Regulatory Roles of Opioid Peptides

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Contents

Regulation of Neuronal Excitability by Opioid Peptides: Intracellular Analysis in Several Brain Areas

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SUMMARY

Four different mechanisms have been postulated to account for the depressant effects of opioid peptides on central mammalian neurons. The evidence for these mechanisms, predominantly obtained from intracellular recordings, is reviewed. We have performed intracellular *in vitro* studies of four different neuron types (hippocampus CA1 pyramidal cells, dentate granule cells, cortical pyramidal cells, and nucleus accumbens neurons). In all these cell types, the predominant effect of low concentrations of receptor selective enkephalin analogues and morphine was reduction of postsynaptic potentials. Depolarizing responses to locally applied L-glutamate were also tested in hippocampal and cortical pyramidal cells and found to be reduced by the enkephalins. Membrane potential and input resistance were little affected by the opioids. These results suggest that, in the four brain regions examined, the predominant mechanism of opiate-induced depressions is reduction of excitatory synaptic transmission. Although presynaptic effects could be involved, the anti-glutamate effect present also in preparations where synaptic transmission was effectively prevented points to a postsynaptic neuromodulatory role for these enkephalins.

1 INTRODUCTION

Electrophysiological research on opiates and opioid peptides has primarily involved extracellular single-unit recordings with iontophoresis, directed at CNS areas with a high density of opiate binding sites, or areas involved in the integration of nociceptive signals. In such extracellular recordings, the preponderant stereospecific action of opiate alkaloids or opioid peptides in most brain regions is depression of spontaneous, synaptically or chemically-evoked neuronal single-unit discharge activity. The depressions are generally blocked by naloxone, suggesting involvement of the empirically defined opiate receptors. The depressions are qualitatively similar throughout the mammalian central and peripheral nervous systems. Some major exceptions to the usual depressions exist, such as the naloxone-reversible excitatory responses seen in hippocampal pyramidal cells (3,4), in spinal cord Renshaw cells (5 and Crain et al., this volume), in the brain stem (6), and in the substantia nigra (7) (see refs. 1,2,8,53 for review and Crain et al., this volume).

In the hippocampus, both *in vivo* studies and *in vitro* intracellular recording revealed excitatory responses of hippocampal pyramidal neurons to opioid agonists. Blockade of GABA responses by bicuculline and transmitter release by Mg^{2+} indicate that excitation may actually be indirect, resulting from a primary inhibitory effect on neighbouring inhibitory interneurons and leading to the excitation of pyramidal cells by disinhibition (4,8; see below). It is possible that the opiate-induced excitations seen in other areas may also derive from disinhibitory opiate actions. However, intracellular recording is usually required to more strictly test the disinhibition hypothesis and is obligatory for determining the possible ionic mechanisms involved in opiate function.

The last decade has seen an increase in intracellular studies, leading to a greater understanding of the mechanisms of opioid function. However, as many as four general types of opioid mechanisms, as schematized in figure 1, have now been described in the literature. The first type shown, presynaptic modulation, may in fact derive from the operation at the presynaptic level of one or more of the other three mechanisms shown. Unfortunately, the difficulty of recording from presynaptic terminals in mammalian CNS generally precludes direct examination of the actual mechanisms involved. However, there have been several studies indicating that opioids can reduce release of synaptic transmitters (9-11; see also the chapters by Cox et al.,

Mulder et al., Illes et al. and Fuder, this volume), thus suggesting a presynaptic site of action.

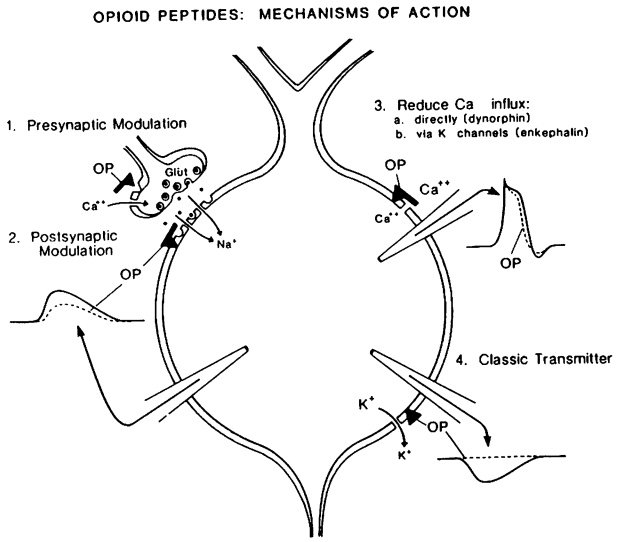

Fig.1 Scheme of possible mechanisms of action of opioid peptides. See text for details.

The second opioid mechanism shown, postsynaptic modulation, was one of the first described (12,13). In this case, we define modulation as an alteration of the action of other transmitters by the opioids, without a direct action on the excitability of the postsynaptic neuronal membrane (see 8). Thus, early studies on the in vivo cat spinal cord showed that intravenous administration of morphine agonists depressed polysynaptic EPSPs, an effect reversed by opiate antagonists (see 12 and 53 for refs.). Later studies involving the intracellular recording of cat spinal cord neurons with simultaneous extracellular microiontophoretic drug application suggested that morphine and opioid peptides did not change the membrane potential or resting membrane resistance but did decrease the magnitude and rate of rise of the EPSPs (12,13). In these in vivo studies opiates and opioid peptides also depressed the glutamate-induced depolarization. Microiontophoretically applied L-glutamate was thought to increase the postsynaptic membrane conductance to Na^+ and K^+. It was therefore postulated that the opiates interfere with the chemically excitable cationic channels

comparable to those also opened by synaptically released excitatory transmitters (12,13). Antagonism of the postsynaptic depolarizing responses to L-glutamate suggests that the opiate receptors involved in this effect are also located on the postsynaptic membrane. To some extent, the anti-glutamate action of the opioid peptides in vivo has been confirmed for mouse spinal neurons grown in culture where a noncompetitive blocking action on the glutamate-activated conductances was observed (14; see also: CEREBRAL CORTEX).

In a recent extracellular study, an antagonism of L-glutamate responses by an enkephalin analogue in substantia gelatinosa neurons recorded in a slice preparation of an adult rat spinal cord has been shown (15). Nonetheless, several intracellular studies on acutely isolated or hemisected spinal cord preparations in vitro did not confirm an opioid antagonism of L-glutamate excitatory actions (16) and/or have recorded opioid-induced hyperpolarizations (16-18). Two of these latter studies involved sucrose gap recordings of hemisected frog spinal cord and measured only small (0.5-1 mV) membrane potential changes. However, a more recent study (18) with intracellular recording of substantia gelatinosa neurons in a rat spinal cord slice measured hyperpolarizations that averaged 8 mV, in about 50% of the cells tested. Opioid activation of a K^+ conductance was suggested (see also below). It was reported at the present meeting that bath application of a μ-preferring agonist (see also below) in concentrations that did not have a measurable effect on resting membrane potential or input resistance produced a depression of the portion of the EPSP induced by activation of small primary afferents in dorsal horn neurons of the rat spinal cord (Jeftinija, this volume). It is possible that some of the discrepancies in the literature with respect to the effects of the opioids on membrane potential and responses to L-glutamate could arise from species, methodological or cell-type differences (see CONCLUSIONS).

The third mechanism, reduced Ca^{2+} influx, is illustrated by studies of cultured dorsal root ganglia neurons, which could also provide insights into the presynaptic effects of opioid peptides (see 19-23; and also Crain et al., this volume). In these neurons (derived from either chick or rat neonates), none of the opioid peptides tested altered membrane potentials, but they did reduce the duration of the (Ca-dependent) action potential (11,20-23). It was initially thought that this reduction was due to interference with a voltage-dependent Ca^{2+} conductance (19,20). However, this opioid effect might arise from the enhancement of some K^+ conductance (e.g., Ca-dependent K^+ conductance or delayed rectifier), as suggested by the recent studies of μ-

and δ-receptor activation in these neurons (22,23). In these studies, the opioid (enkephalin) effect on the action potential duration, but not the response to dynorphin (23), was abolished by intracellular Cs^+ injection, which eliminates most K^+ conductances. Regardless of the primary mechanism, the net effect of such an action would be a reduction of calcium entry into the cell. Such a reduction, if it also occurred in the terminals of these cells in the spinal cord (e.g., in substantia gelatinosa), could provide the mechanism for the reported opioid-induced inhibition of transmitter release (9-11). Unfortunately for this hypothesis, dorsal root ganglion cells acutely isolated from adult rats do not show this opiate effect on (presumed Ca-dependent) action potential duration (24). It is possible that either the relevant somatic opiate receptors disappear entirely with maturity or they migrate down the axons to the terminals in the spinal cord. Also the site of interaction in the terminal region requires revision. Recent histochemical and ultrastructural data suggest a postsynaptic interaction between opioid peptides and primary afferents. However, an effect of diffusing opioid peptides on primary afferent fibers can not be excluded (for details see refs. 8,53).

The fourth major mechanism reported, classic transmitter action, derives from intracellular recordings of locus coeruleus neurons in the brain stem slice in vitro (25), that show hyperpolarizing responses to the opioid peptides associated with an increased conductance. Williams et al. (26) have presented evidence that the opioid-induced hyperpolarization is very sensitive to low naloxone concentrations and that the increased conductance involves K^+. An almost identical series of findings have been reported from the same laboratory for substantia gelatinosa neurons of the rat spinal cord slice preparation (18), again suggesting a classic neurotransmitter-like action for the opioid peptides. More recent data from this laboratory suggests that the K^+ channels involved in the opioid-induced hyperpolarization in locus coeruleus are the same voltage-dependent (inward rectifying) channels activated by several other inhibitory transmitters, and that such activation involves a GTP-binding protein (27).

However, the sum of our findings, derived from intracellular recordings of several diverse brain regions in vitro and carried out in two different laboratories, appears to indicate that the most pronounced effect of low concentrations of the opioid peptides falls into the second category of mechanisms, postsynaptic modulation. These findings are detailed in the following sections.

2 HIPPOCAMPAL CA1 AND CA3 PYRAMIDAL NEURONS

Pyramidal cells of the hippocampus *in vitro* show little or no direct transmembrane effects of morphine or opioid peptides (i.e., ß-endorphin and analogues of leucine- and methionine-enkephalin), in concentrations of up to 5 μM (see 8 and 28 for refs.). However, much lower concentrations (10^{-7} - 10^{-6}M) alter synaptic responses to afferent stimulation (8,16,29-33), which would be consistent with a modulatory action of the opioids (mechanism 2) at the hippocampal pyramidal cell (8). Most studies on the hippocampal slice indicate that the enkephalins and ß-endorphin primarily reduce the size of recurrent and feedforward IPSPs in both CA1 and CA3 cell groups (16,29-33), thus supporting a disinhibitory mechanism (4). However, two other hippocampal slice studies have shown only enhanced EPSPs without changes in the IPSP (30,34), while a third noted a reduction in the size of EPSPs (as well as IPSPs and depolarizing L-glutamate responses) in about 50% of the pyramidal neurons studied (33). *In vitro* studies of explant cultures of rat hippocampus showed both reduced IPSPs and enhanced EPSPs (56,57). The bulk of the extracellular (see refs. 1,8,28,35-37) and intracellular (8,28,29,31-33,37) data thus supports a disinhibitory mechanism of action for the single-unit excitatory (3,4) effects of these opioid peptides in the hippocampus. (See 8 and 28 for reviews of the hippocampus and opiate-related physiology and morphology).

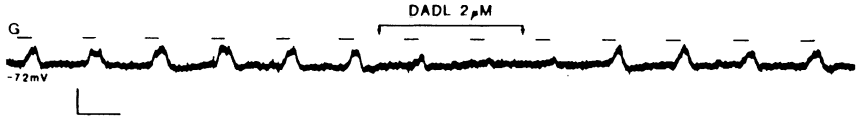

Fig.2 Pen recording of membrane potential in a CA1 pyramidal neuron showing the depression of L-glutamate (G) induced depolarizations by superfusion of 2 μM D-ala^2-D-leu^5-enkephalin (DADL), in the presence of 12 mM Mg^{2+} to block synaptic transmission. L-glutamate applied by pressure (100 kPa) from a micropipette placed in the pyramidal cell layer. Resting membrane potential = -72 mV. Note lack of effect of DADL on membrane potential (Calibration bars 10 mV, 1 min).

Because EPSPs as well as IPSPs were often reduced in our studies of CA1 neurons in hippocampal slices (33), we attempted to determine if

this effect was exerted pre- or postsynaptically by applying excitatory transmitters. Figure 2 shows an example of the blockade by an enkephalin analogue of depolarizing responses to L-glutamate applied locally to a CA1 pyramidal neuron. This effect was seen in 4 of 5 CA1 cells so tested. The anti-glutamate action was clearly a postsynaptic effect, as blockade of synaptic release with high Mg^{2+} concentrations had no effect on this modulation (see also: CEREBRAL CORTEX). The opioid/L-glutamate interactions were antagonized by naloxone, suggesting involvement of true opiate receptors.

It is now apparent that one or more of the COOH-terminally extended versions of leucine^5 -enkephalin, such as dynorphin A or B, or dynorphin A_{1-8}, may constitute a major proportion of mammalian central opioid peptides (see refs. 38,39). Interest has centered on the hippocampal mossy fiber pathway that projects from the dentate granule cells, through the hilus, to CA3 pyramidal cell dendrites. In the rat these fibers (and granule cell sources) were originally thought to contain enkephalin, but subsequent studies showed that these fibers possess considerable immunoreactivity for dynorphin(s) (40). It is interesting that this pathway is generally thought to be excitatory, likely to involve the release of an acidic amino acid such as L-glutamate or L-aspartate. The presence of dynorphins and excitatory amino acids in the same pathway, and possibly in the same fibers, has aroused speculation that these substances may be cotransmitters within the same mossy fiber terminals.

Attempts to define the function of these dynorphin peptides have met with some difficulty. Extracellular single-unit studies with iontophoresis of dynorphin B_{1-13} or dynorphin A_{1-17} have shown both depressant (41,42) and facilitatory (41) actions on CA3 hippocampal pyramidal neurons. Preliminary intracellular studies of dynorphin's actions on CA3 pyramidal neurons in the hippocampal slice preparation are consistent with these extracellular findings: depending on the particular cell studied, either slow hyperpolarizing or depolarizing responses are seen with low concentrations of dynorphin A and B (29). The depolarizing responses were not of the type expected of mossy fiber activation, in that they were slow and not always able to activate spikes. These effects contrast with the effects of enkephalins on these CA3 neurons, where no inhibitory effects are seen and excitatory effects appear to be due primarily to reduction of IPSPs, with little direct change in membrane potential or resistance (29,31).

Further studies are needed to determine whether 1) these dynorphin induced potential changes are due to remote effects on other input

neurons, 2) they are naloxone sensitive, and 3) multiple opiate receptor subtypes are involved. Indeed, a recent field-potential study of hippocampal slices suggests that dynorphins act, like enkephalin, on the μ-receptors in CA1, rather than on κ-receptors as was expected (43). The possible interaction of dynorphin with excitatory amino acids must also be tested to assess the significance of their possible coexistence in the same mossy fiber endings. The mixed effects of dynorphins on CA3 hippocampal pyramidal neurons, and the differences between the effects of the dynorphins and the other opioids, may be manifestations of a functional or receptor heterogeneity in central neurons. However, proof that the dynorphins act as transmitters or as some other neuromessenger type for any given mossy fiber-target cell entity would require the demonstration of identical effects, intracellularly recorded from a single CA3 neuron, in response to exogenous dynorphin and stimulation of the mossy fiber input. However, this has been a difficult task, owing in part to the presumed concomitant release of the excitatory amino acid along with the opioids, as well as the possible release of more than one type of opioid peptide (38).

3 CEREBRAL CORTEX

As in previous studies (44), enkephalin opioids and morphine displayed mainly modulatory actions in neocortical pyramidal cells in the frontal/motor cortex of the rat (n= 20). In these cells (lamina 2 & 3) recorded intracellularly in an *in vitro* slice preparation, the mean resting potential ($\pm$ S.D.) was -77.4 $\pm$ 5.4 mV and mean input resistance ($\pm$ S.D.) was 27.8 $\pm$ 9.8 MΩ. Spike amplitudes ranged from 90-110 mV. There is evidence from previous studies performed in an *in vivo* preparation of the rat cortex that μ- and δ-receptor subtypes of the opioid receptor are colocalized in this species on the same neocortical neuron (45).

Application of the δ-preferring agonist DADL and the μ-preferring agonists morphine and D-ala^2,N-Me-Phe^4,Gly^5-ol (DAGO) by iontophoresis (phoretic currents: 50 - 150 nA), pneumatic ejection from micropipettes or addition to the perfusion medium (10^{-6}- 10^{-8}M) decreased the EPSP amplitude and the amplitude of the L-glutamate induced depolarizations to 20 $\pm$ 18% of control. The EPSPs were most effectively reduced (to 45 $\pm$ 17%) at the lower intensity range of the input/output curve. In none of these cells were changes in membrane potential of more than $\pm$ 4 mV observed. The apparent input resistance of these cells (monitored by constant current pulses or determined via construction of current-voltage curves) was also not changed. The effects

of the enkephalin analogues and morphine on EPSPs and L-glutamate responses (see Fig. 3) were reversed by naloxone (10^{-6}M) added to the perfusion medium. The anti-glutamate action of the enkephalin opioids persisted in Ca-free/high-Mg medium, suggesting that the opioids acted postsynaptically, as synaptic transmission had been effectively blocked (44).

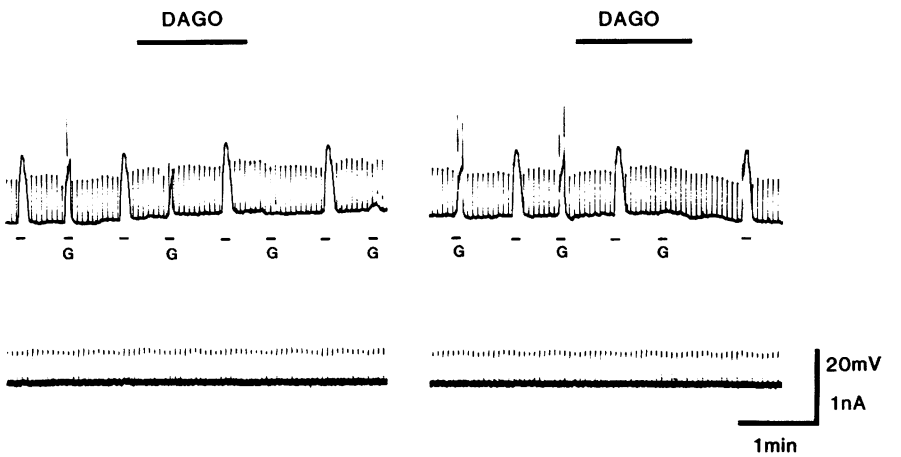

Fig.3 Pen recordings of part of an experiment showing the effects of DAGO iontophoresis on membrane potential and resistance and on responses to L-glutamate (100 nA, G labelled bars) and GABA (80 nA, unlabelled bars) iontophoresis. DAGO reversibly eliminates glutamate-induced depolarizations without significant effects on membrane potential or resistance (brief upward deflections). The latter is monitored by constant depolarizing current pulses (0.4 nA, bottom traces). Gap in record indicates omission of a 4 min interval.

In contrast to L-glutamate and quisqualate evoked depolarizations, N-methyl-D-aspartate (NMDA) induced depolarizations were either not influenced or reduced only slightly by opioids. The depolarizations, elicited by NMDA were blocked by D-2-amino-5-phosphonovaleric acid, which, at the concentrations employed (5-50 μM), did not affect L-glutamate induced depolarizations (46). Single electrode voltage-clamp studies showed that the L-glutamate- and quisqualate-induced inward current is reduced by opioids. Various studies suggest that NMDA and L-glutamate-induced inward currents are triggered through the activation of different receptors, are carried by a different ionic composition and display different ion- and voltage-sensitivities. NMDA receptor activation leads to a current that is carried to a great extent by

Ca^{2+} ions entering along with Na^{+} ions and is markedly potentiated by removal of Mg^{2+}ions. Recent studies suggest that the voltage-dependence of the NMDA-activated conductance is not exclusively determined by Mg^{2+}(46). Due to the voltage-sensitivity of the NMDA response, regenerative currents are induced which increase with depolarization and induce burst-like firing patterns, in contrast to the repetitive firing induced by L-glutamate (see 46,47 for refs.). The responses to iontophoretically applied GABA was not affected by the opioids administered by the various routes (see Fig. 3).

4 DENTATE GRANULE CELL

Interest in opioid effects on granule cells of the dentate gyrus derives from histochemical studies showing a profuse innervation of enkephalin-containing fibers originating from the entorhinal cortex and projecting onto granule cells via the perforant path (40,48). This cell type is morphologically distinct from the pyramidal cell types just discussed, yet to date it appears to respond to the enkephalin peptides like the pyramidal cells.

The 11 dentate granule cells from which we recorded intracellularly showed fairly large resting membrane potentials (mean: -76 mV; range: -60 to -90 mV) with little or no spontaneous spike activity. They responded to stimulation of the perforant path almost exclusively with depolarizing synaptic potentials. These synaptic potentials often showed a biphasic decay, with a faster decaying early portion likely to represent the late phase of an EPSP. A much more slowly decaying later phase (at higher stimulus strengths) is thought to represent a depolarizing IPSP (see 49 and fig. 4).

Superfusion of the slice with a range of concentrations (2-10 μM) of enkephalin analogues (DADL, 8 cells; D-Ala^2, met^5-enkephalin amide: DAMEA, 3 cells) produced only weak and variable effects on membrane potential and input resistance. Of the 11 cells studied, 5 displayed only weak hyperpolarizations (mean: 4 mV) accompanied by slight decreases in input resistance (range 0-16%; mean = 6%). One cell was depolarized and the remaining 5 granule cells showed no change in membrane potential or input resistance. One cell tested with DAMEA (5 μM) showed a 3 mV hyperpolarization, whereas two others registered no change in membrane potential or resistance at 5 and 10 μM DAMEA.

The occasional small changes in membrane potential and resistance generally occurred at the higher enkephalin concentrations (5 - 10 μM). In contrast, 10 of 11 cells exhibited a pronounced reduction (mean: 33%; range: 17-52%) of the magnitude of the evoked synaptic

potentials (Fig. 4), even at low enkephalin concentrations (2 μM). Therefore, these studies, although still ongoing, indicate again a pronounced modulatory effect of the enkephalin analogues on synaptic responses in the dentate gyrus. However, the exact mechanism of this modulation (e.g., presynaptic versus postsynaptic) will require further study.

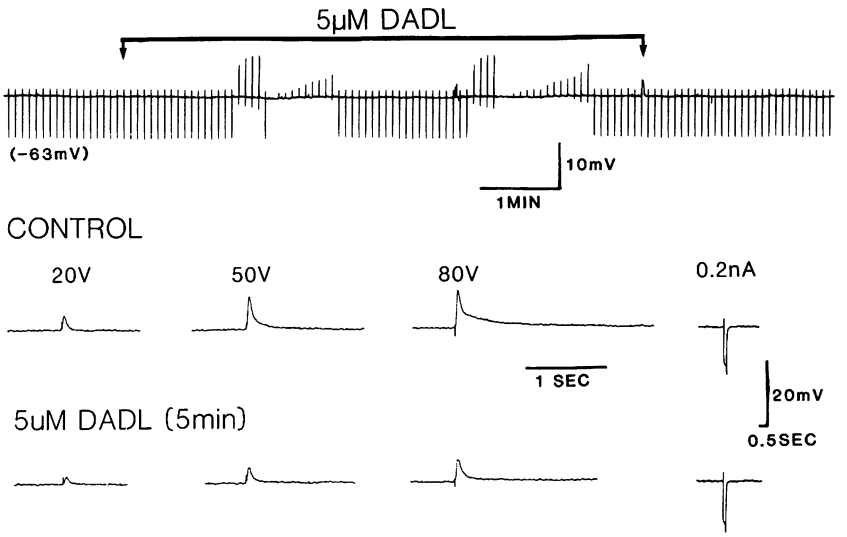

Fig.4 Effects of 5 μM DADL on membrane properties and on evoked postsynaptic potentials of a hippocampal dentate granule cell. Top: pen recording of part of an experiment illustrating the unchanged membrane potential and conductance (downward deflections) during DADL application. Bottom: specimen records of responses to synaptic stimulation (at 3 different stimulus strengths to perforant path) and current pulses in the presence and absence of DADL as indicated. Resting membrane potential = -63 mV. Late slow component of synaptic response is likely an inverted IPSP, also reduced by DADL.

5 NUCLEUS ACCUMBENS

Our interest in the nucleus accumbens (NAcc) stems from immunohistochemical studies showing a profuse enkephalin network in the NAcc (50) and from behavioral studies suggesting that the NAcc may be a key area involved in the reinforcing properties of heroine in a self-

administration paradigm (51). Therefore, to test the effects of opiates and opioid peptides on the neuronal excitability in this area, we have developed an in vitro coronal slice preparation of the NAcc using the vibratome method.

In preliminary studies, we again see evidence for a modulatory effect of the opioid peptides. In 10 cells tested for membrane properties, membrane potentials ranged from -74 to -92 mV (mean: -82 mV) and evoked spikes were 85 - 120 mV. No cells exhibited spontaneous action potential discharge, but all responded to stimulation of the white matter ventral to the NAcc with synaptic potentials. These potentials were always depolarizing and appear to represent EPSPs because of their diminution by membrane depolarization; however, in two cells, hyperpolarizing IPSPs were observed during depolarization of the cells with positive current injection. Most of the cells (80%) studied displayed pronounced inward rectification (increasing apparent input resistance at membrane potentials depolarized from the resting level).

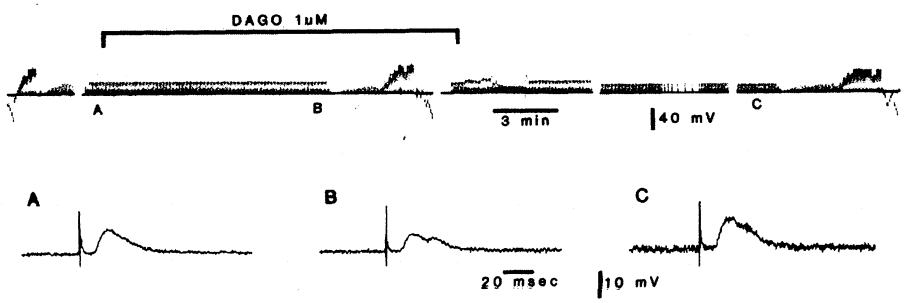

Fig. 5 Effects of DAGO on membrane properties of NAcc neurons. Top: Pen recording illustrating neither significant membrane potential nor conductance changes (upward deflections) during application of DAGO. Bottom: Specimen records (average of 5 sweeps) of evoked EPSPs illustrating the reversible attenuation by 1 μM DAGO. Gaps in record indicate omission of 3-4 min each. Resting membrane potential = -86 mV.

In all of the 7 cells tested with opioids, superfusion of enkephalin analogues (DADL, DAGO) at concentrations of 1-5 μM (all but 1 cell at 1 μM) had no measurable or consistent effect on membrane potential or input resistance. However, in all these cells, the synaptic potentials were reduced (by 20 - 100%) in magnitude by the enkephalin analogues (Fig. 4). Three of these cells could be recorded long enough

to show complete recovery from this effect after washout of the opioid peptides. Further studies are now being pursued to determine the generality of this modulatory effect and the locus and mechanism(s) of action.

6 CONCLUSIONS

The sum of our data indicate that low concentrations of the enkephalins have little direct effect on postsynaptic membrane properties in the four different brain regions studied, but cause pronounced diminution of evoked synaptic potentials. This effect could be exerted either pre- or postsynaptically. Thus, it is possible that the enkephalins could act presynaptically to reduce the release of both excitatory and inhibitory transmitters from nerve terminals. This action could arise from the block of Ca^{2+} currents as suggested from the dorsal root ganglion studies (20-23), or from a hyperpolarization of the nerve terminals due to activation of a K^{+} conductance (18,26). In this regard it is interesting to note that the reduction of Ca^{2+} currents by GABA-B receptor activation, which leads to a marked reduction of synaptic potentials (52) is blocked by elevation of intracellular cyclic AMP (54). Similarly, the net effect of the enkephalins could also depend upon intracellular cyclic nucleotide levels. It was reported in a recent paper (55) that DADL had an inhibitory effect on the Ca^{2+} current in neuroblastoma x glioma hybrid cells. This effect was reduced by pretreatment with pertussis toxin and restored by intracellular injection of G-proteins (G_i and G_o). It was suggested that G_o is involved in the functional coupling of opiate receptors to neuronal voltage-dependent Ca^{2+} channels.

However, our observations of the ability of some receptor subtype selective opioid peptides (DAGO, DADL) and morphine to reduce the depolarizing effects of excitatory transmitters like L-glutamate seems to suggest that at least the effect of the opioids on EPSPs might be exerted postsynaptically. The effects on the IPSPs could then derive from an anti-glutamate effect at the level of the inhibitory interneuron, preventing activation of the interneuron by excitatory input and thus resulting in reduced output of inhibitory amino acid neurotransmitters like GABA.

It is not yet clear why we do not see the pronounced enkephalin-induced hyperpolarizations such as those described for the locus coeruleus and substantia gelatinosa neurons (18,25,26) and the myenteric plexus (1). Perhaps these differences in the opioid responses reflect true differences in cell types and/or their opiate receptors.

It may be argued that the average membrane potential of the various cells recorded could provide the difference in the response type. Thus, if the cells we studied had resting membrane potentials near the K^+ equilibrium potential, we might then be in the range where the driving force would be inadequate to produce a K^+ mediated potential change. Still, some of the cells we recorded had somewhat lower resting membrane potentials (see figs. 2 and 4) or were depolarized by current injection (44); one would expect that at least these cells should be strongly hyperpolarized, but this was not seen. Furthermore, the extensive current-voltage curves obtained in many of the cells we studied should show some change if a K^+ conductance were activated, yet no such changes were seen with the enkephalins.

Another possibility is that the hyperpolarizations result from the enkephalin blockade of tonic glutamatergic, depolarizing input. It might be argued that such an anti-glutamate effect should produce an increase in input resistance rather than the reported decrease. However, a marked inward rectification such as that seen in many central neurons might give an apparent reduction in input resistance with removal of such an excitatory drive.

Another problem to be resolved concerns the inability by some to observe an anti-glutamate or anti-EPSP effect of the enkephalins (16). To some extent these negative findings might derive from the use of different enkephalin analogues that might have preferential binding to different opiate receptor subtypes. Another possibility is that some component of the depolarizations or EPSPs tested might involve an NMDA component, which has been shown not to respond to the opioids (see: CEREBRAL CORTEX). It is therefore possible that previous studies did not detect the anti-EPSP effect because near-maximal stimuli were used to evoke the EPSPs. Our input/output curves constructed for EPSPs of 3 different brain regions (CA1, cerebral cortex and NAcc) show the most pronounced opioid effect at lower to medium stimulus intensities.

In summary, our intracellular data derived from several disparate brain regions suggest that the most potent effect of the enkephalins appears to be the reduction of synaptic potentials, probably exerted postsynaptically via modulation of glutamatergic effects. This action would tend to apply an effective braking mechanism on a large percentage of excitatory neuronal inputs. However, the final outcome of release of endogenous enkephalins may depend upon the local circuitry and whether inhibitory interneurons are also involved. The presence of different levels of the various receptor subtypes will also likely be a factor in determining the final response of the target cell for the endogenous opioids.

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