Time Course and Properties of Late Adaptation in Spinal Motoneurones of the Cat

D. Kernell and A.W. Monster[1,2]

Dept. of Neurophysiology, Jan Swammerdam Institute, University of Amsterdam,
Eerste Constantijn Huygensstraat 20, NL-1054 BW Amsterdam, The Netherlands

Summary. In the spinal cord of anaesthetized cats, motoneurones of m.gastrocnemius medialis were stimulated to repetitive firing by very long-lasting steady currents injected through an intracellular microelectrode (maximum duration 4 min). In such discharges, a gradual decline of impulse frequency was found to occur during several tens of seconds. Most of this "late adaptation" occurred during the first 30 s of firing. Comparisons between the responses of different cells showed that the frequency-drop during late adaptation was strongly correlated to the impulse rate at the beginning of the discharge. For one and the same cell, late adaptation was more prominent at strong than at weaker intensities of stimulation (i.e., at high than at lower initial firing rates). In cells capable of discharging continuously for several minutes, a semi-stationary discharge rate tended to be reached after about 1 min or less.

Key words: Spinal motoneurones – Repetitive firing – Adaptation

Introduction

For the analysis of motor functions, it is important to know how well the motoneurones are capable of maintaining discharges during long-lasting periods of stimulation. Like most other kinds of nerve cells, spinal motoneurones may generate a maintained repetitive impulse discharge in response to a steady stimulating current (Granit et al. 1963a; Kernell 1965a). In such experiments, current injected via an intracellular microelectrode is used as a substitute for the maintained post-synaptic currents that normally would excite these cells in tonic firing (for comparisons between effects of injected and postsynaptic currents, see Granit et al. 1966; Kernell 1969). When activated by a strong step of steady injected current, the firing rate of a motoneurone decreases rapidly during the first second after the onset of stimulation. This "initial adaptation" is particularly pronounced during the first intervals of firing (Granit et al. 1963a; Kernell 1965a). Following the period of initial adaptation, the discharge rate is known to show a further slow decrease during a prolonged period of time (Granit et al. 1963b; Kernell 1965a). The time course of this "late adaptation" has not previously been subjected to any systematic analysis.

In the present paper we will analyze the discharges of spinal motoneurones during periods of constant intracellular stimulation lasting up to 4 min. In a companion paper (Kernell and Monster 1982) we will compare the adaptational properties of motoneurones which innervate different types of muscle fibres. A brief preliminary account has been published (Kernell and Monster 1980).

Methods

The experiments were performed on cats (2.4–4.0 kg) anaesthetized with pentobarbitone (initial dosis 40 mg/kg i.p., supplementary doses given as needed during the course of the experiment). The animals were breathing spontaneously, and their mean blood pressure was ≥ 90 (usually > 100) mm Hg. The nerve to m.gastrocnemius medialis (GM) was prepared for electrical stimulation via a pair of spring-loaded silver electrodes. All other muscles of the same limb were denervated, and the tendon of the GM muscle was attached to a sensitive isometric force transducer. The exposed GM muscle and its nerve were covered with mineral oil. The temperature of this oil pool, the body temperature (subscapular) and the temperature of the mineral oil covering the exposed spinal cord were all kept close to 38° C. Dorsal roots L6–S1 were cut. Motoneurones of GM were penetrated by single-

barrelled glass microelectrodes filled with 2 M potassium citrate. The electrodes typically had their tips broken to a diameter of about 1–2 μm, and their initial resistance was usually less than 10 MΩ. For each motoneurone, the following measurements were made: (i) recording of motor unit twitch, (ii) recording of antidromic action potential (cells with spikes < 60 mV were discarded), (iii) determination of lowest current intensity needed for producing a maintained repetitive discharge ("rhythmic threshold"), (iv) test of late adaptation: stimulation for 4 min (or until cell stopped firing) with constant injected current of supra-threshold intensity (usually 5 or 10 nA above rhythmic threshold), and, for most cells, (v) fatigue test of muscle unit. If the cell remained in good condition, steps (ii)–(iv) were repeated for a number of times with a pause of at least 5 min between each test of late adaptation. Data from measurements of muscle contractions (twitch, muscle unit fatigue) were used for the analysis reported in a subsequent paper (Kernell and Monster 1982).

The rhythmic threshold was determined by aid of the manual operation of a continuous control of current intensity. The accuracy of the determination was typically ±0.5 nA. For the present investigation we have only made use of cells that were capable of discharging continuously for at least 10 s in response to a constant current. Most cells were activated by a current of 5 nA above rhythmic threshold. In gastrocnemius motoneurones a suprathreshold stimulus intensity of 5 nA would produce a main-tained discharge of about 5–10 Hz above the minimum rate of the cell (Kernell 1979). Such a rate increase of 5–10 Hz is relatively small in comparison to the typical extent of the primary range of firing (Kernell 1965b, 1979). As is commonly the case (cf. Kernell 1965a), several cells were encountered that were unable to deliver a maintained discharge of several seconds in response to constant stimulation. These cells could not be used for the present study. There is much evidence indicating, that phasic response properties are commonly caused by some kind of damage inflicted by the penetrating microelectrode (Kernell 1965a; Schwindt 1973).

The resting membrane potential of a penetrated neurone was continuously monitored by a slow pen writer (paper speed 1 cm/min). Current intensities and discharge frequencies were measured and plotted on-line by aid of a digital computer. Furthermore, analog data were also recorded on UV-sensitive paper (bandwidth dc – > 10 kHz) as well as on magnetic tape (bandwidth dc – 2.5 kHz) for later analysis. Mean values are given ± S.D.

Results

Duration of Repetitive Firing

The present study concerns the adaptation during prolonged repetitive discharges. Hence, only cells capable of discharging continuously for at least 10 s of steady stimulation were included into the present material (cf. Methods). The histograms of Fig. 1 show that, even among such "tonic" motoneurones there was a great variation in the duration of discharges evoked by our standard stimuli of 5 or 10 nA above the threshold for rhythmic firing (Fig. 1A, B, respectively). Even when tested in the same cell, the stronger stimulus did not regularly increase the duration of firing. The rhythmic threshold often differed considerably between different cells; its mean value was 11.4 ± 7.8 nA for the 31 cells of Fig.
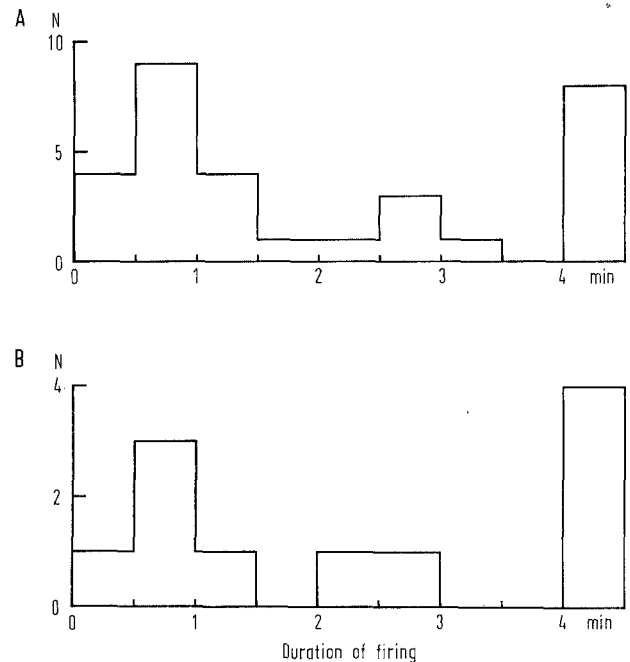

Fig. 1A, B. Histograms showing the distribution of maximum discharge durations for 31 cells stimulated by 5 nA (A) and for 11 cells stimulated by 10 nA (B) above threshold for rhythmic firing. Nine cells are represented in both diagrams. Note difference in ordinate scale between A and B

1A. However, when compared between different cells, the discharge duration produced by a given intensity of suprathreshold current was not signifi-cantly correlated to the threshold of rhythmic firing (for the cells of Fig. 1A: $r = -0.19$, n = 31, $P > 0.1$). If the firing lasted longer than 1–1.5 min, the chance was relatively high that it might continue throughout the 4 min test period (Fig. 1). In three cases, the same current intensity was tested more than once in cells capable of maintained repetitive firing. One of these cells discharged throughout the maximum test period in both trials. In another cell (Fig. 6) the discharge duration was longer for the first than for the second trial, and in a third case the second trial produced a more prolonged discharge than the first one. When calculated for all the values of Fig. 1A there was a weak but statistically significant positive correlation between maximum discharge duration and antidromic spike size ($r = 0.38$, n = 31, $P < 0.05$; spike amplitude 77 ± 7 mV, range 61–90 mV).

For 17 of the cells of Fig. 1A, we investigated how easily repetitive firing might be restarted just after the premature end of a discharge (i.e., cells with discharge duration < 4 min; steady stimulation 5 nA above rhythmic threshold). This was tested by aid of a series of 0.5 s pulses of injected current. In most cases (11 cells), repetitive discharges appeared again
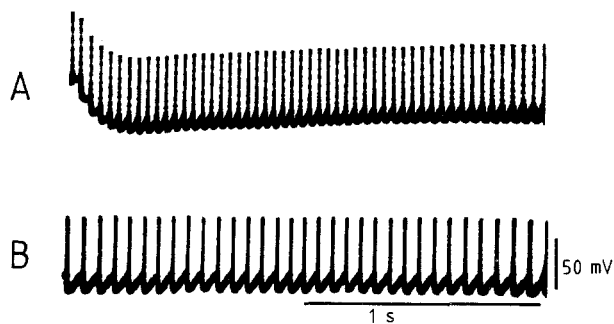
Fig. 2. A Repetitive impulse discharge of motoneurone stimulated by a steady current starting suddenly at the onset of the record. Stimulus intensity 23.6 nA, which was 5 nA above threshold for rhythmic firing. Illustration taken from analog tape record with bandwidth of dc – 2.5 kHz. **B** From later period of same discharge as in **A** (26th–27th s of firing)

Fig. 4. Same discharge as in Fig. 3, but plotted with logarithmic ordinates after subtraction of a value for "final firing rate". This latter value (11.4 Hz) was taken to be 0.1 Hz smaller than the lowest discharge rate encountered in Fig. 3
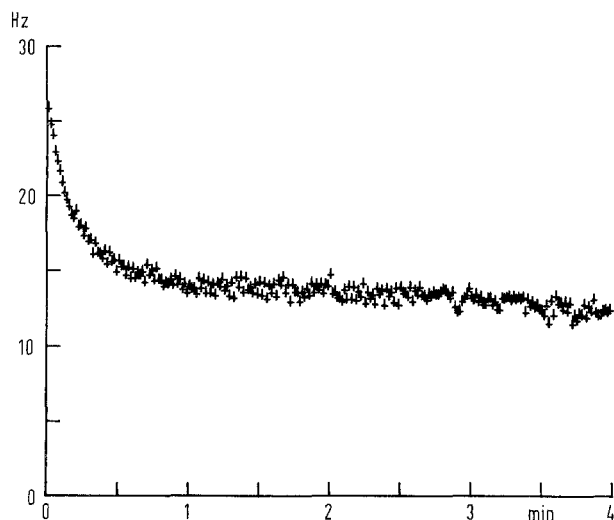

Fig. 3. Plot of firing rate (Hz) versus time (min) for discharge partly shown in Fig. 2. In this and subsequent frequency-time relations, plotted impulse frequencies are the mean values for each consecutive second of discharge

in response to relatively weak test pulses ($\leq$ 3 nA above intensity of preceding steady stimulation). In three cells, however, even an increase to > 23 nA above the intensity of the preceding steady stimulation failed to produce repetitive firing.

Time Course of Late Adaptation

The record of Fig. 2A shows the initial portion of a discharge produced by a current step of 5 nA above threshold. There is an evident drop in instantaneous rate from the first to the second impulse interval, but thereafter the mean frequency seems to stay nearly constant. A study of the same discharge at a longer
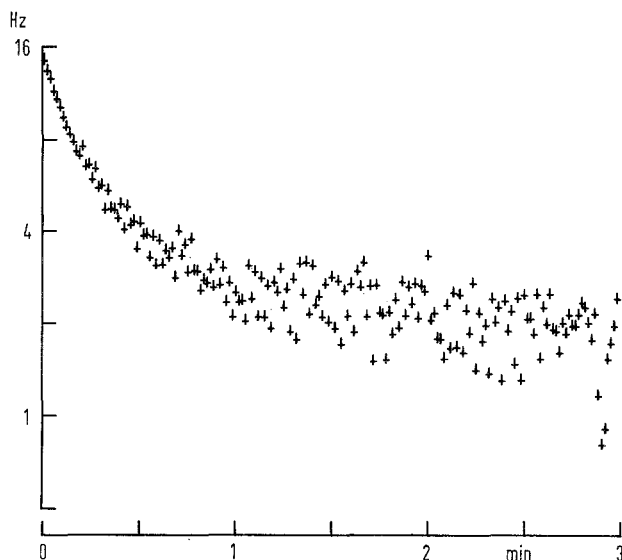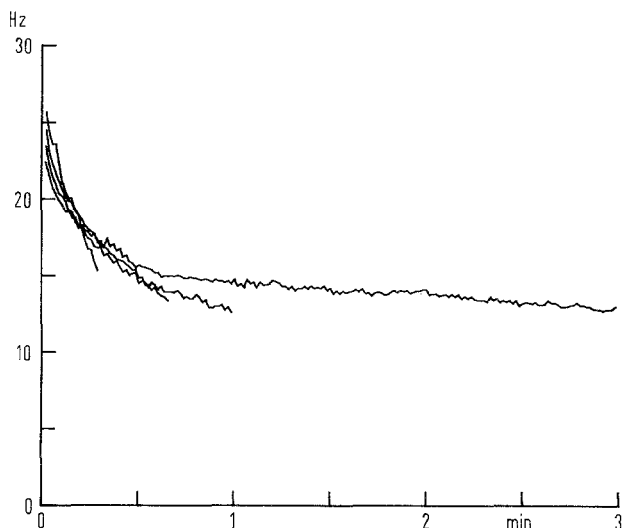

Fig. 5. Mean relation between impulse rate (Hz) and time (min) for four groups of motoneurones with different discharge durations (D_f). All the cells were stimulated by a current of 5 nA above threshold for rhythmic firing. For one group the values are plotted to 18 s (40 s > D_f > 18 s, n = 5) for one group to 40 s (60 s > D_f > 40 s, n = 5) for one group to 60 s (180 s > D_f > 60 s, n = 6) and for one group to 180 s (D_f > 180 s, n = 6). All plots start at 2nd s of firing. For consecutive seconds of discharge, mean firing rates have been connected by straight lines. Several of the neurones of Fig. 1A were not included into the present diagram. Some of the cells were excluded because their total discharge duration was < 18 s, and other cells could not be used because of the appearance of incidental irregularities of discharge rate. For all included cells, firing was regular during the periods for which the illustrated averages were calculated, i.e. for all seconds of firing the standard deviation of the impulse intervals was less than 20% of the mean interval duration (usually much less)
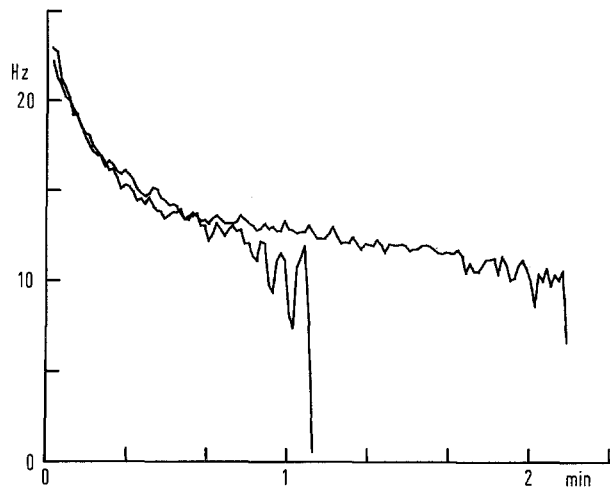
Fig. 6. Impulse rate (Hz) versus time (s) for two periods of steady stimulation in the same cell. Stimulation was in both cases 10 nA above threshold for rhythmic firing

Fig. 7. Drop of discharge rate from 2nd to 26th s of regular firing (Hz) plotted versus rate of 2nd s (Hz) for cells stimulated by 5 nA (*circles*) and 10 nA (*triangles*) above threshold for rhythmic firing. Regression line calculated by method of least squares for all the values of the plot ($r = 0.93$, n = 36, $P < 0.001$). Comparable data for both stimulus intensities included for seven of the cells. For these neurones, the plotted frequency-drop was 11.1±6.8 Hz for the higher and 8.3±4.8 Hz for the lower stimulus intensity. The difference between these two mean values was statistically significant (Wilcoxon matched-pairs signed-ranks test, $P < 0.05$)

time scale reveals, however, that there was a slow but very marked decline in mean discharge rate during several tens of seconds after the onset of stimulation (Fig. 3; cf. Fig. 2A, B).

In Fig. 4, values of Fig. 3 have been plotted with logarithmic ordinates after subtraction of a "final firing rate" (i.e. the lowest discharge rate attained). It is evident that no single time constant can describe the whole time course of late adaptation. This was true also if no "final firing rate" was subtracted from the various impulse frequencies. Furthermore, it is evident from Figs. 3 and 4 that no perfectly constant firing rate was ever attained during the test period of 4 min. The decline in discharge rate was, however, extremely slow at times following the first minute of firing (Fig. 3).

The general findings of Figs. 3 and 4 were characteristic for all the present cells. The time course of the drop in discharge frequency was very similar between groups of cells with different total durations of firing (Fig. 5), and it was repeatable in individual cells (Fig. 6). Compared to frequency-time relations of long total discharge duration, those of briefer ones showed a downward bend only during a short period just prior to the premature end of the discharge (Fig. 6).

Extent of Late Adaptation at Different Levels of Activity

In discharges caused by a steady suprathreshold stimulation of 5 or 10 nA, most of the "late" drop in firing rate occurred within the first 20–30 s (Figs. 3–6). The frequency drop over the period from 2nd
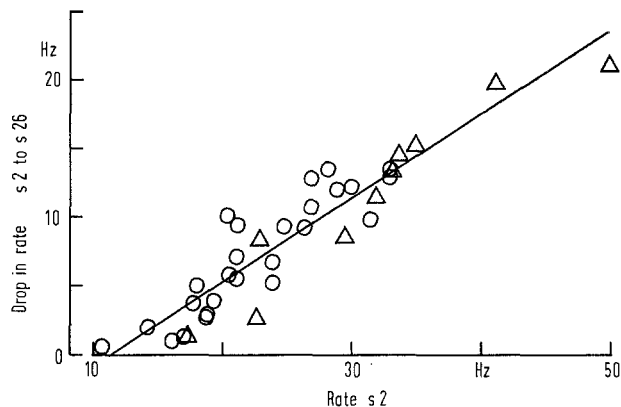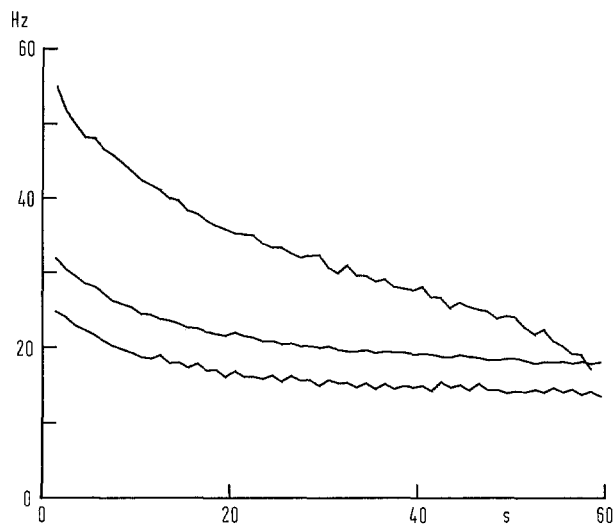

Fig. 8. Impulse rate (Hz) versus time (s) for three periods of steady stimulation in the same cell. Amount of stimulus intensity above threshold for rhythmic firing was 5 nA for lower curve, 10 nA for middle curve and 30 nA for upper curve. Total discharge duration was 240 s (= maximal test period) for the two weaker currents and only 59 s for the stronger one. Same cell as in Figs. 2–4

to 26th s of regular discharge was used as a measure of the extent of late adaptation. Among cells stimulated by the same amount of suprathreshold current, there was a significant positive correlation between the extent of late adaptation and rhythmic threshold (for cells stimulated by 5 nA above threshold: $r = 0.59$, n = 26, $P < 0.01$). An even more striking correlation was found, however, between the extent
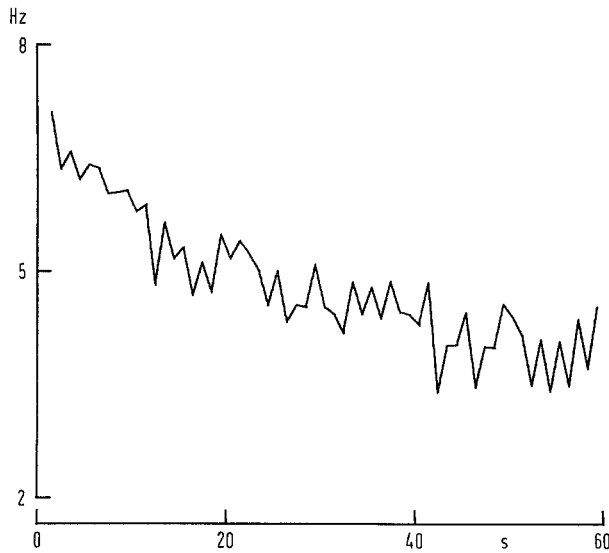
Fig. 9. Difference between the discharge rates of the two lower curves of Fig. 8 (Hz) plotted versus time (s)

of late adaptation and the impulse rate at the beginning of the discharge (Fig. 7): the higher the "onset frequency", the greater the extent of adaptation. This was true also for comparisons between discharges that were elicited in the same cell by different intensities of stimulation (see Legend of Fig. 7). Examples from one neurone are illustrated in Figs. 8 and 9. Due to the fact that the frequency-drop was more marked at high than at lower levels of activity (Figs. 7 and 8), discharge rates produced by weak and stronger currents came progressively closer to each other in the course of late adaptation (Figs. 8 and 9). The late adaptation was not, however, associated with any marked decline in the sensitivity of the cell to abrupt changes in stimulus intensity. Earlier investigations have shown that a recurring stepwise alteration of stimulus intensity elicits much the same changes in discharge rate at different times during the course of late adaptation: the frequency-current relation was found to be shifted in parallel while its slope ("f–I slope") remained approximately constant (Granit et al. 1963b; Kernell 1965a). This was confirmed by our findings. In three cells, stimulus intensity was alternating in a stepwise manner between 5 and 11 nA above rhythmic threshold (step duration 3 s). When tested in this way, the f–I slope changed ≤ 6% during the initial half minute of firing.

Discussion

The present experimental results confirm the existence of a prolonged phase of late adaptation in spinal

motoneurones (Granit et al. 1963b; Kernell 1965a). We have described the time course of this phenomenon for the first time. Several of our observations are consistent with the idea that, in spinal motoneurones, the late adaptation is caused by some kind of cumulative after-effects of many consecutive spikes. In such a case, the after-effects obtained after a certain period of continuous discharge would be expected to be stronger the greater the number of preceding spikes. Thus, in cells activated by steady current, a greater drop of firing rate per unit time would be expected to occur in discharges starting at a high rate than in those starting at a lower rate. Our results show that this actually is the case, both for comparisons between the discharges of different cells (Fig. 7) and for comparisons between different discharges of the same cell (Figs. 7–9). Thus, in a series of long-lasting discharges caused by steady currents, the firing rates produced by different intensities of current will differ more initially than later on (Figs. 8 and 9). Still, the sensitivity of the cell to stepwise changes of current stays nearly constant throughout late adaptation (Granit et al. 1963b; Kernell 1965a; confirmed in present study). This latter observation might be explained by the fact that, in a discharge caused by a recurring stepwise change between a "high" and a "low" stimulus intensity, both these alternating intensities of current will, at corresponding times, have been preceded by approximately the same total number of impulses (i.e., by same amount of "cumulative after-effects").

The mechanisms and properties of the late phase of adaptation, which is acting over tens of seconds of a continuous discharge, are probably different from those responsible for the rapid initial phase of adaptation just after the onset of a current step. The initial phase of adaptation is associated with a marked and rapid decline in the slope of the frequency-current relation, and it is to an important extent caused by the "summation" of the after-hyperpolarizations (AHPs) succeeding the first few spikes (e.g., Kernell 1972; Kernell and Sjöholm 1973; Baldissera and Gustafsson 1974; Baldissera et al. 1978; Barrett et al. 1980). On the basis of results from voltage clamp experiments, it has recently been suggested that also the late adaptation might be caused by changes in the AHP (Barrett et al. 1980). It remains to be experimentally verified how the AHP of motoneurones actually behaves during long-lasting repetitive discharges (in prep.). It is, however, of interest to note that an increase in the duration and/or size of the AHP-conductance would be expected to lead to a decrease in the sensitivity of firing rate to stepwise changes in stimulating current (e.g., Kernell and Sjöholm 1973). During the course

of late adaptation, however, the sensitivity to changes in stimulus intensity remains nearly constant (Granit et al. 1963b; Kernell 1965a; confirmed in present study). Thus, it seems likely that other factors than gradual changes in AHP-conductance are mainly responsible for the late adaptation. The duration of AHP is, however, of major importance for the rate at which a motoneurone will fire when stimulated at an intensity just above threshold (Kernell 1965b). For corresponding intensities of supra-threshold current, gastrocnemius motoneurones with long-lasting AHPs would be expected to fire at slower rates than those obtained in cells with briefer AHPs (cf. Kernell 1979). Hence, motoneurones with long-lasting AHPs would be expected show compara-tively little late adaptation (cf. Fig. 7; for further comments, see Kernell and Monster 1982). Moto-neurones with a long-lasting AHP would be expected to have a comparatively high input resistance and a low rhythmic threshold (Kernell 1966). Thus, the positive correlation between the rhythmic threshold and the amount of late adaptation (see section "Extent of late adaptation" . . .) might mainly be a consequence of the tendency of low-threshold cells to fire at slow rates.

The frequency-drop of late adaptation took place without any marked decrease in the amplitude of the rhythmic spikes (Fig. 2A, B). Thus, a gradual in-activation of the spike-generating mechanisms does not seem to be one of the main causes for the late adaptation in motoneurones. Possibly, the adapta-tion might be due to gradually increasing hyper-polarizing currents generated by an electrogenic sodium pump (cf. crustacean stretch receptor, Sokolove and Cooke 1971) and/or a slowly activated potassium permeability (cf. some molluscan neurones, e.g., Partridge and Stevens 1976; Colding-Jørgensen 1977; for a general discussion, see Jack et al. 1975). In either case, a transient increase of membrane potential would be expected to occur just after the end of the discharge (post-tetanic hyper-polarization; cf. Sokolove and Cooke 1971). Such phenomenae could not, however, be reliably studied in the present experiments because of the polariza-tion artefacts produced when strong currents were injected through the recording microelectrode.

The physiological significance of late adaptation will have to be considered in relation to the contrac-tile effects of motoneuronal firing. Such questions will be discussed in a subsequent paper in connection with experimental studies concerning the properties of late adaptation in motoneurones innervating dif-ferent types of muscle fibres (Kernell and Monster 1982).

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