Pharmacology Biochemistry & Behavior, Vol. 18, pp. 255–262, 1983. Printed in the U.S.A.

Opiates and the Hippocampus: A Review of the Functional and Morphological Evidence

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Received 20 July 1982

CORRIGALL, W. A. *Opiates and the hippocampus: A review of the functional and morphological evidence.* PHARMACOL BIOCHEM BEHAV **18**(2) 255–262, 1983.—In spite of a small number of opiate receptors, recent electrophysiological studies have shown that exogenously applied opiates have a powerful excitatory effect on the principal neurons of the hippocampus. These studies have spawned a reconsideration of opiate binding sites in the hippocampus and investigations of the presence of endogenous opiates within this limbic area. This literature, as well as the relevant behavioral studies, is reviewed, and questions of the role of hippocampal opiates and their binding sites are raised.

Hippocampus Opiates Morphology Function

ACTION ON HIPPOCAMPAL NEURONS, AND MECHANISM

In a chronological sense, electrophysiological studies of opiate action within the hippocampus predated, or at least occurred in parallel with, demonstrations of either binding sites or endogenous ligands in this region, and for this reason studies of effect and mechanism are best dealt with first. The initial recording studies may have been undertaken as much because of the general popularity of the hippocampus for electrophysiology as for any other reason, since receptor populations would not have been a great incentive to examine this area. Whether serendipitously or by design, however, these studies demonstrated a pronounced and unexpected effect of opiates. Subsequently there have been a number of other investigations of an electrophysiological, histological, neurochemical and behavioral nature. The rapid growth of this area of research and, more importantly, the possible functions of an endogenous opioid system in the hippocampus, have prompted this review.

The initial neuropharmacological studies of opiate effects in the hippocampus were in disagreement one with another. Nicoll *et al.* [51] and Chou and Wang [5] both reported consistent increases produced by opiates in hippocampal single unit activity in the rat (with iontophoresis) and in the cat (with intravenous administration) respectively, and antagonism of these increases by naloxone. By contrast, Linseman and Grupp [43] found that hippocampal units in naive rats were relatively unresponsive to low doses of intravenous morphine (although spiking in the hippocampal EEG was observed), whereas naloxone did produce changes in hippocampal units in dependent rats. Segal [56] and Fry *et al.* [20] reported that only a small percentage of hippocampal units were affected by iontophoresis of opiates, with both increases and decreases in rate observed and in general with a lack of naloxone reversibility. The latter study also reported no differentiation of responses to the stereoisomers levorphanol and dextrorphan, and concluded that the observed effects were probably not mediated by stereospecific opiate receptors.

Zieglgänsberger and his co-workers [69] reexamined the action of iontophoretically applied opiates on hippocampal neurons and found more consistent effects, namely, that presumed pyramidal cells were excited by opiates while the spontaneous firing of simultaneously recorded presumed inhibitory interneurons was suppressed. Opiates also blocked evoked inhibition as measured in pyramidal cell poststimulus time histograms. Opiate excitations were antagonized by iontophoresis of bicuculline (antagonist of γ aminobutyric acid, GABA) and of Mg^{++} (to decouple the neurons synaptically), as well as by naloxone. To explain this data, they proposed that opiates augmented the activity of pyramidal neurons by depressing the activity of a population of GABAergic interneurons (the basket cells of the hippocampus) which generate feedback inhibition. At the same time, several laboratories, including our own, were investigating the effects of perfusion-applied morphine or opioid peptides on evoked field potentials in a slice preparation of the hippocampus maintained *in vitro* [7, 8, 15, 38, 45, 53]. These studies all reported that opiates consistently increased the amplitude of the evoked population spike in the CA1 region of the slice, and led to production of additional population spikes as well. The effect on the field potential was corroborated *in vitro* with iontophoretically applied morphine [11]. These observations are in essential agreement with those aforementioned unit studies *in vivo* which reported increased pyramidal cell firing following opiate administration, since the population spike is interpreted as arising from the synchronous generation of action potentials by a number of neurons. In probing the mechanism of these increases in

population spike amplitude, the following observations were made: (1) the population EPSP recorded at the level of the activated synapses onto the pyramidal cells was not affected by morphine, suggesting that the observed augmentation of pyramidal cell activity was not due to increased excitation [8, 15, 45, 53]; (2) the effect was stereospecific [8] and antagonized by naloxone [8, 11, 15, 45], suggesting mediation by opiate receptors; (3) the effect was blocked or reduced by exogenously applied GABA [8] or pentobarbital [53], which prolongs GABA action in the hippocampus, and was mimicked by the GABA antagonist picrotoxin [8, 15]. Dunwiddie *et al.* [15] demonstrated that the magnitude of the enkephalin effect correlated with the extent of inhibition in the tissue as measured by paired-pulse techniques, and moreover that the enkephalin effect could be reduced if the slices were made moderately hypoxic before application of the opiate (the assumption in the latter case being that interneurons are more sensitive than other components to hypoxia). This group also reported that the activity of presumed inhibitory interneurons was depressed by enkephalin [38], similar to the observations *in vivo* [69]. This evidence taken in total supported, or at least did not belie, the hypothesis that opiates act on GABAergic inhibitory interneurons. At one time we entertained the possibility that there might be two separate mechanisms, one for the increase in the amplitude of the primary spike and another for the production of additional population spikes; in fact, we demonstrated that significant tolerance developed to the additional-spike effect but not to the enhancement of the primary spike amplitude [9], a differentiation which is congruent with the idea of two mechanisms. In view of subsequent studies, however, this interpretation appears to be untenable.

If for no other reason, these extracellular studies were useful by virtue of their consistency, documenting a reliable excitation of pyramidal cells by opiates and generating interest in elucidating further the mechanism of this effect. Several laboratories have investigated the effects of opiates using intracellular techniques to record from pyramidal cells and granule cells *in vitro*, either in the slice preparation or in tissue culture. While these studies do provide further information, they are to varying degrees contradictory.

Deadwyler and Robinson [10] reported that morphine depolarized the pyramidal cell membrane; however this study used a single extremely high dose (1 mM) and the possibility of non-specific effects cannot be excluded, particularly in view of the small extent of naloxone reversal obtained. Furthermore, in contrast, a number of other studies discussed below in detail have found that opiates have no effect on membrane potential or input resistance [21, 29, 46, 49, 50, 57] arguing against a direct effect of these agents on the pyramidal cell membrane.

Gähwiler [21] examined the effects of a number of different opiates on presumed pyramidal cells in tissue culture. He observed an increased amplitude and duration of spontaneous slow potentials which could lead to the development of large paroxysmal-like depolarizing shifts and a block of evoked IPSP's. When cells in the cultures were synaptically decoupled with Mg^{++}, spontaneous activity was not altered by the enkephalin compound, suggesting an indirect effect. With a low concentration of either an enkephalin analogue or β-endorphin, the increase of EPSP amplitude and decrease of IPSP amplitude and duration were seen to have their onset before the development of bursting discharges and depolarizing shifts, rather than as a result of altered permeability caused by these epileptic-type events [23]. The conclusion was that the data could be accounted for by attenuation of IPSP's but that facilitation of excitatory transmission, while unlikely to be the sole mechanism, could occur simultaneously. The observation of paroxysmal discharges is in conflict with the study by Dingledine [13], discussed below, in which it was reported that even high (micromolar) concentrations of D-Ala, D-Leu enkephalin did not typically produce depolarizing shifts. This discrepancy does not appear to be due to differences between tissue slice and tissue culture, however, since others have reported paroxysmal depolarizing shifts in the slice with β-endorphin [57] and the analog FK33-824 [29].

Several other of the intracellular studies have also reported a reduction in IPSP's by opiates [46, 49, 50, 57] without direct antagonism of GABA [46, 49, 50]. Siggins and Zieglgänsberger [57] reported that many of the neurons in their study showed reduced EPSP's as well. Nicoll *et al.* [49, 50] compared orthodromically versus antidromically elicited IPSP's and found that the former were attenuated more. They interpreted this as an indirect action of opiates on pyramidal cells more prominently on feedforward rather than recurrent inhibition.

While the disinhibition mechanism of a feedforward and/or feedback system has received the numerical majority of support, there have been other mechanisms put forward. Haas and Ryall [29] observed, in both pyramidal and granule cells, the same increase in size and number of evoked extracellular population spikes and the same lack of effect on membrane potential and resistance as other workers, but in contrast found an increase in amplitude and duration of intracellularly measured EPSP's (and an increase in the extracellular EPSP). They also reported no attenuation of inhibition, either as measured extracellularly from peristimulus time histograms or from intracellular IPSP's. They concluded that enkephalins excite pyramidal and granule cells in the hippocampus by causing an increased presynaptic release of excitatory transmitter.

Another alternative to the disinhibition hypothesis has come from Lynch *et al.* [44] who examined opiate effects in the slice preparation using extracellular paired-pulse techniques to assess the role of recurrent and feedforward inhibition. These workers found an opiate effect on neither of the inhibitory systems. They proposed that opiates might act by changing the active properties of the pyramidal cell dendrites themselves or by affecting the coupling of active dendritic spikes to the somatic spike initiation zone. Evidence against this mechanism is discussed below.

Dingledine [13] has reported a comprehensive investigation of the effects of D-Ala, D-Leu enkephalin (DADL) in the CA1 region of the hippocampal slice. He observed the typical increase in population spike amplitude along with production of additional spikes. In intracellular records he found that the effect of DADL was to increase the size and often the duration of evoked EPSP's, without causing changes in membrane potential, input resistance, spike threshold, or antidromic field potentials. Based on the shape of the membrane changing curve during hyperpolarizing current passage, dendritic membrane resistance also appeared to be unaffected. Input/output functions for population spike versus field EPSP showed higher firing probability for a given excitatory postsynaptic current, while the input/output function for field EPSP versus presynaptic fiber volley was unaffected, in essential confirmation of most earlier investigations suggesting that increased pyramidal cell firing was

not due to an increase in excitatory transmitter release. Recurrent IPSP's generated by antidromic stimulation were not affected by DADL; the hyperpolarization and conductance increase produced by GABA was also unaffected, as was the plot of recurrent IPSP versus membrane potential. Recurrent inhibition still showed up unaltered by the opiate when examined extracellularly in an antidromic-orthodromic paired-pulse design. Similarly feedforward inhibition, tested by double pulse orthodromic stimulation, was unchanged.

Dingledine considered two possible mechanisms for these effects. The first hypothesis was based upon modulation of excitatory transmission by means of some type of signal amplification system, as also suggested by Lynch *et al.* [44]. Against this, however, it has been reported that DADL does not affect calcium spikes [12, 28], and that the effects of dendritic application of the excitatory agents dl-homocysteic acid [29] and N-methyl aspartate [12] were not enhanced by DADL. The alternative possibility suggested by the data was that DADL and other opiates produce an attenuation of some form of dendritic inhibition which is electrotonically remote from the pyramidal cell somata.

In an iontophoretic study of opiate actions in the limbic system, French and Siggins [19] tested whether acetylcholine might be involved in generating excitations in the hippocampus. They found that the muscarinic cholinergic antagonists atropine and scopolamine were ineffective in altering opiate excitations. Furthermore, the opiate excitations were still obtained after septal lesions which interrupt cholinergic innervations of the hippocampus. These findings suggest that opiates do not excite hippocampal pyramidal cells (at least in studies with local application) by inducing presynaptic release of acetylocholine.

Another approach to take in elucidating the mechanism would be to examine directly the neurotransmitter changes produced by opiates. To date, little has been done that could be classified as a direct effect of opiates on hippocampal neurotransmitters. For example Botticelli and Wurtman [3] have shown that intraventricular β-endorphin or enkephalin produced a naloxone-reversible increase in hippocampal acetylcholine, and Vallano and McIntosh [65] have demonstrated that morphine administered *in vivo* led to increased choline uptake into hippocampal synaptosomes, but these effects are most probably due to a primary action of opiates on cholinergic neurons in the septum, and as such the findings are not relevant to an opiate effect obtained *in vitro*, or *in vivo* with iontophoresis or microinjections. However, in a recent report Fan *et al.* [17] examined the effect of an enkephalin analogue on the release of endogenous GABA from hippocampal slices; they found no effect of enkephalin on either spontaneous or potassium-evoked release of GABA. Although the study used but a single dose of the enkephalin, it was high enough to suspect that an opiate effect should occur. The evidence from this study of course does not auger well for the disinhibition hypothesis, at least as it relates to GABAergic inhibitory interneurons.

Discrepancies between effects observed in early studies may have been due to a number of factors. In the case of single unit studies, recordings may have been from a mixed sample of interneurons and pyramidal cells and, if the disinhibition via interneuron hypothesis were true, the sample would have shown both depression and excitation by opiates. This however, seems to be an unlikely possibility in any large number of cases. There may have been interactions between the opiates tested and the anesthetic used (see for example [39] and [64]). Alternatively, the technique of assessing effects of iontophoretically-applied opiates on a background of glutamate-evoked activity may also be problematic; we have observed [7] that the amount of naloxone reversibility obtained depended upon the extent of activation of the pyramidal cells (by electrical stimulation rather than glutamate application, but the extrapolation is not unreasonable). Other difficulties with antagonists applied iontophoretically have been noted [19].

In spite of the initial questions of effect per se, subsequent studies have shown that opiates produce a consistent augmentation of pyramidal cell activity. Furthermore, these cellular effects produced in the hippocampus may be related to the seizure activity which has been reported to occur in the EEG following intraventricular [30] or intrahippocampal [16] injections of opiates, as well as in "EEG" recorded from regio superior explants of hippocampal tissue matured in the anterior chamber of the eye [62]. For example, we have observed that changes in hippocampal CA1 field potentials can in some cases be accompanied by high amplitude events in the EEG [41]. The simple conclusion regarding the mechanism of the cellular effects, however, is that it remains unclear; we know the effects of morphine, β-endorphin, enkephalin and synthetic opiate analogs on the activity of the principal neurons of the hippocampus, but we do not know with certainty how these opiates exert their effect. Certain observations have been common, however, across at least a majority of studies. For example, there seems to be no direct antagonism of GABA, there appear to be no direct effects on the somatic membrane, and presynaptic release of excitatory transmitter is not increased. A conflict remains, however, between studies on one hand which point toward an effect of opiates on feedforward and/or feedback inhibition, and those which report no action on these inhibitory systems. Initial hypotheses of opiate effects on inhibition were based upon extracellular data, particularly evoked field potentials and presumed interneurons that were suppressed by opiates. In the latter case the identification of the interneurons was not absolute; in the former case, while it is not clear what reasons account for discrepancies (c.f. [15] and [44]), it is clear that extracellular data cannot solely be relied upon to provide the mechanism of the opiate effect. However, discrepancies between intracellular studies which show an apparent opiate-produced reduction in IPSP's and those which show unaltered IPSP's are particularly worrisome and cannot be reconciled in any facile manner: on both sides the studies have used the *in vitro* hippocampal slice preparation, with which one has substantial experimental control. Nonetheless there are variables between studies, only some of which may be put aside. For example, Nicoll *et al.* [49] used pentobarbital in the bathing medium to potentiate IPSP's, while Dingledine [13] did not; however, a subsequent report by Nicoll *et al.* [50] claimed that their findings do not depend upon the presence of pentobarbital (nor for that matter on whether chloride-containing electrodes or chloride-substituted electrodes are used). Furthermore, although several different opiate agonists have been used in the intracellular studies, there is enough overlap to rate as unlikely the possibility of different agonists acting by different mechanisms. However, what may prove to be a significant variable is the lack of consistent ionic concentrations in the bathing media used in these studies. In terms of percent changes from study to study this is particularly true with respect to calcium, magnesium and potassium. Electrical events in hippocampal neurons depend, in ways not yet fully understood, upon conductance changes to various ions. If

the mechanism of opiate action upon hippocampal neurons depends upon conductance changes involving these ions, consistency of results and interpretation will not be forthcoming until this issue is addressed. In spite of problems and discrepancies, it seems not unduly expansive to suggest that the search to pinpoint the mechanism of the opiate effect in the hippocampus will entrain new insights into synaptic physiology in general.

In concluding this section on mechanism and effect, two qualifying points should be made. One is that the existing studies have been primarily of the CA1 region whereas, as discussed below, there is strongest evidence for a complete endogenous opiate system in the mossy fiber to CA3 pathway. Nevertheless, a few recording studies have examined the CA3 region as well, and observed effects similar to those in CA1 [29, 46]. The second point is that, in contrast to the effects in CA1, Tielen *et al.* [63] have recently reported that an enkephalin analog depressed the perforant path-evoked population spike in the dentate granule cells *in vitro*. This, however, is in disagreement with other observations which have shown an increased dentate population spike both in the slice preparation ([29], also Corrigall and Linseman unpublished observations) and *in vivo* [41].

BINDING SITES AND ENDOGENOUS LIGANDS

Both binding studies with microdissected homogenates [36] and autoradiographic examinations [1] have shown the hippocampus to contain a low number of opiate receptors. In parallel with this, the absolute amounts of endogenous opiate ligands are not particularly striking as compared to other areas of the brain (see for example [54, 68]). However, because of the prominent effects of exogenously applied opiates on hippocampal physiology, one is led to the conclusion that the receptors present must at least be located strategically if not numerously. Recent studies have shown that there are notable local densities of receptors in various hippocampal subfields and across the somatic/dendritic extent of these subfields. Equally important, investigations have begun to show that there are circumscribed distributions of endogenous ligands within the hippocampus, and that these distributions match known fiber projections.

Using immunohistofluorescence to survey enkephalin-containing areas of the central nervous system, Sar *et al.* [55] reported that positively labelled fibers/terminals were to be found in the CA2 area of the hippocampus; however this observation was not replicated by Wamsley *et al.* [66]. Hong and Schmid [33] measured the concentration of met-enkephalin in the hippocampus. They found that the temporal hippocampus contained roughly three times the concentration of met-enkephalin as the septal region, and that the CA3 and dentate areas each contained approximately twice the concentration as CA1, CA2 or the subicular regions. Both mechanical and chemical lesion studies suggested that at least some of the enkephalin was intrinsic to the hippocampus.

A more detailed morphological picture of enkephalin systems in the hippocampus has come from three independent immunohistochemical studies all of which are in substantial agreement one with the other, in spite of their use of tissue from three different species [18, 25, 59]. These studies have shown that the mossy fiber system displays enkephalin-like immunoreactivity. The immunoreactivity matched the topographical localization of zinc, which has been a classical marker for the mossy fibers, and was evident in the granule cells within the dentate gyrus, fibers within the hilus and regio inferior, and the mossy fiber swellings; furthermore it was eliminated in appropriate regions of the mossy fiber projection area by hilar lesions. In addition to the apparent enkephalin content of the mossy fibers, one of the studies [25] also identified a second fiber system with enkephalin-like reactivity which appeared to arise extrinsically to the hippocampus, passing by way of the retrohippocampal/subicular region to distribute in CA1 within stratum lacunosum-moleculare and in the distal third of the molecular layer of the dentate gyrus (the latter projection matching the distribution of afferents from the lateral entorhinal cortex). Both Gall *et al.* [25] and Fitzpatrick and Johnson [18] documented various reactive cells in regio superior between strata radiatum and lacunosum-moleculare, in stratum radiatum, and in stratum pyramidale. It is possible that some of these neurons represent the endogenous source of opiate for the effects which have been observed in CA1 with exogenous application of enkephalins and morphine.

Regarding the distribution of opiate binding sites, several studies have noted that labelling of the hippocampus in autoradiographs is stratified, being localized in and around the pyramidal cell layer but falling off in intensity in stratum radiatum and the molecular layer [14, 27, 31, 47]. Goodman *et al.* [27] made the further discrimination that it is predominantly a μ-type receptor population which is localized to the pyramidal cell layer, with δ-type receptors being distributed more diffusely and with lower density. Chang *et al.* [4] also reported a greater number of morphine than enkephalin binding sites in the hippocampus. Both of these latter two studies were with rodent tissue. Opposite findings have been reported for bovine tissue however [52]; in this case, the dentate gyrus and hippocampus proper were found to have high ratios of δ to μ binding sites, and in fact showed up as two of only several areas which registered a predominance of δ sites. Meibach and Maayani [47] examined the distribution of tritiated dihydromorphine binding sites across the various subfields of the hippocampus. The CA2 area was seen to have the greatest binding, compatible with the observation of opiate-containing fibers in this area [55], followed by CA1, CA3 and the dentate gyrus. Examination within subfield pointed out the perisomatic concentration in CA1, while in CA2 the binding was high from the cell body region out to the molecular layer. The binding densities in CA3 and dentate were uniform but lower than in CA2 or in the peak somatic area of CA1. Duka *et al.* [14] compared the binding of tritiated etorphine to that of DADL. The former labelled the pyramidal cell layer throughout CA1 to CA4 with more moderate labelling in oriens, radiatum and molecular layers, while the enkephalin bound most prominently in CA2 and, more moderately, in CA3 and the subiculum.

Functionally there has been limited investigation of the binding sites for opiates within the hippocampus. By examining the threshold concentrations of hippocampal μ, δ and κ agonists necessary to obtain burst discharges in hippocampal cultures, Gähwiler and Maurer [24] concluded that μ receptors were primarily responsible for pyramidal excitations. Gähwiler [22] also observed desensitization to the effects of the μ agonist FK33-824 which carried over to DADL. Dingledine [13] showed with iontophoretic mapping that DADL sensitive sites in the slice preparation were concentrated in pyramidale and oriens. Since DADL has limited specificity as a δ agonist [4], DADL effects per se and its lack of effect in tissue desensitized with FK33-824 likely arise because DADL binds to the μ population. These functional

observations are then in fundamental agreement with binding studies in terms of receptor type and distribution.

A few studies have examined the plasticity of the opiate systems in the hippocampus. This is an important question, since, if endogenous opiate systems actually function within hippocampus, one might expect to be able to demonstrate changes in ligand levels or in binding sites as a function of changes in the state or behavior of an animal. A step in this direction has been taken by Hong *et al.* [34]. These workers have shown that intrahippocampal kainic acid caused recurrent motor seizures which correlated with a post-seizure increase in hippocampal met-enkephalin content lasting days to weeks. The increase was specific to the hippocampus, specific for met-enkephalin (compared to substance P, GABA, and glutamate), also occurred with electroconvulsive shock or intraseptal, intrastriatal or intraventricular kainic acid injections, and was blocked if convulsions were blocked by pretreatment with phenobarbital. Gall *et al.* [25] reported that the immunoreactivity of the mossy fibers was abnormally intense in animals with hilar lesions, both in the fibers on the side contralateral to the lesion and in surviving fibers on the ipsilateral side. Both hilar lesions and intraventricular kainic acid were shown subsequently to produce similar increases in enkephalin-like immunoreactivity localized to the mossy fiber system [26], suggesting that the findings of Hong *et al.* [34] with kainic acid-induced seizures may apply to the mossy fiber system. Finally, it has been shown that zinc ions (among other ions which reduce receptor binding) decrease the binding of ^{3}H-enkephalinamide in the hippocampus through a decrease in receptor affinity [60]. This data may signify a physiological interaction between zinc and enkephalin in the hippocampus; that is, zinc, which is known to be present in the mossy fiber system, may regulate enkephalin binding in CA3.

PHYSIOLOGICAL RELEASE AND ROLE OF HIPPOCAMPAL OPIATES

There is some evidence to support the concept that the endogenous ligands for μ and δ receptors may be met- and leu-enkephalin respectively [27]. In the hippocampus the predominance of μ sites (at least in rodent hippocampus) is in keeping with the greater content of met- than leu-enkephalin [68]. In addition, it has been suggested that μ receptors mediate the analgesic effects of opiates, while other effects such as epileptic and behavioral events are due to δ sites (see, for example [27]). However, in a preliminary study, we have assessed analgesia and various other behavioral indices such as temperature changes, openfield behavior, etc. following microinjections of morphine into the dorsal hippocampus (Linseman and Corrigall, unpublished observations). Neither analgesia nor changes in other behavioral indices were observed. The only behavioral change that we noticed was stillness following drug infusion which may have been due to seizures or spreading depression [58]. These findings then are either not compatible with the observed predominance of μ receptors in the hippocampus, or with the idea of μ receptors mediating analgesia and δ mediating epileptic effects.

An alternative possibility for function that we questioned was whether an endogenous opiate system might have a role in potentiation in the hippocampus [40]. Logically, our jumping off points were that opiates do alter hippocampal field potentials in a fashion similar to potentiation, and the demonstration by Hong and co-workers noted above that ligand levels are changed by seizures (the link between seizures and kindling/potentiation tacitly underlies this rationale). In addition, we had evidence from our studies with the slice preparation that at the mid to high end of the stimulus/response function there was a lack of full reversal of the opiate-effect by naloxone [7]; this data suggested to us a possible interaction between stimulation and opiate which could lead to a persistant change remaining after removal of the agonist from the receptor. However, notwithstanding these arguments, our investigation showed no effect of pretreatment with naloxone on potentiation of CA1 responses, leading us to conclude that endogenous opiates are not involved in this phenomenon. In our study the potentiation was induced stepwise over the course of several hours, and it would be worthwhile to reexamine this question using a kindling type of approach wherein actual seizures are induced over a number of days of stimulus presentation, this being perhaps more in line with the kainic acid induced seizures. In addition, it would be useful to investigate potentiation/kindling electrophysiologically in the mossy fiber-CA3 pathway, since it is this pathway which has been shown to exhibit enkephalin immunoreactivity, and it may be that the seizure-induced alteration in ligand levels occurs here.

Other investigations have focussed on the mossy fiber to CA3 system. Collier *et al.* [6] have reported that granule cell stimulation occurring during a delay period in a spatial memory task disrupted performance, but that pretreatment with naloxone prevented this deficit. In contrast to these findings, we [42] have observed no amelioration by naloxone of performance deficits caused by perforant path stimulation during the delay period in a similar although not identical spatial memory task. Since perforant path stimulation at the parameters we used should lead to a substantial activation of the mossy fiber system, our study does not agree with that of Collier *et al.* [6], and leaves unanswered the question of a role for an endogenous opiate system in working memory. In the same study we also obtained electrophysiological evidence that opiates are not released by activation of the mossy fibers or indeed of fibers in stratum radiatum or the perforant path, since the field potential responses evoked by stimulating these pathways were unaffected by naloxone.

Of course the mossy fiber system is not solely enkephalinergic, if indeed it utilizes an opiate at all as neurotransmitter. Rather these fibers are thought to use an amino acid as transmitter [61], although recent evidence suggests neither glutamate nor aspartate are involved [37, 48]. It appears that the mossy fibers do not utilize enkephalin as transmitter at least in a simple way, however, and they therefore belong to the growing category of neurons containing both a peptide and a "classical" neurotransmitter [32]. It may be that the "classical" transmitter is regularly used for neurotransmission, while the peptide has a more specialized role. The obvious and well-used example is one in which the peptide serves more a modulatory role, setting a level of excitability upon which the action of the neurotransmitter may be superimposed. In this respect, observations of the slow onset of peptide effects and their protracted timecourse in the hippocampus are relevant (e.g., see [13]). The idea of a modulatory function in part led us to examine whether other brain regions might affect hippocampal neurotransmission by causing release of opiates [42]. To date these studies are preliminary and have examined the changes in hippocampal transmission arising from prestimulation of three different areas, the septum, the midbrain reticular formation, and the raphe nucleus during slow wave sleep. Stimulation of any of these areas is known to augment hippocampal responses [2,

35, 67], but naloxone was not effective in reducing the enhancement due to prestimulation. Obviously other sites of prestimulation will have to be examined before definitive conclusions are possible regarding opiate release by this type of mechanism.

Clearly our knowledge of how the endogenous opiates within the hippocampus are released, and of what these opiates might effect when released, is not far advanced. Intrahippocampal opiate microinjections as a probe for what endogenously-released ligands might cause behaviorally suffers from being a very open-ended approach. On the other hand, further search for an opiate dependence in behavioral tasks which are known to depend upon hippocampal function appears to be a necessary route to follow. A problem with this approach, as well as with studies which attempt to reverse putative opiate-mediated electrophysiological responses, is that alteration of the behavior or of the responses by naloxone or another antagonist is required. Although the endogenous ligands in the hippocampus appear to be enkephalin-like in immunohistochemistry, they may be in fact structurally and pharmacologically very distinct. The choice of the appropriate antagonist is therefore somewhat arbitrary; certainly negative findings with an antagonist in these types of studies should be viewed within this context.

CONCLUSIONS

Overall, there appear to be two primary opiate "systems" identified in the hippocampus. One of these, in CA1, has been described extensively with electrophysiological studies and to some degree with labelled agonist binding, but immunohistochemistry has not shown any substantial projection to the area. A relevant question is, therefore, what are the endogenous presynaptic sources of opiate ligand for the powerful excitation known to occur with exogenously applied opiate? Is the ligand localized to the neurons shown to be enkephalin immunoreactive in this area, and if so, is it released by stimulation of afferents to the hippocampus? Or is the ligand not enkephalin but another opiate whose projection has yet to be elucidated? Alternatively, in view of the relative scarcity of labelled fibers in the CA1 area, the possibility must be entertained that opiate receptors in this area are not involved in synaptic functions, but rather are extrajunctional and subserve the binding of opiates released from sources other than local hippocampal fibers. The other putative opiatergic "system" in the hippocampus is the mossy fiber-CA3 projection which has been identified in immunohistochemical studies as well as in investigations of binding sites and effects of exogenously-applied opiates in CA3. So far, however, this "system" has eluded physiological and, generally, behavioral attempts to demonstrate opiate neurotransmission.

Although it seems likely that the changes produced by opiates at the cellular level integrate at the EEG level to seizure-like activity, it is unlikely, or at least difficult to reconcile, that the role of any endogenous system would be epileptogenic. It is possible of course that opiate-induced seizures might be an artefact of exogenous application either because of relatively high concentrations compared to what would be released physiologically or because of wide-spread activation. A question relative to either system, therefore, is its role in behavior. Are opiates participants in regulatory functions, or in the more elusive functions of learning and memory ascribed to the hippocampus? Perhaps opiatergic systems in the hippocampus are involved primarily with euphoric effects; this may become clearer with investigation of whether either local exogenous administration or activation of the endogenous system is reinforcing. Related to this, because of the plasticity of this limbic area, do abused opiates acquire any of their addictive characteristics because of interaction with the hippocampus?

ACKNOWLEDGEMENTS

I would like to thank Mrs. June Shepperd for typing the manuscript and Ms. RoseMarie Lucato for commenting on it. I also wish to acknowledge the valuable discussions with my colleague, Dr. M. A. Linseman. This work was supported by the Addiction Research Foundation of Ontario.

NOTE ADDED IN PROOF

Recent evidence as yet published only in preliminary form suggests that there may be overlap of the enkephalin and dynorphin systems in the mossy fiber projection (McGinty, J. F. *et al., Soc Neurosci Abstr* **8:** 98, 1982; Watson, S. J. *et al., Soc Neurosci Abstr* **8:** 98, 1982). Although questions of the pharmacological action of exogenously applied dynorphin and, as with enkephalin, its release by stimulation of the mossy fibers remain to be answered definitively, at the present time one must assume that the dynorphin family of opioid peptides may figure in our understanding of hippocampal opiate systems.

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