus-linked spikes of different amplitudes while retaining the advantage of recording from a population of fibres. As shown in Fig. 6, data have been obtained from fibres with conduction velocities ranging from less than 2 to more than 7 m/sec. Since the responses from a population of fibres were recorded, it was possible to observe the recruitment of larger nerve fibres at stronger stimulus strengths. As a result, threshold of frog peripheral temperature-sensitive receptors have been shown to be directly related to conduction velocities of afferent fibres.

Such a size principle is known for vertebrate and invertebrate muscle fibre systems (Henneman, Somjen & Carpenter 1965; Davis, 1971) and was proposed many years ago (Bullock, 1953) as a principle of sensory organization. Nevertheless, the only previous demonstration of this phenomenon is in the phasic-tonic dichotomy of some sense organs (cf. Yatsuki, Yoshino & Chen, 1951).

Impulses recorded in the dorsal cutaneous nerves had, as a consequence of thermal stimulation, conduction velocities averaging 4 m/sec and spike heights of 16 μV, in contrast to the slowly adapting mechanoreceptors, which had conduction velocities of 7 m/sec and spike heights of about 28 μV. This (and antidromic data) corresponds roughly with Catton's distinction between '*b*' and '*c*' fibres. As stimulus strength (slope or magnitude of the temperature ramp) increased, the mean amplitude of the responsive fibres increased (Fig. 5), demonstrating the recruitment

of slowly adapting mechanoreceptors (type '*b*') into this response (Figs. 7, 8).

Since higher threshold thermoresponsive fibres responded to mechanical as well as to thermal stimuli (Fig. 8), their role in thermal detection may be questioned. The temperature sensitivity of mammalian mechanoreceptors has long been recognized (Hensel & Zotterman, 1951), but such sensitivity has been regarded as functionally unlinked with behavioural or physiological thermoregulation (cf. Iggo, 1969). Evidence of thalamic (Poulos & Benjamin, 1968) and trigeminal (Poulos & Lende, 1970*a*, *b*) convergence of temperature- and mechanical-dependent receptor activity has prompted the assignment of some thermoregulatory importance to these receptors, at least the greater stimulus strengths (Poulos & Lende, 1970*b*; Burton *et al.* 1972).

An increase in fibre size (as judged by increased spike amplitude and its clearly linear relationship to conduction velocity: Figs. 6, 7, 8) of the receptor axons was found as the strength of the thermal stimuli was increased. The conduction velocity of these receptors places them in the range of temperature-responsive mechanoreceptors. In agreement with the findings of Poulos & Lende (1970*b*), the data reported above showed that a recruitment of mechanoreceptors occurs at higher thermal stimulus strengths. These data further suggest that at higher stimulus strengths information might be transmitted via temperature-sensitive mechanoreceptors.

The demonstration that strong, sudden stimuli activate larger fibres than do light, steady stimuli does not contradict the finding (Wall, 1960; Hunt & McIntyre, 1960) that large mechanoreceptors have lower thresholds than do small mechanoreceptors. These are compatible both because of the smaller size of fibres in the present populations and because the light weight was steadily applied to the skin and the heavy weight was suddenly added.

Sympathetic stimulation

Graded sympathetic stimulation produced correspondingly graded increase in dynamic thermal sensitivity (Figs. 9, 10*a*). This demonstration of sympathetic modulation of thermal receptors stands in marked contrast to the results of mammalian experiments (Dodt & Walther, 1957; Gallegos, 1966), and the contrast probably stems from the present advantage of working with a poikilotherm.

Sympathetic stimulation is known to modulate activity in a large variety of other cutaneous receptors, but it is not known whether this modulation is through some direct effect on the receptor or through intermediate effect on vasomotor tone or tissue metabolism (Curtis, 1963;

Paintal, 1964). Working with frog skin, Habgood (1950) showed that facilitation could be demonstrated in one nerve following conditioning stimuli applied to an adjacent nerve. Evidence that this effect was chemical derived from a demonstration that antidromic stimulation of one nerve with its receptive field closely opposed produced an enhanced tactile response in receptors of the opposed skin. Loewenstein (1956) showed that this effect was due to sympathetic stimulation by demonstrating that both frequency and duration of tactile-evoked discharges increased following stimulation of the sympathetic chain. Similar enhancement of glossopharyngeal activity evoked by gustatory (and perhaps thermal) stimuli and phasic activity of mechanoreceptors were shown in frogs following sympathetic stimulation by Chernetski (1964).

The studies of Loewenstein (1956), Chernetski (1964), and Habgood (1950) all demonstrated sympathetic enhancement of tactile receptors in frog skin. All experiments were performed on what Catton (1958) considered rapidly adapting '*a*' mechanoreceptors, while the present study involved only Catton's types '*b*' and '*c*' receptors.

Sympathetic fibres responsible for enhancements of the experiments of Loewenstein (1956) and Chernetski (1964) had conduction velocities of 0·4 and 0·8 m/sec. Pick (1970) regards these as forming the bulk of sympathetic outflow in dorsal cutaneous nerves of amphibia. This is consistent with the high voltage and long duration necessary to stimulate the sympathetic fibres in the present study (0·2 V, 5 msec).

Drug effects

There is some question about the identity of the amphibian sympathetic transmitter. In the heart, epinephrine certainly predominates (Östlund, 1954; Falck, Häggendal & Owman, 1963); but large amounts of circulating norepinephrine are present in the blood of cold-acclimated animals, and it has been suggested that the normal mammalian roles of the two major catecholamines might be reversed in anurans (Segura & D'Agostino, 1964).

Epinephrine and norepinephrine both increased maximum dynamic sensitivity in concentrations as low as 10^{-8} g/ml (Fig. 10*b*, *c*). Norepinephrine-induced facilitation of maximum dynamic sensitivity above 10^{-7}–10^{-6} g/ml. thereafter approaching 150% control (Fig. 10*c*). This is precisely the degree of enhancement shown with sympathetic stimulation (Fig. 10*a*). Epinephrine increased maximum dynamic sensitivity to more than 200% at 10^{-4} g/ml., the largest dose administered. It should be noted that 10^{-7}–10^{-6} g/ml. epinephrine produced 150% control response, the asymptotic enhancement produced by both norepinephrine and sympathetic stimulation. In bullfrogs, the level of epinephrine in the heart resulting from sympathetic stimulation is about 5×10^{-7} g/ml. (Azuma,

Binia & Visscher, 1965). The concentration of norepinephrine in the sympathetic ganglia is about 3×10^{-6} g/ml.

Recruitment of larger fibres was shown to account for the increased response. This recruitment was identical to that observed with increased stimulus intensity (slope or magnitude) in the control animal.

Previous authors have demonstrated effects of epinephrine and norepinephrine similar to those produced by stimulation of the sympathetic nerves. Loewenstein (1956) applied concentrations of epinephrine 10^{-6} g/ml. and norepinephrine to the inner surface of frog skin and found that mechanical threshold was reduced about 10%, that additional fibres were being recruited, and that adaptation was very much slowed. This response to epinephrine disappeared very quickly, resulting in a tonic change in spontaneous activity after 60 sec. The minimum dose required was 10^{-8} g/ml. Chernetski (1964) confirmed the enhancement of frog phasic mechanoreceptor activity by epinephrine and norepinephrine (10^{-5} g/ml.), finding doses in the same range effective modulators of glossopharyngeal activity. Similar effects have been reported in cat (Douglas & Gray, 1953; Nilsson, 1972).

Ephedrine characteristically mimicked the actions of epinephrine and norepinephrine on frog cutaneous cold receptors, but peaked in effectiveness at 10^{-6} g/ml. Tachyphylaxis is a common clinical finding with ephedrine (Goodman & Gilman, 1970), and finding it in frog skin is not surprising.

Since adrenergic agonists were effective facilitators of maximum dynamic sensitivity, antagonists were applied to the skin. The sympathetic enhancement of cold receptor activity was blocked by both α- and β-blocking agents (Fig. 13). In addition, the maximum dynamic sensitivity of the unstimulated preparation decreased with progressive doses of the β-blocker. β-blocking agents depress local metabolism (Goodman & Gilman, 1970), and this might account for the decreased excitability of temperature receptors.

α-blockade of the sympathetic effect is supported by the demonstration of vasoconstriction mediated by alpha-adrenergic receptors in frogs (Erlijk, Centrangolo & Valdez, 1965). Furthermore, phenotolamine blockade of the sympathetic effect has been shown for other systems (Nilsson, 1972).

Mechanism of action

Loewenstein (1956) suggested that epinephrine interacted with stretch of the skin, modifying the generator potential of the receptor. This hypothesized modification of generator potential was confirmed (Loewenstein & Altamirano-Orrego, 1956). Both rate of rise and amplitude of the generator

potential of cat Pacinian corpuscle were shown to increase after treatment with epinephrine.

The only receptors histologically identified in frog skin are 'free nerve endings' (Rubin & Syrocki, 1936; Whitear, 1955). The similarity of the pharmacological responses of these endings and those of the carotid body chemoreceptors is stressed by Paintal (1964). Biscoe (1971) showed that free nerve endings can act as local metabolic sensors, and it is tempting to speculate that such a metabolic mechanism might transduce cutaneous sensations. At least one metabolic inhibitor (dinitrophenol) is known to depress tactile excitability in cat (Catton & Ueda, 1962). Furthermore, Dobromyslova & Slov'ea (1960) showed that both increased glucose and the application of 10^{-4} epinephrine decreased latency of the Türk reflex, while latency increased following monoiodoacetic acid, sodium fluoride, and dinitrophenol. This reflex is therefore facilitated by increasing carbohydrate supply and depressed by blockers of glycolysis and oxidative phosphorylation. Chernetski (1964) further suggested that epinephrine-induced phosphorylase activation might account for sympathetic enhancement in his preparation. Unfortunately, application of 2′5′ adenosine phosphate to the preparation has no effect upon the response, attributed by him to a permeability barrier.

The vascular theory of thermoreceptor function (Kenshalo & Nafe, 1963) does not fit the present data. Epinephrine iontophoresis in humans produces little effect on human cool threshold at low adapting temperatures (Dawson, 1964; Kenshalo, 1969) and decreases cool threshold at high adapting temperatures. Clearly the present study has shown that thermoreceptor activity is enhanced by sympathetic stimulation and the application of epinephrine to the receptive fields. It is once again suggested that previous studies of cutaneous sensory threshold have been confused by variations in blood flow, and that re-evaluation of the effects of drugs on thermoreceptor function should be undertaken using a preparation which does not require an intact circulation.

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