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Research Note

Ca^{++} dependent bistability induced by serotonin in spinal motoneurons

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Summary. The plateau potential, responsible for the bistable state of spinal motoneurons, recently described in the decerebrate cat, was suggested to depend on serotonin (Hounsgaard et al. 1984). In an in vitro preparation of the spinal cord of the turtle we now show that serotonin, applied directly to the bath, transforms the intrinsic response properties of motoneurons, uncovering a plateau potential and voltage sensitive bistability. The changes induced by serotonin were blocked by Mn^{++}, while the plateau potential and the bistability remained after application of tetrodotoxin. We conclude that serotonin controls the expression of a Ca^{++} dependent plateau potential in motoneurons.

Key words: Spinal cord – Motoneurons – Bistable state – Serotonin

Introduction

The translation of synaptic potentials to axonal firing in motoneurons is the final processing of motor behaviour in the vertebrate central nervous system. Recent experiments on spinal motoneurons in the unanesthetized, decerebrate cat showed this translation to be more complex than previously assumed (Hounsgaard et al. 1984). In particular, it was demonstrated that short bursts of synaptic potentials could shift motoneurons between two stable states of activity due to initiation and termination of a plateau potential. The electroresponsive properties responsible for the bistability of motoneurons were suggested to be serotonin dependent. The serotonergic innervation of spinal motoneurons in a wide selection of vertebrates (Kojima and Sano 1983) indicated that the control of intrinsic membrane properties by this neurotransmitter might be a general mechanism in motoneurons. The present experiments on motoneurons in the isolated spinal cord of the turtle support this conjecture and extends the analysis of serotonin dependent response properties by exploiting the advantages of an in vitro preparation.

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Methods

Transverse slices (1–2 mm thick) dissected from the lumbar and cervical enlargement of the turtle spinal cord were placed on the bottom of a lucid chamber and superfused with standard solution (NaCl 120 mM, KCl 5 mM, $NaHCO_3$ 30 mM, $MgCl_2$ 2 mM, $CaCl_2$ 3 mM, glucose 10 mM) at a rate of 1 ml per minute. All experiments were performed at room temperature (20–22° C). In some experiments the solution was modified by replacing $CaCl_2$ with $MnCl_2$, by addition of serotonin (10^{-4} M) and tetrodotoxin (10^{-6} M) to the reservoire. Intracellular recording from neurons in the ventral horn was performed with glass electrodes filled with potassium acetate. The electrode resistance ranged from 30 to 80 MΩ. Motoneurons were identified by antidromic invasion following stimulation of the ipsilateral ventral root with a suction electrode and by their low input resistance and characteristic response pattern to injected depolarizing current. Data was stored on magnetic tape for later analysis and display.

Results

The marked effect of serotonin on the response properties of turtle motoneurons is illustrated by the records in Fig. 1. In normal medium the rhythmic firing, induced by a depolarizing current pulse, adapted from a high firing frequency at the onset to a lower steady state level (Fig. 1A). Turtle motoneurons share these properties with motoneurons in the frog spinal cord in vitro (Barrett and Barrett 1976) and in the anesthetized cat (Kernell 1965; Kernell and Monster 1982). This response pattern

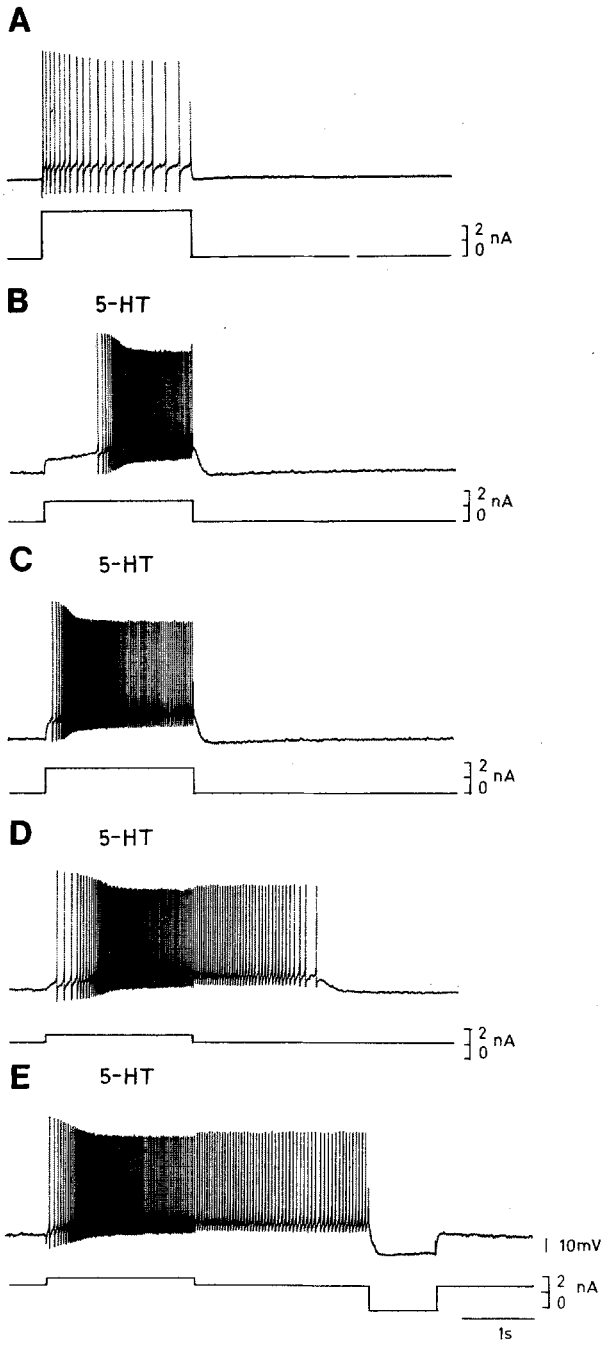

Fig. 1A–E. Serotonin induced changes in response properties of turtle motoneurons. **A** Adaptation of rhythmic firing in normal medium. **B and C** Accelerated firing and plateau potential evoked from resting membrane potential in serotonin containing medium. Note low threshold and the late onset of firing in **B** and the shift in onset in **C** in response to a current pulse of higher amplitude. **D** Acceleration and longer lasting plateau potential exceeding the firing level evoked from depolarized membrane potential in the presence of serotonin. **E** Illustrates the sustained firing induced by a depolarizing current pulse and terminated by hyperpolarizing current pulse at a membrane potential slightly depolarized from the level in **D.** All records from the same cell. **A–C** 0 nA bias current. **D** 1.2 nA bias current. **E** 1.4 nA bias current

changed in the presence of serotonin. From the resting membrane potential a depolarizing current pulse now induced a train of action potentials, accelerating from a low initial firing frequency to a higher steady state level (Fig. 1B and C). Termination of the pulse was followed by a brief plateau potential.

Serotonin also reduced the threshold and at low stimulus intensity (Fig. 1B) spike initiation was preceded by a considerable delay during which a gradual depolarization was observed. At higher stimulus intensity there was a progressively earlier onset of firing (Fig. 1C). The duration of the plateau potential following a suprathreshold depolarizing current pulse depended on the background level of the membrane potential. From a more depolarized membrane potential, maintained by a persistent subthreshold current, a superimposed depolarizing current pulse generated a plateau potential of longer duration, exceeding the firing level of the motoneuron (Fig. 1D). At still higher levels of depolarization brief depolarizing and hyperpolarizing current pulses shifted the level of motoneuronal activity between two stable states (Fig. 1E). These results showed that serotonin induced a voltage dependent, bistable state in turtle motoneurons.

The in vitro conditions allowed the ionic mechanism of the changes induced by serotonin to be explored in some detail. Tetrodotoxin, added to serotonin containing medium eliminated the generation of action potentials, but left a plateau potential (Fig. 2B). Compared with a control sweep prior to addition of tetrodotoxin (Fig. 2A) the duration of this plateau potential corresponded to the phase of accelerated firing during and after the pulse. The onset and duration of the tetrodotoxin insensitive plateau depended on the membrane potential from which it was generated. At membrane potentials increasingly depolarized from rest, by steady bias currents, the plateau potential evoked by a superimposed depolarizing current pulse had an increasingly earlier onset and longer duration. Finally, from a membrane potential 10–20 mV depolarized from rest, brief depolarizing and hyperpolarizing current pulses initiated and terminated the plateau and thus shifted the membrane potential between two stable states (Fig. 2C). This bistable state was Ca^{++} dependent since the plateau potential was abolished when Ca^{++} was replaced with Mn^{++} in the medium (Fig. 2D). These experiments indicated that a Ca^{++} conductance was involved in the changes induced by serotonin.

This was supported by experiments in which Ca^{++} was replaced by Mn^{++} in serotonin containing

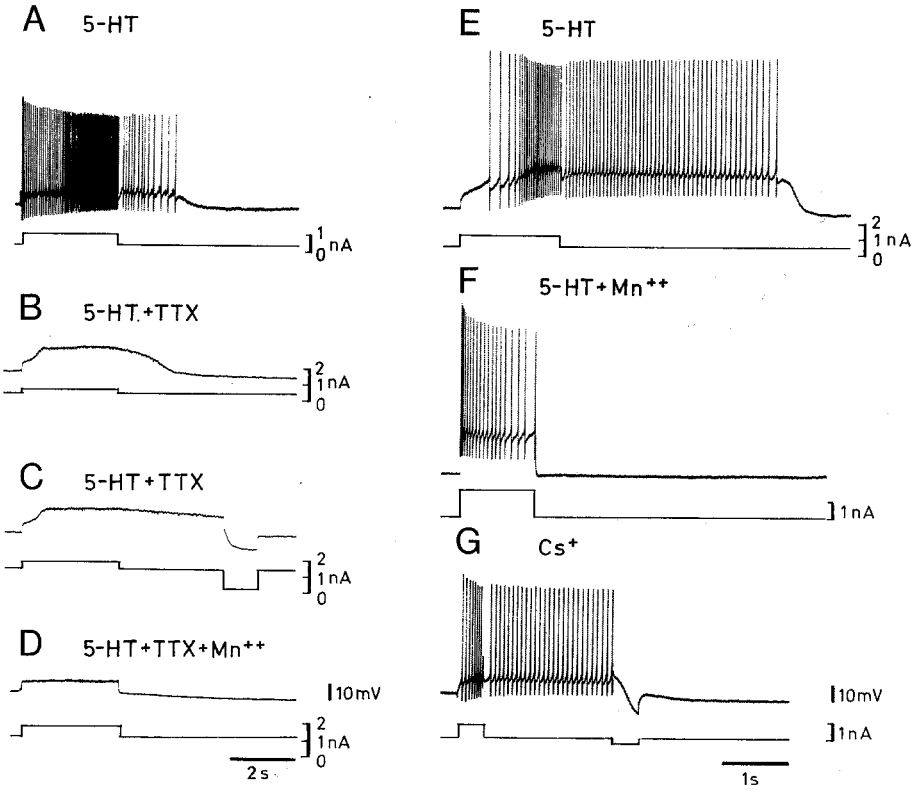

Fig. 2A–G. Ionic mechanism of serotonin induced changes in response properties of turtle motoneurons. **A** Control sweep in serotonin containing medium. **B** Tetrodotoxin resistant plateau potential in the presence of serotonin. **C** Sustained plateau potential initiated by depolarizing current pulse and terminated by hyperpolarizing current pulse from a membrane potential depolarized relative to the level in **B**. **D** Response to depolarizing current pulse in the presence of Mn^{++}, tetrodotoxin and serotonin. **E** Accelerated firing and prolonged plateau potential generated by depolarizing current pulse, serotonin containing medium. **F** Adaptation of firing frequency during depolarization, serotonin and Mn^{++} containing medium. Note the similarity with the response in normal medium in Fig. 1A. **G** Bistable state induced by Cs^{+}. **A–D, E–F** and **G** from three different cells

medium without tetrodotoxin. As illustrated in Fig. 2E and F all serotonin induced changes in the response properties of turtle motoneurons were abolished by Mn^{++}.

Discussion

The view that the functional state of vertebrate motoneurons may be controlled by serotonergic synaptic activity is strengthened by the present experiments. In the cat and the turtle the response properties of motoneurons in the bistable state are closely similar despite the phylogenetic distance. The dependence on serotonin for the bistable behaviour of motoneurons in the decerebrate cat rested on indirect evidence; the bistable behaviour was abolished after interrupting the descending pathways by spinalization, but could be restored by intravenous injection of the serotonin-precursor, 5-hydroxytryptophan (Hounsgaard et al. 1984). Our findings here show that the response properties, that characterize bistable motoneurons in the cat, are induced in turtle motoneurons in vitro by direct exposure to serotonin. A plateau potential appears to explain all the changes in the behaviour of motoneurons induced by serotonin in both species. Both Ca^{++} and Na^{+} conductances are known to mediate plateau potentials in vertebrate neurons (Llinás and Sugimori 1980a, b). The experiments presented here show that the plateau potential in turtle motoneurons is predominantly mediated by a Ca^{++} conductance.

While the permissive effect of serotonin for plateau potentials in motoneurons is clear, the mechanism is not. In addition to the possibility that serotonin affects Ca^{++} conductance directly, other mechanisms must be considered. Plateau potentials conceivably imply a balance between inward and outward currents at a depolarized voltage level relative to the resting membrane potential. In Purkinje cells maintained plateau potentials can be induced not only by the increased inward currents in the presence of Ba^{++} but also by reducing K^{+} conductance with TEA (Llinás and Sugimori 1980a). In line with this, a reduction of K^{+} conductance mimic the effect of serotonin on motoneurons. The acceleration of firing and the bistability is readily observed in presence of 2 mM CsCl in normal medium (Fig. 2G). Tetra-ethylamonium chloride has a similar effect (unpublished observation, see also Werman et al. 1982; Schwindt and Crill 1980b). It is therefore of interest that serotonin actually reduce a K^{+} conductance in some invertebrate neurons (Deterre et al. 1981, Klein and Kandel 1980). In vertebrates a similar reduction of K^{+} conductance produced by serotonin has been suggested for facial

motoneurons (VanderMaelen and Aghajanian 1980) and myenteric neurons (Johnson, Katayama and North 1980). Finally, plateau potentials have been recorded in motoneurons after reduction of Cl^- conductance (Katz and Miledi 1963; Schwindt and Crill 1980a). Experiments to determine the mode of action of serotonin are currently under way.

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