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Discharge pattern analysis suggests existence of a low-threshold calcium channel in cold receptors

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Summary. The regular periodic activity patterns of mammalian cold receptors have been quantitatively studied. Analysis of the timing of either single impulses or impulse groups demonstrated that the periodic receptor process is maintained independently of impulse generation and continues to operate under conditions when afferent impulses are not initiated. These results imply that the underlying conductances must be operational at threshold potentials related to impulse generation. In addition to temperature, the periodic process is considerably sensitive to calcium, which affects mainly the probability of impulse generation during each cycle. Reduction of external calcium and application of calcium entry blockers with relative selectivity for low-threshold calcium channels are similarly effective in modulating cold receptor activity. The data imply the existence of a low-threshold calcium conductance at the sensory terminal.
Key words. Cold receptor; periodic discharge pattern; transducer process; low-threshold calcium channel.

Relatively little is known about the nature of the cellular processes which in cold receptors convert patterns of heat energy into afferent neuronal signals. These sensors are considered to be 'free' nerve endings [1] and their small size and low distribution density have so far not allowed us to study their transducer processes directly. However, periodic components have been observed in the temporal pattern of afferent activity in all mammalian cold receptor populations [2]. Several studies provide evidence that a temperature- and calcium-sensitive receptor potential oscillation generates afferent impulses when it exceeds a threshold value [3–5]. Since the stimulating effect of reduced external calcium can be exactly mimicked by menthol [6], which selectively impairs calcium channel conductance [7, 8], the existence of a specific calcium channel at the sensory terminal has been postulated [6, 9]. Menthol interferes with calcium entry through two of the calcium channels present in dorsal root ganglion (DRG) cells [7]. These conductances differ in their physical and pharmacological characteristics [10, 11]. Here we present evidence that the receptor potential oscillation is maintained at threshold potentials related to impulse generation, indicating the existence of a low-threshold channel. This view is supported by the observation that calcium entry blockers affecting preferentially low-threshold channels are effective in modulating cold receptor activity.

Material and methods

Isolated preparations of the tongues of cats were prepared and perfused as previously described [12]. The normal perfusing medium contained 118.40 mM NaCl, 26.60 mM $NaHCO_3$, 2.83 mM KCl, 1.32 mM KH_2PO_4, 1.46 mM $MgSO_4$, 1.53 mM $CaCl_2$, and 11.60 mM glucose. In some experiments, the calcium concentration was changed to 0.5 mM. Calcium channel modulators were applied by addition to the perfusing medium. The lingual nerve was dissected into fine strands, which were placed on a platinum electrode for the recording of single unit activity. Cold receptors were stimulated by a water-

circulated thermode[12]. The findings reported here are based on recordings from 38 single cold receptors. Data were analyzed off-line with a microcomputer system, using a program developed for analysis of neuronal burst discharges[13]. To calculate either the oscillation period or the number of impulses per period, we used the data of interspike intervals as described in detail previously[6].

Results and discussion

Two experimental approaches were used to determine the physical and pharmacological properties of the calcium channel involved in sensory transduction. In the main series of experiments we analyzed the timing of impulse generation to prove whether the cyclic afferent activity is controlled by an independent receptor process, oscillating at threshold potentials, or if the initiated impulses themselves contribute substantially to the maintenance of the cyclic pattern. Generally, we observed regular groups of impulses at 10 to 25 °C[3], and irregular activity at 35 or 40 °C. However, the interspike intervals of these 'irregular' discharges were not randomly distributed, but were clustered at values of approximately integer multiples of the shortest distribution maximum, when represented as an interval-distribution histogram (fig. 1A). Such a pattern indicates that the periodic receptor process occasionally fails to initiate the appropriate impulse. Impairing the calcium entry by either reducing the calcium concentration in the perfusing medium from 1.5 (control) to 0.5 mM (n = 10), or by adding 10 to 50 µM menthol (n = 3), reversibly stimulated afferent activity. In accordance with earlier observations[5,6,9], the effects of calcium and menthol were qualitatively identical within the temperature range of 10 to 40 °C; both affected the frequency of oscillation as well as the probability of each cycle to generate impulses. Generally, the oscillation frequency was markedly accelerated above 30 °C and slightly decelerated below 25 °C, whereas the number of impulses per cycle was increased throughout the whole temperature range. Thus the long intervals of discharges with a multimodal interval distribution were completely replaced by short ones of uniform duration (fig. 1A), whereas single impulses were replaced by impulse groups (c.f. fig. 2). If the unit was silent prior to the change of medium, 'irregular' discharges were induced, which also showed a highly ordered interval distribution (fig. 1A). Obviously calcium does not act as a current carrier, since impairing the calcium entry does not result in inhibition but in excitation. All evidence[5,6,12] points to the existence of a calcium-controlled outward current which, upon impaired activation, offsets the receptor potential to a more depolarized level.

Based on the interval-distribution data of 4 receptors exhibiting such multimodal distributions, we calculated the frequency of the underlying periodic receptor process, and compared these values with the experimental data (table). The average relative deviation between

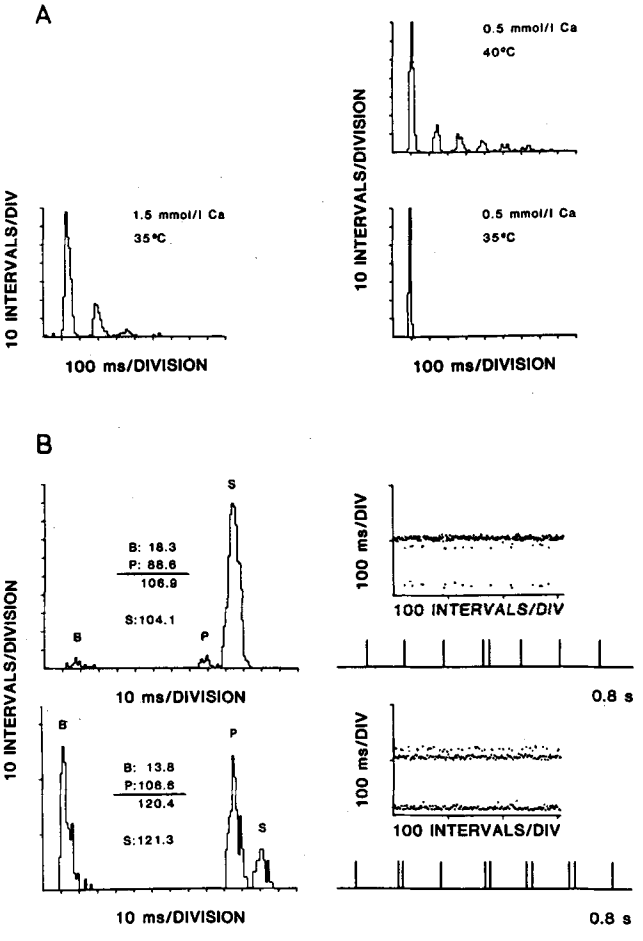

Figure 1. Temporal pattern of afferent discharge of single cold receptors at maintained temperatures. *A* Interval-distribution histograms of a single cold sensor at 35 and 40 °C. Left diagrams, control conditions (1.5 mM calcium); right diagrams, reduced external calcium conditions (0.5 mM calcium). Under control conditions, the unit is not active at 40 °C. Under low calcium conditions, at 40 °C the unit is activated, and at 35 °C the activity pattern changes to a uniformly spaced impulse discharge, resulting in a narrow unimodal interval-distribution. *B* 2 examples of afferent activity at 35 °C maintained temperature. In both cases, single impulses as well as doublets are generated randomly. Left, interval-distribution histograms; right, impulse discharge for 0.8 s. Insets, duration of successive interspike-intervals. B, P, S have the following meaning: interval within a doublet, interval following a doublet, interval following a single impulse. The relative deviation between S and the sum of B and P (related to S) is 2.7 and −0.7%, respectively.

calculated and experimental values for all multiples was 1.11% (range: −0.9 to 4.8%), indicating a plain linear relationship. On the average, there was a consistent deviation of −4.9% between the calculated and experimental values which directly represent the oscillation period. The nature of this deviation is at present unclear. However, these data show unequivocally that the oscillating receptor process is capable of precisely generating afferent impulses at intervals up to 10 times the oscillation period (table).

If single impulses and impulse groups are initiated together, their timing is of the same high accuracy. We found that intervals following single impulses approxi-

Average interval duration of different maxima of interval-distribution histograms (4 units) [1]

	35 °C [2,4]			35 °C [2]			40 °C [3,4]			40 °C [3]			40 °C [3]		
n	e	c	d	e	c	d	e	c	d	e	c	d	e	c	d
1	146.1	149.9	−2.5	92.6	97.9	−5.2	115.5	121.6	−5.0	122.8	129.8	−5.4	115.7	123.6	−6.4
2	309.1	299.8	3.1	193.8	195.9	−1.0	250.8	243.3	3.0	259.4	259.6	−0.07	245.3	247.2	−0.9
3	458.0	449.7	1.8	299.3	293.9	2.2	376.3	364.9	3.1	393.6	389.4	1.0	370.8	370.8	0.0
4				400.7	391.9	2.2	498.5	486.5	2.4	522.4	519.2	0.6	503.3	494.4	1.8
5				502.6	489.9	2.6	614.5	688.2	1.0	651.3	649.0	0.3	619.3	618.0	0.2
6				596.2	587.9	1.4	735.4	729.8	0.7	786.0	778.8	0.9	737.9	741.6	−0.5
7				699.4	685.9	1.9				899.7	908.6	−0.9	883.5	865.3	2.1
8				800.0	783.8	2.0									
9				–	881.8	–									
10				1027.0	979.8	4.8									

n, number of distribution maximum; e, experimental values; c, calculated values; d, relative deviation between c and e (in percent of c). [1] For method of calculation [3,15]; [2] control conditions (1.5 mM Ca); [3] reduced Ca conditions (0.5 mM Ca); [4] data of unit shown in fig. 1A.

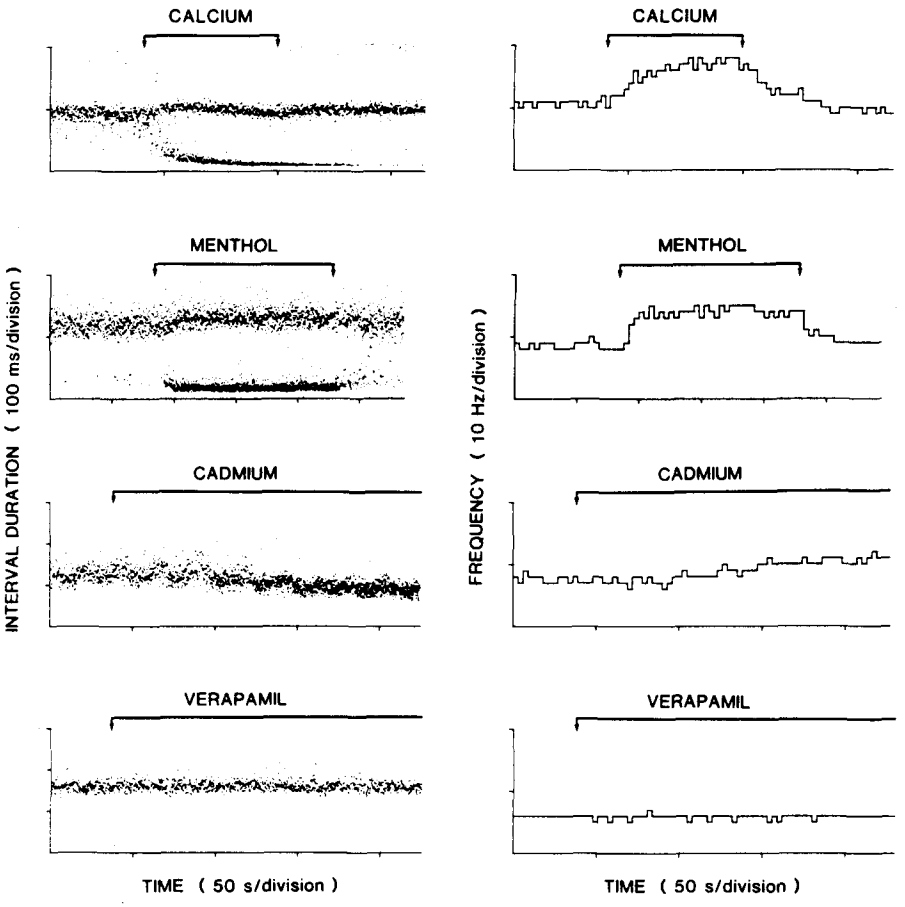

Figure 2. Effect of various calcium entry blockers on discharge rate and discharge pattern of cold receptors. Four different units. Stimulus temperature, 30 °C except for cadmium, 35 °C. Left diagrams, duration of successive interspike-intervals (intervals shorter than 50 ms are intragroup intervals); right diagrams, mean discharge rate (bin width, calcium, 2 s; menthol, 4 s; cadmium, 3 s; Verapamil, 3 s). The perfusing medium contained: calcium, 0.5 mM (control, 1.5 mM); menthol, 10 μM; cadmium, 100 μM; Verapamil, 10 μM. Bar indicates test conditions. Identical data in left and right diagrams. Oscillation frequency: calcium, control 10.7 s^{-1}, test 9.0 s^{-1}, recovery 10.1 s^{-1}; menthol, control 8.0 s^{-1}, test 6.5 s^{-1}, recovery 7.7 s^{-1}; cadmium, control 8.6 s^{-1}, test 11.8 s^{-1}, recovery 7.5 s^{-1} (not shown).

mately equal the sum of intragroup intervals and intervals following the group. Figure 1B shows 2 examples of afferent activity consisting of various proportions of doublets among single impulses. The relative error of timing of either single impulses or doublets is in the same order as that reported above for multimodal interval distributions representing single impulse activity. In addition, earlier investigations of the pattern of impulse group generation have shown that the oscillation period is not modified when a varying number of impulses is initiated during one cycle [3].

These observations strongly suggest that the oscillating receptor process is independent of the spike-generating process, and that it continues to oscillate under condi-

tions when action potentials are not initiated. Therefore, these results imply that the conductances, which maintain the oscillation, must be operational at rather negative membrane potentials, i.e. at subthreshold potentials related to spike generation.

In a supplementary series of experiments, we investigated the effectiveness of several substances, known to interfere with calcium entry, to modify cold receptor activity. Perfusion of the isolated preparation with either menthol-containing medium (10 to 50 μM, n = 8) or medium of reduced calcium concentration (0.5 mM, n = 22) resulted in reproducible, reversible, and dose-dependent changes of discharge rate and pattern (fig. 2). Cadmium reversibly stimulated cold receptor activity when added to the perfusing medium in a concentration of 100 μM, whereas 10 μM were practically without effect (n = 3, fig. 2). Verapamil did not affect cold receptor discharges at 10 μM, but inhibited activity completely at 100 μM in all receptors tested (n = 8, fig. 2). In a different preparation (facial cold receptors of the rat; K. Schäfer and M. Heinz, unpublished observations), nickel and menthol proved to be equally effective, when applied in equimolar concentrations.

One difficulty encountered in attempting to separate calcium channel types based on their pharmacological characteristics is the lack of agents that selectively affect the low-threshold channel[14]. In DRG cells, this channel is markedly depressed by menthol (100–500 μM)[7] and by nickel (10–100 μM)[15], whereas cadmium (20–50 μM)[11] is less effective. In the same preparation, higher concentrations of nickel (> 100 μM)[15] and cadmium (100–200 μM)[10,11] progressively reduce low- and high-threshold currents. Verapamil (20-400 μM) depresses the high-threshold, but not the low-threshold type in sensory neurons[14,16]. In an isolated baroreceptor preparation, the inhibiting effect on the receptor response of verapamil (10 μM) was ascribed to interference with an inward Na^+ current[17].

In conclusion, our analysis of the timing of periodic afferent activity does not support the view that a high-threshold calcium conductance, which is activated during action potentials, is involved in sensory transduction of mammalian cold receptors. Instead our data indicate the contribution of a low-threshold calcium conductance with biophysical properties resembling those of the T-type current of sensory neurons. This current is activated at threshold potentials of impulse generation, and has been assumed to be involved in rhythmic neuronal activity[14,18]. It can only be activated from membrane potentials negative to −60 mV[11,14]. At resting conditions, the membrane potential of mammalian myelinated sensory nerve fibers is in the range of approximately −78 mV[19]. T channels are also operational at voltages achieved during the action potential[10], but the comparatively long time needed to obtain the peak conductance[10] makes an appreciable contribution to the inward current during the afferent impulse unlikely. On the other hand, the rather long inactivation time constant[10] accords very well with the impulse group duration of cold receptors at similar temperatures (20–25 °C)[3]. Our observations on the efficacy of several calcium entry blockers to modulate cold receptor activity support these views. Compounds with a relative selectivity for the low-threshold current all were effective, and there was no indication that under our experimental conditions the effects of calcium and menthol were mediated by channels other than calcium channels[6–8]. It seems therefore justified to assume that a low-threshold calcium channel contributes to signal transduction in cold receptors.

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