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Modulation of Cutaneous Cold Receptor Function by Electrolytes, Hormones and Thermal Adaptation

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Summary

The response properties of feline cold receptors were analyzed under control conditions, during conditions of altered external calcium concentrations and during application of menthol, catecholamines and ouabain. Afferent activity was extracellularly recorded from cold fibres of an isolated preparation of the tongue. Reduced calcium levels (0.5 mM) generally enhanced and elevated calcium levels (5.0 mM) suppressed cold fibre activity. The effects of menthol (10^{-5} M) on cold receptors were qualitatively similar to those of reduced calcium. Application of adrenaline and noradrenaline (10^{-6} M) were predominantly inhibiting. In cold receptors, the mean discharge rate is determined by the frequency of an oscillating receptor process and the probability of each cycle of this process to initiate afferent impulses. All measures mainly affected the probability of impulse generation rather than the oscillation frequency. Application of ouabain (10^{-6} M) resulted in excitatory responses, caused by an increase of both probability of impulse generation and frequency of the oscillating receptor process. It is concluded that cold receptor function is based on a specific combination of common neuronal elements rather than on specific sensory processes.

Key words

Cold receptor function – Calcium – Menthol – Catecholamines – Ouabain

Introduction

Temperature receptors convert patterns of heat energy into afferent neuronal signals. Information from these receptors is processed into conscious temperature sensations and into thermoregulatory responses. Relatively little is known about the cellular processes which are involved in the transduction of temperature signals. However, by analysis of the temporal pattern of afferent discharge under various experimental conditions, in cold receptors some of the underlying sensory processes could be identified. There is evidence that a temperature- and calcium-sensitive receptor potential oscillation generates the afferent action potentials (Braun *et al.* 1980, Schäfer *et al.* 1982); the calcium channel involved seems to be of the low-voltage activated type (Schäfer *et al.* 1991). An electrogenic sodium pump has been discussed as the primary temperature sensing element (Schäfer and Braun 1990). These processes are implicated in the cellular function of a variety of neurones, and can be interfered with by several compounds (Bean 1989, Glynn 1984). Therefore it might be speculated that cold receptor function under certain conditions might be subjected to physiological modulation. To what extent the changes of receptor function during long-term thermal adaptation (Hensel and Schäfer 1982) are also related to such mechanisms has so far not been discussed.

We therefore studied the potency of various substances known to affect the function of calcium channels and of electrogenic sodium pumps to modify cold receptor response properties. To prevent

interference by regulatory measures of the organism, cold receptors of a perfused isolated organ preparation were investigated.

Methods

Adult cats were initially anaesthetized with intraperitoneal sodium pentobarbital (35 mg/kg). If necessary, supplemental doses were given during the experiment. Isolated preparations of the tongue were prepared and perfused as previously described in detail (Schäfer 1987). In some experiments, the calcium concentration was changed from 1.53 mM (control) to 0.5 and 5.0 mM, respectively. Menthol, catecholamines and ouabain were applied by addition to the perfusing medium. For the method of recording and stimulation, see Schäfer (1987). Data were stored on magnetic tape and analysed off-line with a microcomputer system, using a program developed for analysis of neuroneal burst discharges (Hirsch *et al.* 1990). Note that the mean discharge rate is determined by the frequency of the receptor potential oscillation and by the probability of each cycle to generate afferent impulses (Braun *et al.* 1980). The parameters are the oscillation frequency (burst frequency) and the number of impulses initiated during each cycle.

Results

During the experiments, 115 cold receptors were identified and their thermal responses recorded. All receptors showed the periodic discharge pattern consisting of repetitive activity at higher and grouped discharges (bursts) at lower temperatures, which had been analyzed in detail in several previous investigations (Braun *et al.* 1980, Schäfer *et al.* 1982). In addition to control conditions, the receptors were studied at elevated or lowered external calcium concentrations (n = 22), during perfusion with 10 to 50 μM menthol (n = 8), during perfusion with 10^{-6} M adrenaline or noradrenaline (n = 60), and during perfusion with 10^{-6} M ouabain (n = 25). With the exception of ouabain, the effects of these compounds were fully reversible.

Generally, 5 mM calcium depressed and 0.5 mM stimulated cold receptor activity compared to control conditions. The effect was temperature-dependent, since calcium was more effective in the upper temperature range; below 15 °C calcium was ineffective or the effect was even inversed (Fig. 1). There was a linear inverse relationship between the mean discharge rate and the logarithm of the

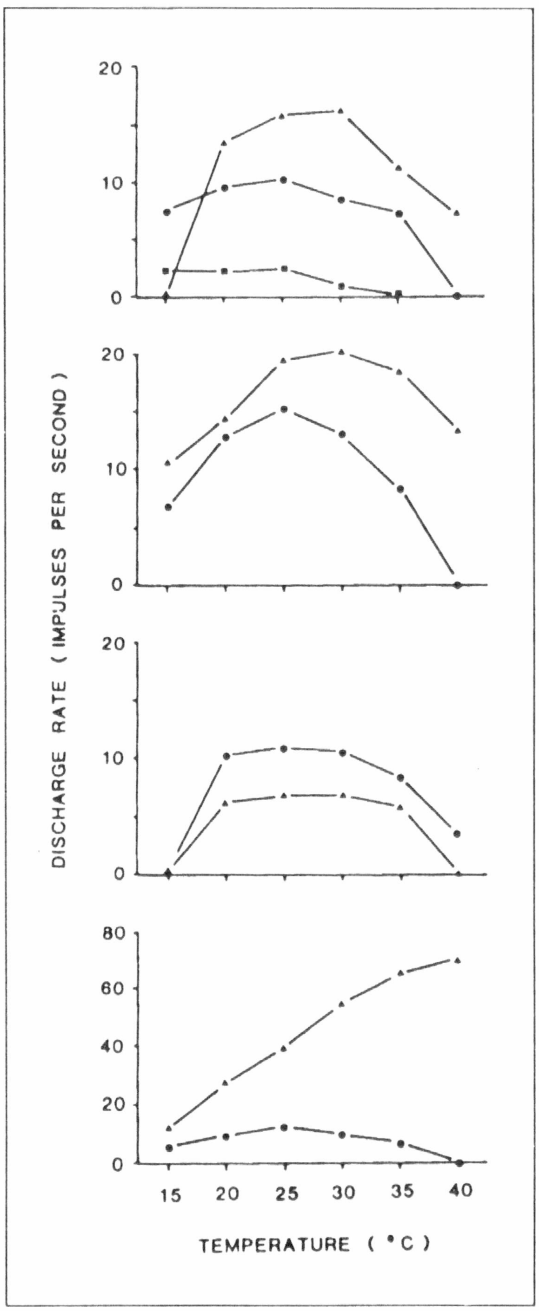

Fig. 1

Modulation of lingual cold receptor activity by various compounds at constant temperatures. Isolated perfused tongue preparation. Circles represent control conditions; triangles and squares test conditions. From top to bottom, effect of altered external calcium concentration (control, 1.53 mM; triangles, 0.5 mM; squares, 5.0 mM); effect of 10^{-5} M menthol; effect of 10^{-6} M adrenaline,

and effect of 10^{-6} M ouabain calcium concentration at static temperatures between 25 and 35 °C. The changes of afferent activity were based on specific changes of the cyclic discharge pattern. The frequency of the periodic pattern, which increases with raising static temperatures, remained almost unaffected, except for temperatures above 35 °C; at 35 and 40 °C, reductions of the calcium concentration increased the oscillation frequency. On the other hand, the probability of impulse generation, which can be determined as the number of impulses generated during each cycle of the periodic receptor process, increased throughout the whole temperature range during perfusion with reduced external calcium. Thus the changes of mean discharge rate were primarily caused by a modulation of the number of impulses induced during each cycle of the cold receptor potential oscillation.

There is good evidence that a low-voltage activated calcium conductance is involved in the signal transduction of cold receptors (Schäfer *et al.* 1991). It has reecently been demonstrated that menthol interferes quite specifically with calcium channel currents (Swandulla *et al.* 1986, 1987). Perfusing the isolated tongue preparation with solutions containing 10 to 50 μM menthol stimulated cold receptor activity; the effect was similar to that induced by reducing external calcium (Fig. 1). During menthol application, the mean discharge rate maintained a new steady-state value which was dose-dependent. Again the changes of mean activity were based on changes of the periodic discharge pattern, which were similar to those observed during low calcium conditions: the stimulating effect of menthol was mainly caused by an increase of the probability of impulse generation.

The effects of adrenaline on cold receptor activity were less homogeneous than those of calcium and menthol, but were predominantly inhibiting (Fig. 1). During perfusion with 10^{-6} M adrenaline, the discharge rate was reduced in 18 experiments and slightly stimulated in 11 experiments, when compared to control conditions. During perfusion of the isolated preparation with 10^{-6} M noradrenaline, an inhibiting effect was observed in 15 experiments and a slightly stimulating effect in 7 experiments. Similar results were obtained during infusion experiments with adrenaline and noradrenaline on whole animals. In most cases, the inhibiting effect was based on a reduced probability of impulse generation, i.e. the number of impulses per oscillation period was decreased. Thus, adrenaline modulated the same parameters of the periodic discharge pattern as calcium and menthol, but with an opposite sign.

During perfusion of the isolated organ preparation with 10^{-7} to 10^{-6} M ouabain, a maintained new steady-state level of activity was not achieved. After a lag of several minutes the mean activity began to increase and continued to do so, until a peak value was reached (Fig. 1). The cold receptor then fell silent and commonly remained inhibited, but in several receptors these driven discharges appeared repeatedly, and even at various adapting temperatures. The responses to ouabain were based on an initial increased probability of impulse generation, whereas the oscillation frequency remained remarkably unaffected. Within a few seconds, the oscillation frequency then attained a peak value of considerable magnitude, which was associated with a concomitant sharp decline of the firing probability, i.e. the number of impulses per oscillation period was reduced from relatively high values (8-16) to one or less. Finally, the oscillation frequency gradually fell to low values until activity ceased completely.

Discussion

Although discharge pattern analysis and isolated organ preparation have been proved to be very useful tools in the study of cold receptor function, one has to admit that our present knowledge about the underlying cellular processes is still rather limited. However, studies employing these tools have enabled us to identify at least two basic processes of signal transduction, an electrogenic sodium pump as the primary thermal sensor (Schäfer and Braun 1990), and an oscillator which is controlled by a low-voltage activated calcium channel (Schäfer *et al.* 1991). Interference with the conductance of this channel obviously displaces the receptor potential. Both mechanisms contribute to the cellular function of a variety of excitable cells (Bean 1989, Glynn 1984) and the findings on cold receptors have strengthened the earlier idea that the temperature sensitivity of cold receptors is not an unique property of these cells but might rather be based on mechanisms

common to most excitable cells (Carpenter 1981). If signal transduction in fact is a function of a specific combination of "standard" neuroneal elements, then it must be considered that these elements will retain their general properties, including the possibility that they might be susceptible to endogeneous modulation.

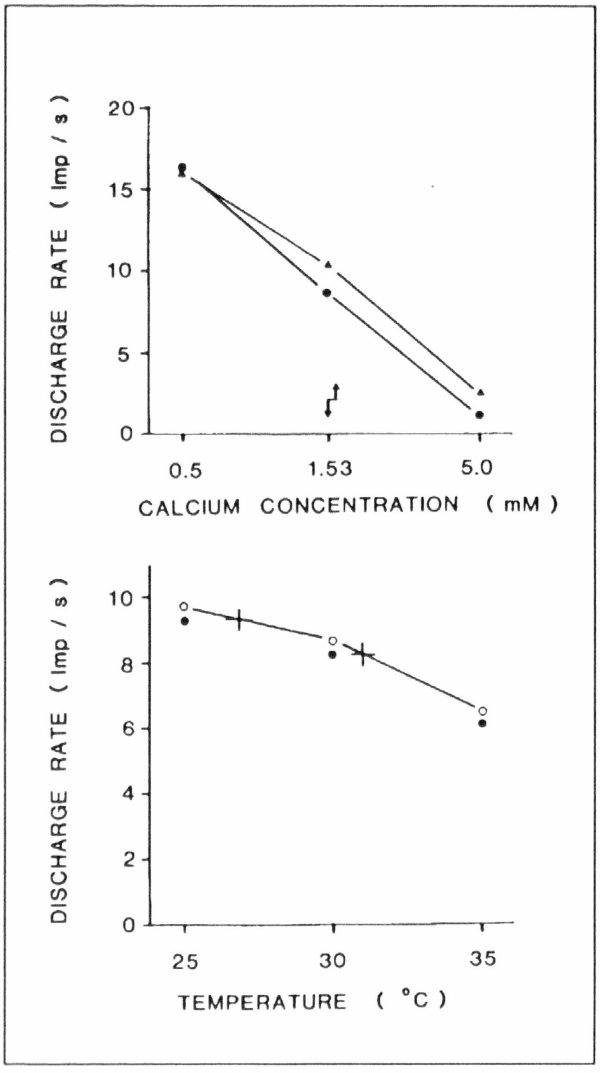

Fig. 2

Dependence of cold receptor activity on external calcium concentration. Top diagram, cold receptor activity at 25 °C (triangles) and 30 °C (circles). Arrows indicate elevation of external calcium concentration by 0.1 mM. Bottom diagram, decrease of cold receptor activity by a 0.1 mM increase of external calcium. Open symbols, control (1.53 mM); closed symbols, elevated calcium (1.63 mM). Crosses indicate shift of temperature signal (+1.81 °C and 0.97 °C, respectively).

The data indicate that an electrogenic sodium pump is present in cold receptors, determining mainly the probability of impulse generation. These pumps are normally active below their maximum capacity and are controlled by the intracellular sodium concentration; physiological variations of the extracellular potassium concentration are of little effect (Rossier *et al.* 1987). Neuroneal sodium pumps are stimulated by catecholamines (Rossier *et al.* 1987); the resulting hyperpolarization inhibits cellular electrical activity. It might be speculated that this mechanism is responsible for the suppression of cold receptor activity during application of adrenaline and noradrenaline; it is unlikely that the effect is mediated by calcium channels since catecholamines commonly inhibit conductance through these channels (Marchetti *et al.* 1986).

The high sensitivity of cold receptors to the external calcium concentration is remarkable, and it is tempting to assume that it might be of functional significance. Increases of 0.1 mM of plasma ionized calcium have been observed to occur during physical work (Greenleaf *et al.* 1979), and a calcium-dependent additional factor has been suggested to affect thermoregulation during exercise (Greenleaf 1979). Due to the flat slope of the mean activity–temperature relation of cold receptors at relevant physiological temperatures, small variations of external calcium produce relative large shifts of the temperature signal: our data (Fig. 2) indicate a positive shift of almost 1 °C at 30 °C skin temperature (1.8 °C at 25 °C). The marked sensitivity of cold receptor function to calcium is confirmed by the effect of menthol application. Whereas peak calcium currents of sensory neurones are reduced to a half-maximum value by 3×10^{-4} M menthol (Swandulla *et al.* 1987), cold receptor activity is considerably enhanced by even 10^{-5} M. Several findings suggest that the effect is the result of a specific interaction of menthol with calcium channels (Swandulla *et al.* 1986, 1987), and the molecular requirements for the thermal effect (Watson *et al.* 1978) indicate a specific ligand-receptor interaction.

If such modulatory mechanisms in fact are operative under certain conditions in temperature receptors, then quite possibly it must be assumed that their effects are small and difficult to be detected. Moreover, the nature of the mechanisms implies that the

effects are rather short- than long-termed, so that only temporal adjustments of sensory function are to be expected. Generally, the demonstration of such processes being present and operative gives no evidence that they are involved in ordinary regulatory activity; they may be viewed as merely representing various states of environmental noise. At present it is not meaningful to relate the complex changes of cold receptor response properties during long-term thermal adaptation (Hensel and Schäfer 1982) to these mechanisms.

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