A Low Threshold Calcium Spike Mediates Firing Pattern Alterations in Pontine Reticular Neurons

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Intracellular electrical recordings in an in vitro slice preparation of the brainstem medial pontine reticular formation, a region thought to be important in mediation of desynchronized sleep phenomena, demonstrate a population of neurons that have a calcium-dependent, low threshold spike. This low threshold spike was inactivated at relatively depolarized membrane potential levels and, when this spike was deinactivated, it induced a burst of action potentials. The membrane potential dependence of the spike may underlie changes in action potential firing patterns associated with behavioral state change because the baseline membrane potential in neurons of the medial pontine reticular population depolarizes during passage from waking and slow wave sleep to desynchronized sleep, which is characterized by the absence of burst firing.

SINCE THE FIRST RECORDINGS OF neurons during the behavioral state alterations of the sleep-wake cycle were made, a central neurophysiological problem has been to define the mechanisms controlling the different firing patterns and neuronal responsiveness in different behavioral states (*1*). Alterations in firing pattern may result not only from changes in synaptic input but also from intrinsic membrane properties of the neuron. Both statistical analysis and intracellular recordings in vitro suggest that the altered firing patterns and responsiveness of thalamic neurons during slow wave sleep are due to a low threshold spike (LTS) that is deinactivated during the baseline membrane potential hyperpolarization of slow wave sleep (*2*).

Although the baseline membrane potential of medial pontine reticular formation (mPRF) neurons depolarizes 7 to 10 mV on passage from waking and slow wave sleep to desynchronized sleep (*3*), the consequences of this change for neuronal firing patterns and responsiveness and their underlying mechanisms are not known, probably because of the lack of a suitable experimental preparation. Nevertheless, there is substantial electrophysiological and pharmacological evidence that the mPRF mediates essential events of desynchronized sleep, including ponto-geniculo-occipital waves, the rapid eye movements, and the muscle twitches (*4*). We have used an in vitro preparation, the mPRF brainstem slice of young rats, to demonstrate that the firing pattern of one population of mPRF neurons is altered with changes in the baseline membrane potential as a result of the presence of a LTS.

Brainstem slices were made (500 μm) with an ultrasonically vibrating blade and were totally submerged in a chamber (*5*). Sprague-Dawley rats (7 to 10 days old) were used because the behavior of interest, desynchronized sleep, is present in fivefold greater amounts than in adults, and neurons more readily survived the slicing procedure. The slices were perfused with oxygenated modified Ringer solution containing 124 m*M* NaCl, 26 m*M* $NaHCO_3$, 1.2 m*M* KH_2PO_4, 2 m*M* KCl, 2.4 m*M* $CaCl_2$, and 1.3 m*M* $MgCl_2$ and maintained at 30°C. Anatomical landmarks were easily identifiable and recordings were made within the mPRF from the level of VI nucleus to 1 mm rostral. Recording sites were mechanically lesioned and their anatomical localization reconfirmed after Nissl staining of the slice. Intracellular recordings and current injections were made with a bridge amplifier and glass microelectrodes filled with KCl (2*M*) (resistance 60 to 90 megohms). Current pulses were delivered at 10-second or greater intervals. Only cells with a stable resting membrane potential more negative than −55 mV and an action potential overshoot greater than 5 mV were considered healthy and included in this study. As judged by these criteria, the preparation was viable for longer than 9 hours.

On the basis of their response to intracellularly applied current, 17 neurons of the mPRF were divided into two classes—those displaying an inactivating LTS (n = 9) (Fig. 1, A and B) and neurons without LTS (n = 8) (Fig. 1, C and D). The average resting membrane potential was −67 ± 9 mV (SD), and the average input resistance was 96 ± 52 megohms (SD). In LTS neurons, when the membrane potential was maintained at −75 mV or more negative by DC current injection, depolarizing current pulses evoked a burst of action potentials superimposed on a long duration LTS. Larger amplitude depolarizing current pulses evoked a burst of action potentials followed by a tonic pattern of repetitive firing (Fig. 1A). Application of depolarizing current when the membrane potential was more positive than −65 mV resulted in only

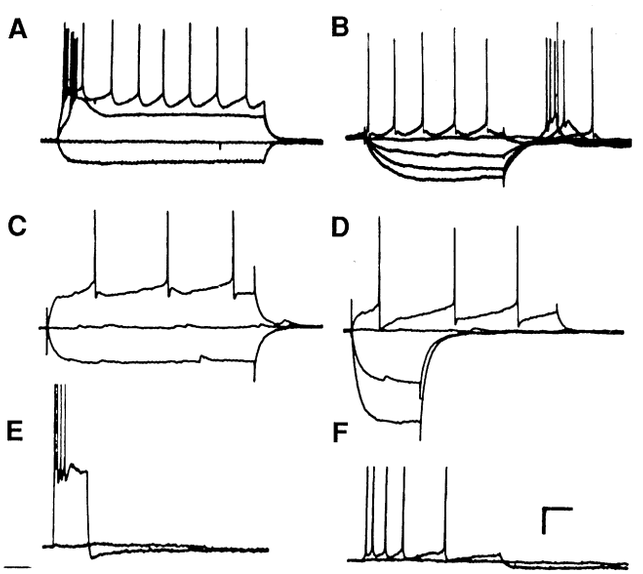

Fig. 1. Three distinct repetitive firing patterns in response to depolarizing current input. (A) Typical voltage traces of an LTS burst response to intracellularly injected constant current pulses of 900-msec duration and −300-, 0-, +300-, and +600-pA amplitude. With greater current, a tonic pattern followed the burst. Baseline membrane potential was −78 mV. (B) Five voltage traces with a baseline membrane potential of −64 mV. Hyperpolarizing current pulses of 400-msec duration and 100-, 200-, and 300-pA amplitude can evoke rebound LTS bursts. Note that as hyperpolarization increases so does the LTS amplitude. The depolarizing current pulse of 300-pA amplitude evokes only a tonic firing pattern due to LTS inactivation. (C) and (D) are typical voltage traces of a response from a neuron without LTS to current pulses of 300-msec duration, and +400- and −300-pA amplitude (membrane potential is −80 mV) for (C) and 350- or 150-msec duration and +200-, −400-, −800-pA amplitude (membrane potential is −63 mV) for (D). The depolarizing current pulses evoke only a tonic firing pattern. (E) Typical voltage responses from CA1 hippocampal neurons (from 7- to 10-day-old rats) showing the long duration after hyperpolarization and (F) a firing pattern of accommodation in response to excitatory current pulses of 1-second and 750-msec duration and 500- and 300-pA amplitude for (E) and (F), respectively. Calibration bars: 20 mV for all except (E) which is 10 mV; 120 msec for (A) and (F); 80 msec for (B); 40 msec for (C) and (D); 400 msec for (E).

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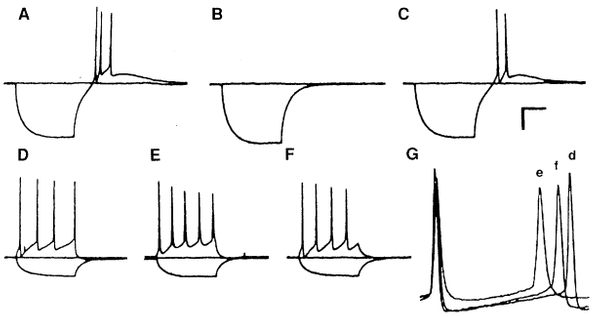

Fig. 2. Calcium effects on burst and tonic modes of repetitive firing. All traces are typical voltage records taken from the same neuron before (A and D), during (B and E), and after (C and F) perfusion with media containing 10 m*M* Mg^{2+} but no Ca^{2+}. Baseline was −62 mV. (A) to (C) are burst responses that follow the removal of LTS inactivation by hyperpolarizing current pulses of 400-pA amplitude and 150-msec duration. (D) to (F) are tonic responses to current pulses of +200-pA (−200-pA pulses show unaltered input resistance) amplitude and 150-msec duration. (G) Three overlapping traces with expanded time and voltage scale of the second and third action potentials from (D) to (F) (marked as d, e, and f, respectively). Calibration bars are 20 mV, 40 msec for all but (G) which are 10 mV, 5 msec.

a tonic firing pattern; this was due to LTS inactivation, which was removed by hyperpolarization (Fig. 1B). The response to depolarizing current in neurons without LTS was always a tonic firing pattern, even when the membrane potential was more negative than −75 mV; there was also no posthyperpolarization rebound (Fig. 1, C and D). The frequency versus current relation showed a simple linear proportionality, possibly reflecting the neuron's immaturity (*6*).

The dependence of the LTS on Ca^{2+} flux across the membrane was examined. In control perfusion media a hyperpolarizing current pulse removed LTS inactivation with a resultant posthyperpolarization rebound activation of an LTS burst response (Fig. 2A). This excitatory response could be reversibly blocked by a 10-minute application of perfusion media containing 10 m*M* Mg^{2+} and no Ca^{2+} (n = 3) (Fig. 2, A to C), demonstrating the Ca^{2+} dependence of the response. The fast Na^+-dependent action potentials were not blocked by this perfusate (Fig. 2, D to G). The addition of tetrodotoxin (0.3 μ*M*) (n = 3) to the bath abolished the fast action potentials. The LTS, however, was unaffected (Fig. 3). Replacement of Ca^{2+} with 10 m*M* Mg^{2+} in the perfusate blocked the LTS as it did when tetrodotoxin was not present.

We also evaluated the effects of Ca^{2+} on tonic firing because repetitive firing is known to be strongly influenced by Ca^{2+} flux across the membrane in both invertebrate (*7*) and vertebrate (*8–10*) central nervous system neurons. Bath application of perfusion fluid containing 10 m*M* Mg^{2+} and no Ca^{2+} to mPRF neurons increased the excitability as measured by the number of action potentials generated by an excitatory current pulse starting from the same membrane potential (n = 5) (Fig. 2, D to F). This increase was always observed despite the increased divalent cation concentration, which can decrease neuronal excitability (*11*). The resting input resistance was unaffected. A similar result followed bath application of medium containing Cd^{2+} (200 μ*M*) (n = 2) a Ca^{2+} current antagonist (*12*) or tetraethylammonium (TEA) (5 m*M*) (n = 3), an antagonist of Ca^{2+}-dependent K^+ current (*13*). Examination on an enlarged time scale revealed a reduction of the after hyperpolarization, an increase in action potential duration, and a reduction of action potential amplitude (Fig. 2G). The latter two became more prominent with successive action potentials. This suggests that the increase in action potential duration could have resulted from inactivation of the delayed rectifier potassium current (I_k) revealed in the absence of Ca^{2+}-dependent K^+ current (*14*) and that the reduction of after hyperpolarization could have led to Na^+ current inactivation (*15*) and the observed reduction of action potential amplitude. These effects were the same for the tonic mode of firing in both LTS neurons and neurons without LTS.

Still another class of repetitive firing behavior has been described in CA1 and CA3 (*8*) hippocampal neurons as an accommodation of action potential firing to a constant excitatory current pulse, associated with a long duration after hyperpolarization that may be mediated by a distinct late Ca^{2+}-dependent K^+ conductance (*16*). Neither accommodation nor a late afterhyperpolarization was observed in mPRF neurons. To examine the possibility that this was a function of neuronal immaturity, we tested CA1 pyramidal cells from 7- to 10-day old rats for accommodation and the presence of a late afterhyperpolarization. In all cases (n = 7; input resistance 42 to 76 megohms; resting membrane potential 68 to 75 mV) both were observed (Fig. 1, E and F) and the magnitude of the late afterhyperpolarization was 3.6 ± 1.1 mV (SD).

Finally, an early transient outward rectification was apparent, especially when excitatory current was applied from membrane potentials negative to −70 mV (compare the delay to threshold in Fig. 1C to that in Fig. 1D, despite similar interspike intervals). Consistent with the existence of an A current (*7*), this rectification was antagonized by 4-aminopyridine (200 to 400 μ*M*) (n = 3) (*17*), and the number of action potentials generated by an excitatory current pulse was increased without affecting input resistance.

The electrophysiological responses of LTS neurons and of neurons without LTS may reflect recordings from two kinds of neurons. Another possibility, suggested by work in thalamus and inferior olive (*10*), is that the LTS response class may have been derived from somatic recordings and the response class without LTS from dendritic recordings. It is likely that the two response classes do not reflect different developmental stages since (i) there was no correlation between age and prevalence of either of the two classes and (ii) there was no age-associated gradation of the LTS. Also qualitative changes affecting the class of repetitive firing after differentiation and migration are complete have not been reported.

In conclusion, our findings demonstrate two classes of mPRF neuronal response to excitatory current input. In one class the presence of an inactivating, Ca^{2+}-dependent LTS allows either a burst or tonic mode of repetitive firing depending upon the membrane potential. The other class shows only a tonic mode, irrespective of the membrane potential. The tonic mode of both classes appears to be influenced by both voltage- and Ca^{2+}-sensitive outward currents but, in contrast to hippocampal pyramidal neurons,

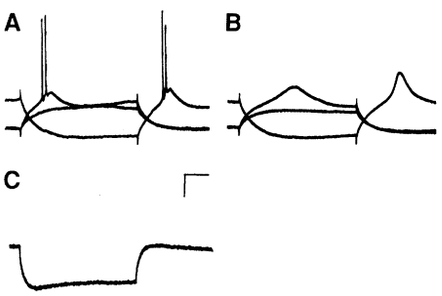

Fig. 3. The low threshold calcium spike in the absence of fast sodium currents. (A) consists of three voltage traces taken in normal perfusion media. Two depolarizing current pulses (400-msec duration) were applied from a baseline membrane potential of −78 mV. The +200-pA amplitude pulse was subthreshold and the +300-pA pulse was suprathreshold to evoke an LTS with two fast action potentials. At a baseline membrane potential of −61 mV, a hyperpolarizing pulse of 300-pA amplitude is followed by an LTS. (B), from the same neuron, is identical to (A) except that tetrodotoxin (0.3 μ*M*) was added to the perfusate to antagonize fast Na^+-dependent action potentials. When exposed to 10 m*M* Mg^{2+} and no Ca^{2+} (C), the LTS was abolished. Calibration bar is 20 mV and 80 msec.

there is no evidence for accommodation in association with a late afterhyperpolarization.

The electrophysiological properties responsible for these firing behaviors are likely to play integral roles in saccade generation and somatic motor acts since mPRF neurons have mono- and poly-synaptic input to neurons of the occulomotor system (*18*) and to lower motoneurons (*19*). For example, in the waking state, the LTS may allow quick induction of action potential firing after the state of relative membrane potential hyperpolarization frequently observed in waking (*20*). The presence of an LTS also suggests that mPRF may play a more complex role in information processing than that of a simple premotor relay. Finally, numerous lines of evidence have implicated mPRF neurons as important in generation of desynchronized sleep events, a time when both the membrane potential is tonically depolarized by 7 to 10 mV and when detailed action potential firing pattern analysis (*21*) indicates the absence of burst discharges. One mechanism for this behavioral state bias toward nonburst discharges may be a change of firing mode for LTS neurons.

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22. Supported by the Veterans Administration and NIMH grant MH 39,683. We thank B. Isenring and B. Rageth for their technical assistance and M. Fairbanks for her assistance in manuscript preparation.

4 June 1986; accepted 24 September 1986

A Pseudoautosomal Gene in Man

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The X/Y homologous gene *MIC2* was shown to exchange between the sex chromosomes, thus demonstrating that it is a pseudoautosomal gene in man. *MIC2* recombines with the sex-determining gene(s) *TDF* at a frequency of 2 to 3 percent. It is the most proximal pseudoautosomal locus thus far described and as such is an important marker for use in studies directed towards the isolation of *TDF*.

HALF A CENTURY AGO KOLLER AND Darlington observed partial meiotic pairing of sex chromosomes in male rats and proposed that recombination might occur between the morphologically distinct sex chromosomes (*1*). If genes are exchanged between the sex chromosomes they would not show classical sex linkage and the descriptive term "pseudoautosomal" was proposed for such loci (*2*). In the mouse, the abnormal *Sxr* locus is pseudoautosomal and the gene *STS* may also exchange between the X and Y chromosomes (*3*). In man, however, *Sts* is not a pseudoautosomal gene (*4*) and a search for pseudoautosomal disease loci proved unsuccessful (*5*). Recent molecular analysis of the human Y chromosome has shown that in man a pseudoautosomal region does exist (*6*). In this report we describe a human pseudoautosomal gene and present evidence that it is also the closest known marker distal to the sex-determining gene(s) (*TDF*) in man.

The monoclonal antibody 12E7 recognizes a cell surface antigen that is encoded by the *MIC2* gene (*7*). By means of somatic cell genetics, *MIC2* genes were found on both human sex chromosomes (*7*). Biochemical analysis suggested that the products of the two genes are very closely related or identical (*8*) and subsequent isolation and analysis of a complementary DNA (cDNA) clone, corresponding to an *MIC2* gene, pSG1, confirmed the close relationship between the X- and Y-located *MIC2* genes at the DNA level (*9*). By means of pSG1 as a probe for in situ hybridization, the *MIC2* genes were localized to the short arm of the Y chromosome and the tip of the short arm of the X chromosomes (*10*). This localization was consistent with *MIC2* being pseudoautosomal in nature (*2, 6*). Several different blocks of homologous sequences are, however, shared between the human sex chromosomes, in addition to those in the pseudoautosomal region (*11*) and formal identification of the *MIC2* locus as pseudoautosomal required demonstration of exchange between the X and Y chromosomes during male meiosis.

Like several other pseudoautosomal sequences that have been described (*6*), pSG1 detects multiple restriction fragment length polymorphisms (RFLP's). The 1.0-kb pSG1 sequence detects a number of apparently independent RFLP's in genomic DNA's that have been digested with Taq I, Msp I, Hind III, Pvu II, and Pst I. The pattern of hybridization observed in Taq I– (Fig. 1a), Hind III–, Pvu II– and Pst I–digested DNA's was very complex with several constant (C) and polymorphic (V) bands of varying intensity, while in Msp I digestions a simple two-allele system was revealed (Fig. 1b, a_1 and a_2). The details of the RFLP's detected by pSG1 will be described (*12*). The extensive variability detected at the *MIC2* locus by pSG1 should make the cloned sequence a highly informative marker for use in family studies; however, because of the complexity of the pattern of constant

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