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BISTABILITY OF α-MOTONEURONES IN THE DECEREBRATE CAT AND IN THE ACUTE SPINAL CAT AFTER INTRAVENOUS 5-HYDROXYTRYPTOPHAN

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SUMMARY

1. In the preceding paper (Crone, Hultborn, Kiehn, Mazieres & Wigström, 1988) it was shown that a short-lasting synaptic excitation ('on' stimulus) of extensor motoneurones (primarily triceps surae) in the decerebrate cat often resulted in a maintained excitability increase, which could be reset by a short-lasting inhibitory stimulus train ('off' stimulus). In the present experiments intracellular recording from triceps surae motoneurones and the electroneurogram (ENG activity) from triceps surae nerve branches were performed in parallel.

2. Sustained firing of individual triceps surae motoneurones was most often recorded in parallel with the maintained ENG activity following a synaptic 'on' stimulus. When the motoneurone was silenced, by a hyperpolarizing current through the microelectrode, there was no sign of on-going synaptic excitation during the maintained ENG activity following an 'on' stimulus. It was therefore suggested that voltage-dependent intrinsic properties of the motoneurones themselves could be responsible for the maintained firing.

3. In confirmation of this hypothesis it was found that short-lasting depolarizing current pulses through the recording microelectrode could trigger a self-sustained firing in the motoneurone provided that the bias current (i.e. the holding potential) was kept within certain limits. Hyperpolarizing current pulses terminated the firing. When the spike-generating mechanism was inactivated (by long-lasting excessive depolarization) similar depolarizing and hyperpolarizing current pulses could initiate and terminate plateau potentials in the motoneurones. By grading the depolarizing current pulses it was found that the plateau potentials were of all-or-none character, typically around 10 mV in amplitude. The two levels of excitability which can be triggered by short-lasting excitation and inhibition of the motoneurones is referred to as 'bistable' behaviour of the motoneurones.

4. After an acute spinal transection, in the unanaesthetized cat, the bistable behaviour of the motoneurones disappeared. However, it reappears following intravenous injection of the serotonin precursor 5-hydroxytryptophan (50–120 mg/kg).

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5. Individual triceps surae motor units were recorded by selective EMG electrodes during tonic stretch reflexes in the decerebrate preparations. Based on an analysis of their firing pattern during lengthening and shortening (or vibration) of the muscle it is suggested that plateau potentials in motoneurones are recruited during the tonic stretch reflex. Furthermore, it is argued that a quantitatively important part of the depolarization of motoneurones during the tonic stretch reflex indeed originates from these plateau potentials.

6. It is suggested that the plateau potentials are due to a voltage-dependent non-inactivating Ca^{2+} conductance, which is revealed during active serotonergic raphe–spinal innervation of the motoneurones (cf. Hounsgaard & Kiehn, 1985).

INTRODUCTION

In the accompanying paper Crone, Hultborn, Kiehn, Mazieres & Wigström (1988) described that a short-lasting synaptic excitation of extensor motoneurones, e.g. by a train of monosynaptic Ia EPSPs (an 'on' stimulus) may trigger a long-lasting motoneuronal output, which is maintained until terminated by short synaptic inhibition, e.g. by a train of volleys in the peroneal nerve (an 'off' stimulus). Initially the maintained excitability increase was interpreted as being due to a maintained polysynaptic excitation of the motoneurones supported by reverberating activity in closed neuronal loops (Hultborn, Wigström & Wängberg, 1975; Hultborn & Wigström, 1980). Curiously, in most cases they did *not* find the expected maintained depolarization during intracellular recordings from motoneurones; following the synaptic depolarization during the 'on' stimulus the membrane potential instead rapidly returned to the original level. However, in some cases a large maintained depolarization was recorded as illustrated by Hultborn & Wigström (1980, their Fig. 4; reproduced by Baldissera, Hultborn & Illert, 1981) and the noise on the top of this depolarization was interpreted as additional evidence for an on-going synaptic excitation.

In the beginning of the present series of experiments we observed that a postsynaptic inhibition, which is selective to motoneurones, could effectively terminate the maintained activity in the peripheral motor nerve following an 'on' stimulus (presented in the preceding paper, Crone *et al.* 1988). That observation strongly indicates that the motoneurones themselves are responsible for the maintenance of different excitability levels. This possibility was certainly strengthened with the demonstration by Schwindt & Crill (1980*a*) of a bistable behaviour of α-motoneurones during penicillin-induced seizures.

In the present work we have performed parallel recordings of the mass activity in the peripheral motor nerve (the ENG activity) and of the membrane potential from individual motoneurones following repeated synaptic 'on' and 'off' stimuli. We demonstrate that these 'on' stimuli trigger all-or-none plateau potentials in the motoneurones, which may support a maintained firing. It is concluded that the maintained motor activity described in the preceding paper (Crone *et al.* 1988) reflects an intrinsic bistable behaviour of α-motoneurones.

Some of the results have been reported in a short communication (Hounsgaard, Hultborn, Jespersen & Kiehn, 1984) and a review (Hounsgaard, Hultborn & Kiehn, 1986).

METHODS

Preparation. The experiments were performed on sixteen unanaesthetized decerebrate cats (2·0–3·2 kg). Five of them were in addition acutely spinalized. The protocol for anaesthesia and anaemic decerebration (the animals were kept anaesthetized during the operation), the dissection of nerves and the spinal cord as well as the maintenance of the preparation are identical to the procedure described in the previous paper by Crone *et al.* (1988). The nerves to the following muscles were dissected free on the left side: posterior biceps–semitendinosus, anterior biceps–semimembranosus, medial gastrocnemius, lateral gastrocnemius–soleus, plantaris–flexor digitorum longus, tibialis posterior, peroneus brevis and tertius as well as the deep peroneal nerve and tibialis anterior. The sural nerve and the superficial peroneal nerve were used to stimulate cutaneous afferents.

Recording and stimulation. Intracellular recordings from motoneurones were obtained with glass capillary microelectrodes filled with 3 M-potassium acetate or 2 M-potassium citrate. The tips were broken to a diameter to around 1·5–2·0 μm. The nerves were stimulated with square pulses (0·1 ms) either as single pulses or in trains of 200–300 Hz. The strength of the stimulus was expressed in multiples of threshold ($\times T$) for the lowest threshold afferent fibres. Nerve volleys were recorded from the surface of the spinal cord. The nerve recordings (ENG) were rectified and filtered (RC integrated) for reproduction. In three experiments activity of individual motor units was recorded with fine selective EMG electrodes (single-fibre electrodes, DISA) in parallel with gross EMG recording with thin copper wires inserted in the same muscle. Data were stored on a 7-channel Racal tape-recorder (DC to 2·5 kHz) for off-line analysis on a PDP 11/34 computer.

Drugs. A 1% solution of 5-hydroxy-DL-tryptophan (5-HTP) was obtained by dissolving the substance (Sigma) in 0·9% NaCl under warming and stirring. Following spinalization at Th12 level 5-HTP (50–140 mg/kg) was given. In one experiment the dose of 5-HTP was reduced to 12 mg/kg following pre-treatment with the decarboxylase inhibitor carbidopa (2 mg/kg, a gift from Merck Sharp & Dohme, Copenhagen). Pre-treatment with carbidopa (which does not cross the blood–brain barrier) reduces the 5-HTP decarboxylation in the periphery and therefore minimizes actions upon systemic blood pressure and respiration, while reducing the dosage of 5-HTP required to elicit central effects. In the decerebrate cats without spinal lesions 5-HTP (5–50 mg/kg) was often given during the experiments when sustained nerve discharge in response to short-lasting nerve stimulation began to fail.

RESULTS

(1) *Sustained discharge and plateau potentials in motoneurones of the decerebrate cat initiated and terminated by short-lasting synaptic inputs*

This series of experiments started with an intracellular analysis of the motoneuronal response during the long-lasting excitability shifts evoked by short-lasting synaptic excitation ('on' stimulus) and inhibition ('off' stimulus). Intracellular recordings were obtained from motoneurones to lateral gastrocnemius–soleus or medial gastrocnemius, while the general excitability level in the pool of motoneurones was monitored by simultaneous recording of the ENG activity from the corresponding nerve (cf. Fig. 1*A*). Figure 1*B–D* shows such parallel recordings from a motoneurone (lateral gastrocnemius–soleus, upper traces) and the corresponding ENG activity (rectified, lower traces). The short train of group I volleys in Fig. 1*B* ('on' stimulus) triggered a maintained ENG activity (lower trace) and a sustained discharge of the motoneurone (upper trace), both of which were terminated by a train in the nerve from peroneus brevis and tertius ('off' stimulus; $10 \times T$). If the sustained firing of motoneurones was supported by a maintained synaptic excitation it should be possible to visualize the EPSPs, when a continuous hyperpolarizing current kept the motoneurone below firing threshold. The records in Fig. 1*C* were obtained with a continuous hyperpolarizing current which prevented

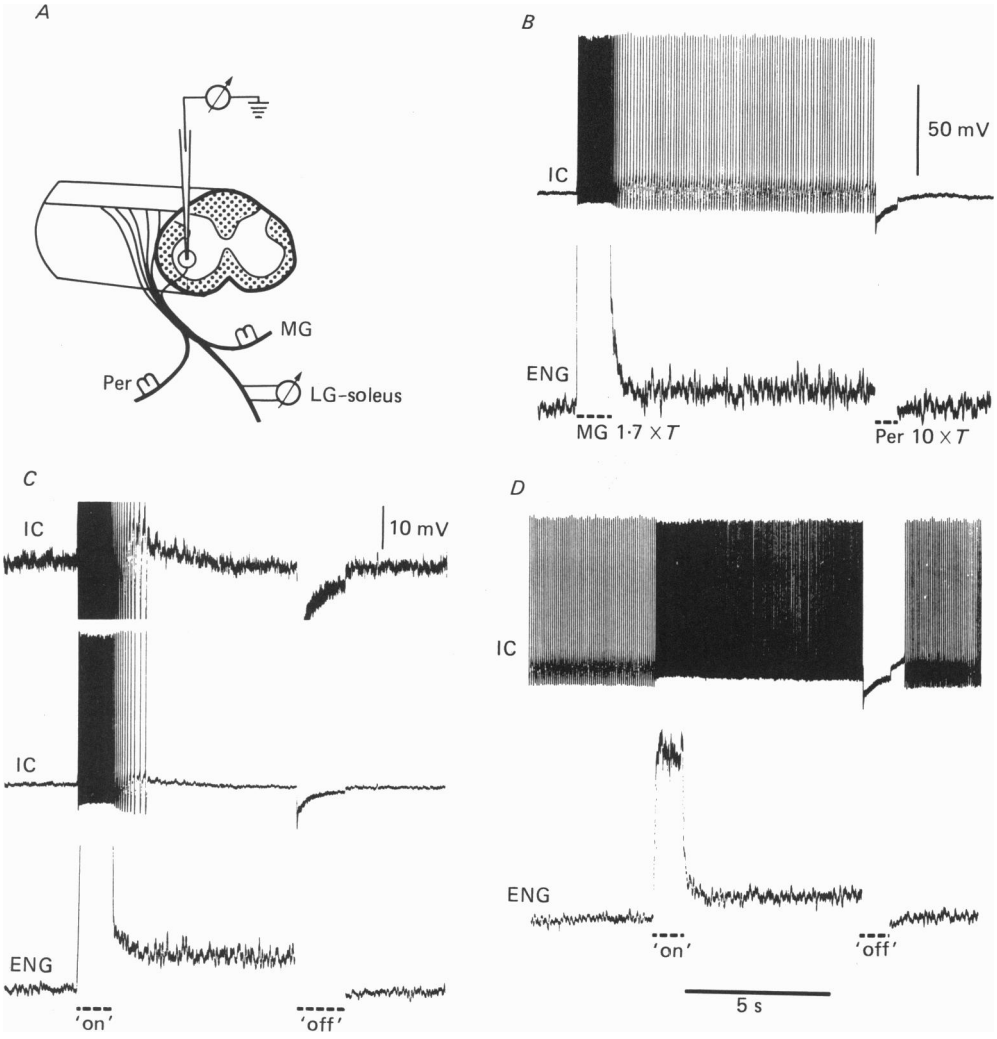

Fig. 1. Sustained shifts in excitability of α-motoneurones in decerebrate cat triggered by postsynaptic excitation and inhibition. *A*, experimental arrangement (applies for Figs 1–5): unanaesthetized anaemically decerebrate cat. The nerves to medial gastrocnemius (MG) and peroneus brevis–tertius (Per) were cut and mounted for stimulation and lateral gastrocnemius–soleus nerve (LG–soleus) for recording (ENG). *B–D*, simultaneous recording from a lateral gastrocnemius–soleus motoneurone (intracellular, IC, upper trace) and the corresponding ENG (rectified and filtered, lower trace). The excitability increase was induced and terminated by short trains of stimuli to medial gastrocnemius ('on'; MG $1{\cdot}7 \times T$, 300 Hz) and peroneal ('off'; Per $10 \times T$, 200 Hz) nerve, respectively. The timing of the 'on' and the 'off' stimuli are marked by interrupted lines below the ENG. In *C* the intracellular record is shown with high (calibration in *C*) and low amplification (calibration in *B*). Time calibration applies for all records. All records from same cell. Bias current: −6 nA (*B*), −1 nA (*C*), −8 nA (*D*). 5-HTP (20 mg/kg) was administered I.V. during the experiment.

firing during the phase of maintained ENG activity (originating from other firing motoneurones). Intracellular high-gain recording (uppermost record) shows that the discharge during the 'on' stimulus is followed by a transient discharge and depolarization *after* the 'on' stimulus. The after-depolarization soon declined towards the membrane potential seen before the 'on' stimulus. With a stronger hyperpolarizing bias current (other motoneurones, not illustrated) the transient after-discharge and the after-depolarization were completely suppressed. Thus, there were no signs of on-going synaptic excitation of the recorded motoneurone during the phase of maintained ENG activity.

When a continuous depolarizing bias current produced a low-frequency steady firing the 'on' stimulus caused an increased firing frequency (Fig. 1*D*, from 14 to 24 impulses/s), while the 'off' stimulus returned the firing frequency to the original slow rate. The 'on' stimulus always caused a stable change in firing frequency; the firing increase thus could not be graded by changing the strength or duration of the 'on' stimulus (cf. further below). However, with a stronger bias current producing higher tonic firing frequency the 'on' stimulus did not cause an additional increase (not illustrated). With the bias current adjusted to give a slow tonic discharge the mean increase in firing frequency produced by an 'on' stimulus was $14{\cdot}5 \pm 2{\cdot}7$ impulses/s (mean $\pm$ S.E.M., five motoneurones).

The 'on' and 'off' stimuli thus revealed a bistable firing behaviour, either shifting between silence and low-frequency firing (Fig. 1*B*) or between two levels of tonic discharge frequencies (Fig. 1*D*). Since no synaptic excitation matching the time course of the ENG activity could be seen when the motoneurone was kept hyperpolarized it seems most likely that the synaptic excitation during the 'on' stimulus triggers a voltage-dependent conductance increase causing a maintained inward (depolarizing) current (cf. below). The bistable firing behaviour in response to short-lasting synaptic excitation and inhibition was demonstrated in nineteen triceps surae motoneurones (six cats) in the decerebrate preparation. In six motoneurones it was not possible to demonstrate this phenomenon although simultaneous recording of the ENG activity demonstrated bistable behaviour of other motoneurones in the same motor pool.

In order to visualize the mechanism underlying the sustained firing by the 'on' stimulus it would be advantageous to block the action potentials. With excessive positive current injection (40–100 nA for 1–2 min) the sodium spikes are inactivated and usually remain so for a (short) period even when the membrane potential is returned to the previous threshold level. Figure 2*A* illustrates the response to 'on' and 'off' stimuli under such conditions. The 'on' stimulus triggered a long-lasting depolarization, a plateau potential, of about 7 mV, which is terminated by the 'off' stimulus.

The plateau potentials were of an all-or-none character. When the 'on' stimulus was decreased (stimulus intensity decreased, or duration of the stimulus train decreased; see also further below), the plateau potential failed to appear. The membrane potential then rapidly returned to the level prior to the 'on' stimulus. Changes in bias current also had important effects; with hyperpolarization the threshold for the plateau potential was not reached and with too strong positive bias

it seemed that the plateau potential was continuously activated ('on' stimuli did not cause additional depolarization) while the 'off' stimuli failed to return the membrane potential to the resting level.

Plateau potentials in motoneurones with inactivated spikes, triggered by synaptic excitation in the decerebate preparation, were recorded in seven triceps surae motoneurones. The mean value of their amplitude was $9{\cdot}4 \pm 0{\cdot}83$ mV (mean $\pm$ S.E.M.).

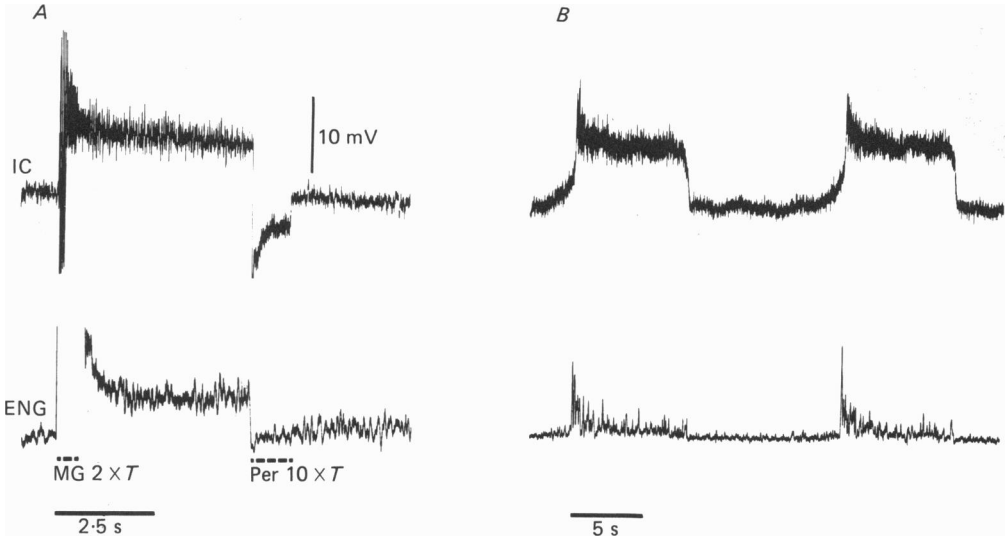

Fig. 2. Bistable behaviour of α-motoneurones in decerebrate cat. *A* and *B* show simultaneous recordings from a lateral gastrocnemius–soleus motoneurone (IC, upper traces) and from the corresponding nerve (ENG, lower traces). *A*, after prior inactivation of the spike-generation mechanism by excessive depolarization a sustained shift in membrane potential was initiated by synaptic excitation ('on'; the medial gastrocnemius nerve MG $2 \times T$, 300 Hz) and terminated by synaptic inhibition ('off'; the peroneal nerve, Per $10 \times T$, 200 Hz). Bias current, +15 nA. *B*, spontaneous long-lasting shifts in membrane potential (same cell as in *A* a few minutes later). Bias current, +15 nA. Voltage calibration as in *A*. Time calibrations are different in *A* and *B*.

Some decerebrate preparations show a slow spontaneously recurring motor activity as illustrated by the ENG recording in Fig. 2*B* (a few minutes later than records in *A*, same motoneurone). The intracellular recording shows that the spontaneously generated synaptic excitation seemingly triggers long-lasting plateau potentials in the motoneurone. The plateau is terminated at the same time as the ENG activity and this suggests that a central synchronized synaptic inhibition of all motoneurones is responsible.

(2) *Self-sustained firing and plateau potentials triggered by direct stimulation of the motoneurones through the recording microelectrode*

If it is correct that long-lasting activity following synaptic activation is due to intrinsic motoneuronal properties it should be possible to mimic the effects of synaptic 'on' and 'off' stimuli by direct intracellular current pulses in depolarizing

and hyperpolarizing direction respectively. This is indeed the case as will be demonstrated in this section.

Figure 3*A* shows self-sustained firing initiated and terminated by depolarizing and hyperpolarizing current pulses respectively. Following inactivation of the fast sodium spikes similar intracellular pulses triggered and terminated the underlying plateau potential (Fig. 3*B*; mean value was 10·0±0·57 mV (mean±S.E.M.) in six neurones). The intracellular record in Fig. 3*B* also illustrates the pronounced

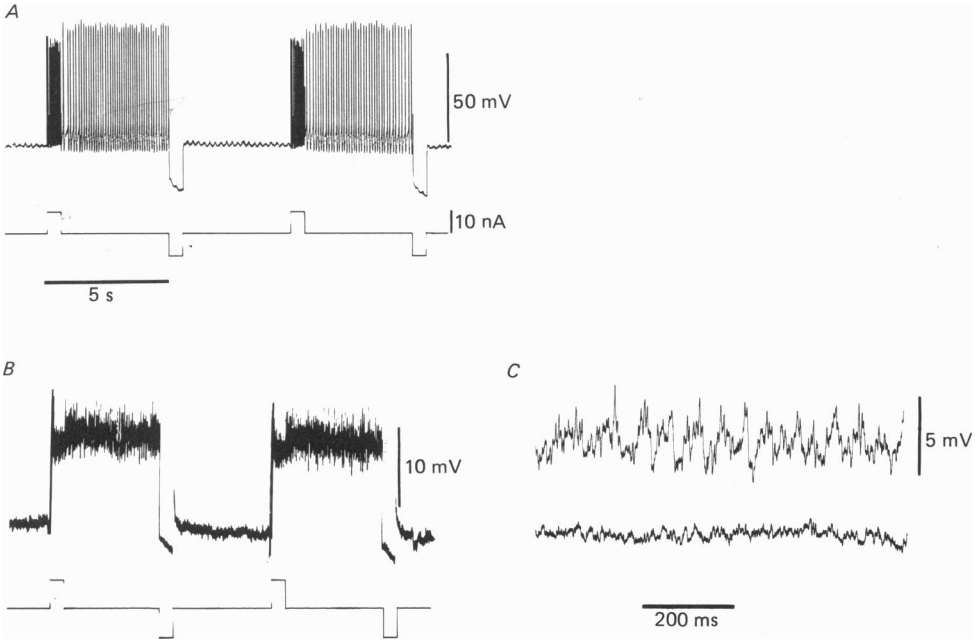

Fig. 3. Sustained shifts in excitability of α-motoneurones in decerebrate cat triggered by depolarizing and hyperpolarizing currents injected intracellularly. Intracellular recordings (IC) from a plantaris–flexor digitorum longus motoneurone in upper traces and injected current in lower traces. *A* illustrates sustained repetitive firing initiated by a short depolarizing current pulse and terminated by a short hyperpolarizing current pulse (the intracellular signal was passed through a 5 Hz filter for reproduction). *B*, in this example the spike-generating mechanism was inactivated (see Fig. 2). Current pulses now evoked and terminated a plateau potential. *C* shows in expanded time scale two parts from *B* before (lower trace) and after (upper trace) triggering the plateau. Note the increased noise level during the plateau. Different voltage calibration for intracellular recordings in *A*, *B* and *C*. Time and current calibrations in *A* apply for *B*. The steady bias current was −2 nA and +8 nA in *A* and *B* respectively.

potential fluctuations ('noise') superimposed on the plateau. The high 'noise' level on the plateau as compared to rest may be better appreciated from the time-expanded samples in Fig. 3*C*. Since the plateau is evoked by an intracellular depolarizing pulse it is very unlikely that the 'noise' is due to synaptic input. It is more probable that it relates to opening and closure of the ionic channels responsible for the generation of the plateau potential.

In order to further illustrate the generation of plateau potentials and their effects

on firing frequency we have analysed the firing pattern *during* as well as *after* rectangular current pulses (Fig. 4). Experiments on anaesthetized (intact or spinal) cats have shown that the discharge frequency following a step current injection is initially high and then rapidly decreases to attain a 'steady' level after a few spikes ('initial adaptation', Granit, Kernell & Shortess, 1963; Kernell 1965; Baldissera &

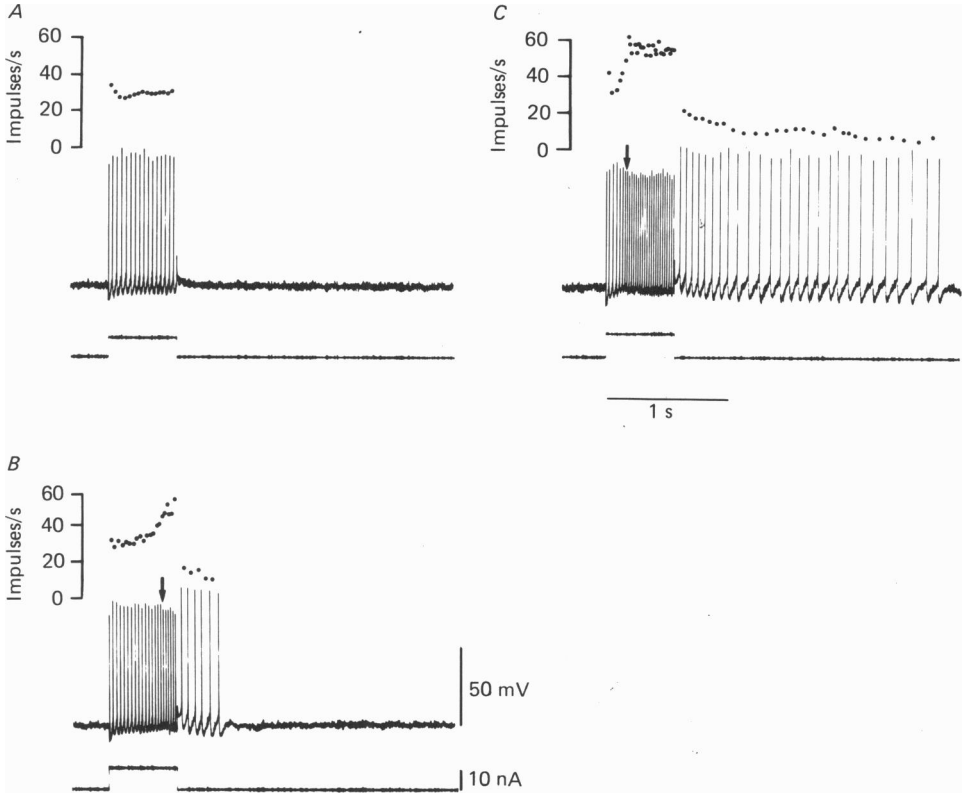

Fig. 4. Response of an α-motoneurone to injection of rectangular current pulses. *A–C*, intracellular recordings (IC; successive sweeps from same cell) from a medial gastrocnemius motoneurone (middle traces) and injected current (lower traces). Instantaneous frequency, corresponding to spike intervals plotted in upper traces. The amplitude of current was +10, +11 and +12 nA in *A*, *B* and *C* respectively, and the steady bias current was −7 nA in all records. Voltage and current calibrations in *B* and time calibration in *C* apply for all records.

Gustafsson, 1974). The initial adaptation was also seen in the present investigation, although it is particularly small in the illustrated example (see, however, Fig. 7). In the *unanaesthetized* decerebrate preparation there is in addition an acceleration of firing later during the depolarizing pulse which is followed either by a transient after-discharge (Fig. 4*B*; note the instantaneous frequency plotted above the intracellular record) or a maintained self-sustained discharge (Fig. 4*C*). The bias current (−7 nA) is the same for all trials in Fig. 4, while the pulse amplitudes (lower records) were 10, 11 and 12 nA in *A*, *B* and *C* respectively. A true acceleration is seen with 11 nA (*B*), but it starts earlier, develops more rapidly and reaches a steady level with 12 nA (*C*).

The firing frequency at which the acceleration started was in the range of 17–81 impulses/s (data from three neurones in decerebrate cats and eleven neurones in acute spinal cat plus 5-HTP) with a mean value of 50·6 ± 4·7 impulses/s (mean ± S.E.M.).

The underlying plateau potential develops slowly (hundreds of milliseconds) and the onset and velocity of development depends on the amplitude of the stimulating current pulse. A similar relation can also be seen with stepwise increases in depolarizing bias currents, while the rectangular current pulse is kept constant. It is concluded that the generation of the plateau potential (or the resulting frequency acceleration) depends both on the amplitude and the duration of the 'on' stimulus and, furthermore, that the holding potential is also essential.

The acceleration in firing frequency could also be studied with slow triangular current pulses passed through the microelectrode as illustrated in Fig. 5*A*. The ramp increase in injected current during a single trial provides the same information that can be gained from a large number of trials with rectangular current pulses of different amplitudes.

The intracellular recording first shows a continuous steady increase in firing frequency, but suddenly (arrow, Fig. 5*A*) a jump in firing frequency occurs. On observing this frequency jump the polarity of the current ramp was reversed manually as soon as possible.

In the graph of Fig. 5*B* the instantaneous frequency was plotted against injected current (frequency–current relation, *f–I* relation); filled circles refer to the ascending part of the triangular current pulse and open circles to the descending part. It is immediately seen that the *f–I* relation shows a counter-clockwise hysteresis, i.e. for any current amplitude the firing frequency is higher during the descending phase than during the ascending (open circles above closed circles). The frequency acceleration at the peak of the triangular pulse is a prerequisite for the counter-clockwise hysteresis. If no acceleration has occurred at the peak of the stimulating triangular pulse (i.e. if the ascending phase is manually terminated at a smaller current amplitude) the frequency of the descending phase either overlaps with the ascending phase or has lower frequencies, i.e. the *f–I* relation shows a clockwise hysteresis (not illustrated, but cf. Fig. 8*A*). This is most probably related to the 'late adaptation' seen during prolonged (several seconds) tonic firing (Kernell & Monster, 1982). The graph in Fig. 5*B* reveals an additional feature. The motoneurone keeps firing with much less depolarizing current during the descending phase; the rheobase at the beginning of the triangular pulse is +7 nA, while firing ceases only when the current reaches −3 nA. The difference of injected current for similar firing frequencies in the ascending and descending phases was in the order 3–10 nA ($n = 6$).

With triangular current injections it is easy to estimate the threshold for the plateau potential, especially in relation to the threshold of the normal action potential. In the example of Fig. 5 the frequency acceleration occurs after a linear increase to a level of about 37 impulses/s (17 nA). In twelve motoneurones (three neurones from decerebrate cats and nine neurones from spinal cats plus 5-HTP) the frequencies at which the acceleration took off ranged between 16 and 50 impulses/s (with a mean of 32·4 ± 2·9 impulses/s (mean ± S.E.M.)). It is important to notice that

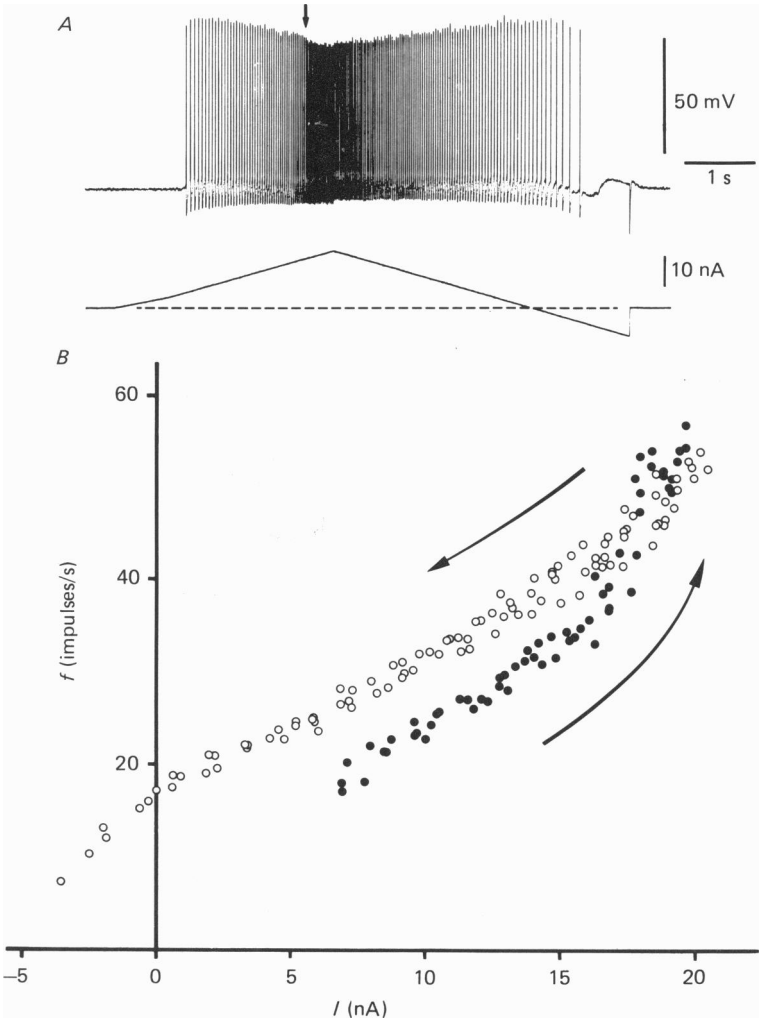

Fig. 5. Response of an α-motoneurone to a triangular current pulse injection. *A* illustrates an intracellular recording (IC) from a lateral gastrocnemius–soleus motoneurone (same cell as in Fig. 1) in upper trace and injected current in lower trace. The intracellular signal was passed through a 5 Hz filter for reproduction and the steady bias current was −6 nA. *B*, the instantaneous frequency *f* (impulses/s) measured in the cell in *A* is plotted against current *I* (the direction of arrows indicate the ascending (●) and descending (○) phase of the triangular waveform). The frequency–current relation shows a counter-clockwise hysteresis.

the counter-clockwise hysteresis was seen even with very low firing frequencies in some cells. This corresponds well with the observation that an 'on' stimulus to a quiescent motoneurone (synaptic excitation as in Fig. 1 or depolarizing pulses as in Fig. 3) could trigger a maintained slow tonic firing. Thus, in some motoneurones the plateau potentials seem to have threshold close to that of the fast sodium spikes.

Thirty motoneurones out of fifty-two recorded in the unanaesthetized decerebrate preparation, showed bistable behaviour judged by one or (usually) several of the

following criteria: (1) plateau potentials following intracellular pulses, (2) self-sustained discharge following intracellular pulses, (3) the acceleration in firing during rectangular pulses, or (4) counter-clockwise hysteresis during triangular current pulses.

The high positive ratio for bistability in triceps surae motoneurones (twenty-seven out of forty-three neurones) may reflect that motoneurones were only accepted when the simultaneous nerve recording showed a maintained ENG activity following a synaptic 'on' stimulus; thus it was known that at least some motoneurones in the triceps surae pool were 'positive' at the time of intracellular recording. Since bistability could be tested by direct stimulation through the recording electrode motoneurones innervating other muscles than triceps surae were also investigated.

TABLE 1. Summary of bistable behaviour in different species of motoneurones in the unanaesthetized decerebrate cat and in the acute spinal cat following 5-HTP. Compare Table 1 in Conway *et al.* (1988)

	Decerebrate		5-HTP	
	Positive	Negative	Positive	Negative
Triceps surae	27	16	15	3
Semimembranosus–anterior biceps	1	2	5	4
Plantaris–flexor digitorum longus	2	0	—	—
Posterior biceps–semitendinosus	0	4	1	2
Total	30	22	21	9

However in such cases the presence of maintained ENG activity in the corresponding nerve to synaptic 'on' stimuli was not tested. Table 1 provides a summary of the distribution of positive cells. In addition to this material we briefly recorded, about twenty flexor motoneurones (posterior biceps–semitendinosus or pretibial flexors), which did not display any bistable behaviour. Accordingly there is an obvious dominance of extensor motoneurones among motoneurones which show bistable properties in the decerebrate preparation.

(3) *Bistable properties of motoneurones in the acute spinal cat following I.V. administration of 5-hydroxytryptophan*

In the preceding paper (Crone *et al.* 1988) it was demonstrated that the maintained increase in motoneuronal activity following short-lasting synaptic excitation disappeared after an acute spinal transection in the decerebrate cat, but reappeared after intravenous injection of large doses of the serotonin precursor 5-HTP. It will now be shown that following spinal transection motoneurones lack bistable properties and that administration of 5-HTP allows bistable behaviour to reappear. Twenty-eight motoneurones (twenty-three extensors) were recorded in unanaesthetized decerebrate cats with an additional spinal transection (five cats). All motoneurones were tested for self-sustained firing after intracellular current pulses, a frequency acceleration during rectangular pulses and for acceleration and counter-clockwise hysteresis in the *f–I* relation on presentation of triangular current pulses. Only one of these neurones (an extensor) showed a slight tendency for acceleration during a square pulse, while the rest started with a high initial firing rate which rapidly

adapted to a slow steady-state firing during a square pulse. They all responded with a clockwise hysteresis (see Fig. 8*A*) during triangular current injections. In conclusion, there was no sign of bistable properties in the motoneurones following an acute spinal transection.

After I.V. administration of 5-HTP (50–140 mg/kg) the characteristics of bistability seen in the decerebrate cat (sections (1) and (2)) developed in twenty-one motoneurones, while nine neurones were negative in that respect. Table 1 provides a summary of the distribution of positive cells.

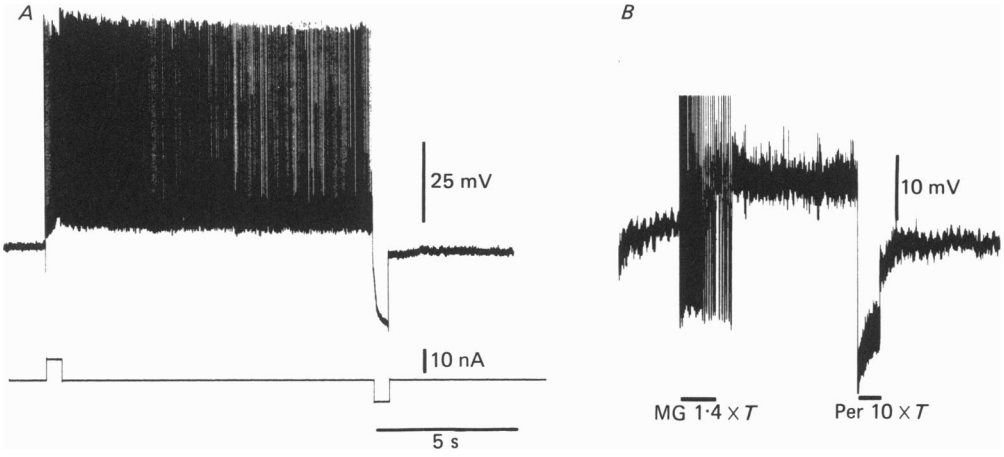

Fig. 6. Sustained shifts in excitability of α-motoneurones in the acute spinal cat following administration of 5-HTP. *A* and *B*, upper traces show intracellular recordings from two different lateral gastrocnemius motoneurones. *A* illustrates sustained repetitive firing initiated and terminated by short-lasting current injection. *B*, in this cell the spike-generating mechanism was inactivated. Following synaptic excitation ('on'; the medial gastrocnemius nerve, MG 1·4 × *T*, 300 Hz) a plateau potential was triggered. The plateau was terminated by a synaptic inhibition ('off'; peroneal nerve, Per 10 × *T*, 200 Hz). Voltage calibration different in *A* and *B*. The steady bias current was 0 nA in *A* and +16 nA in *B*. 5-HTP (10 mg/kg) and Carbidopa (5 mg/kg) were given I.V.

Figure 6 demonstrates self-sustained firing and plateau potentials in two lateral gastrocnemius–soleus motoneurones in an acute spinal cat after administration of 5-HTP. The self-sustained discharge (Fig. 6*A*) was initiated and terminated by short intracellular current pulses. In the other motoneurone (Fig. 6*B*) the spike-generating mechanism was partly inactivated by excessive depolarization and short-lasting synaptic excitation and inhibition evoked and terminated a depolarizing plateau potential with an amplitude of nearly 10 mV (data from four neurones: 7·3 ± 1·6 mV (mean ± S.E.M.)). These results seem identical to the results obtained in the unanaesthetized decerebrate cat (Figs 1, 2 and 3).

Figure 7 illustrates the frequency acceleration during rectangular current pulses in a flexor digitorum longus motoneurone. As described in connection with Fig. 4 the firing frequency accelerated during the current pulse and jumped to a higher level (arrows) when the amplitude of the current steps was increased (17, 18 and 19 nA in *A*, *B* and *C* respectively). A very brief after-depolarization (Fig. 7*A*), a transient

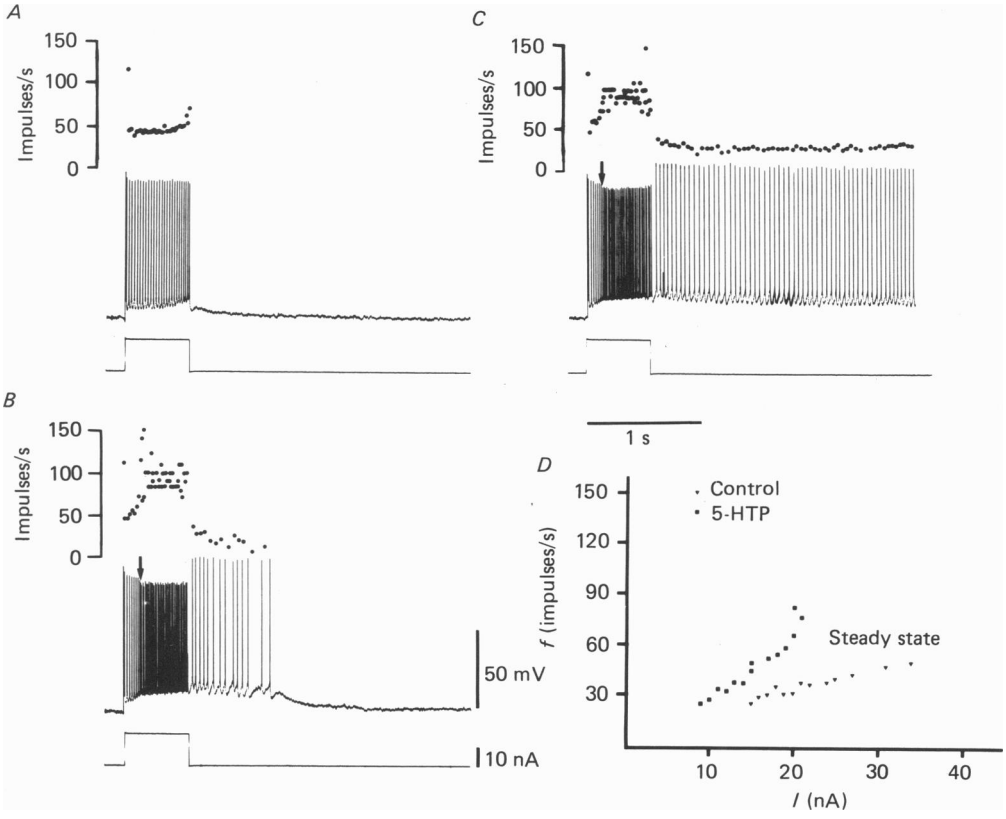

Fig. 7. Response of an α-motoneurone to injection of rectangular current pulses in an acute spinal cat after 5-HTP administration. Instantaneous firing frequency, intracellular recording (IC; plantaris–flexor digitorum longus motoneurone) and injected current are illustrated in *A–C*. The amplitudes of the current pulses were +17, +18 and +19 nA in *A*, *B* and *C* respectively and the steady bias current was 0 nA in all records. 5-HTP (65 mg/kg) was given I.V. before the records were taken. Voltage, time and current calibrations apply for all records. *D* shows a steady-state frequency–current relation (0·5 s after the beginning of the current injection) before (▼; control) and after 5-HTP (■; 75 mg/kg). Same neurones as in Fig. 8. Note that the curve after 5-HTP has been shifted to the left indicating an increase in excitability. The steady bias current was 0 nA in control and −6 nA after 5-HTP. The bias currents are added to the current pulses in the plot.

after-discharge (Fig. 7*B*) or a self-sustained discharge (Fig. 7*C*) followed the termination of the square pulse. Note that the initial adaptation is still present.

Mechanical instability induced by the amount of fluid used to dissolve 5-HTP and the changes in blood pressure induced by this drug reduced the chance of getting stable long-lasting recordings from motoneurones. Therefore successful recording from the same motoneurone before and after administration of 5-HTP was only achieved twice. Figure 8 illustrates the firing response to triangular current pulses for one of these motoneurones before (*A*) and after (*B*) 5-HTP. Before 5-HTP the *f–I* relation illustrates the clockwise hysteresis, i.e. for the same current the firing frequency is lower during the descending than during the ascending phase. As

mentioned above this behaviour would be expected from the 'late adaptation' described by Kernell & Monster (1982). After the 5-HTP administration the *f-I* slope becomes steeper and furthermore shows a frequency acceleration at the peak of the ascending phase (i.e. at a frequency of about 40 impulses/s). Above all, a distinct counter-clockwise hysteresis has now developed similar to the intact decerebrate preparation (cf. Fig. 5). The small decrease in rheobase (12 nA before (*A*) and 9 nA after 5-HTP (*B*)) may be caused either by an increase in input resistance and/or a depolarization of the cell.

The *f–I* slope is mostly evaluated from the instantaneous frequency during rectangular current pulses of different amplitudes. The graph in Fig. 7*D* illustrates the relation between the amount of injected current and the instantaneous frequency at the end of 500 ms rectangular pulses (i.e. close to steady state). This experiment was performed before and after 5-HTP administration (same motoneurone as in Fig. 8). It reveals that the rheobase was decreased and the *f–I* slope became steeper after 5-HTP. The very high frequency with the strongest current after 5-HTP (the last two points) shows that frequency acceleration has occurred (cf. records in Fig. 7*A–C* from another motoneurone). The linear parts of the *f–I* slopes (excluding the two high-frequency points after 5-HTP) were 1·3 impulses s^{-1} nA^{-1} in the control condition and increased to 3·6 impulses s^{-1} nA^{-1} after 5-HTP. The *f–I* slopes of the other neurone which was recorded before and after 5-HTP increased from 1·6 to 2·7 impulses s^{-1} nA^{-1}.

(4) *Discharge pattern of individual triceps surae motor units during muscle stretch and vibration*

In the previous sections we have described bistable properties of motoneurones during intracellular recording in paralysed preparations. It would be of interest to record the effect of plateau potentials on the firing pattern of non-penetrated motoneurones and to determine the contribution of the plateau potentials to some motor activity in the non-paralysed preparation, e.g. the classic tonic stretch reflex. In three experiments we have therefore recorded the activity of individual motor units (in soleus or lateral gastrocnemius) by selective EMG electrodes.

Figure 9 shows the direct recording of a motor unit (middle trace), its instantaneous frequency (top) and the length of the lateral gastrocnemius–soleus muscles (bottom trace). In the experiment of Fig. 9*A* the muscle was stretched slowly until the motor unit was recruited (arrow a) and was then kept at this length (the electromagnetic puller was manually controlled). Despite the constant length a frequency jump occurred spontaneously at arrow b. About 8 s later (arrow c) the muscle was released beyond the initial length at which the unit was silent. There was indeed some decrease in firing frequency from the high level, but the most striking feature is the maintained firing despite extensive shortening. In the light of the results described in the previous sections it seems likely that the frequency jump (arrow b) reflects recruitment of the plateau potential, which can support a sustained firing despite decreasing synaptic excitation when the muscle is relaxed.

The motor unit in Fig. 9*B* was recorded during lengthening/shortening excursions of different amplitudes. During the first cycle of small amplitude the firing frequency is increasing and decreasing rather symmetrically (proportionally) with the changes

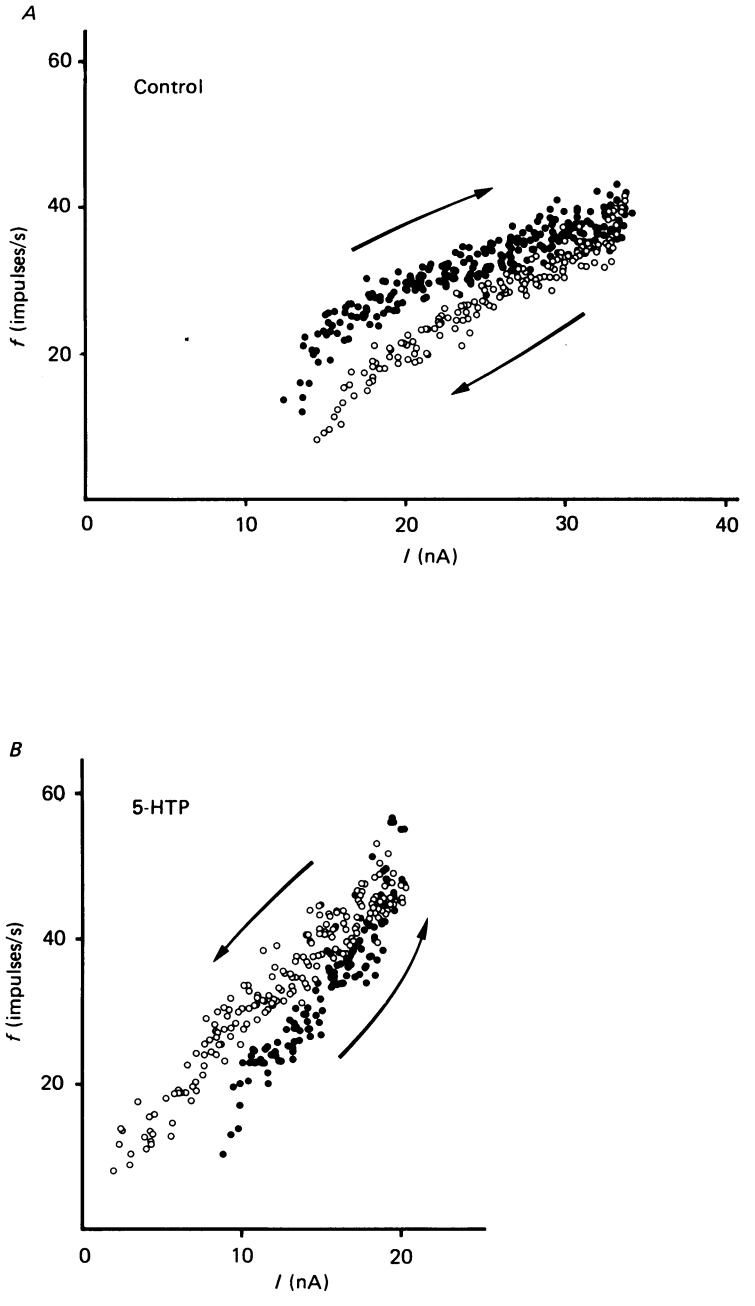

Fig. 8. Response properties of an α-motoneurone to a triangular current pulse injection before (*A*) and after (*B*) 5-HTP administration (75 mg/kg). In *A* and *B* the instantaneous firing frequency is plotted against current. The arrows indicate the ascending (●) and descending (○) phase of the triangular waveform. *A*, before 5-HTP the frequency–current relation shows a clockwise hysteresis. *B*, after 5-HTP the frequency–current relation shows a counter-clockwise hysteresis. The steady bias current was 0 nA in *A* and −11 nA in *B*. The slope of current injected was 2·6 nA/s in *A* and *B*.

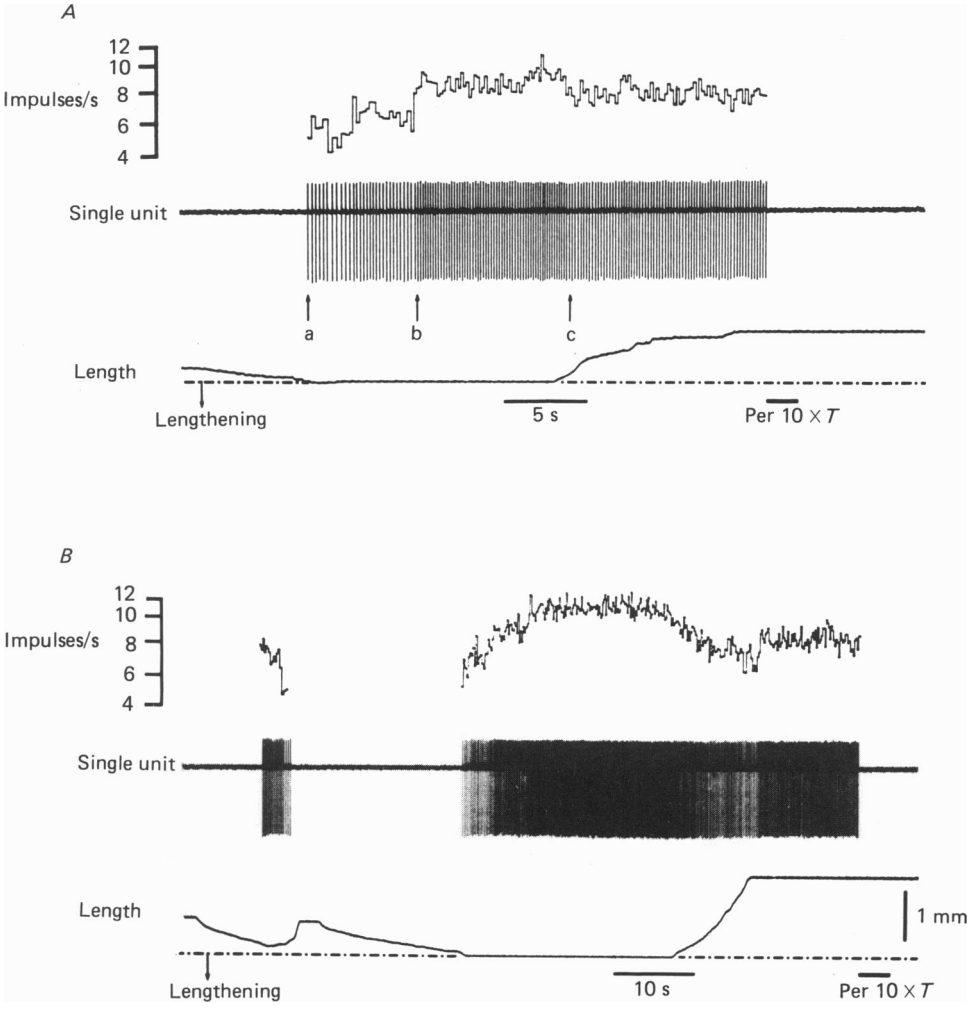

Fig. 9. Discharge pattern of individual soleus motor units during muscle stretch. Experimental arrangement as in Fig. 10. *A* and *B*, upper traces show the instantaneous frequency (impulses/s) corresponding to interspike intervals from the single-unit recordings in middle traces. Lower traces show muscle length; downward deflections indicate lengthening of the muscle ('stretch'). The firing of the motor unit was terminated by an 'off' stimulus train (peroneal nerve, Per $10 \times T$, 300 Hz) in *A* and *B*. Length calibration in *B*. Different time calibration in *A* and *B*.

in muscle length. With the second more extensive stretch there is a sudden frequency acceleration, and the frequency remains high despite the subsequent shortening of the muscle; this obviously resembles the counter-clockwise hysteresis seen with triangular current pulses (cf. Fig. 5).

The frequency jump is illustrated further in Fig. 10. In this case the muscle was slowly stretched until a motor unit was recruited with a very slow tonic firing rate. With the muscle length kept constant – and with the motor unit firing at 4–7 impulses/s – a frequency jump could be triggered by a short-lasting vibration of

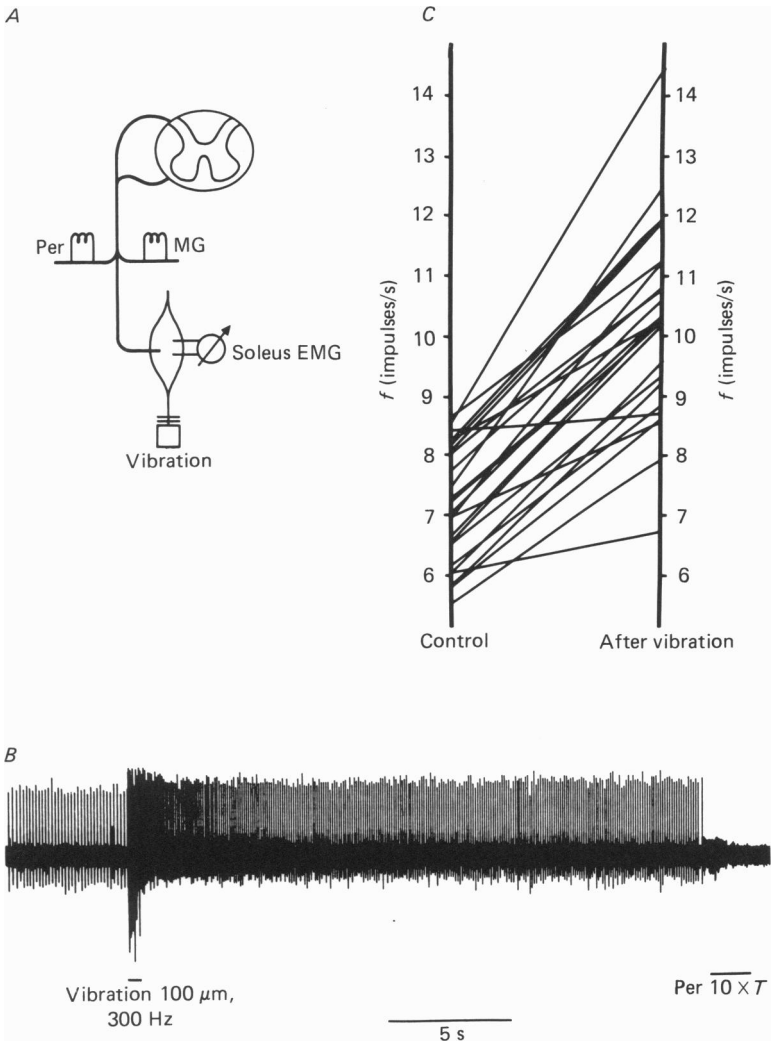

Fig. 10. Instantaneous discharge rate of soleus motor units before and after a short vibration of the muscle (constant length). *A*, experimental arrangement (applies also for Fig. 9): unanaesthetized anaemically decerebrate cat. The single-unit recordings in Figs 9 and 10 were obtained with a modified single-fibre electrode (see Methods). *B*, recording from single motor unit to illustrate the maintained increase in firing frequency following a short-lasting vibration (100 μm, 300 Hz). *C*, diagram showing results from twenty-four lateral gastrocnemius–soleus motor units. Firing frequency before vibration (Control; the low tonic discharge rate was maintained by a minimal muscle stretch) is shown on the left side, while the firing frequency following vibration (100 μm, 300 Hz) is shown on the right side. Each line represents a motor unit.

lateral gastrocnemius–soleus (Fig. 10*B*) or electrical stimulation of the medial gastrocnemius nerve (300 Hz, 1·4 × $\overline{T}$). The tonic firing frequency increased from 7·4 to 12·4 impulses/s in the example in Fig. 10*B*. The graph in Fig. 10*C* summarizes this frequency jump for twenty-four motor units from two experiments. The increase in

discharge rate by the short-lasting vibration ranged from 0·3 to 5·9 impulses/s (3·2 ± 0·22 impulses/s (mean ± S.E.M.)). These results strongly suggest that plateau potentials are triggered at low firing frequencies in slow tonic motor units.

DISCUSSION

Relation between plateau potentials in motoneurones and maintained excitability shifts following short-lasting synaptic excitation and inhibition

In the preceding paper (Crone *et al.* 1988) it was demonstrated that a short-lasting synaptic excitation of motoneurones (especially triceps surae) could trigger a maintained excitability increase which could be reset by a short period of synaptic inhibition. The plateau potentials in α-motoneurones described in this paper are obviously causal for this phenomenon. Indeed several pieces of qualitative and quantitative observation suggest that the plateau potentials may be the *exclusive mechanism* for the maintained motor output under the given experimental conditions. Firstly, synaptic inhibition which is specific for motoneurones (and some inhibitory interneurones, i.e. the Renshaw inhibition) serve as an effective 'off' stimulus resetting the excitability level (Crone *et al.* 1988); if on-going excitation has contributed significantly to the maintained excitability increase one would expect it to survive a temporary inhibition to the motoneurones. Secondly, direct recordings from motoneurones do not reveal any sign of long-lasting synaptic excitation when the holding potential is negative enough to prevent the triggering of plateau potentials. Thirdly, the presence of plateau potentials in motoneurones as well as the maintained changes in EMG (or ENG) activity were seen in the decerebrate preparation; both were abolished by an additional spinal transection and both reappeared following I.V. administration of 5-HTP.

Some of the experimental observations by Crone *et al.* 1988 may seem difficult to explain by the 'all-or-none' behaviour of the plateau potentials. (i) It was shown that the maintained EMG activity could be graded and was related to both the strength and duration of the 'on' stimulus train (Fig. 1, in Crone *et al.* 1988). With intracellular current pulses we have shown that the activation of plateau potentials is a slow process (hundreds of milliseconds), and that the actual latency depends both on the stimulus amplitude and the holding potential (cf. Figs 4 and 7). Since both the EPSP amplitude and the holding potential may vary widely among different neurones in a motoneuronal pool, it is easy to understand that a gradual increase in the synaptic 'on' stimulus (strength as well as duration) will cause a gradual increase in the number of recruited motoneurones (up to certain limits). (ii) Repeated stimuli – at intervals of, for example, 10 s – caused a *stepwise* increase in the summated EMG response. As shown in the preceding paper (Crone *et al.* 1988) the stepwise increase in the summated EMG was explained by recruitment of new motor units (showing sustained discharge), while already recruited units remained at the same firing frequency (see Fig. 5 in Crone *et al.* 1988). Recruitment of new motor units with repeated 'on' stimuli of constant stimulus strength at intervals of 10 s could possibly be explained by small fluctuations of excitability which, by chance, make some motoneurones more responsive at the time of the second, third or fourth stimulus train than at the first train. The alternative explanation, that short-lasting (less than 500 ms) depolarizations, which are subthreshold for evoking plateau potentials, nevertheless would increase the probability of triggering the plateau 10 s later, seems very unlikely.

Plateau potentials in different types of motoneurones

The experiments on the frequency jump during stimulation with intracellular current pulses (either rectangular or triangular; Figs 4, 5, 7 and 8) suggest that the threshold for the plateau potential is reached when the firing frequency is in the range of 20–50 impulses/s. However, as mentioned in section (2) the counter-clockwise hysteresis in firing seen with triangular current pulses could be seen even with very low frequencies in some cells. It therefore seems likely that the hysteresis in firing frequency in triceps surae motor units (recorded with selective EMG electrodes) to small lengthenings and relaxations of that muscle indeed reflect recruitment of plateau potentials even at firing frequencies below 10 impulses/s. It would thus be of interest to know if the threshold for the plateau potential is lower in slow motoneurones participating in the stretch reflex (Burke, 1968) than in fast motoneurones. It is known that the duration of the post-spike after-hyper-polarization correlates with motor unit type (slow and fast) and twitch contraction time (see Burke, 1981, for references). In the present material (not described) positive signs of bistability have been found in motoneurones with after-hyperpolarizations of all durations. Although it seems safe to conclude that both fast and slow motoneurones show bistability the present material does not allow a correlation between motor unit type (or duration of after-hyperpolarization) and threshold of the plateau potential.

The material in section (1), where gross ENG activity was correlated with recordings from individual motoneurones, consists exclusively of triceps surae motoneurones. In the following sections where plateau potentials were evoked by direct injection of current pulses also other species of motoneurones were tested. It is difficult to obtain an unbiased view on the prevalence of plateau potentials in different species of motoneurones on the basis of the numbers in the Results on positive and negative cells as the criteria for investigating different motoneurones changed during the course of the investigation. In general we made every effort to show bistability in triceps surae motoneurones, since the parallel recording of the EMG and ENG response indirectly proved their presence. On the other hand knee flexor motoneurones (posterior biceps–semitendinosus) proved negative even with intense testing in the beginning of the series and therefore testing was brief and rather superficial in the later experiments. However, such difficulties cannot obscure an obvious difference between extensor (triceps surae, plantaris, flexor digitorum longus) and flexor (posterior biceps–semitendinosus) motoneurones both in the intact decerebrate preparation and in the spinal 5-HTP-treated preparation. This could be explained either by real differences in intrinsic properties between flexor and extensor motoneurones (either in relation to serotonergic receptors or the ionic channels responsible for the plateau) or rather reflect the higher excitability level of extensors than of flexors at least in the decerebrate cat. It may be difficult to compensate for this difference with stronger depolarizing bias currents to flexor motoneurones as the injected current mainly affects the soma region, while a tonic synaptic excitation may influence the whole soma–dendritic membrane. In the following paper (Conway, Hultborn, Kiehn, Mintz, 1988) plateau potentials will be

demonstrated in flexor motoneurones in acute spinal cats following I.V. administration of the noradrenaline precursor L-DOPA (L-β-3,4-dihydroxyphenylalanine). Therefore it is obvious that the basic mechanisms underlying the bistable properties are present in both flexor and extensor motoneurones.

Mechanisms underlying the plateau potentials

The presence of plateau potentials implies that a membrane depolarization (by current injection or synaptic excitation) triggers a non-inactivating conductance leading to a net inward current. Schwindt & Crill (1980*a*) originally observed plateau potentials and bistable behaviour in motoneurones during penicillin-induced spinal seizures. They suggested that the plateau potentials were generated by a persistent Ca^{2+} current and that this was uncovered when outward currents were reduced (Schwindt & Crill, 1980*b*, *c*). It is quite likely that this mechanism also underlies the plateaux studied here, in particular since serotonin-induced plateau potentials in spinal motoneurones of the turtle have been shown to be mediated by a non-inactivating Ca^{2+} conductance (Hounsgaard & Kiehn, 1985).

Several results indicate that the plateau potentials in the decerebrate cat are promoted by serotonin liberated from raphe–spinal motoneurones. Engberg, Lundberg & Ryall (1968) found that suppression of flexor reflexes in the decerebrate state was partly blocked by methysergide. Crone *et al.* (1988) extended this observation to include the maintained discharge following an 'on' stimulus which is likely to be a reflection of plateau potentials. These observations indicate that the serotonergic raphe–spinal projection is active in the decerebrate preparation. In line with this we have shown here that plateau potentials in motoneurones in decerebrate cats disappear after spinal transection, but reappear after administration of a serotonergic precursor. In addition *in vitro* experiments have shown that serotonin induces plateau potentials in turtle motoneurones (Hounsgaard & Kiehn, 1985).

While the permissive effect of serotonin for plateau potentials in motoneurones is clear, the mechanism is not. This question is now being addressed in experiments on turtle motoneurones (Hounsgaard & Kiehn, 1985 and J. Hounsgaard & O. Kiehn, unpublished observations).

Relative importance of plateau potentials in the tonic stretch reflex

A general discussion on the tentative functions of plateau potentials at the level of 'the final common pathway' will be given in the following paper (Conway *et al.* 1988). Here we will deal exclusively with the importance of the plateau potentials for the tonic stretch reflex.

It is now established that the tonic stretch reflex is dependent both on an excitatory input to the motoneurones from the muscle spindles and a particular set of descending activities from the brain stem facilitating both α-motoneurones and static γ-motoneurones as well as inhibiting transmission in pathways mediating autogenic inhibition from the stretched muscle (see Matthews, 1972, and Houk & Rymer, 1981, for references). In addition to the well-known monosynaptic projections to motoneurones from Ia afferents connections (although weaker) from muscle spindle group II afferents have more recently been established (Kirkwood & Sears, 1975; Stauffer, Watt, Taylor, Reinking & Stuart, 1976; Lüscher, Ruenzel,

Fetz & Henneman, 1979; Munson, Sypert, Zengel, Lofton & Fleshman, 1982). As discussed in detail in the preceding paper (Crone *et al.* 1988; see also Matthews, 1972; Houk & Rymer, 1981) there have been several suggestions on excitation supplied by polysynaptic pathways from both muscle spindle group Ia and group II afferents, but in no case was the evidence direct or conclusive.

From available data it is possible to obtain an approximate estimation of the depolarization in soleus motoneurones (during the tonic stretch reflex) caused by the monosynaptic EPSPs from muscle spindle Ia afferents (see the small-print section). It turns out that the tonic depolarization by the monosynaptic Ia EPSPs following a 5 mm stretch will be 0·4–1·8 mV, corresponding to an increase in firing frequency of 0·2–0·9 impulses/s.

The mean depolarization (mean dep.) can be estimated from the amplitude of the aggregated Ia EPSP (A), the mean firing frequency of the Ia afferents (f) and the membrane time constant (t):

$$\text{mean dep. (mV)} = A\text{(mV)}f\text{(impulses/s)}\,t\text{(ms)}. \quad (1)$$

The maximal homonymous Ia EPSP in soleus motoneurones was shown to be 5·6 mV in barbiturate-anaesthetized cats (Eccles, Eccles & Lundberg, 1957). It may be somewhat larger in unanaesthetized preparations (cf. Burke, 1968), but should not exceed 10 mV. The sensitivity of Ia afferents to stretch in the anaesthetized decerebrate state is 2–5 impulses s^{-1} (mm stretch)$^{-1}$ (see Matthews, 1972 for references). With a moderate 5 mm stretch the mean firing frequency of the Ia afferents will thus increase by 10–25 impulses/s. The membrane time constant in slow motoneurones is about 7 ms (see Burke, 1981). When these values are applied to eqn (1), it turns out that the mean depolarization supplied by the monosynaptic Ia EPSPs following a slow 5 mm stretch will be 0·4–1·8 mV (dependent on whether minimal or maximal values are chosen). Assuming that the slow motoneurones have an input resistance of about 2 MΩ (see Burke, 1981) this depolarization will correspond to a 'synaptic current' of 0·2–0·9 nA. This transformation is of interest as it is known that the firing frequency of motoneurones increases by about 1 impulse s^{-1} nA^{-1} (Kernell, 1966). If a motoneurone is recruited at the onset of a slow ramp-and-hold stretch of 5 mm it can then be expected that the increase in firing rate by the increasing monosynaptic Ia input during the slow ramp will be 0·2–0·9 impulses/s.

During slow ramp stretches of muscles in the decerebrate cat it has been noticed that the firing frequency of individual motor units rapidly increase for the first few intervals following recruitment (from 5 to about 10 impulses/s), but that the frequency hardly increases with further muscle stretch (Grillner & Udo, 1971; Cordo & Rymer, 1982). The initial pronounced rate modulation in combination with the apparently 'clamped' frequency with further muscle extension seems paradoxical. From the quantitative calculations in the small-print section it is obvious that the monosynaptic excitation from muscle spindles cannot account for the rapid frequency acceleration in relation to the recruitment (which occurs almost without further stretch). However, the negligible frequency increase with further stretch is easily understood by the minute contribution from the Ia EPSP. The pronounced initial frequency acceleration is most probably not directly caused by a synaptic excitation, but rather reflects the development of a plateau potential; the results given in section (4) strongly imply the presence of plateau potentials and their amplitude matches the frequency jump quite well.

The intracellular recordings have shown that the plateau potentials have an amplitude of 5–15 mV and this is much larger (by a factor of ten) than the tonic synaptic excitation following a 5 mm stretch. Nevertheless it is the synaptic

excitation which triggers the plateau potential. It seems likely that the high dynamic sensitivity of the muscle spindle Ia afferents, especially at the beginning of the stretch (cf. Matthews, 1972), causes a strong, but brief, synaptic depolarization from which the plateau potential can be generated. Given a sufficient background excitation the plateau potential can support the firing even with some loss of synaptic excitation, when the muscle spindle discharge is adapting (see Fig. 9 in this paper, and Fig. 8 in Crone *et al.* 1988).

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