Neuropeptides 5: 241-244, 1984

ACTIONS OF D-ALA^2-D-LEU^5-ENKEPHALIN AND DYNORPHIN A (1-17) ON NEOCORTICAL NEURONS IN VITRO

B. Sutor and W. Zieglgänsberger

Department of Clinical Neuropharmacology, Max-Planck-Institute for Psychiatry, Kraepelinstrasse 2, D-8000 Munich 40, F.R.G., (reprint requests to BS)

ABSTRACT

Intracellular recordings were made from neocortical neurons in vitro. Application of D-Ala^2-D-Leu^5-enkephalin (DADL) by different methods produced a decrease in EPSP amplitude and in the amplitude of L-glutamate-induced depolarizations without changes in membrane potential or membrane input resistance. The DADL effects were blocked by naloxone and persisted when synaptic transmission was depressed, suggesting DADL acts on postsynaptically located opiate receptors. With dynorphin A (1-17), depolarizations, hyperpolarizations, decreases and increases in EPSP were observed, but never an anti-glutamate effect.

INTRODUCTION

The opioid peptides methionine- and leucine-enkephalin and dynorphin A (1-17) have been localized in the rat neocortex by immunohistochemistry (1,5). In addition, the existence of opiate receptors on cortical neurons was demonstrated (2,3). Previous electrophysiological studies (4,6) showed that opiates and opioid peptides stereoselectively inhibit the activity of cortical neurons. In the present study, we investigated the action of D-Ala^2-D-Leu^5-enkephalin (DADL) and dynorphin on neocortical neurons in vitro by means of intracellular recordings.

METHODS

Wistar rats (140-160 g) were anesthetised, decapitated and the brains removed. Coronal slices, prepared from the frontal/motor cortex, were transferred to the recording chamber. The temperature in the chamber was 36.5°C. The slices were superfused (1 ml/min) with a solution consisting of (in mM): NaCl 124; KCl 5; NaH_2PO_4 1.25; $CaCl_2$ 2.5; $MgSO_4$ 1.3; glucose 10; sucrose 5 (added to adjust the osmolarity to 321 mOsm) and $NaHCO_3$ 26. The solution was saturated with oxygen containing 5% CO_2 to keep the pH at 7.4. Recording electrodes (40-90 M-Ohm) were filled with 4M K-acetate (pH 7.0). Stimulation was performed with bipolar silver electrodes placed in the superficial layers of the cortex. Conventional methods were employed for recording and storage of signals. Drugs were applied either by addition to the bath solution (DADL, dynorphin, naloxone), by iontophoresis (L-glutamate, 1M, pH 8.0; DADL 5 mM or

10 mM, pH 5.5; currents were automatically compensated), or by pressure application from multibarrelled pipettes (DADL, dynorphin).

RESULTS

The action of DADL was investigated on 62 neurons recorded intracellularly from superficial cortical layers. The electrophysiological properties of these neurons were (mean ± S.D.): membrane potential, -75.4 ± 6.7 mV; membrane input resistance, 14.1 ± 3.9 M-Ohm; membrane time constant, 14.2 ± 8.6 ms; action potential amplitude, 94.6 ± 17.2 mV.

DADL, applied by iontophoresis (50-300 nA for 0.5-2 min), by pressure (with pipettes containing a 10 nM or 1 µM DADL solution, 10-35 kPa for 0.5-2 min), or by addition to the bath solution (10 nM to 1 µM), neither altered the membrane potential nor the membrane input resistance of these neurons. In all neurons tested (n=38), DADL reversibly decreased the amplitude of the stimulus-evoked EPSP by 30-100%. In the same neurons, no effect of DADL on the IPSP could be detected. The action of DADL on the EPSP was independent from the method of application. Bath-applied naloxone (1 µM) reversibly blocked the effects of DADL on the EPSP (n=3). Iontophoretically applied L-glutamate (L-GLU, 10-100 nA for 10-35 s) depolarized the membrane potential of all neurons tested. This effect was still present when synaptic transmission was blocked by removing Ca^{2+} from the bath solution and increasing the Mg^{2+} concentration to 9 mM. Under these conditions, the EPSP was completely depressed. DADL decreased or abolished the L-GLU-induced depolarization by 20-100% in all investigated neurons (Fig. 1, n=21). This anti-glutamate effect of DADL was dose-dependent, independent from the mode of application, naloxone-reversible, and persisted when synaptic transmission was depressed.

In addition to not altering resting membrane potential or input resistance, DADL also had no detectable effect when the membrane potential was displaced by DC-current injection (Fig. 1). On the other hand, when neurons were depolarized by continuous L-GLU application (20-40 nA for 2-4 min), DADL produced a dose-dependent hyperpolarization (Fig. 1, n=10). The amplitude of this hyperpolarization was dependent on the amplitude of the L-GLU-induced depolarization and also occurred without resistance change. Phoretically administered Na^+ ions with comparable current intensities never evoked a hyperpolarization (n=3).

The effects of dynorphin A (1-17) were investigated on 15 neurons. Depolarizations, hyperpolarizations, and both decreases and increases in the EPSP amplitude were observed. In some neurons, dynorphin A produced transient short-lasting depolarizations which evoked burst discharges. In contrast to DADL, there was never an anti-glutamate effect with dynorphin A (1-17).

DISCUSSION

The presented results show that DADL has no direct effect on any membrane conductance of cortical neurons that is detectable in the soma, since it does not change membrane potential and membrane input resistance. In these neurons, DADL depresses excitatory input as evidenced by decrease in the amplitude of the EPSP and the amplitudes of L-GLU-induced depolarizations. Direct excitation of the neurons by DC current injection remains unaffected, whereas depolarizations induced by continuous L-GLU application are either reversibly re-

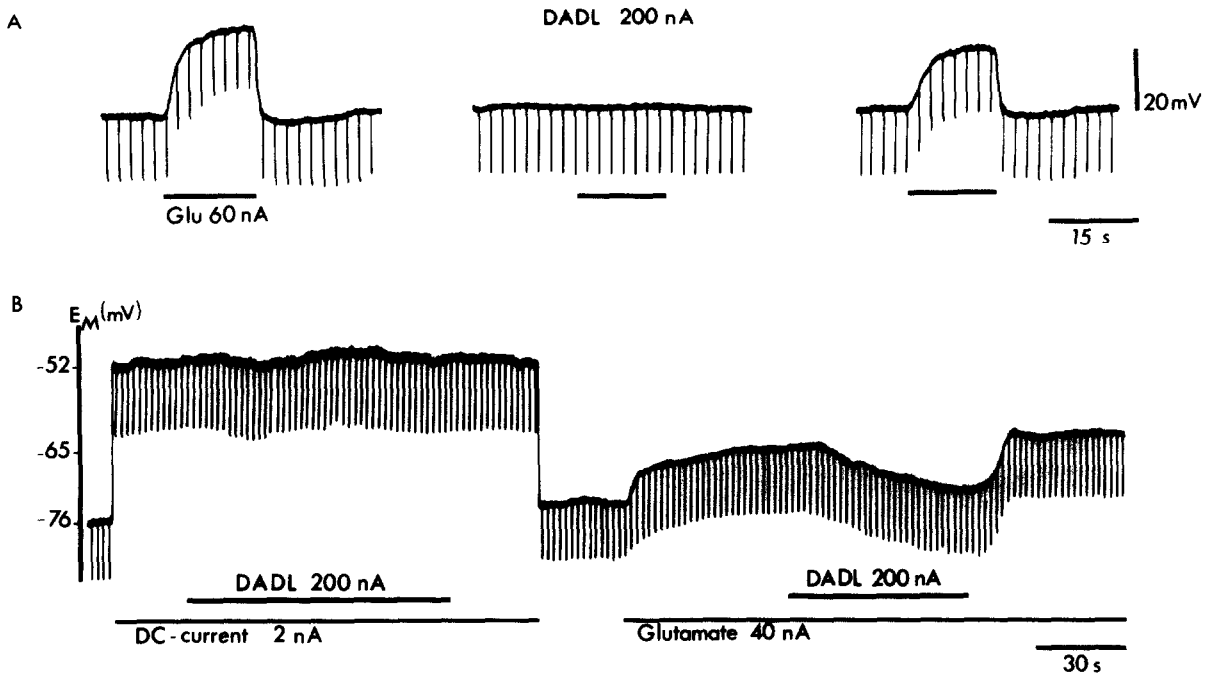

FIG. 1 Intracellular recordings from a neocortical neuron in vitro (membrane potential -76 mV). A: Chart writer recordings from the neuron during application of 60 nA glutamate (GLU) for 15 s. Iontophoretically administered DADL with 200 nA for 2 min reversibly abolished the glutamate-induced depolarization (interval between the subsequent recordings 5 min). Note the unchanged membrane potential and membrane resistance during the action of DADL (resistance measurement: 100 ms hyperpolarizing current pulse with 1 nA). B: Chart writer recording from the same neuron. Application of DADL with 200 nA during DC-current depolarization with 2 nA had no effect. In contrast, the same dose of DADL produced a hyperpolarization without resistance change, when the neuron was depolarized by continuous application of glutamate with 40 nA.

duced in amplitude or shifted to membrane potential values measured before L-GLU application. This disfacilitatory effect of DADL occurs without resistance change, indicating a modulatory effect of DADL on the L-GLU-induced depolarization. The persistence of this anti-glutamate effect of DADL when synaptic transmission is blocked indicates that the receptors activated by DADL are located on the cell recorded from. Naloxone (1 μM) completely and reversibly inhibits the effects of DADL on the EPSP and on the L-GLU-induced depolarization, suggesting DADL acts via opiate receptors.

One possible reason (inter alia) for the lack of a unique action of dynorphin A (1-17) could be the partial inactivation of the peptide by peptidases in the slice and the resulting generation of cleavage products with a different activity pattern than dynorphin A (1-17) (for example leu-enkephalin). For the moment, it seems to be most important to reveal changes in cellular

behaviour due to selective activation of kappa-receptors. Therefore, experiments with exogenous kappa-selective agonists and antagonists are in progress.

The described results with DADL accord with previous studies on cat motoneurons and dorsal horn neurons in vivo (9,10), but are in contrast to data derived in vitro from rat locus coeruleus (7) and substantia gelatinosa neurons (8). One possible reason for this discrepancy could be that opiates are acting via different mechanisms in different regions of the brain. For the moment, the mechanism underlying the disfacilitatory effects of DADL on cortical neurons is unestablished, but initial investigations suggest that neither Ca^{2+} nor K^{+} are involved in the action of the opioid peptide on cortical neurons.

REFERENCES

1. Khachaturian, H., Lewis, M.E., Höllt, V. and Watson, S.J. (1983). Telencephalic enkephalinergic systems in the rat brain. Journal of Neuroscience 3: 844-855.

2. Lewis, M.E., Pert, A., Pert, C.B. and Herkenham, M. (1983). Opiate receptor localization in rat cerebral cortex. Journal of Comparative Neurology 216: 339-358.

3. Quirion, R., Bowen, W., Herkenham, M. and Pert, C.B. (1982). Visualization and solubilization of rat brain opiate receptor with a "k" ligand selectivity pattern. Cellular and Molecular Neurobiology 2:333-346.

4. Satoh, M., Zieglgänsberger, W. and Herz, A. (1976). Actions of opiates upon single unit activity in the cortex of naive and tolerant rats. Brain Research 115: 99-110.

5. Weber, E., Roth, K.A. and Barchas, J.D. (1982). Immunohistochemical distribution of alpha-neoendorphin/dynorphin neuronal systems in rat brain: evidence for colocalization. Proceedings of the National Academy of Sciences USA 79: 3062-3066.

6. Williams, J.T. and Zieglgänsberger, W. (1981). Neurons in the frontal cortex of the rat carry multiple opiate receptors. Brain Research 226: 304-308.

7. Williams, J.T., Egan, T.M. and North, R.A. (1982). Enkephalin opens potassium channels on mammalian central neurones. Nature 299: 74-77.

8. Yoshimura, M. and North, R.A. (1983). Substantia gelatinosa neurones hyper polarized in vitro by enkephalin. Nature 305: 529-530.

9. Zieglgänsberger, W. and Bayerl, H. (1976). The mechanism of inhibition of neuronal activity by opiates in the spinal cord of cat. Brain Research 115: 111-128.

10. Zieglgänsberger, W. and Tulloch, I.F. (1979). The effects of methionine- and leucine-enkephalin on spinal neurones of the cat. Bran Research 167: 53-64.