Postinhibitory Rebound During Locomotor-Like Activity in Neonatal Rat Motoneurons In Vitro

SANDRINE BERTRAND AND JEAN-RENÉ CAZALETS
Centre National de la Recherche Scientifique, Neurobiologie et Mouvements, 13402 Marseille Cedex 20, France

Bertrand, Sandrine and Jean-Rene Cazalets. Postinhibitory rebound during locomotor-like activity in neonatal rat motoneurons in vitro. J. Neurophysiol. 79: 342–351, 1998. The aim of this study was to establish how a membrane property contributes to the neuronal discharge during ongoing behavior. We therefore studied the role of the postinhibitory rebound (PIR) in the bursting discharge of lumbar motoneurons intracellularly recorded in newborn rat in vitro brain stem/spinal cord preparation. The PIR is a transient depolarization that occurs after a hyperpolarization. We first investigated how it was expressed during experimentally induced hyperpolarizations. Its amplitude increased with the inhibition and was voltage dependent. The Ca2+ channel blockers Mn2+ and Co2+ partly suppressed the PIR in a few of the motoneurons tested. When hyperpolarized, the motoneurons exhibited a sag that was associated with the PIR. Adding caesium to the bath abolished both sag and rebound, which suggested that the PIR in the lumbar motoneurons was mainly due to the activation of the inward rectifying current I0. In the second part, we studied the physiological involvement of PIR during fictive locomotion induced by bath application of N-methyl-d-l-aspartate and serotonin. We established that experimentally induced PIR could initiate or modulate the bursting discharge of motoneurons during fictive locomotion. We then studied whether the firing patterns of the motoneurons were correlated in one way with the synaptic inhibition. When the monosynaptic inhibitory input to the motoneurons was abolished with the glycinergic blocker strychnine, these neurons stopped discharging (although they still were depolarized rhythmically). The firing of action potentials was restored by applying negative current pulses. This study provides evidence as to how one membrane property in mammals is involved in a complex type of behavior, namely locomotion.

INTRODUCTION

The discovery made during the past 15 years that vertebrate neurons not only summate synaptic information but share many membrane properties with their distant parents the invertebrates (Brownstone and Hultborn 1992; Llinas 1988) has modified our view of how the nervous system works. Assuming that all the neuronal types, from cortical neurons (Connors and Gutnick 1990) to motoneurons (Hounsgaard et al. 1988) possess a whole palette of properties, the question now arises as to how these single cell properties are integrated within a functioning network? To date, it is mainly in primitive nervous systems that it has been possible in some cases to answer this question and to demonstrate that these neuronal properties participate in generating or shaping the motor output (Arshavsky et al. 1993; Grillner et al. 1995; Harris-Warrick et al. 1992; Hartline and Graubard 1992; Lukoviak 1991; Satterlie 1985; Selverston and Moulins 1985). Although there are various studies in mammals (Burke 1982; Gustafsson and Pinter 1984; Henneman et al. 1965; Hounsgaard et al. 1988; McCormick and Pape 1990; Smith et al. 1991), the gap between the data collected on the cellular properties of elementary parts of the CNS and those collected at a more integrated level still remains. This is due to the fact that a system in which behavior is expressed is generally not accessible to cellular investigations. The aim of this study was to provide some evidence that one particular membrane property may participate in the pattern of neuronal activity produced during ongoing motor behavior. For this purpose, we used an isolated newborn rat brain stem/spinal cord preparation, which can be used to analyze motor behavior with the advantages and convenience of an in vitro preparation, and we obtained evidence that the locomotor output partly depends on a given membrane property of the motoneuron that we describe here, namely the postinhibitory rebound (PIR) (Dean et al. 1989; Roberts and Tunstall 1990; Satterlie 1985).

The newborn rat in vitro brain stem/spinal cord preparation has been developed to study the cellular mechanisms underlying respiration (Funk et al. 1995; Monteaup and Hilaire 1991) and locomotion (Cazalets 1995; Kudo and Yamada 1987; Smith et al. 1988). Locomotor-like activity can be generated in this preparation by bath-applying excitatory amino acids and/or serotonin (Cazalets et al. 1992; Scaglioni-Houssaini et al. 1993). This activity can be recorded in the ventral roots (Cazalets et al. 1992) or the hindlimb muscles (Kiehn and Kjaerulf 1996). Using semi-intact preparations, it has been observed that the activities recorded in vitro share many similarities with the locomotor activity recorded in intact adult animals (Kiehn and Kjaerulf 1996; Kudo and Yamada 1987). Using a double-bath technique with petroleum jelly (Vaseline) walls, we recently established that a locomotor network located in the rostral part of the lumbar spinal cord (i.e., segments L1/L2) can generate a perfectly well-organized locomotor pattern in the whole lumbar spinal cord (Cazalets et al. 1995). This was confirmed by other groups (Cowley and Schmidt 1997; Kjaerulf and Kiehn 1996; Kremer and Lev-Tov 1997), which reported that the more caudal segments could produce a weak rhythmic activity when isolated. The network located in L1/L2 sends a biphasic (excitatory and inhibitory) monosynaptic drive to the motoneurons distributed along the lumbar cord (Cazalets et al. 1996). Moreover, it is possible with the partitioned spinal cord to selectively and independently modify the functioning of the locomotor network and that of its target motoneurons. Using this technique, we attempted to establish how a neuronal property, the postinhibitory rebound depending on the inhibitory drive, contributes to setting up the motor pattern. In the first part of this study, we analyzed the voltage...
and ionic dependence of the PIR based on previous works (Berger and Takahashi 1990; Takahashi 1990a,b). It is suggested that rebound is sustained by low-voltage-activated (LVA) calcium channels and an inward rectifier current. We then investigated the role of rebound by analyzing how the synaptic inhibition may interact with the motoneuron discharge during locomotor-like activity.

**Methods**

Experiments (*n* = 41) were performed on Wistar rats aged 1–5 days. The animals were anesthetized with ether before decapitation. The skin of the back was removed and the vertebrae were cut dorsally. The spinal cord was removed and placed in a recording chamber. The isolated brain stem/spinal cord was superfused with an oxygenated (95% O₂-5% CO₂) physiological saline [composition (in mM): 130 NaCl, 4 KCl, 3.75 CaCl₂, 1.3 MgSO₄, 0.58 NaH₂PO₄, 25 NaHCO₃, and 10 glucose], adjusted to pH 7.4 with HCl. The solution temperature was kept constant at 25°C. The dissection procedure adopted and the experimental methods used to prepare the isolated in vitro brain stem/spinal cord have been described extensively in previous papers (Sqalli-Houssaini et al. 1991).

**Extracellular recordings and partitioning of the lumbar spinal cord**

The locomotor-like activity was recorded using extracellular stainless steel pin electrodes placed in contact with lumbar roots and insulated with Vaseline. The same electrodes could be switched from recording to stimulation. The neurograms were amplified, displayed, and stored using classical electrophysiological devices. To superfuse the locomotor network located in the rostral lumbar cord independently from the caudal motoneurons, Vaseline barriers were built at the T₁₂/T₁₃ and L₂/L₃ levels (Cazalets et al. 1995). In all experiments, locomotor-like activity was induced by bath applying a mixture of serotonin (5-HT; 5 × 10⁻⁵ M or 10⁻⁴ M) and N-methyl-D-aspartate (NMA) (1.5 × 10⁻³ M) (Sqalli-Houssaini et al. 1993).

**Intracellular recordings**

To gain access to the motoneurons, the pia was removed. Glass micropipettes were pulled using a Sutter PC-80 micropipette puller and filled with 2 or 4 M K-acetate (35–60 MΩ). The intracellular potentials were amplified by an Axoclamp 2A in the bridge mode. The motoneurons were identified by their antidromic action potential in response to extracellular stimulation of the ventral roots. Only neurons with an antidromic potential of ≥70 mV in amplitude and with a membrane resting potential of at least −60 mV were included in the analysis. During locomotor-like activity induced by bath-applying NMA/5-HT, the motoneurons were depolarized (Cazalets et al. 1996).

Intracellular potentials were collected by an Instech ITC-16 interface connected to a Macintosh Quadra 650 computer. Data were collected from 48 motoneurons with resting potentials of −68.6 ± 0.9 mV (mean ± SE) and input resistance of 24.4 ± 1.5 MΩ. We recorded motoneurons with axons running in the ventral roots of the left lumbar 3 segment (IL₃, Mn.), of IL₄ segment, and of IL₅ segment. No significant variations in the parameters analyzed, i.e., membrane potentials, input resistances, and mean PIR amplitudes were observed between motoneurons of different segments.

**Pharmacology**

All the drugs used [5-HT, NMA, tetrodotoxin (TTX), strychnine] and the various salts (CoCl₂, CsCl, MnCl₂) were obtained from Sigma (St. Louis, MO). Serotonin, NMA, TTX, and strychnine usually were prepared at 10⁻² M and frozen before being diluted to the appropriate concentration before their bath application. To block Ca²⁺ channels, the preparation was superfused with a saline in which Ca²⁺ was replaced by Mn²⁺ (2 mM) with no PO₄³⁻ to prevent precipitation. Mn²⁺ and Cs²⁺ were added to the saline from a 1 M stock solution.

**Data analysis**

Standard stimulation protocols were delivered by the computer to the neurons tested. Each protocol consisted of five stimulation loops. The amplitude of the PIR was measured at the maximum peak depolarization, and the left part of the trace before the stimulation was taken as the baseline. The period value of the fictive locomotion cycles was calculated from the onset of one burst of spikes to the onset of the following burst of spikes. The phase at which a given event occurred within a cycle was calculated as its latency in the cycle divided by the cycle period (see Cazalets et al. 1990). The software programs used for the data acquisition and analysis were from Axon Instrument, Foster City, CA. Measurements could be performed on cycles normalized by interpolation (using an Axograph plug-in). The onset of the sag was defined as the peak of the hyperpolarization during the application of a negative current pulse. We used *t*-test for significance, which was assumed when *P* < 0.05.

**Results**

**Presence of postinhibitory rebound in neonatal rat motoneurons**

The postinhibitory rebound is the depolarization that occurs at the offset of an hyperpolarizing event before the membrane potential returns to its initial value. In the neonatal rat motoneurons, this rebound response was observed under several conditions. First, it occurred in response to the injection of negative steps of current. In this case, depending on the amplitude of the hyperpolarizing stimulus, it was either subthreshold (Fig. 1A₁) or gave rise to an action potential (Fig. 1A₂). When the motoneurons were depolarized, not only a single spike but a whole burst of spikes that could last for ≤2 s could be generated (Fig. 1B). The rebound activity also was observed frequently in the case of physiologically evoked hyperpolarizations, such as those evoked by sensory afferent stimulation. Figure 1C shows that in the motoneuron tested, the inhibitory postsynaptic potential triggered by electrically stimulating lumbar dorsal root 5 (L₅) was immediately followed by an action potential.

We first studied the conditions under which the rebound response is expressed in lumbar motoneurons. To obtain accurate measurements, we generally triggered rebound depolarizations that were below the threshold at which action potentials are generated. We investigated the changes that occurred in the rebound size when the amplitude of the negative pulses was varied (Fig. 2A₁, see also Fig. 1A₂). It emerged that the amplitude of the PIR increased with the injected current (Fig. 2A₂). We also observed that the rebound response was voltage dependent, i.e., that there existed a close relationship between the voltage and the PIR amplitude when the neurons were depolarized either by continuously injecting positive current or under transmitter evoked depolarization. In all these cases, the neurons produced a more pronounced rebound response at more depolar-
ionic conductance underlying postinhibitory rebound in lumbar motoneurons

The changes that occurred in the input resistance during the rebound were measured by repeatedly injecting brief hyperpolarizing current pulses into the motoneurons (Fig. 2B1). Figure 2B2 shows that there was a decrease in the membrane resistance during the rebound, which was observed in all 10 neurons tested (mean $-7.6 \pm 2, 7\%$ of the control value, with pulses of 30 ms). We first investigated the possible involvement of Ca$^{2+}$ channels or of voltage-dependent sodium channels and, second, the involvement of an inward rectifying current because previous studies have shown that these currents are present in newborn rat motoneurons (Berger and Takahashi 1990; Takahashi 1990a,b).

Because in previous studies the PIR often was reported to be due to the activation of low-threshold voltage-activated Ca$^{2+}$ channels (LVA Ca$^{2+}$) (Janssen and Llinas 1984), we examined the effects of the inorganic blocking of Ca$^{2+}$ channels by Mn$^{2+}$ (2 mM) or Co$^{2+}$ (2 mM) in 0 Ca$^{2+}$ saline. The blocking effects of these modified salines on Ca$^{2+}$ channels were checked previously by testing them on the synaptic transmission between sensory afferents and motoneurons. As shown in Fig. 2C1, PSPs disappeared within 10 min under bath application of the saline containing 0 Ca$^{2+}$, Mn$^{2+}$ (this effect was observed in the 10 neurons tested). Under these conditions and at the same membrane potential (Fig. 2C2), the rebound response was depressed in only 3 of the 10 cells tested with Mn$^{2+}$ ($-40.6 \pm 5.7\%$). In the remaining ones, it was not affected. For the four motoneurons perfused with Co$^{2+}$, we could not see any change in the PIR amplitude. This partial dependency of PIR on Ca$^{2+}$ channels was observed at all the membrane potentials tested for the 3 cells of the 14 tested.

We also checked whether fast sodium channels were involved in the rebound response. For this purpose, we bath-applied TTX ($5 \times 10^{-7}$ M, $n = 5$ neurons). The spikes (whether orthodromic or antidromic) disappeared within 5–10 min under TTX bath application, while at the same time, no change occurred in the PIR amplitude (data not shown). Even at higher TTX concentrations ($10^{-6}$ M, $n = 2$), no significant variations in the rebound amplitude were observed. These results demonstrate that the PIR was partly due to the activation of Ca$^{2+}$ channels in some neurons, but another current seemed also to be involved in the rebound genesis in most of the lumbar motoneurons.

We observed that the motoneurons that expressed a rebound during prolonged hyperpolarizing pulses also developed a sag with time. Because sag and PIR are both induced by inhibition, a detailed analysis (Fig. 3) of the relationships between the sag and the rebound was performed (in 11 motoneurons) to determine whether there existed a clear-cut correlation between the two phenomena. The sag (gray curve of Fig. 3A1) corresponds to the fact that during the application of negative current pulses, the membrane potential reaches a maximum at $69.5 \pm 4.7$ ms ($n = 11$ neurons) after the onset of current and thereafter declines gradually to a steady-state level. The location of this peak (Fig. 3A1) did not depend on the pulse amplitude (Fig. 3A1). Figure 3A2 gives the I-V curve recorded at the onset of the sag and the I-V curve recorded in the steady state with negative current pulses. This shows that at the beginning of the pulse (Fig. 3A2, ●), the voltage increased linearly with the current, while it underwent a rectification in the steady state (Fig. 3A2, ○). We further examined the effects of the development of sag on PIR. For this purpose, we applied to the motoneuron negative pulses with various durations (30–100 ms, Fig. 3B). It was observed that the neuron exhibited a rebound only when the inhibition was long enough to induce a sag.

In a previous study, Takahashi (1990b) reported that the activation of an inward rectifying current (described under voltage clamp conditions), which he called $I_{IR}$, produced a sag in neonatal rat lumbar motoneurons. This conductance was affected by bath application of caesium. To test the hypothesis that the present sag and the PIR might be of the same ionic origin, we added caesium to the saline. In all the 10 neurons tested, both the sag and the PIR amplitude decreased at concentration of 4 mM Cs$^+$ $\sim 65.4 \pm 5\%$ ($n = 10$; Fig. 3C). In the best case recorded (Fig. 3C), the rebound was decreased by 75%. There was a concomitant increase in the input resistance under Cs$^+$. This effect of caesium was partly reversed during a 15-min wash out with normal saline (Fig. 3C). At concentrations of $>4$ mM, we found that Cs$^+$ modified firing behavior of the neurons by lengthening the shape of action potential and by increasing the input resistance, as it also was observed previously by Walton and Fulton (1986).
Physiological involvement of postinhibitory rebound during fictive locomotion

The above results have shown that the lumbar motoneurons exhibit postinhibitory rebound. Nevertheless, it still remains to be determined whether the rebound is involved actively in the motoneuronal discharge during locomotor activity. To address this question, we used complementary approaches. First of all, we examined whether the same hyperpolarization-induced by injecting negative current pulses to initiate rebound in a quiescent motoneuron also might initiate or modulate the motoneuronal discharge during ongoing rhythmic activity. For this purpose, we analyzed the changes in the firing pattern elicited by inducing rhythmic artificial hyperpolarizations during locomotor-like activity (Fig. 4). Negative current pulses were applied randomly to motoneurons during fictive locomotion, which was initiated by bath-applying NMA/5-HT to the whole preparation (Fig. 4A). The action potentials were counted during 11 successive trials within 10 s cycles and were plotted as a function of time (for details, see the legend of Fig. 4). Figure 4B, 1 and 2, gives the results obtained with two different membrane potentials. At -60 mV, the motoneuron discharged only at a very low rate (although it received a consistent, mainly subthreshold locomotor drive). In this case, the rebound induced a short burst of spikes (Fig. 4B1). At -52 mV, although the motoneuron discharged regularly during locomotor-like activity, the application of a negative current pulse still led to the expression of a greater number of action potentials (Fig. 4B2). All the neurons (n = 7), tested in this way during locomotor-like activity exhibited a modulation of their bursting discharge by experimentally induced hyperpolarizations able to promote PIR.

In another set of experiments, we attempted to evaluate the influence of synaptic inhibition and to analyze the relationships that exist between the naturally occurring inhibitory drive and the firing behavior of the motoneurons. The first question arose because of the finding that neurons (such as the 1 presented in Fig. 5A1) exhibited spontaneous firing concomitant with the release from synaptic inhibition. In six neurons, we statistically tested if there was a lower probability of obtaining spikes in the later part of the excitatory drive. For each individual cell, the mean instantaneous frequency was calculated in normalized cycles (~60 locomotor cycles). It then was compared during the first 20% and the last 20% of the excitatory drive. For these six motoneurons, this value was significant (0.0422 < P < 0.0001) and was greater at the onset of the excitatory drive than at the offset. We also examined the effects of short negative current pulses randomly applied to the neuron (Fig. 5A2) to see whether, under these conditions, the neuron might generate action potentials during some preferential phase of the cycle. Changes in various parameters were analyzed in relation with the phase cycle (see METHODS for phase calculations).
The onset of a burst in right L₂ ventral root (rL₂) was taken as the baseline to calculate the phase at which the inhibition occurred during one normalized cycle (Fig. 5B, gray area). It appears that spikes were emitted preferentially at the offset of the inhibition (i.e., 0.5, Fig. 5B1), whereas the probability of obtaining a spike in the late part of the excitatory drive was much lower. We checked whether this asymmetrical pattern was due to parallel changes in either the shape of the excitatory drive or to changes in the input resistance. The mean membrane potential (Fig. 5B2, calculated over 90 cycles) thus was plotted as a function of the phase. The shape of the depolarizing phase did not reflect the changes obtained in the spiking discharge shown in Fig. 5B1 because the excitatory phase (from 0.55 to 1) showed a plateau. Nor were the changes in the input resistance of the neuron (Fig. 5B3) correlated with the variations in the spiking activity, its maximum being reached later in the cycle (at 0.75). All in all, these results suggest that the increase in the firing activity may be attributable to the inhibitory process, which increases the ability to generate spikes.

We then attempted to interfere selectively with the naturally occurring inhibition that the motoneurons receive during locomotor activity. It recently has been established that the motoneurons in the in vitro neonatal brain stem/spinal cord preparation received a biphasic (excitatory plus inhibitory) and monosynaptic drive (Cazalets et al. 1996). Using a partitioned spinal cord, it was observed that the inhibitory part of the drive could be blocked with the glycine receptor blocker strychnine. We used this protocol to check whether blocking the inhibition might modify the lumbar motoneuronal discharge during locomotor-like activity. Fictive locomotion was initiated by bath-applying NMA/5-HT to lumbar segments L₁ and L₂ only (Cazalets et al. 1995). Under these conditions, we recorded a regular alternating activity in L₂ ventral roots, left (IL₂) and right (rL₂) (Fig. 6A). Simultaneously, we intracellularly recorded a IL₂ motoneuron, which produced rhythmic depolarizations with an average spike discharge of 5.3 ± 0.27 per cycle (Fig. 6B1). Figure 6B2 gives an expanded detail showing the membrane fluctuations during locomotor-like activity. The depolarizations occurred in phase with the activity of IL₂ and alternated with inhibitions. Bath-applying the glycine receptor blocker strychnine (3 × 10⁻⁶ M) to the caudal lumbar segments (L₁, sacrum) abolished the inhibitory postsynaptic potentials received by the motoneurons (Fig. 6C2). As shown in Fig. 6C1, at the same time, when the inhibition was blocked by strychnine, the depolarizations generated only one or two spikes if any (stars). These changes in the motoneuronal discharge were not attributable to changes in the membrane potential [-66.5 ± 3.5 mV (mean ± SD) under control conditions and -66 ± 2.8 mV with strychnine in the bath, n = 6 neurons] or to the input resistance, since it was increased by strychnine (+14.1 ± 10% of the control value). For the six neurons tested (in different experiments) in which the effects of strychnine were analyzed, we always observed either a considerable decrease in the burst discharge (as in the Fig. 6, mean number of spikes: 4 ± 1 in control and 1.3 ± 1 with strychnine in the bath, n = 4 neurons) or a complete cessation (in 2 neurons) of the motoneuronal firing. These results suggest that the hyperpolarization of the cell before phasic excitation favors the bursting activity recorded under control conditions.

To confirm that the cessation of motoneuronal firing was directly due to the lack of rhythmic glycine receptor inhibition
and only to this factor, we checked whether an imposed inhibition, which mimicked the missing glycinergic inhibition, could restore a phasic discharge. We therefore, under strychnine condition (Fig. 7A1), applied pulses of negative current to the motoneuron to induce postinhibitory rebound. As shown in Fig. 7A2 (in which the neuron tested was the same as in Fig. 6), these hyperpolarizations could restore a bursting discharge in the presence of strychnine. Under the same conditions (Fig. 7B1), repeatedly applied negative pulses of current did not only elicited a single burst of spikes but restored a rhythmic bursting discharge in the motoneuron. This was the case for the six neurons tested under this conditions. Note that in the experiment of Figs. 7B the neuron tested discharged bursts of action potentials under control conditions (not shown), and that the subsequent addition of strychnine to the saline completely abolished this spiking activity.

In the experiments we have just described (Fig. 6), we tested the importance of the inhibition by preventing it. To check, however, whether during locomotor-like activity, PIR controls the action potential discharge via the same ionic mechanisms as those described above (Fig. 3), we tested the effects of Cs⁺ (2 mM) during fictive locomotion (n = 3 neurons). As shown in Fig. 7B2, subsequently adding of Cs⁺ to the saline containing strychnine finally blocked the firing activity elicited by repeatedly injecting of negative current pulses of the three neurons tested. Moreover in Fig. 7B2, we even increased the current as compared with Fig. 7B1. The remaining rebound observed was probably due to the activation of LVA channels (see Fig. 2C and DISCUSSION). It was not possible, however, to test this hypothesis during fictive locomotion because bath-application of Ca²⁺ blockers would abolish all synaptic activity. These data therefore suggest that the inward rectifying current may par-

FIG. 4. Modulation of the motoneurons discharge by hyperpolarizing current pulses during fictive locomotion. A: locomotor-like activity was induced by bath applying N-methyl-D-aspartate (NMA)/serotonin (5-HT) to the whole preparation. Every 10 s, pulses of −1.25 nA were applied to the motoneuron (Mn). Action potentials were counted during 500-ms bins 5 s before and 5 s after the hyperpolarization pulse the beginning of which corresponds to the time 0. B: 1 and 2: mean number of spikes per cycle produced by the motoneuron at 2 different potentials (values at top of the graphs, n = 11 trials for each potential). Rebound consistently increased the neuronal firing regardless of their basic discharge.

FIG. 5. Influence of the inhibitory drive on the bursting pattern of motoneurons. A1: during fictive locomotion induced by bath-applying NMA/5-HT, the motoneuron (−50 mV) recorded produced spikes only during the offset of the inhibitory phase of the locomotor cycle. A2: spike discharge was elicited occasionally by randomly injecting negative current pulses (−0.7 nA, 100 ms). B1: plot of the mean number of action potentials per cycle related to the cycle phase showing that spikes were triggered preferentially at the offset of the inhibition (II). B2: plot of the mean drive amplitude (n = 90 cycles) during 1 normalized cycle. B3: graph of the input membrane resistance as a function of the cycle phase (n = 82 cycles).
In their pioneering work in mammals, pulses is likely to occur and be expressed during locomotion. In vivo may express sag and PIR in response to negative current hyperpolarizing current pulse applied to evoke both a sag
Xenopus mollusks (Satterlie 1985) to various vertebrates such as the The first question that arises here is whether the rebound participates in the bursting discharge of motoneurons during locomotion.
mechanisms that can give rise to this phenomenon. These other mechanisms, two of them may explain the results we obtained when considering the pharmacology of the PIR in neonatal rat lumbar motoneurons: LVA channels (Fig. 2C) and an inward rectifying current (Fig. 3). This would be compatible with previous studies in which it was demonstrated that both LVA channels (Berger and Takahashi 1990) and inward rectifying current (Takahashi 1990b) are present in these neurons. It seems, however, that in neonatal rat, these two types of current may not contribute equally to the genesis of the rebound. In fact, the reduction of PIR by the Ca \(^{2+}\) blockers (Mn \(^{2+}\) or Co \(^{2+}\)) was observed only in some neurons in which, in addition, it did not completely abolish the rebound. On the other hand, the rebound depolarization may be mainly due to a current being blocked by Cs \(^{+}\). Takahashi (1990b) has shown that inward rectification in newborn rat motoneurons is due to a mixed cationic K \(^{-}\) and Na \(^{+}\) current called \(I_{nr}\), which is similar to the \(I_{ik}\) (Maccabelli and MacBrain 1996; Mayer and Westbrook 1983) and \(I_{q}\) currents (Hulliwell and Adams 1982). These currents are all sensitive to the application of caesium. The fact that \(I_{nr}\) underlies PIR is not a specific property of newborn rat motoneurons because it has been observed in other systems and in adult animals, as well as in the trigeminal motoneurons (Chandler et al. 1994), in the nucleus ambiguous neurons (Johnson and Getting 1991) of the adult guinea pig, and in newborn mouse brain stem (Rekling et al. 1996). In those cells, as in neonatal rat motoneurons (see Fig. 3), the sag and rebound were mediated by the \(I_{ik}\) or \(I_{q}\) current. This is not always the case, however, because Dekin (1993) has reported that bulbospinal neurons of the nucleus tractus solitarius exhibit a pronounced inward rectification, which does not sustain rebound depolarization. Finally, it has been established (Bayliss et al. 1994) that in the course of ontogenesis a marked increase in the amplitude of the inward rectifying current occurs in the hypoglossal motoneurons, which suggests that the postinhibitory rebound may play an even more important role in adults. It is not yet possible to explain why the \(I_{nr}\) current plays a predominant role in these neurons as regards to the LVA current although both types of currents are present in the motoneuron membrane. In addition, the possibility cannot be ruled out that even some other mechanisms, such as the anode break phenomenon, also may contribute to PIR in the lumbar motoneurons of newborn rats.

**Discussion**

**Ionic nature of PIR in neonatal rat lumbar motoneurons**

Postinhibitory rebound is a commonly observed feature in many species, from crustaceans (Barrio et al. 1994) and mollusks (Satterlie 1985) to various vertebrates such as the lamprey (Grillner et al. 1995) and the Xenopus (Roberts and Tunstall 1990). In their pioneering work in mammals, Ito and Oshima (1965) reported that cat motoneurons in vivo may express sag and PIR in response to negative current pulses. Probably due to this diversity, its underlying ionic mechanisms exhibit a high degree of variability. In a previous study, Johnson and Getting (1991) reviewed the various mechanisms that can give rise to this phenomenon. These include M current (Barrio et al. 1994; Constanti et al. 1981), low-threshold Ca \(^{2+}\) (LVA) channel (Jahnsen and Llinas 1984; Onimaru et al. 1996; Tell and Bradley 1994), anode break excitation (Hodgkin and Huxley 1952), Ca \(^{2+}\)-activated Cl \(^{-}\) current (Owen et al. 1984), and inward rectifying currents (Chandler et al. 1994; Johnson and Getting 1991; Marder and Calabrese 1996; Pape 1996). Among these various mechanisms, two of them may explain the results we obtained when considering the pharmacology of the PIR in neonatal rat lumbar motoneurons: LVA channels (Fig. 2C) and an inward rectifying current (Fig. 3). This would be compatible with previous studies in which it was demonstrated that both LVA channels (Berger and Takahashi 1990) and inward rectifying current (Takahashi 1990b) are present in these neurons. It seems, however, that in neonatal rat, these two types of current may not contribute equally to the genesis of the rebound. In fact, the reduction of PIR by the Ca \(^{2+}\) blockers (Mn \(^{2+}\) or Co \(^{2+}\)) was observed only in some neurons in which, in addition, it did not completely abolish the rebound. On the other hand, the rebound depolarization may be mainly due to a current being blocked by Cs \(^{+}\). Takahashi (1990b) has shown that inward rectification in newborn rat motoneurons is due to a mixed cationic K \(^{-}\) and Na \(^{+}\) current called \(I_{nr}\), which is similar to the \(I_{ik}\) (Maccabelli and MacBrain 1996; Mayer and Westbrook 1983) and \(I_{q}\) currents (Hulliwell and Adams 1982). These currents are all sensitive to the application of caesium. The fact that \(I_{nr}\) underlies PIR is not a specific property of newborn rat motoneurons because it has been observed in other systems and in adult animals, as well as in the trigeminal motoneurons (Chandler et al. 1994), in the nucleus ambiguous neurons (Johnson and Getting 1991) of the adult guinea pig, and in newborn mouse brain stem (Rekling et al. 1996). In those cells, as in neonatal rat motoneurons (see Fig. 3), the sag and rebound were mediated by the \(I_{ik}\) or \(I_{q}\) current. This is not always the case, however, because Dekin (1993) has reported that bulbospinal neurons of the nucleus tractus solitarius exhibit a pronounced inward rectification, which does not sustain rebound depolarization. Finally, it has been established (Bayliss et al. 1994) that in the course of ontogenesis a marked increase in the amplitude of the inward rectifying current occurs in the hypoglossal motoneurons, which suggests that the postinhibitory rebound may play an even more important role in adults. It is not yet possible to explain why the \(I_{nr}\) current plays a predominant role in these neurons as regards to the LVA current although both types of currents are present in the motoneuron membrane. In addition, the possibility cannot be ruled out that even some other mechanisms, such as the anode break phenomenon, also may contribute to PIR in the lumbar motoneurons of newborn rats.

**Physiological involvement of PIR in the fictive locomotion**

The first question that arises here is whether the rebound that is induced experimentally by injecting negative current pulses is likely to occur and be expressed during locomotion. We have established here that the minimum duration of a hyperpolarizing current pulse applied to evoke both a sag and a rebound is \(\sim 60\) ms (Fig. 4C). This minimal inhibitory value always is reached during actual behavior. One can actually assume that the system reaches its fastest speed during galloping in the adult rat. In this case, the minimum cycle period is \(\sim 200\) ms (Cohen and Gans 1975). As the inhibition corresponds to about one-half of a cycle, this 100-
ms value is far more than the minimum required. In the present study, we did not systematically analyze the PIR voltage dependency because it was observed that the values of these parameters were very similar to those previously reported in detail (Dean et al. 1989; Johnson and Getting 1991). The membrane potential at which voltage-dependent rebound is expressed is also compatible with the voltage recorded during ongoing behavior because when sequences of locomotor activity are elicited, the motoneurons are depolarized (Jordan 1983). In any case, this depolarization would favor the expression and the participation of the rebound during locomotor activity.

We have established that PIR can be expressed in all the motoneurons when they are quiescent. It was also possible in some favorable cases to demonstrate and to highlight the role played by PIR during locomotor-like activity (Figs. 4–7), but it is impossible to say yet whether all the motoneurons in this preparation behave in the same way. In particular, we could not fully evaluate the exact contribution of the synaptic inhibition and of the resulting PIR, which might shape the firing pattern of the whole motoneuronal population. This was partly attributable to the heterogeneity of the motoneuronal population and mainly to the variations in the synaptic drive from one neuron to another.

Postinhibitory rebound has been described in many systems, and some studies have demonstrated that it contributes to shaping the pattern of cell firing. In the lobster pyloric neurons (Hartline and Graubard 1992), PIR was found to produce a phase shift in the stomatogastric ganglion. In C. clione, Satterlie (1985) showed that a simple neuronal network can produce an alternating cyclic activity in the absence of tonic drive if the neurons involved exhibit postinhibitory rebound. In Xenopus (Roberts and Tunstall 1990), it also has been suggested that rebound plays a key role in the genesis of rhythmic motor pattern. In the stomatogastric ganglion of the spiny lobster, Harris-Warrick et al. (1995) demonstrated that the manipulation of PIR by dopamine changes the phase relationships of the pyloric rhythm. In the lamprey, Matsushima et al. (1993) suggested that LVA calcium current, which in part generated the rebound depolarization, would be of particular importance for neurons mediating the reciprocal inhibition between the hemisegments in the spinal cord.

It is not easy to demonstrate the involvement of membrane properties of this kind in behavior. The in vitro spinal cord preparation of the rat appears to be a suitable model for research on these lines because it is possible with this preparation to uncouple the functioning of the network located in lumbar segments L₁/L₂ from the activity of follower motoneurons in more caudal segments. When fictive locomotion is induced by locally bath-applying NMA/5-HT to L₁/L₂, it becomes possible to distinguish between the synaptic information originating from the locomotor network and the integrative capacities of the motoneuron (Cazalets et al. 1995, 1996). When the inhibition was suppressed in this way with strychnine, the motoneurons stopped rhythmically discharging (Fig. 6). This relationship between the inhibition and the motoneuronal discharge was not directly linked to any change in the membrane potential or in the input resistance because these parameters were not significantly affected by strychnine. In addition, the neurons kept their ability to produce action potentials in response to direct depolarization with positive current. The fact that hyperpolarizing current pulses reinducte rhythmic firing (Fig. 7B) confirms the existence of this direct relationship. In this context, strychnine seems to have rather paradoxical effects because on withdrawing an inhibition, one might normally expect the discharge to increase. Moreover, it would have been interesting to analyze the effect of Cs⁺ alone during fictive locomotion. This was not possible because when Cs⁺ was
applied at concentrations that considerably reduce the rebound, it also induced abnormal discharges, similar to those observed with other K⁺ channel blockers (4-AP and tetraethylammonium) (Cazalets, unpublished observation).

The present study shows what a key role inhibition can play by regulating the general level of a system. If inhibition plays a role of this kind, i.e., adjusting the firing in a positive way, it presumably will show great flexibility particularly as this is necessary to keep the system in control.

We thank Drs. Barthe, Clarac, and Vinay for critical reading of an early version of the manuscript. The authors also thank Drs. Cattaert and Jamon for helpful comments on the manuscript and R. Navarro for nursing the pups.

This research was supported by a grant from Institut de la Recherche sur la Moelle Épineure and Institut National de la Santé et de la Recherche Médicale Grant 920803. S. Bertrand received a fellowship from the Ministère de l’Enseignement Supérieur Français.

Address for reprint requests: S. Bertrand, CNRS, NBM, 31 chemin Joseph Aiguier, BP 71, 13402 Marseille Cedex 20, France.

Received 17 April 1997; accepted in final form 10 September 1997.

REFERENCES


