PRIMARY AFFERENT DEPOLARIZATION INDUCED BY γ-AMINOBUTYRIC ACID INJECTED INTO THE CENTRAL CANAL OF THE CAT SPINAL CORD

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UDC 612.831.014.46:615.31.547.466.3

KEY WORDS: spinal cord; perfusion of central canal; GABA; presynaptic inhibition.

Presynaptic inhibition of spinal reflexes is mediated by γ-aminobutyric acid (GABA), which is liberated from axon terminals of special interneurons and acts on primary afferent terminals, weakening their excitatory synaptic action [3, 5, 6]. It has been suggested that the action of GABA is accompanied by membrane depolarization of the afferent terminals which, spreading electrotonically, causes the appearance of slow negative potentials in the dorsal roots [3, 5, 6]. Various technical difficulties are encountered in the study of mechanisms of presynaptic action of GABA in the mammalian spinal cord. Application of GABA to primary afferent endings is complicated by the fact that GABA passes only with difficulty through the blood–brain barrier [5]. It has not yet proved possible to use the technique of microiontophoretic application of GABA in conjunction with simultaneous stable derivation of the membrane potential of the afferent fiber by means of an intracellular microelectrode. In our own investigations of the action of GABA on primary afferent fibers we attempted to "bypass" the blood–brain barrier by injecting GABA into the central canal of the spinal cord. Changes in potential of afferent fibers arising under these circumstances were then monitored by recording the dorsal root potentials (DRPs).

EXPERIMENTAL METHOD

Experiments were carried out on cats weighing 2.0-4.0 kg anesthetized with chloralose (70-90 mg/kg intravenously). The spinal cord was divided at the level of segment L_2. On the left side the ventral roots of segments L_6-L_2 and a bundle of fibers (filaments) of the dorsal root L_6 were divided distally. Cutaneous and muscular branches of the sciatic nerve were isolated and divided in the ipsilateral hind limb. The portion of spinal cord caudally to the level of transection was perfused continuously through the central canal with artificial CSF. The technique of perfusion was described by the writers previously [1]. The standard CSF had the following composition (in mM): NaCl 138, KCl 3.3, $CaCl_2$ 1.3, $MgCl_2$ 1.2, $NaHCO_3$ 24.5, NaH_2PO_4 0.58, $(NH_4)_2CO_3$ 2.0; glucose 0.6 g/liter. In some experiments calcium-free CSF also was used, with the $MgCl_2$ concentration increased to 20 mM. The normal osmolarity of the CSF was preserved by a corresponding reduction in the NaCl concentration. GABA was applied by perfusing the brain with CSF containing GABA (GABA-CSF), in one of the following concentrations: 5, 10, or 50 mM. The perfusion time with GABA-CSF was 2 min. The central canal was then perfused with standard CSF again. The change of perfusing fluid was carried out by means of a T-shaped cock (Fig. 1). Afferent nerves of the knee flexors, posterior biceps, and semitendinosus (PBST) or of the ankle flexor m. peroneus profundus (PP) were stimulated by square pulses of current 0.1 sec in duration. Reflex electrical discharges were derived from ventral roots of segments L_7 or S_1 by means of platinum wire electrodes. Potentials were derived from filaments of dorsal root L_6 by means of nonpolarizing Ag–AgCl electrodes, connected to a dc amplifier, using the sucrose gap technique (Fig. 1). Details of the design of the sucrose gap, developed to record DRPs of the spinal cord *in situ* will be described in a separate communication.

EXPERIMENTAL RESULTS

The effect of GABA on DRP was studied in 24 experiments. In 10 experiments perfusion of the central canal with CSF containing GABA in a concentration of 5-50 mM had no effect. In

Department of Pathological Physiology, N. I. Pirogov Second Moscow Medical Institute.
(Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 92, No. 12, pp. 703-705, December, 1981. Original article submitted July 15, 1981.

 0007-4888/81/9212-1678$07.50

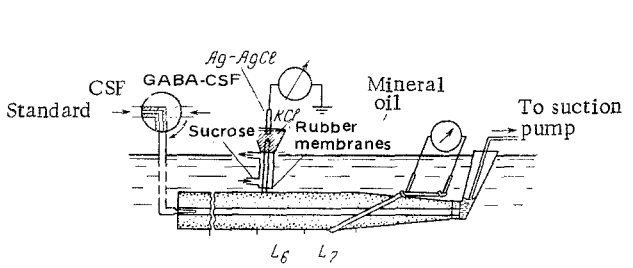

Fig. 1

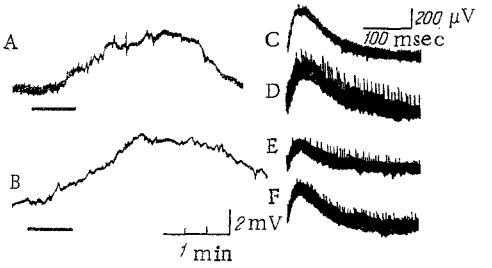

Fig. 2

Fig. 1. Scheme of perfusion of central canal of lumbar spinal cord with simultaneous recording of DRPs by sucrose gap method.

Fig. 2. Action of GABA on DRPs of spinal cord. A) Depolarization of dorsal root induced by injection of GABA (50 mM) into flow of standard CSF perfusing spinal cord; B) injection of GABA in same concentration into flow of Mg-CSF; C-F) DRPs of L_6 evoked by stimulation of PBST by series of four pulses (250 Hz) of maximal strength for group I afferents; C) before injection of GABA; D) 1.5 min after beginning of perfusion with GABA (50 mM); E, F) 5 and 30 min respectively after end of perfusion with GABA. Places in A and B obtained during repeated stimulation of PBST nerves with volleys of four pulses (250 Hz) of maximal strength for group I fibers every 5 sec. Amplitude of DRPs evoked by stimulation of CST was reduced by GABA (A). Perfusion with Mg-CSF completely blocked DRPs (B).

14 experiments the use of GABA-CSF was accompanied by definite primary afferent depolarization (PAD). The threshold dose of GABA for PAD was 5 mM (two experiments), 10 mM (eight experiments), and 50 mM (four experiments). In all cases when two or three of the different concentrations of GABA used were effective, the amplitude of PAD increased with an increase in GABA concentration. During perfusion of the spinal cord for 2 min with CSF containing GABA in a concentration of 50 mM, PAD was found 30-45 sec after introduction of GABA into the central canal. Depolarization reached a maximum 1-2 min after the end of GABA perfusion and gradually diminished during the next 6-15 min. The maximal amplitude of PAD to GABA in a dose of 50 mM fluctuated in different experiments from 3 to 6 mV. A characteristic example of PAD to GABA is shown in Fig. 2A. PAD induced by GABA was usually accompanied by the appearance of a long burst of spike activity in the dorsal roots (Fig. 2D). Parallel with development of PAD the amplitude of the electrotonic negative DRPs arising in response to stimulation of nerves PBST or PP decreased (Fig. 2E). Bicuculline, a competitive antagonist of GABA if injected intravenously in a dose causing seizure spike discharges in the ventral roots of the spinal cord (2 mg/kg), appreciably reduced the amplitude of DRPs. There was a parallel decrease in the amplitude of PAD induced by intraspinal injections of GABA.

The depolarizing action of GABA could be either the result of the direct effect of GABA on the membrane of primary afferent endings or the results of a change in activity of the interneurons forming synaptic contacts with primary afferent fibers. To determine which of these mechanisms plays a role in PAD production, the action of GABA was investigated when synaptic transmission in the spinal cord was blocked by magnesium ions. Blocking chemical synapses by magnesium ions is widely used in experiments on surviving preparations of the vertebrate CNS *in vitro*: on the isolated spinal cord of frogs [4], newborn rats [2], etc. In the present experiments CSF with Mg^{++} ion concentration increased to 20 mM (Mg-CSF) was used to inhibit synaptic transmission. Complete reversible blockade of DRPs induced by peripheral nerve stimulation took place after continuous perfusion of the central canal with Mg-CSF for 20-30 min. Addition of GABA in a concentration of 50 mM to the Mg-CSF evoked PAD of similar amplitude and time course to the PAD induced by addition of GABA in the same concentration to standard CSF (Fig. 2B).

The results show that GABA, if injected into the central canal of the cat spinal cord, gives rise to dose-dependent primary afferent depolarization. The depolarizing action of GABA is largely depressed by bicuculline and, consequently, it is brought about at least partially through the participation of bicuculline-sensitive GABA receptors. The method of

long-term perfusion of the central canal of the spinal cord, as elaborated by the present writers, enabled the effects of GABA to be studied when synaptic production of PAD was blocked by Mg^{++} ions. Preservation of the depolarizing action of GABA under those conditions must be regarded as a result of the direct effect of GABA on the primary afferent membrane. These data confirmed the role of GABA as mediator of presynaptic inhibition. The results are also of special interest because they show that the method of perfusion of the central canal of the spinal cord *in situ* can be used to study the action of neuromediators on intraspinal primary afferent endings in adult cats.

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