ELECTRICAL CHARACTERISTICS OF SENSORY NEURONS OF Hirudo medicinalis

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During intracellular polarization of identified sensory neurons of the leech by square pulses of hyperpolarizing current electrical parameters of the cell membranes were determined: input resistance of the neuron R_n, time constant of the membrane τ, the ratio between conductance of the cell processes and conductance of the soma ρ, the resistance of the soma membrane r_S, the input resistance of the axon r_a, capacitance of the membrane C_S, and resistivity of the soma membrane R_S. The results obtained by the study of various types of neurons were subjected to statistical analysis and compared with each other. Significant differences for neurons of N- and T-types were found only between the values of τ, C_S, and R_S ($P < 0.01$). These parameters also had the lowest coefficients of variation. The surface area of the soma of the neurons, calculated from the capacitance of the membrane (the specific capacitance of the membrane was taken as 1 $\mu F/cm^2$) was 7-10 times (N-neurons) or 4-6 times (T-neurons) greater than the surface area of a sphere of the same diameter. The resistivity of the soma membrane R_S was 35.00 $k\Omega \cdot cm^2$ for cells of the N-type and 19.50 $k\Omega \cdot cm^2$ for T-neurons. The reasons for the relative stability of this parameter compared with the input resistance of the cell (coefficient of variation 22-7 and 53-31% respectively) are discussed. The possible effects of electrical characteristics on the properties of repeated discharges in neurons of different types also are discussed.

INTRODUCTION

Altogether 14 sensory neurons have been identified in the segmental ganglia of Hirudo medicinalis and, depending on differences in their electrical responses to mechanical stimulation of the skin and to passage of a direct current through the cell body they can be distinguished into three groups: T, P, and N [18]. The identified neurons can easily be recognized in each segmental ganglion by their position and electrical responses. The sharpest differences between them are manifested in the character of responses to the action of long stimuli (direct current, mechanical stimulation of the skin). Neurons of N-type are characterized by a low maximal firing rate (up to 1-5 spikes/sec) and a long duration of after-hyperpolarization (AH) after the single excitation wave (the "time constant" of AH is about 200 msec). The maximal firing rate of the T- and P-neurons is 200 and 70-80 spikes/sec respectively. The duration of AH in these cells is substantially less than in cells of the N-type (about 10 msec in T-neurons and about 20 msec in P-neurons) (Fig. 1). As experiments on a series of objects showed, the differences in the frequency of repeated discharges can be attributed, first, to differences in the duration of the after-decrease of excitability in each type of cell after the passage of a single action potential (AP) and, second, to different rates of rise of potentials appearing as on-responses to depolarizing currents [11, 13]. Depolarization of the cell to the threshold level is determined by the rate of rise of the "passive" shift of potential and the active local reaction of the membrane. Differences in the duration of the after-decrease of excitability in cells of N- and T-types are mainly due to differences in the temporal course of restoration of conductance for potassium ions when increased after passage of the AP [15]. However, differences in the "passive" characteristics of the cell membrane may also be reflected in the character of the firing pattern of the neuron.

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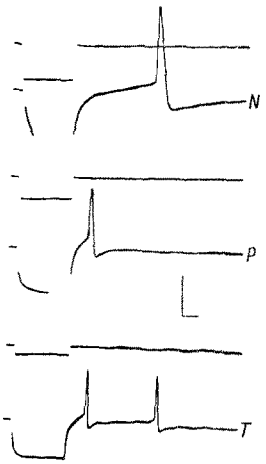

Fig. 1. Action potentials in sensory neurons of N-, P-, and T-types arising as off-responses to hyperpolarizing current. Calibration: 40 mV, $4 \cdot 10^{-9}$ A, 10 msec.

The object of this investigation was to compare the electrical characteristics of different types of sensory neurons of Hirudo medicinalis distinguished by marked differences in repetitive discharges in response to prolonged stimuli.

EXPERIMENTAL METHOD

Intracellular potentials of sensory neurons of the leech were recorded by glass microelectrodes with a resistance of 30-50 mΩ, filled with 2 M potassium citrate. The neurons were polarized through the same microelectrode by means of a bridge circuit. Neurons of the different types were identified both by the response of the cell to puncture of its membrane by the microelectrode [18] and by the shape of the antidromic AP during stimulation of the lateral or dorsal root of the ganglion. The electrical characteristics of the cell membrane were determined by analysis of the transitional process after application of a hyperpolarizing current of 1-2 nA. The following parameters were calculated: input resistance R_n, time constant τ, dendritic dominance factor ρ, capacitance of the soma membrane C_S, resistivity of the soma membrane of the neuron R_S, resistance of the soma membrane r_S, and input resistance of the axon r_a.

The methods used to calculate these parameters are adequately described elsewhere [2, 16, 20]. The curves were analyzed by graphic differentiation [5]. The surface area of the soma was determined from photographs of the neurons injected with the dye Procion yellow [19]. According to electron-microscopic investigations [8], neurons of the leech have a folded outer membrane. To estimate the "true" surface area of the cell body, the capacitance of the membrane was taken to be 1 $\mu F/cm^2$ (as in many animal tissue cells), and on this basis the area of the membrane (S_{calc}) was calculated as the ratio between the input capacitance of the neuron soma and the specific capacitance of the membrane. The resistivity of the soma membrane was calculated without allowing for folding of the membrane ($R_{S.meas}$ in Table 1) and allowing for the calculated surface area of the neuron soma ($R_{S.calc}$). For each parameter for the N- and T-neurons the arithmetic mean and coefficient of variation (CV) were determined. The significance of differences between the parameters was assessed with the aid of Student's criterion. The P-neurons were not compared with other cells. Only four neurons of this type were investigated and the parameters for them showed great variability, evidently because of the heterogeneity of the P-neurons in the ganglion (four such cells were identified in each segmental ganglion).

RESULTS AND DISCUSSION

The results of measurement of the passive characteristics of neurons of N-, T-, and P-types are given in Table 1.

The input resistance of the different types of neurons in these experiments was 10-54 mΩ. Jansen and Nicholls [15] consider that an input resistance of the order of 50-100 mΩ is normal for cells of the N-type, but this applies only to exceptional cases. Sensory neurons of the leech are comparatively small (30-50 μ) and are covered by a dense glial membrane. Even if microelectrodes with a resistance of 30-50 mΩ are used it is not always possible to avoid damaging the cell. The passive characteristics were calculated only for neurons in which stable responses were observed during the 15-20 min after insertion of the microelectrode. Assessment of the input resistances from records illustrating several papers [5, 6, 19] showed that in most cases the investigations were carried out on neurons whose resistance also was 15-40 mΩ. The wide scatter of the values of the input resistances in the present experiments (CV = 53-31%) made it difficult to compare this parameter in neurons of different types. Differences in the sizes of the neurons and in their position in the ganglion, affecting successful introduction of the microelectrode, could also lead to additional errors. However, even allowing for these obstacles, neurons of the leech have a much higher input resistance than more widely studied objects (giant neurons of Anisodoris [17] and Aplysia [14], Betz cells of the cat motor cortex [16], cat motoneurons [10]). This is due both to the small size of the neurons and the resistivity of the membrane (see below).

To determine the resistivities of the membrane more accurately, allowance was made for the increase in total conductance of the cell because of shunting of part of the applied current by the processes

TABLE 1. Electrical Characteristics of Different Types of Sensory Neurons of H. medicinalis

Type of cells	Surface area of soma, S_{meas}	Surface area of soma, S_{calc}, $cm^2 \times 10^{-4}$	R_n, Ω (input) ($\times 10^6$)	ρ	r_s, Ω ($\times 10^6$)	ra, Ω ($\times 10^6$)	C_s F ($\times 10^{-10}$)	τ, msec	$R_{s.meas}$, $\Omega \cdot cm^2$ ($\times 10^3$)	$R_{s.calc}$, $\Omega \cdot cm^2$ ($\times 10^3$)
N-neurons	$d=64\ \mu$	7,55	24,00	1,09	50,16	45,90	7,55	38,00	6,45	38,00
	12 860 μ^2	7,85	18,50	0,86	34,40	40,10	7,85	27,00	4,42	27,00
	$1,286 \cdot 10^{-4}$ cm^2	9,44	20,00	0,59	31,80	53,90	9,44	30,00	4,09	30,00
		7,45	43,00	0,28	55,00	196,00	7,45	41,00	7,07	41,00
		7,45	21,00	1,94	61,70	31,80	7,45	46,00	7,93	46,00
		8,80	16,00	0,98	31,70	32,40	8,80	28,00	4,08	28,00
M ± m		8,09±0,34	23,75±5,21	0,96±0,23	44,13±5,36	66,68±26,04	8,09±0,34	35,00±3,18	5,67±0,69	35,00±3,18
CV		10%	53%	59%	29%	95%	10%	22%	29%	22%
T-neurons	$d=52\ \mu$	4,62	19,50	1,00	39,00	39,00	4,62	18,00	3,31	18,00
	8190 μ^2	4,46	14,00	2,20	44,80	20,40	4,46	20,00	3,80	20,00
	$0,8490 \cdot 10^{-4}$ cm^2	3,05	27,00	1,31	62,40	47,60	3,05	19,00	5,30	19,00
		3,86	36,50	0,49	54,40	111,00	3,86	21,00	4,62	21,00
		3,38	24,50	1,17	53,20	45,50	3,38	18,00	4,52	18,00
		3,31	24,50	1,59	63,50	39,90	3,31	21,00	5,39	21,00
M ± m		3,76±0,27	24,33±3,08	1,29±0,23	52,88±3,93	50,57±12,70	3,76±0,27	19,50±0,56	4,49±0,33	19,50±0,56
CV		17%	31%	44%	18%	62%	17%	7%	18%	7%
P-neurons	$d=64\ \mu$	2,94	28,00	1,06	57,70	54,60	2,94	17,00	7,42	17,00
	12 860 μ^2	15,70	10,50	0,51	15,90	31,20	15,70	25,00	2,04	25,00
	$1,286 \cdot 10^{-4}$ cm^2	3,52	54,00	0,47	79,40	169,00	3,52	28,00	10,21	28,20
		12,80	10,00	1,26	22,60	17,90	12,80	29,00	2,91	29,00
M ± m		8,74±3,24	25,60±10,35	0,82±0,20	43,90±15,10	69,68±33,35	8,74±3,24	24,70±2,86	5,65±1,91	24,70±2,86

of the cell [20]. This increase in conductance was expressed as the ratio between the conductance of the cell processes and the conductance of the soma (the factor ρ). If the value of ρ is small (as it is, for example, in mollusk neurons) it can be determined from the slope of the electrotonic potential curve at the time of application of the polarizing current [2]:

$$\left.\frac{dV}{dt}\right|_{t=0} = \frac{V_\infty}{\tau}(\rho + 1),$$

where V_∞ is the electrotonic potential of the soma after the end of the transitional process. The value of ρ varied in the present experiments from 0.28 to 2.2. Differences between the mean values for T- and N-cells were not significant. The coefficients of variation of ρ in the cells of the two types were much greater than CV for the input resistances of the neurons (especially for T-neurons). Injury to the cell membrane by the microelectrode can substantially modify the ratio between the conductances of soma and processes, for the soma membrane suffers the most damage and its resistance is reduced correspondingly. An improvement in recording methods must therefore lead to some increase in the value of ρ. The results agree with those obtained for giant neurons of Planorbis corneus [2]. The value of ρ in these cells varied from 0.0 to 1.4. However, considering the possibility of a marked change in ρ because of damage to the neuron soma by the microelectrode, the real value of ρ could be as much as 2-3 (also in view of the fact that the input resistances of the cells may reach 50-100 mΩ) [15]. In that case the value of ρ in the leech neurons comes closer to the results obtained for the neurons of Anisodoris [17].

The time constant of the membrane was determined from the slope of the curve $\ln (dV/dt) = f(t)$ relative to the ordinate. The mean value of τ for six N-neurons was 35.00 msec, with CV 22%; for six neurons of the T-type it was 19.50 msec, CV = 7%. The time constant in the P-neurons was 24.70 msec (four neurons).

The duration of after-hyperpolarization potentials recorded from the N- and T-cells differed sharply (200 and 10 msec respectively), whereas the sensory neurons of the leech had comparable values of τ (35.00 and 19.50 msec respectively). Nevertheless these differences are statistically significant ($P < 0.01$) and cells with AH of longer duration (neurons of N-type) had a greater time constant.

However, the observed parallel cannot be regarded as proof that the duration of AH depends on the value of τ. The time constant of the neuron soma membrane can determine the course of AH only if there is a significant difference between the values of τ and the "time constant" of AH; moreover, an essential condition must be satisfied: τ must be very large compared with the duration of AH. The ratios between these values differed in the neurons of different types studied. In N-cells the duration of AH (200 msec) was much much greater than the value of τ (35 msec) and for that reason the course of AH was entirely determined by the time of recovery of normal permeability for potassium ions (the "potassium" character of AH in leech neurons was demonstrated by Nicholls and Baylor [18]). Opposite relationships were observed in neurons of the T-type. The mean value of τ for six neurons was 19.5 msec but the "time constant" of AH was about 7-10 msec; in the T-cells the value of τ may therefore have some effect on the rate of recovery of MP after passage of the AP. The possibility of a reduction in τ because of increased conductance of the cell membrane in the course of AH must also be considered (this leads to a decrease in the effect of τ on the duration of AH). P-Neurons (if the sample of data obtained by the investigation of four neurons can be assumed to be representative) had about equal values of τ and AH (24.7 and 20 msec respectively) and the effect of τ on the course of AH was about the same as in the T-cells.

Comparison of the parameters of the N- and T-neurons showed the presence of significant differences ($P < 0.01$) only in the values of C_S, τ, and $R_{S.calc}$. The coefficients of variation of these parameters also were comparatively low (from 7 to 22%). Relative constancy of the capacitance characteristics of the membrane has been demonstrated for many objects [9, 10, 16], and even during the development of excitation processes the capacitance of the membrane does not change by more than 20% [9], whereas conductance increases by hundreds of times. When neurons are identified by their electrophysiological parameters, most attention should therefore be paid to these most stable characteristics. Capacitance of the membrane (C) is determined by the area of the membrane and the value of the specific capacitance. Since the specific capacitance of the membrane in most animal and plant cells is about 1 $\mu F/cm^2$, the surface area of the soma of identifiable neurons can be compared with respect to the value of C_S. Such a comparison is particularly necessary for the neurons of many invertebrates because of the considerable folding of the soma membrane surface [1, 4, 7, 8]. The real surface area of the soma in the giant neurons of Planorbis corneus can exceed the surface area of a sphere of the same diameter by 10-25 times [2].

This ratio for N-neurons was 7-10 times, and for T-neurons 4-6 times. Differences in the values of the calculated areas were significant ($P < 0.01$). Calculation of the resistivities of the soma membrane of the neurons allowing for folding of the membrane showed a considerable difference between these values in neurons of N- and T-types (35.00 and 19.50 $k\Omega \cdot cm^2$ respectively, $P < 0.01$). Those values differ somewhat from those obtained for neurons of Aplysia (2500-11,300 $\Omega \cdot cm^2$) [14] and Helix (1000-8300 $\Omega \cdot cm^2$) [3] and those calculated without regard to folding of the membrane. However, allowing for the folded structure of the membrane, very close values of the resistivity have been obtained for the neurons of Helix pomatia (23,400 $\Omega \cdot cm^2$) [12].

When the resistivity was determined without regard to folding of the membrane, similar values were obtained for N- and T-neurons (5670 and 4490 $\Omega \cdot cm^2$), i.e., disregarding the relief of the cell surface can not only lead to a decrease in the value of the resistivity, but it can also mask possible differences in its value in different neurons.

It is also interesting to examine the relative stability of the resistivity of the membrane ($R_{s.calc}$) in T- and N-cells. The coefficient of variation for them was 7 and 22%. Meanwhile the coefficient of variation for the input resistance of the cells was 31 and 53%. Differences between the resistivities of the membrane were significant ($P < 0.01$) but the difference between the input resistances was not significant.

The variability of the input resistances is due to differences in the degree of injury to the cell membrane by the microelectrode. Only a small area of the membrane around the microelectrode tip was injured, but this had a strong shunting effect on the current passed through because of its proximity to the tip of the polarizing and recording electrode. The properties of the greater part of the surface membrane, however, were only slightly affected, with consequent stability of the cell responses to external stimuli for a period measured in tens of minutes and relative constancy of the basic electrical characteristics of the membrane (C_S and R_S).

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