NEW EVIDENCE FOR DEPRESSION OF DEPOLARIZATION OF PRIMARY AFFERENTS BY AMMONIUM IONS

Yu. S. Sverdlov, T. Yu. Ruchinskaya, and G. A. Erzina

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In experiments on cats, perfusion of the central canal of the lumbar segments of the spinal cord with artificial CSF with the addition of ammonium ions – depressed slow negative electrotonic dorsal root potentials (DRP). Depression of DRP developed parallel with depression of postsynaptic inhibition of monosynaptic reflexes, but it was not connected with depression of mono- or polysynaptic reflex discharges. Subsequent perfusion of the central canal in normal CSF led to full recovery both of DRP and of inhibition of the test reflexes. It is suggested that depression of DRP by ammonium ions could be the result of blockade of the chloride pump, acting in afferent terminals and creating an emf for the outward transmembrane chloride current, producing depolarization of afferent fibers.

KEY WORDS: spinal cord; ammonium ions; perfusion of the central canal.

In experiments on cats, ammonium acetate, injected intravenously in convulsant doses, depressed mechanisms of primary afferent depolarization (PAD) [1, 2]. Depression of PAD took place parallel with depression of postsynaptic inhibition of monosynaptic reflexes and was explained by blockade of the transmembrane chloride pump. However, interpretation of the results was made difficult by the fact that injection of convulsant doses of ammonium was accompanied by changes in arterial blood pressure and, in some cases, by cardiac arrest.

In the present experiments the action of ammonium ions on the spinal centers was accordingly studied by a method of perfusion of the central canal of the spinal cord, so that complications connected with systemic administration of ammonium salts could be avoided.

EXPERIMENTAL METHOD

Experiments were carried out on cats (2-4 kg) anesthetized with chloralose (80-100 mg/kg, intravenously). Cutaneous and muscular branches of the sciatic nerve to the left hind limb were isolated and divided distally. After laminectomy on vertebrae T12-S2 the spinal cord was divided at the level of segment L1. The ventral roots of all segments caudally to L5 were ligated distally and divided below the ligatures. The caudal end of the spinal cord was isolated for a distance of 3-6 mm and divided where its diameter was about 1 mm. After secure fixation of the animal, the surface of the cord was covered with a thick layer of mineral oil at a temperature of 36-38°C and the central canal of the spinal cord was perfused with artificial CSF. The general scheme of the method of perfusion (Fig. 1) was taken from Morton et al., [9]. By means of a micromanipulator, an injection needle 0.5 mm in diameter was inserted into segments L2 or L3 in the median plane at an angle of 60-80° to the horizontal (Fig. 1A). The needle was connected through a three-way tube to glass reservoirs (Fig. 1B, C) from which the perfusion solutions gravitated into the spinal cord. The fluid level in the reservoirs was 10-15 cm above the level of the spinal cord. The depth of insertion of the needle varied between 2 and 3 mm. Insertion of the needle ceased when CSF began to flow out of the divided caudal end of the spinal cord. To collect the escaping CSF, the authors of the technique in [9] recommended division of the spinal cord at level S1 and insertion of a cannula about 1 mm in diameter into the central canal. In the present experiments a special curved polyethylene cannula (Fig. 1D), into which the caudal end of the spinal cord was drawn by means of a syringe, was used to collect the CSF. In the present writers' view, this last method of collection of CSF is less traumatic and is easier to carry out. CSF accumulating in the cannula was drawn off continuously by means of a water-jet pump. The central canal was perfused with artificial CSF of "normal" com-

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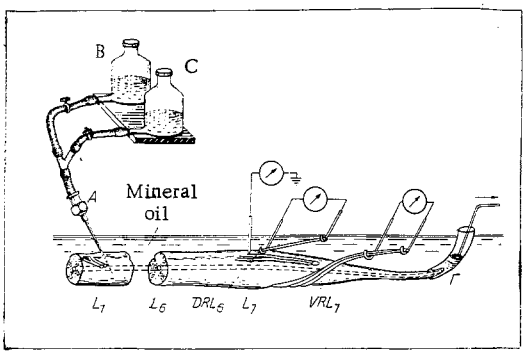

Fig. 1

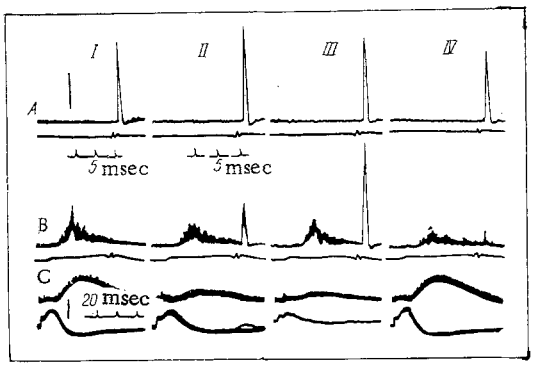

Fig. 2

Fig. 1. Scheme of perfusion of central canal of spinal cord. Explanation in text.

Fig. 2. Action of ammonium ions on spinal cord potentials. A) Monosynaptic reflex discharges in ventral root L7 (above) evoked by stimulation of nerve to gastrocnemius by single electrical stimuli of supermaximal strength for group I fibers; B) effect of preceding afferent volleys evoked by application of single stimuli of nine times threshold strength to the sural nerve on the same discharges; C) DRP of L6 (above) and DCP (below) evoked by application of single stimuli of nine times threshold strength to sural nerve. I) All traces obtained 50-55 min after beginning of spinal cord perfusion with normal CSF; II) 20-25 min later, III) 30-35 min after perfusion of central canal with CSF in which NaCl replaced by NH_4Cl; IV) 60 min after beginning of perfusion of central canal of spinal cord with normal CSF. Calibration: for ventral root potentials in III, A, B 600 μV, elsewhere 400 μV, for DRP 200 μV, for DCP 600 μV. Each frame represents 5-8 sweeps of beam.

position [5] and with CSF containing ammonium ions. The composition of the normal CSF was (in g/liter) NaCl 8.1, KCl 0.25, $CaCl_2$ 0.14, $MgCl_2$ 0.11, $NaHCO_3$ 1.76, NaH_2PO_4 0.07, urea 0.13, glucose 0.61; pH 7.4. To prepare the "ammonium" CSF, in some cases the NaCl in normal CSF was replaced by an equimolar concentration of NH_4Cl, and in other cases NH_4Cl or ammonium acetate was added to normal CSF in concentrations of 5 to 15 g/liter. The CSF entering the spinal cord was warmed as it passed through the needle, which was immersed in warm mineral oil. The rate of perfusion was 0.1-0.2 ml/min.

The afferent nerves of the hind limb were stimulated with square pulses 0.1 msec in duration. Reflex electrical discharges were recorded from ventral root L7 and electrotonic potentials were recorded from a bundle of fibers isolated from the dorsal root L6. The dorsal cord potentials (DCP) were recorded by a needle electrode at the level L6 (Fig. 1). Amplifiers with a time constant of 0.3 sec and a dual-beam oscilloscope with camera attachment were used to record potentials.

EXPERIMENTAL RESULTS

The traces illustrated in Fig. 2A and B illustrate the action of ammonium ions on inhibition of monosynaptic reflex discharges of motoneurons of the extensor muscles of the ankle (gastrocnemius) caused by volleys of afferent impulses in fibers of the cutaneous sural nerve. This inhibition is a well known example of postsynaptic inhibition. Volleys of impulses in the sural nerve 50-55 min after the beginning of perfusion of the central canal with normal CSF caused definite inhibition (a decrease in amplitude) of the test responses. Replacement of the normal CSF by CSF containing an equimolar concentration of NH_4Cl instead of NaCl led to gradual depression of this inhibition, which was completely restored again 60 min after the beginning of perfusion of the central canal with normal CSF. The traces in Fig. 2C illustrate the action of ammonium ions on depolarization of central endings of primary afferent fibers evoked by volleys in the sural nerve. Parallel with depression of postsynaptic inhibition the amplitude both of the slow negative electrotonic dorsal root potential (DRP) and of the positive wave of the DCP, which is known to reflect the electric field potential arising during depolarization of primary afferent endings, was reduced.

In the writers' previous investigations [1, 2] intravenous injection of ammonium ions in effective doses was accompanied with a marked increase or, sometimes, complete suppression of monosynaptic reflex discharges, which suggested nonspecific blockade of PAD by ammonium ions. By contrast with this, in the present experiments depression of postsynaptic inhibition and PAD evoked by injection of ammonium ions to the central canal of the spinal cord was accompanied by a marked increase (by 30-50%) in the amplitude of mono-

synaptic reflex responses (compare the calibration markers for ventral root potentials in Fig. 2, I, A and C). As a rule, besides monosynaptic, polysynaptic discharges also were increased.

Intravenous injection of convulsant doses of ammonium salts, as was done in all previous investigations aimed at studying the action of ammonium on central neurons, led to cardiovascular distrubances, the most serious of which was cardiac arrest. The method of perfusion of the central canal, which was used in the present case, enabled disturbances of the systemic circulation to be avoided.

The results of the experiments with perfusion of the central canal thus confirm the earlier conclusion [1, 2] that "ammonium" blockade of PAD is due to the specific action of ammonium ions on spinal neurons.

Selective depression of PAD by ammonium ions is of definite interest in the discussion of ionic mechanism of PAD. There is evidence that the onset of PAD is due to increased membrane permeability of afferent terminals for chloride ions, as a result of which these ions rush from the inside to the outside of the fibers, thus creating an electric current which depolarizes the presynaptic membranes [3, 4, 6]. It is thus logical to suggest the presence of a transmembrane gradient of chloride ions from inside to outside, unbalanced by the membrane potential, which is maintained through the constant activity of a transmembrane chloride pump in the direction from outside to inside. Accordingly special importance attaches to the fact that depression of postsynaptic inhibition of central neurons by ammonium salts is due to blockade of the active transmembrane transport of chloride ions (admittedly in the direction from inside outward), creating an emf for the chloride current producing the IPSP [7, 8, 10]. The assumption that a chloride pump, similar in its mechanism to the chloride pump in motoneurons but acting in the opposite direction, exists in afferent terminals readily explains both the sensitivity of PAD to ammonium ions and the parallel observed between the decline and recovery of postsynaptic inhibition of monosynaptic reflexes and of DAP processes after administration of ammonium ions.

The reversibility of changes in spinal potentials following the addition of ammonium ions to the CSF is evidence that perfusion of the central canal with artificial CSF itself causes no serious functional distrubances, at least within a limited period of time. This conclusion is also confirmed by the results of special preliminary experiments, in which continuous perfusion of the central canal with artificial CSF for 3-4 h caused no visible changes in reflex electrical discharges in the ventral roots of the perfused segments. There is no doubt that the method of perfusion of the central canal can be used successfully to study the action of the spinal cord of agents which, if administered systemically, caused undesirable cardiovascular and other distrubances.

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