Brain Research, 128 (1977) 569–574

Membrane resistance, monosynaptic EPSPs, and the epileptogenic action of penicillin in spinal motoneurons

PATRICK F. DUNN* and GEORGE G. SOMJEN

Department of Physiology and Pharmacology, Duke University Medical Center, Durham, N.C. 27710 (U.S.A.)

(Accepted February 23rd, 1977)

Penicillin is one of the drugs most widely used to induce experimental seizures yet its mode of action is not well understood. When applied to various so-called 'model' systems, it has been reported to cause changes of membrane resistance or synaptic effectiveness[1,8,10]. Such actions, if demonstrable in the mammalian central nervous system, could explain the occurrence of spontaneous seizure activity.

Our studies concerned membrane resistances and afferent-evoked excitatory postsynaptic potentials (EPSPs) of spinal motoneurons before and after the induction of seizures by the injection of penicillin. In high spinal cat preparations, penicillin has been shown to induce epileptiform seizures[11,13]. However, its mechanism of action has not been elucidated.

Functionally decapitate adult (2–4 kg) cats were prepared as described elsewhere[17]. Motoneurons located in lumbosacral segments L7 and S1 were identified by antidromic stimulation of various leg nerves, and impaled with beveled glass microelectrodes of 1–10 MΩ resistance (0.5–2.5 μm tip diameter), usually filled with 0.5 *M* potassium citrate or less commonly with 1.0–2.0 *M* KCl. The single microelectrode switching circuit, originally developed by Wilson and Goldner[18] and adapted for use in mammalian systems by Dunn and Wilson[7], was utilized to both inject and record current and the resultant membrane potential via one microelectrode. Such recordings were independent of microelectrode resistance and without any capacitive artifact in the voltage recording in cases in which the microelectrode response time was sufficiently smaller than the membrane time constant. When this approach was not successful, a conventional bridge balance, which was incorporated into the single microelectrode switching circuit, was used. Peripheral nerves or dorsal roots were stimulated to evoke EPSPs in penetrated motoneurons. Penicillin was administered intravenously after a cell was held at a stable resting potential for a minimum of 3 min. The initial dose was 10^6 IU/kg body weight, or up to an additional 0.5×10^6 IU/kg

* Present address: Engineering Division, Building 310, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Ill. 60439, U.S.A.

body weight until seizure activity ensued. Continuous recordings were taken from ventral roots to monitor seizure activity[13].

The input resistance of motoneurons was measured in two ways. In order to follow changes in resistance over extended periods of time, a constant intensity current pulse was injected at regular intervals, and the resultant membrane potential change taken as an index of input resistance. Prior to penicillin administration, and again from time to time thereafter, this routine was interrupted and a series of at least 10 hyper- and depolarizing current pulses of various intensities were introduced in order

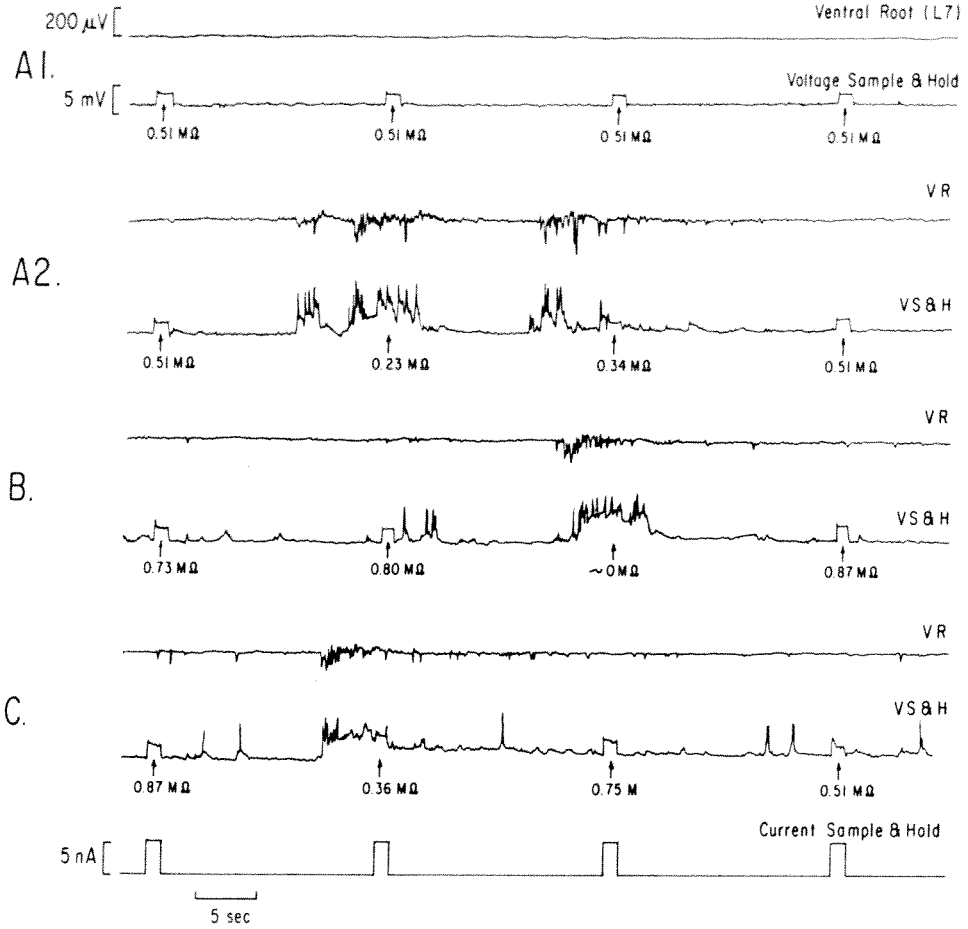

Fig. 1. Membrane resistance of various motoneurons for the same preparation before and after administration of a convulsant dose of penicillin, in both seizure-free and seizure intervals. Records A1 (pre-penicillin) and A2 (post-penicillin) are from one cell, B and C, from two others. Magnitude and timing of current injections are identical for all 4 voltage traces, as shown at the bottom of the figure. Ventral root recording indicates occurrence of seizure activity in segment L7 in which motoneurons were located. 'Voltage sample-and-hold' (cf. Dunn and Wilson[7]) registers intracellular voltage, 'current sample-and-hold', the amount of current injected. Calculated input resistance is written below voltage tracing for each current injection. Resting membrane potential —72 mV for A, —75 mV for B and —74 mV for C. Dose of penicillin, 10^6 IU/kg body wt. Tracings were copied by hand from polygraph recording. Spikes were not recorded due to the frequency response of the pen.

to determine the relationship between the varying current intensity and resultant membrane potential. Input resistances were then computed via a least-squares linear regression analysis, following examination of the data for possible non-linear current–voltage regions. 'Resting' input resistances reported here for the penicillin state were calculated only from measurements taken during seizure-free intervals, and in a

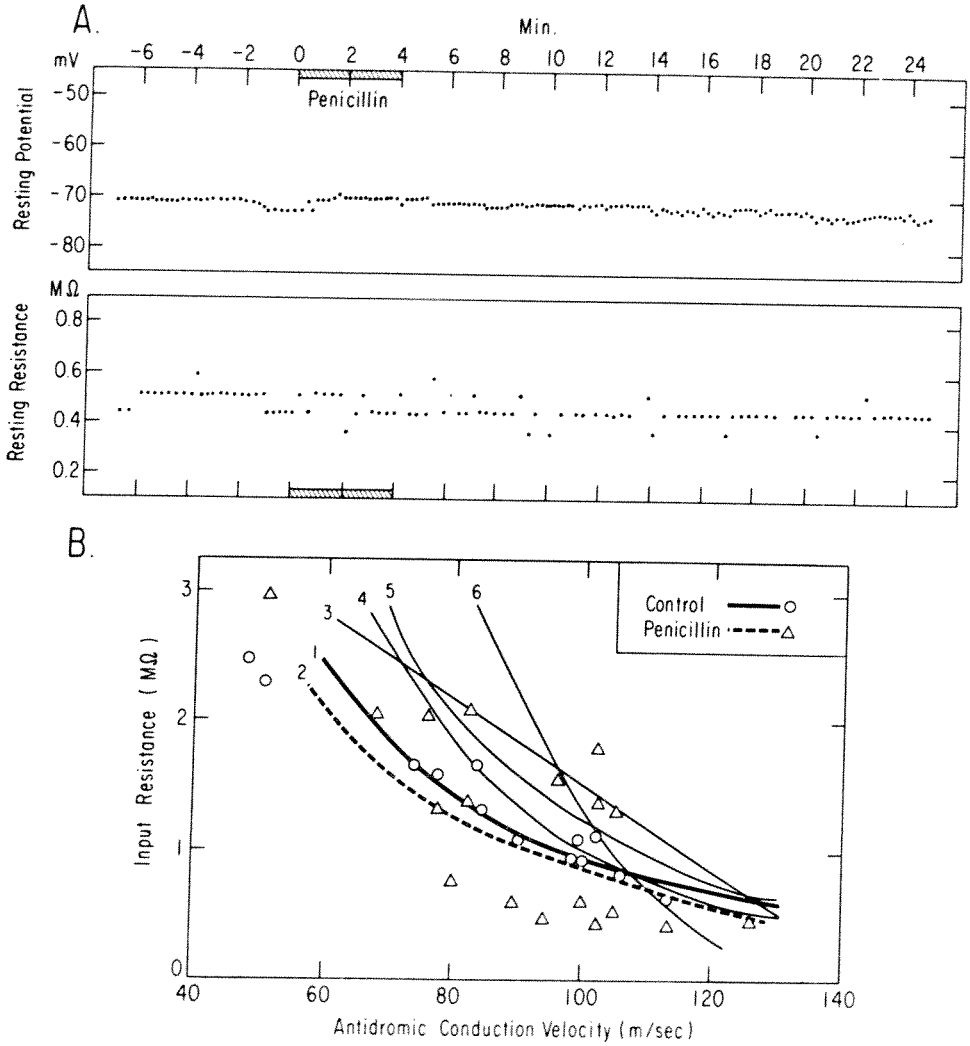

Fig. 2. Resting input resistance of motoneurons before and after the administration of a convulsant dose of penicillin. A: data from one neuron before, during and after the administration of 10^6 IU/kg body wt. penicillin. B: input resistance versus antidromic conduction velocity of the axon, for cells found in the control state, and after a convulsant dose of penicillin, but measured in seizure-free intervals. The curves fit the data to the least-squares deviation for the function: $R_i = aV_c^b$; (R_i = input resistance; V_c = conduction velocity; a, b = constants). 1, for our control data (correlation coefficient = —0.90 and n = 13); 2, for our data obtained after penicillin (correlation coefficient = —0.47 and n = 18); 3, for data from Barrett and Crill[2]; 4, data from Burke[3]; 5, from Burke[4]; 6, from Kernell[12].

majority of the cases could not be measured at potentials more than approximately 10–15 depolarizing mV from resting potential.

Fig. 1 displays recordings from various motoneurons in pre- and postpenicillin states, obtained using the single microelectrode switching circuit in the current-clamp mode. Membrane potential changes for the penicillin state in quiescent intervals were always found to be of the same order as those recorded in the control state (see recordings A1 and A2, Fig. 1). When input resistance was measured during paroxysmal activity, it was invariably found to be greatly reduced (see recordings A2, B and C, Fig. 1).

Fig. 2A presents in chronological sequence measurements of one motoneuron's resting potential and input resistance prior to, during and following penicillin administration. It illustrates that 'resting' input resistance did not change by the administration of penicillin in an amount sufficient to induce seizure activity. In all, 5 other cells were followed completely before, during, and after the injection of penicillin, and an additional 6 before, during and only immediately following (less than 1 min after) administration of the drug. None showed a consistent change in input resistance that could be attributed to the injection. In cases where some change in input resistance did ensue, it occurred relatively suddenly, well after completion of the injection, and was associated with a simultaneous change in membrane potential either in the depolarizing or in the hyperpolarizing direction.

In addition to the cells from which recordings were obtained both before and after injection of penicillin, input resistance was measured for a larger number of neurons in the control state, and of another group after the administration of penicillin. For these cases, antidromic conduction velocities were also measured to provide for a meaningful comparison of input resistances among cells, since input resistance is dependent on cell size. Fig. 2B shows the results of such measurements. Regression functions fitted to data obtained by other investigators for the control case are also illustrated for comparison. Even though the variation of data was considerably greater, the least-squares regression curve after penicillin administration was almost identical to the control regression curve. It is possible that the occasional enhanced background synaptic bombardment observed in such preparations could be responsible for the wide scatter of input resistance values.

Monosynaptic EPSPs were measured before, during and after the administration of penicillin in 5 cells. No change in the amplitude of the EPSPs attributable to the penicillin was found. If a change occurred, it could be traced to a simultaneous change of membrane potential and resistance, presumably indicating an artifact due to impending dislodgement of the electrode. Such events occurred without regularity and had no consistent relationship to the time of injection. A disynaptic IPSP was recorded in one cell. It waxed and waned in the expected manner with membrane potential. The function relating IPSP amplitude to membrane potential remained unchanged after penicillin injection.

No obvious direct effect of penicillin on motoneurons in the spinal cord was revealed by these observations. Seizure activity may perhaps originate in populations of interneurons which drive the motoneurons, thereby producing the depolarizing

shifts which appear simultaneously in most motor cells within a convulsing segment[14]. The large reduction in membrane resistance noted during such events is consistent with their synaptic origin. Or it could be, as Schwindt and Crill[16] suggest, that the mechanism of depolarizing shifts of most motoneurons may be similar to that of the oscillatory potentials of gastropod neurons. It should be noted, however, that it has been our experience that neurons subject to these paroxysms on a majority of occasions could not be induced by injected current to fire in a bursting pattern in seizure-free intervals, as would be expected if their membranes were inherently unstable (negative resistance).

Hochner et al.[10] have reported a decrease of chloride conductance of the crustacean muscle membrane, and Pellmar and Wilson[15] have found that chloride-dependent postsynaptic inhibition was markedly and selectively blocked in neurons of *Aplysia*. Why then have not such effects been demonstrated in the mammalian spinal cord? Possibly, since the resting chloride conductance of spinal motoneurons is low, its changes remain undetected when total membrane resistance is measured. Disynaptic postsynaptic inhibition in the spinal cord is generally regarded as being in part at least chloride-mediated, and was shown to be unchanged by convulsant doses of penicillin by Davidoff[6], Lothman and Somjen[14], Davenport et al.[5], and also in this work. On the other hand, penicillin has been shown to somewhat depress recurrent inhibition[14] and also to alter polysynaptic IPSPs[5]. Further, when comparing results obtained in vitro to those obtained in vivo, the possible variation in actual tissue concentration for the same dose level applied should be considered. The 2 m*M* concentration of penicillin used by Hochner et al.[10] and by Pellmar and Wilson[15], corresponding to 1.2×10^6 IU/liter, would be remarkably close to the convulsant dose in cats if the drug dissolved evenly in body water. The blood–brain barrier however is regarded as being relatively impermeable to penicillin. For example, Goodman and Gilman[9] state that, for 50 IU/ml present in plasma, only 0.5 IU/ml appear in the cerebrospinal fluid. Nevertheless, chloride conductance changes caused by penicillin merit further attention, since the susceptibility of mammalian tissue may be greater than that of invertebrates.

In conclusion, our findings show no effect of penicillin upon motoneuron resting input resistance, and are in agreement with Davenport et al.[5] that monosynaptic potentials evoked by afferent input are not influenced by penicillin. The somewhat unpredictable enhancement of monosynaptic reflex transmission attributed to this drug[14] thus remains also unexplained. Possibly irregular subliminal depolarizations evoked by excitatory background are responsible for the enhanced reflex transmission, which usually was small.

This work was supported by PHS Grant NS 11933. Dr. Dunn was the recipient of a stipend from NIMH Training Grant MH-08394.

1 Ayala, G. F., Spencer, W. A. and Gumnit, R. J., Penicillin as an epileptogenic agent: Effect on an isolated synapse, *Science*, 171 (1971) 915–917.

2 Barrett, J. N. and Crill, W. E., Specific membrane properties of cat motoneurons, *J. Physiol. (Lond.)*, 239 (1974) 301–324.
3 Burke, R. E., Motor unit types of cat triceps surae muscle, *J. Physiol. (Lond.)*, 193 (1967) 141–160.
4 Burke, R. E., Firing patterns of gastrocnemius motor units in the decerebrate cat, *J. Physiol. (Lond.)*, 196 (1968) 631–654.
5 Davenport, J., Schwindt, P. C. and Crill, W. E., Postsynaptic potentials in penicillin-induced spinal myoclonus in cat, *Neurosc. Abstr.*, 2 (1976) 259.
6 Davidoff, R. A., Penicillin and inhibition in the cat spinal cord, *Brain Research*, 45 (1972) 638–642.
7 Dunn, P. F. and Wilson, W. A., Development of the single microelectrode current and voltage clamp for central nervous system neurons, *Electroenceph. clin. Neurophysiol*, in press.
8 Futamachi, K. J. and Prince, D. A., Effect of penicillin on an excitatory synapse, *Brain Research*, 100 (1975) 589–597.
9 Goodman, L. S. and Gilman, A., *The Pharmacological Basis of Therapeutics*, 1970.
10 Hochner, B., Spira, M. E. and Werman, R., Penicillin decreases chloride conductance in crustacean muscle: A model for the epileptic neuron, *Brain Research*, 107 (1976) 85–103.
11 Kao, L. I. and Crill, W. E., Penicillin-induced segmental myoclonus. I. Motor responses and intracellular recording from motoneurons, *Arch. Neurol. (Chic.)*, 26 (1972) 156–161.
12 Kernell, D., Input resistance, electrical excitability and size of ventral horn cells in cat spinal cord, *Science*, 152 (1966) 1637–1640.
13 Lothman, E. and Somjen, G., Motor and electrical signs of epileptiform activity induced by penicillin in the spinal cords of decapitate cats, *Electroenceph. clin. Neurophysiol.*, 41 (1976) 237–252.
14 Lothman, E. and Somjen, G., Reflex effects and postsynaptic membrane potential changes during epileptiform activity induced by penicillin in decapitate spinal cords, *Electroenceph. clin. Neurophysiol.*, 41 (1976) 337–347.
15 Pellmar, T. C. and Wilson, W. A., Penicillin effects on iontophoretic responses in *Aplysia californica*, *Brain Research*, in press.
16 Schwindt, P. C. and Crill, W. E., Penicillin-induced membrane conductance changes in cat spinal motoneurons, *Neurosci. Abstr.*, 2 (1976) 266.
17 Somjen, G. G., Evoked sustained focal potentials and membrane potential of neurons and of unresponsive cells of the spinal cord, *J. Neurophysiol.*, 33 (1970) 562–582.
18 Wilson, W. A. and Goldner, M. M., Voltage clamping with a single microelectrode, *J. Neurobiol.*, 6 (1975) 411–422.