Neuroscience 137 (2006) 659–670

FICTIVE LOCOMOTOR PATTERNS GENERATED BY TETRAETHYLAMMONIUM APPLICATION TO THE NEONATAL RAT SPINAL CORD *IN VITRO*

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Abstract—Intrinsic spinal networks generate a locomotor rhythm characterized by alternating electrical discharges from flexor and extensor motor pools. Because this process is preserved in the rat isolated spinal cord, this preparation *in vitro* may be a useful model to explore methods to reactivate locomotor networks damaged by spinal injury. The present electrophysiological investigation examined whether the broad spectrum potassium channel blocker tetraethylammonium could generate locomotor-like patterns. Low (50–500 μM) concentrations of tetraethylammonium induced irregular, synchronous discharges incompatible with locomotion. Higher concentrations (1–10 mM) evoked alternating discharges between flexor and extensor motor pools, plus large depolarization of motoneurons with spike broadening. The alternating discharges were superimposed on slow, shallow waves of synchronous depolarization. Rhythmic alternating patterns were suppressed by blockers of glutamate, $GABA_A$ and glycine receptors, disclosing a background of depolarizing bursts inhibited by antagonism of group I metabotropic glutamate receptors. Furthermore, tetraethylammonium also evoked irregular discharges on dorsal roots. Rhythmic alternating patterns elicited by tetraethylammonium on ventral roots were relatively stereotypic, had limited synergy with fictive locomotion induced by dorsal root stimuli, and were not accelerated by 4-aminopyridine. Horizontal section of the spinal cord preserved irregular ventral root discharges and dorsal root discharges, demonstrating that the action of tetraethylammonium on spinal networks was fundamentally different from that of 4-aminopyridine. These results show that a potassium channel blocker such as tetraethylammonium could activate fictive locomotion in the rat isolated spinal cord, although the pattern quality lacked certain features like frequency modulation and strong synergy with other inputs to locomotor networks. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: motoneuron, burst, central pattern generator, spinal network, 4-aminopyridine.

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Abbreviations: ANOVA, analysis of variance; APV, D-amino-phosphonovalerate; CCF, correlation coefficient function; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CPCCOEt, 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester; CPG, central pattern generator; CV, coefficient of variation; DR, dorsal root; l, left; NMDA, *N*-methyl-D-aspartate; r, right; S.D., standard deviation; TEA, tetraethylammonium; Th, threshold; TTX, tetrodotoxin; VR, ventral roots; 4-AP, 4-aminopyridine; 5-HT, serotonin; Φ, mean phase.

0306-4522/06$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2005.09.025

Recent approaches to neurorehabilitation after spinal cord injury have proposed to exploit accessory spinal networks to perform the generation of locomotor programs damaged by lesions to interneurons (collectively termed central pattern generator, CPG) responsible for initiating and maintaining locomotion (Barbeau and Fung, 2001; Grasso et al., 2004; Parker, 2005). Emerging concepts in the field of spinal plasticity applied to locomotion suggest that propriospinal circuits accessory to the CPG may be activated by pharmacological modulators: hence, appropriate training and coincidence of afferent signals and pharmacological agents can produce a concerted interaction to re-activate, at least in part, the lost locomotor function (Edgerton et al., 2004).

Within this framework, a few studies have been focused on the use of the potassium channel blocker 4-aminopyridine (4-AP) that has been suggested to improve the outcome of rehabilitation programs (Grijalva et al., 2003). Initially (Hansebout et al., 1993) it was supposed that 4-AP could mainly act by blocking potassium channels of axons unmasked by a demyelinating lesion, thus restoring conduction of action potentials. Recent studies have indicated that low concentrations of 4-AP facilitate the operation of the locomotor CPG, though 4-AP alone cannot activate the locomotor program (Taccola and Nistri, 2004). These observations actually raised the question whether other potassium channel blockers could mimic the action of 4-AP or even improve upon it by direct stimulation of the CPG activity.

Former studies have shown that tetraethylammonium (TEA), a broad spectrum potassium channel blocker, could unmask bursting by spinal motoneurons and interneurons by promoting the activation of various depolarizing conductances and by augmenting excitatory synaptic inputs (Schwindt and Crill, 1980; Takahashi, 1990). These results suggested to us that TEA could be an interesting agent to investigate its ability to induced locomotor-like patterns. A previous report has suggested that low concentrations of TEA can speed up the cyclic activity of the locomotor CPG activated by NMDA and serotonin (5-HT; Cazalets et al., 1999). In the present study we examined how a wide range of TEA concentrations could affect the ability of spinal networks to evoke rhythmic discharges, whether these had the characteristics of fictive locomotion, the contribution of motoneurons to this activity and the locus of its origin within the spinal cord *in vitro*.

EXPERIMENTAL PROCEDURES

All methods have been recently described (Taccola and Nistri, 2004, 2005). In brief, spinal cords were removed from neonatal

Wistar rats (0–5 days old) under urethane anesthesia (0.2 ml i.p. of a 10% w/v solution) and isolated from mid-thoracic level to cauda equina. The experiments were run in accordance with the National Institutes of Health guidelines and the Italian act Decreto Legislativo 27/1/92 n. 116 (implementing the European Community directives n. 86/609 and 93/88): all efforts were made to reduce the number of animals used and to minimize animal suffering.

Cords were superfused (6.5 ml min^{-1}) with oxygenated Krebs solution of the following composition (in mM): NaCl 113, KCl 4.5, $MgCl_2$ 1, $CaCl_2$ 2, NaH_2PO_4 1, $NaHCO_3$ 25, glucose 11, pH 7.4, at room temperature. Drugs were applied via the Krebs solution. Horizontal lesions to separate dorsal from ventral parts of the spinal cord were performed using a surgical razor blade as indicated by Bracci et al. (1996).

Ventral and dorsal root (VR and DR, respectively) activity was recorded by means of suction electrodes, and stored on digital cassette. Fictive locomotor patterns were obtained from electrical recordings of the activity from left (l) and right (r) L2 VRs (mostly flexor motor-pool commands to the hindlimbs) and l and r L5 VRs (mostly extensor motor-pool commands to the hindlimbs) (Kiehn and Kjaerulff, 1998). A typical indicator of fictive locomotion (see Butt et al., 2002) is the double alternation between homosegmental l and r VRs, and between L2/L5 on the same side.

Single or trains of electrical stimuli (0.1 ms duration) were delivered to one DR via a bipolar miniature suction electrode. Intensity of stimulation was referred in terms of VR response threshold (Th).

For intracellular recordings, antidromically identified lumbar (L4 or L5) motoneurons (Fulton and Walton, 1986) were impaled with 3-M-KCl-filled microelectrodes under current-clamp conditions. The input resistance of motoneurons was obtained by delivering hyperpolarizing current steps (0.05–0.55 nA). Current/voltage plots were linear within the voltage range recorded.

The analysis of rhythms was carried out as indicated by Taccola and Nistri (2004, 2005). The discharge period (T) was measured as the time between the beginning of two subsequent oscillations (calculated as average of 20 responses). The coefficient of variation of the period (CV=standard deviation (S.D.)×$mean^{-1}$) was used to quantify the regularity of bursting. Signal correlation between pairs of roots was expressed as the correlation coefficient function (CCF) using pCLAMP 9.2 software (Molecular Devices, Union City, CA, USA). While CCF $>+0.5$ indicates synchronicity between two roots, CCF <-0.5 shows alternation. As for the analysis of fictive locomotor-like rhythm induced by 10 mM TEA on VRs, 10 min traces were analyzed by fast Fourier transform that gave two different mean frequencies, corresponding to synchronous and alternated rhythm. All data were expressed as mean±S.D., with "*n*" as the number of experiments. Rayleigh test and circular statistic were used to represent phase coupling between roots (Drew and Doucet, 1991) as previously reported (Taccola and Nistri, 2004). The length of vectors in the polar plots indicates the strength of signal coupling, while the direction shows the concentration of phase values around the mean phase (Marchetti et al., 2001). The Φ value, expressed in angular degrees, corresponds to the time span from the onset of a cycle in one root to the onset of the corresponding cycle on the other root, divided by the period. A $\Phi=180°$ means that the two cycles are completely alternated, while a Φ value of 0° or 360° describes a fully coincident phase (Kjaerulff and Kiehn, 1996).

After performing a normality test to distinguish between parametric or non-parametric data, different statistical approaches

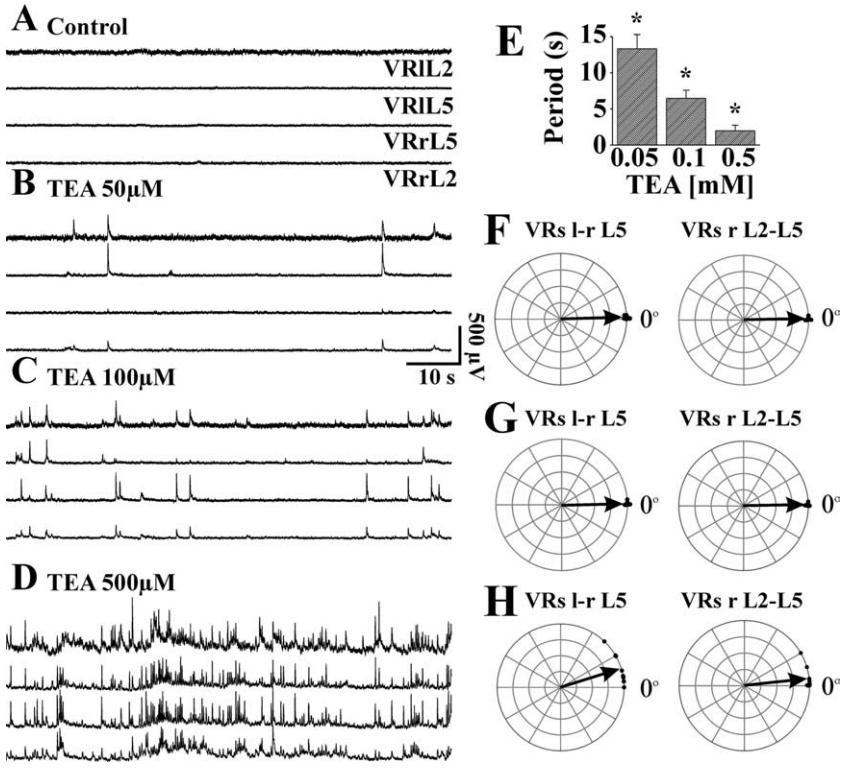

Fig. 1. Synchronous oscillations induced by 50–500 μM TEA. (A) Control records taken from four VRs (identified by their abbreviations at the end of the corresponding trace); note lack of activity under resting conditions. (B) Synchronous discharges of short duration and irregular period appear on all VRs. (C) TEA at 100 μM increases synchronous discharges. (D) Intense oscillatory activity observed in the presence of 500 μM TEA. A–D are from the same preparation. Scale bars apply to A–D records. (E) Histograms indicating period values for three concentrations of TEA, $n=6$. Asterisks=$P<0.05$. (F–H) Polar plots of VR oscillations shown in B, C and D. Clustering of data points around 0° to indicate synchronicity. Length of signal vector indicates strength of phase coupling between VRs.

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