NEUROANATOMICAL AND PHARMACOLOGICAL CORRELATES OF THE BEHAVIORAL MANIFESTATIONS OF INTRAVENTRICULAR ADMINISTRATION OF KAINIC ACID IN THE RAT

By
THOMAS HERBERT LANTHORN

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA
1978
ACKNOWLEDGEMENTS

Words cannot express the heart-felt thanks I give to all those who have made this life possible. Nevertheless, thank you.

There are a few people who must be singled out from the many because of their direct contribution to insuring that this dissertation was a successful event.

First, SueAnne, from whom all life draws its meaning; my friend and my wife.

Dr. Robert L. Isaacson; who always seemed to understand my needs and to give direction when I didn't see any. I could not do better than to have half his breadth of foresight.

Dr. Carol Van Hartesveldt; a true critical scientist worthy of being imitated, to whom this final written work owes much.

The rest of my committee; Drs. Adrian Dunn, Charles Vierck, L. James Willmore, and Marc Branch, who received my work with an interest I hope to deserve.

Mrs. Virginia Walker; who, in the final analysis, made this dissertation a reality--a right hand and a friend.

And last, SueAnne, who put up with more than could be expected of anyone, who brought me joy and beauty, and to whom the rest of my life is dedicated.
<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Glutamate</td>
<td>1</td>
</tr>
<tr>
<td>Kainate</td>
<td>7</td>
</tr>
<tr>
<td>DOSE-RESPONSE STUDY OF BEHAVIOR INDUCED BY KAINATE</td>
<td>18</td>
</tr>
<tr>
<td>Introduction</td>
<td>18</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>18</td>
</tr>
<tr>
<td>Results</td>
<td>21</td>
</tr>
<tr>
<td>Discussion</td>
<td>24</td>
</tr>
<tr>
<td>DOSE-RESPONSE STUDY OF LESIONS INDUCED BY KAINATE</td>
<td>26</td>
</tr>
<tr>
<td>Introduction</td>
<td>26</td>
</tr>
<tr>
<td>Methods and Materials</td>
<td>26</td>
</tr>
<tr>
<td>Results</td>
<td>27</td>
</tr>
<tr>
<td>Discussion</td>
<td>28</td>
</tr>
<tr>
<td>CHOICE OF KAINATE-INDUCED BEHAVIORS FOR INTENSIVE STUDY</td>
<td>30</td>
</tr>
<tr>
<td>TIME-COURSE OF KAINATE-INDUCED WET-DOG SHAKES</td>
<td>33</td>
</tr>
<tr>
<td>Introduction</td>
<td>33</td>
</tr>
<tr>
<td>Methods and Materials</td>
<td>33</td>
</tr>
<tr>
<td>Results</td>
<td>33</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>34</td>
</tr>
<tr>
<td>SUSCEPTIBILITY OF KAINATE-INDUCED WET-DOG SHAKES TO GLUTAMATE</td>
<td>36</td>
</tr>
<tr>
<td>RECEPTOR BLOCKAGE</td>
<td></td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Introduction</td>
<td>36</td>
</tr>
<tr>
<td>Methods and Materials</td>
<td>37</td>
</tr>
<tr>
<td>Results</td>
<td>37</td>
</tr>
<tr>
<td>Discussion</td>
<td>37</td>
</tr>
<tr>
<td>EFFECTS OF REPEATED INJECTIONS OF KAINATE</td>
<td>39</td>
</tr>
<tr>
<td>Introduction</td>
<td>39</td>
</tr>
<tr>
<td>Methods and Materials</td>
<td>40</td>
</tr>
<tr>
<td>Results</td>
<td>41</td>
</tr>
<tr>
<td>Discussion</td>
<td>44</td>
</tr>
<tr>
<td>LOCALIZATION OF KAINATE-INDUCED WET-DOG SHAKES.</td>
<td>46</td>
</tr>
<tr>
<td>Intraventricular versus Intracisternal Injection</td>
<td>46</td>
</tr>
<tr>
<td>Kainate-Induced Lesion versus Kainate WDS</td>
<td>51</td>
</tr>
<tr>
<td>Selective Lesions of Hippocampal Subfields</td>
<td>53</td>
</tr>
<tr>
<td>COMPARISONS WITH OTHER WDS-INDUCING SITUATIONS</td>
<td>63</td>
</tr>
<tr>
<td>Introduction</td>
<td>63</td>
</tr>
<tr>
<td>Opiate Withdrawal</td>
<td>64</td>
</tr>
<tr>
<td>Serotonin</td>
<td>68</td>
</tr>
<tr>
<td>Methionine-Enkephalin</td>
<td>71</td>
</tr>
<tr>
<td>Ketocyclazocine</td>
<td>73</td>
</tr>
<tr>
<td>Sodium Valproate</td>
<td>75</td>
</tr>
<tr>
<td>Ice Water</td>
<td>77</td>
</tr>
<tr>
<td>GENERAL DISCUSSION</td>
<td>79</td>
</tr>
<tr>
<td>Summary</td>
<td>79</td>
</tr>
<tr>
<td>Implications</td>
<td>80</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>84</td>
</tr>
<tr>
<td>BIOGRAPHICAL SKETCH</td>
<td>90</td>
</tr>
</tbody>
</table>
Kainic acid (kainate) is believed to be an agonist of the putative neurotransmitter, glutamate. Intraventricular injection of kainate was found to induce dose-related changes in the behavior of rats and to result in selective lesions in the brain. One constellation of behaviors was induced by doses that did not appear to be neurotoxic. This set of behaviors included 'wet-dog' shakes (WDS), diarrhea and excessive salivation. It appeared to be the same constellation of behaviors that occurs during morphine withdrawal in the rat. This set of behaviors was chosen for further study because they were considered to be the most likely to have resulted from activation of normal mechanisms of neurotransmission.

A series of studies investigated the neuroanatomical and pharmacological correlates of this behavioral manifestation of kainate administration. The evidence from studies which examined the effects
of restricted ventricular injection of kainate and those which investigated the effects of selective lesions of the brain on kainate-induced behavior suggest that kainate induces this constellation of behaviors by actions on the CA3 and CA4 pyramidal neurons of the hippocampus.

A lesion, induced by kainate itself, was found to prevent the occurrence of this constellation of behavior by subsequent injections of kainate. The effect of this lesion on the occurrence of these behaviors induced by agents other than kainate was examined. The data suggests the existence of two distinct mechanisms for the induction of these behaviors. One mechanism reacts the same as morphine withdrawal to pharmacological manipulations, and the other, sensitive to kainate, reacts in an opposite manner to the same manipulations. Evidence is presented to suggest that the kainate-sensitive mechanism is mediated by a second opiate receptor, the kappa (K)-receptor.
INTRODUCTION

Glutamate

Evidence for a neurotransmitter role

A strong case can be made that glutamate is a neurotransmitter in the central nervous system. Iontophoretically applied glutamate can depolarize neurons (1-4). On vertebrate neurons a hyperpolarizing effect has been reported only once—in cerebellar slices (5). Nearly every neuron tested has been found to be sensitive to glutamate. This led some investigators to propose that glutamate may be a non-specific membrane depolarizing agent (1,2). Several experimental results are not consistent with this hypothesis. It has been reported that iontophoretically applied glutamate depolarizes neuronal soma or dendrites, but not axons (3). The depolarizing effect on soma or dendrites is restricted to extracellular application; intracellular ejection has little effect (6). Furthermore, glutamate does not depolarize most olfactory bulb cell bodies (7), indicating that it is not nonspecific.

In addition, some neurons are more sensitive to glutamate than others. McLennan et al. (8) probed the thalamus with micropipettes and found regional differences in the amount of response to glutamate. These differences appeared to be coexistent with known nuclei, that is, neurons of the ventrolateral nucleus were more responsive to glutamate than the neurons of adjacent nuclei.

Neurons have been shown to possess uptake mechanisms for glutamate (9-12). In fact, two mechanisms appear to exist. One is a glutamate
specific, Na+-dependent, mechanism present in synaptosomes. The other is a nonspecific, non-Na+-dependent mechanism. The glutamate-specific sodium-dependent mechanism has a much higher affinity than the nonspecific binding site. The sodium-dependent mechanism corresponds to a transmitter reuptake mechanism (a specific, fast-transport mechanism), while the nonspecific binding site is probably just the general uptake mechanism present in all cells.

A third binding site for glutamate also exists. It has an even higher affinity for glutamate than the specific reuptake mechanism, but is not sodium dependent. It is very specific for glutamate (versus GABA or glycine, for example) and most of its specific activity is in synaptosomes. This probably represents a postsynaptic glutamate receptor (12-15).

Looking at just one area, the mossy fibre bundle, for one example, a high concentration of glutamate and glutamate dehydrogenase, its synthetic enzyme, is found (16). The specific, Na+-dependent transport mechanism is also found in this region of the hippocampus (17). Stimulation of the mossy fibre bundle excites the pyramidal neurons postsynaptic to it (8), and also results in a large increase in extracellular (presumably, released) glutamate (16). The excitation of the post-synaptic neurons is rapid in onset and very powerful; effects which are mimicked by iontophoretic application (19). Thus, many of the usual criteria for defining a substance as a neurotransmitter have been satisfied at the mossy fibre bundle terminal field. The major piece of data missing is to show that the effects of tract stimulation can be blocked by glutamate receptor antagonists. This criteria has been met in the corticostriatal tract (20) and the perforant pathway (21), but other criteria have not been satisfied in these terminal fields. Therefore, by all the usual criteria, though not all have been shown at one synapse, glutamate is a neurotransmitter.
It seems fairly reasonable then to carry out research as if it were a transmitter, always cognizant that it may fail at some point to fit the definition.

Use of glutamate as an experimental tool

Although glutamate is the endogenous compound of interest, several factors make it undesirable for experimental use. First of all, the detection and measurement of glutamate directly involved in neurotransmission is severely hampered because glutamate is a natural amino acid used by all cells of the body, both as a constituent of protein and as part of the Krebs cycle, the energy mechanism of all neurons and glia (22). Thus the brain contains a large amount of glutamate which is not directly related to neurotransmission.

The brain goes to extraordinary lengths to keep the extracellular concentration of glutamate low. The only areas which allow passage of measurable amounts of glutamate from the blood into the nervous system are the circumventricular organs which do not possess the primary blood-brain barrier--capillaries whose lining cells have tight junctions between them (23,24).

Within the brain itself, extracellular concentrations of glutamate are kept very low by active uptake processes. The intracellular concentration is quite high and this imbalance contributes to the polarization of neuronal membranes. In an attempt to study the distribution of glutamate synapses, McLennan (25) injected tritiated glutamate into the brains of rats. The autoradiographs showed that essentially all the radioactivity (glutamate) ended up in glial cells. In a study of the metabolism of glutamate by the brain, Mao, Guidotta, and Costa (26) injected glutamate into the lateral ventricles, killed the animals as
soon as five minutes after the injection, and assayed the brains for increases (relative to controls) in glutamate and various metabolites. Even though the amount of glutamate injected was equal to about 30% of the total normal glutamate content of the whole rat brain, they could find no increase in glutamate content after only five minutes. Metabolites, like glutamine, were greatly increased. These two studies underscore the fact that the brain itself has very powerful mechanisms for sequestering and catabolizing glutamate. Furthermore, such mechanisms also occur in the glial cells so that neuronal mechanisms need not be affected. Thus, the direct injection of glutamate is not likely to have much direct effect upon neurons unless very high local concentrations can be achieved, as they are in iontophoretic application.

In an effort to overcome this handicap, large amounts of glutamate have been administered by various routes. The results from the injection of glutamate, by any route, seem to fall into two classes. The first category consists of little or no effect. The second category consists of convulsions, toxic behaviors and neural degeneration (26,31). For example, the intraventricular injection of 1 μmole of glutamate had little effect on the general behavior or operant responding of rats. It generally lowered the rate of responding but did not alter the acquisition, pattern, or extinction of the operant response. Ten μmoles of glutamate resulted in tonic seizures (32). Thus, exogenous glutamate is not a very useful neuromodulator. The toxic, convulsive dose is very high relative to behaviorally active doses of other transmitters, suggesting that convulsions are not the normal function of glutamate.

The neural degeneration induced by systemically injected glutamate is notable since it selectively destroys the inner layers of the retina.
and those parts of the brain immediately adjacent to circumventricular organs (CVO), such as the arcuate nucleus of the hypothalamus and the area postrema (24). Thus peripheral injections of glutamate may be useful as a tool for inducing certain lesions. The pattern of degeneration after systemic or direct, intracranial injection is also interesting. The lesion is restricted to those neurons whose soma or dendrites are within the injected area. Axons passing through and afferent terminals are unaffected (23,24,33,34). Thus, glutamate may be useful as a tool for inducing lesions in areas containing major fiber bundles, such as the striatum and the lateral hypothalamus. While it may become useful for producing lesions, glutamate is of little use in understanding the normal function of glutamate as a neurotransmitter.

Even if glutamate did induce interesting behavioral changes there would still be a problem in interpretation. There appear to be two receptors with which glutamate can interact. One is also well-suited for aspartate, another excitatory amino acid, which is slightly shorter than glutamate. The glutamate molecule, itself, can fold to some extent and fit this same receptor. However, there also appears to be another receptor which is best suited for a fully extended molecule of glutamate and for which aspartate is too short. The first receptor may be termed the general excitatory amino acid receptor (GEAAR) and the second, the glutamate-preferring receptor (35-37). (See Figure 1). The first receptor would have specificity bestowed on it in the brain because either a glutamergic or aspartergic terminal would synapse with it. However, interpretation of the results of exogenously applied glutamate would be limited to actions possibly mediated endogenously by glutamate.
<table>
<thead>
<tr>
<th>Glutamate-Preferred Receptor Agonists</th>
<th>Kainate</th>
</tr>
</thead>
<tbody>
<tr>
<td>(extended)</td>
<td></td>
</tr>
<tr>
<td>COOH</td>
<td>COOH</td>
</tr>
<tr>
<td>CH−CH₂−CH₂−COOH</td>
<td>CH−CH−CH₂−COOH</td>
</tr>
<tr>
<td>NH₂</td>
<td>NH</td>
</tr>
<tr>
<td></td>
<td>CH−C−CH₃</td>
</tr>
<tr>
<td></td>
<td>CH₂</td>
</tr>
<tr>
<td></td>
<td>CH₂</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(folded)</th>
<th>Aspartate</th>
</tr>
</thead>
<tbody>
<tr>
<td>COOH</td>
<td>COOH</td>
</tr>
<tr>
<td>CH</td>
<td>CH−CH₂−COOH</td>
</tr>
<tr>
<td>NH₂</td>
<td>NH</td>
</tr>
<tr>
<td>CH₂</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1

Structures of Aspartate, Glutamate and Kainate
Kainate as a glutamate agonist

Recently, a number of chemicals have been recognized to be analogues of glutamate and tests have shown some to be agonistic and a few others antagonistic by iontophoretic application. The agonists have gained particular attention because some of them are as much as one thousand times more potent than glutamate itself. In particular, domoate, quisqualate, and kainate, are very potent glutamate agonists on mammalian neurones (35,38,39).

Kainate is of particular interest. By far the most attractive attribute of kainate was that it appeared to be a very poor substrate for the glutamate reuptake mechanism (12,40). One report showed that an excess of kainate strongly displaced glutamate from the high affinity, Na+-independent binding site in the synaptosome fraction of locust neuromuscular junction, but had almost no effect on the binding of glutamate to the lower affinity, Na+-dependent binding site (12). The two sites probably correspond to the postsynaptic receptor and the neuronal reuptake mechanism, respectively. This noninteraction with the fast transport mechanism would result in kainate being active for a prolonged period of time. Iontophoretic studies of kainate have shown that it is one to two orders of magnitude more powerful than glutamate (35,38,39). This could be accounted for by the paucity of kainate uptake though no direct evidence is available.

Recently this simple agonistic mode of action of kainate has been challenged. Singh, McGeer, and McGeer (41) reported that after kainate injection, the Na+-dependent reuptake of glutamate was increased, an effect which seemed to be dissociable, temporally, from postsynaptic receptor degeneration. This dissociation suggested a direct effect of
kainate upon the presynaptic elements. In a following study, McGeer, McGeer, and Singh (42) destroyed the corticostriatal tract, presumably the glutamergic input to the striatum, prior to striatal injection of kainate. This loss of presynaptic input eliminated the neurotoxic effect of kainate in the striatum. Further in vitro data showed that kainate, at fairly high concentrations (mM), strongly inhibits the Na+-dependent glutamate reuptake mechanism (42,43). This suggests that kainate's effects at these doses may be due to presynaptic actions, such as blockade of reuptake or increased release of glutamate.

Like glutamate, the effect of kainate on mammalian neurons is depolarization. Hyperpolarization has never been reported as a result of kainate application. The depolarization occurring after application of kainate is long-lasting. Excessive or prolonged depolarization is neurotoxic and kainate is indeed a very potent neurotoxin (44).

Systemic (oral, sc, or ip) administration of kainate has three notable effects—convulsions, neural degeneration, and death. All three effects occur at the same doses so that these effects cannot be separated. A dose of 0.15 mmol/kg (30 mg/kg) of kainate induces convulsions and neural degeneration in mice. This makes kainate two hundred times more potent than glutamate in inducing neurotoxicity (24). The pattern of neuronal destruction succeeding kainate is identical to that induced by systemic glutamate. The kainate lesion, not unexpectedly, extends slightly further out from the CVOs than glutamate-induced degeneration. Since death often results after a neurotoxic dose of kainate, systemic administration of it is not a very useful experimental tool. One study, however, is interesting. Polc and Haefely reported that 0.3 mg/kg kainate, iv, increased monosynaptic, though not polysynaptic, reflexes (45), that is, increased the amount of muscle contraction induced by
submaximal stimuli. This may be consistent with the hypothesis that glutamate is a transmitter of primary sensory afferents to the spinal cord.

The binding of \(^3\text{H}\)-kainate to the brain has been studied by Simon, Contrera, and Kuhar (46). Their studies show that kainate binds to grey matter, but not white matter, and most strongly to the crude synaptosomal fraction. In terms of structures, the striatum had the most dense binding. Cerebral cortex, hippocampus and cerebellum had similar densities of binding which were about half that of the striatum. The midbrain, thalamus and pons-medulla all were similar and were one-fifth to one-fourth of the density shown by the striatum.

The binding of kainate was most easily displaced by kainate and quisqualate, suggesting a similarity of these two neuroexcitatory agents. Of all the other agents examined glutamate was the most effective agent in displacing kainate. Glutamic acid dimethyl ester (GDME) and glutamic acid diethyl ester (GDEE) were also reasonably effective displacing agents. However, aspartate was a rather poor displacing agent. Other putative neurotransmitters, specifically glycine, GABA, serotonin, norepinephrine, dopamine, and acetylcholine, were completely ineffective even when the concentration of these chemicals was a couple of thousand times that of kainate. These results indicate that kainate and glutamate bind at the same site, a site which is quite specific for their chemical structure.

Kainate is even more interesting as a glutamate agonist because of its chemical structure. As shown in Figure 1 it is locked into its configuration by the presence of a ring structure and some bulky side-chain groups. Thus, it is an analogue of the extended conformation of glutamate (35). The functional realization of this specificity has been shown
in the differential sensitivity of Renshaw and non-Renshaw spinal interneurones to aspartate and glutamate in cats. Various studies have shown that glutamate is more concentrated in the dorsal horn, while aspartate is concentrated in the more ventral regions. This has suggested to some authors that primary afferent terminals release glutamate while spinal interneurones use aspartate (47). In the cat, at least, Renshaw cells do not appear to receive primary afferent terminals, while most other spinal interneurones do (48,49). The Renshaw cells appear to be more sensitive to iontophoretically applied aspartate than glutamate, while the converse is true of the other spinal interneurones (50). Kainate applied to these same neuron populations is more effective on spinal interneurones other than Renshaw cells. The difference is greater with kainate than with glutamate suggesting that the difference is in fact due to the presence of two different receptors, the GEAAR and the glutamate-preferring receptors. In the rat, Renshaw cells appear to receive primary afferent terminals and the differential sensitivity to glutamate and aspartate is not seen (51).

Aspartate was a poor antagonist of kainate binding (46), a finding which is consistent with the hypothesis that kainate specifically interacts with the glutamate-preferring receptor. Furthermore, two agents known to antagonize responses to aspartate, diaminopimelic acid (DAP) and diaminoadipic acid (DAA), were completely ineffective in displacing kainate from its binding site (46).

Use of kainate as an experimental tool

A relative mountain of studies of the effects of intracranial injection of kainate have appeared in the last two years. Three research groups are responsible for the vast majority of these studies: E.G. McGeer, P. L. McGeer, T. Hattori et al.; J. T. Coyle, R. Schwarcz et al.; and J. W. Olney et al.
The primary locus of these studies has been kainate injection into the striatum of rats. With concentrations of kainate ranging from 2.5 - 15 mM (0.5 - 1 µl) the following have been consistently reported. Within three to five days after unilateral injection, neurons of the injected striatum degenerate (52,56). When 10 mM (1 µl) of kainate is used, over 90% of the neurons totally degenerate. The degeneration can be seen within a couple of hours of the injection. Histological examination has revealed several important facts. The first elements affected are neuronal soma and dendrites. The axons of the degenerating neurons begin to degenerate in due course (56). However, up to three weeks after injection, the longest period studied, axons of passage and afferent terminals are unaffected (57). This is particularly interesting in the caudate because the internal capsule fibers course through it. These would be destroyed by most lesion techniques. Hattori, McGeer, and McGeer (58) labeled the axon terminals of the corticostriatal tract with $^3$H-proline and then injected kainate into the striatum. They found that the labeled terminals were unchanged by the injection while the relevant postsynaptic structures degenerated. This result is doubly interesting because of the evidence that glutamate is the transmitter released by these terminals.

Biochemical markers of synaptic integrity have also been analyzed. The results are consistent with the histological data. Neurons of the striatum are believed to use two transmitters, ACh and GABA. Intrinsic neurons use ACh and GABA, while the principal efferent neurons use GABA. As expected, the kainate-induced lesion greatly reduces the content of these transmitters, their synthetic enzymes, and their specific reuptake mechanisms. GABA content is also reduced in the substantia nigra which
is consistent with the striatonigral tract being GABAergic. ACh content of the substantia nigra is unaffected (53,55).

Several other transmitters are afferent to the striatum. Dopamine input to the striatum is believed to arise from neurons in the pars compacta of the substantia nigra. After injection of kainate into the striatum, neither the content nor the specific reuptake mechanism for dopamine is changed. The synthesis of dopamine is, by distinction, greatly increased. This appears to be due to an increased number of tyrosine hydroxylase molecules (53). These results are consistent with unaffected presynaptic terminals and a loss of postsynaptic receptors.

Serotonin input to the striatum comes from neurons of the raphe nuclei. After a kainate-induced lesion, the activity of the specific reuptake mechanism for serotonin is unchanged, while receptor binding is decreased (59). These results are similar to those seen with dopamine.

The status of glutamate in the striatum is less clear. One possible glutamergic input is the corticostriatal tract.* After lesion in the striatum with kainate, glutamate receptor binding is decreased. However, the glutamate content is unchanged and the reuptake mechanism is slightly increased (63). The study by Hattori, McGeer, and McGeer (58), mentioned earlier, showed that the corticostriatal terminals stayed intact and had the same concentration of vesicles after kainate as in controls. This evidence strongly suggests that glutamate in the striatum arises from extrinsic neurons.

*Lesion in this tract selectively reduces the glutamate content of the striatum (relative to other amino acids) and the terminals of this tract possess a glutamate reuptake mechanism. Stimulation of the corticostriatal tract excites striatal neurons and this stimulation-induced excitation can be blocked by glutamic acid diethyl ester (GDEE), a glutamate receptor blocker (20,60-62).
These biochemical and histological changes are very similar to those found in human patients who have died of Huntington's chorea. The kainate-induced lesion may provide an animal model of Huntington's chorea and research is moving in that direction.

Finally, lesion in the basal ganglia by kainate (5 mM, 1 μl) lowers the met-enkephalin content by 50% (64). This is half the dose of kainate that destroyed over 90% of the striatal neurons. This suggests that the enkephalinergic input to the striatum arises from basal ganglia neurons. It has been suggested that these are located in the globus pallidus.

In the doses used to produce most of these biochemical changes (10 mM, 1 μl) destroy over 90% of the striatal neurons. However, in most cases synaptic integrity, as measured by biochemical methods, is reduced by 60-60%. This difference is disconcerting, but may be partly explained: by a recent report by Olney and de Gubareff (57). They found, by EM examination, that although neurons had totally degenerated, the postsynaptic densities were still attached to many of the intact presynaptic terminals. These densities, which probably contain the receptors, appeared to be intact up to three weeks after kainate injection. Longer postinjection periods were not examined. If the receptors are intact, then binding could still occur and explain the presence of binding in the absence of postsynaptic neurons.

In their first report on biochemical changes McGeer and McGeer (55) reported that 2.5 nmol (> 0.5 μg) induced significant changes, but that 1 nmol (0.21 μg) did not. Coyle and Schwarcz (52) reported that they tried 0.1 - 10 μg of kainate. The lowest dose for which data was reported was 0.5 μg and it induced minimal changes. These studies suggest the possibility that some concentrations of kainate may not be neurotoxic.
Unilateral injection of kainate, in neurotoxic concentrations, into the striatum results in changes in behavior. In rats, grooming stops and diarrhea occurs nearly immediately (55). The animals turn contralaterally and go into tonic-clonic convulsions. With more than 2 µg (10 mM, 1 µl), death occurs frequently (53,55). The contralateral turning occurs for several hours and is replaced by ipsilateral turning. The timing of this change correlates well with neuronal degeneration and may represent excited and then inhibited (absolutely) glutamocceptive neurons in the caudate (53). Only one study has examined the effects of bilateral injection. Neurotoxic doses (6 mM, 1µl) were injected into each caudate. The rats were tested later for changes in general activity, and acquisition and memory of a step-down passive avoidance task. No changes were seen in spontaneous locomotor activity. However, kainate-injected animals were significantly impaired in both acquisition and one-day retention of the passive avoidance task (65).

Specificity of kainate to glutamate receptors has been tested by injecting various agents prior to or along with kainate. Neither agonists nor antagonists of dopamine, acetylcholine, GABA, or serotonin, nor chronic morphine treatment protected against kainate toxicity. Some analogs of kainate and glutamate did, e.g., diethyl kainate, α-methylglutamate, diaminopropionic acid, and diaminobutyric acid (66).

Several studies have examined the effects of injections into the substantia nigra (53,67). Histological examination reveals that the pars reticulata is the primary site of degeneration. Biochemical markers of presynaptic activity indicate that GABA is unaltered. Thus, GABA is probably an extrinsic transmitter; from the striatum. Acetylcholine is also unchanged. Dopamine content is reduced, though only about 40-50%
at a high concentration of kainate (12 mM, 0.3 μl). This suggests that nondopaminergic, glutamoceptive neurons exist in the substantia nigra, primarily localized in the pars reticulata.

Injection of kainate into one substantia nigra results in ipsilateral turning by the animal for several hours. This is succeeded by contralateral turning in concert, at least temporally, with neuronal degeneration. These results are consistent with the effects of unilateral electrolytic lesion and opposite to the results with 6-OHDA-induced lesion. The kainate effects are not affected by 6-OHDA lesions. This strongly suggests that a nondopaminergic, glutamoceptive neuron population also controls turning in rats, independently of dopaminergic neurons, and opposite in behavioral results (67).

Only one study has examined the effects of thalamic injection. The injection of 5 mM kainate (1 μl) was directed into the ventrolateral nucleus. Behaviorally, these unilateral injections result in the cessation of grooming, the appearance of circling, and various convulsive behaviors. Biochemical changes were relatively minor, consisting of a 28% decrease in glutamic acid decarboxylase (GAD) activity in the thalamus and no change in choline acetyltransferase (CAT) or tyrosine hydroxylase (TH) activity. No change in the activity of these enzymes was seen in either the striatum or substantia nigra subsequent to thalamic injection (55).

Herndon and Coyle (68) injected kainate (10 mM, 1 μl) into the cerebellum. They found, histologically, that all the neurons of the cerebellar cortex degenerate except the granule cells. This result is crucial to the claim that kainate acts via glutamoceptive neurons. The granule cells give rise to the parallel fibers which are the primary excitatory input to the cerebellar neurons. The boutons of the parallel
fibers synapse on the Purkinje, Golgi, stellate and basket neurons, that is, all the other neurons of the cerebellum. However, no granule-granule synapse has been reported. Several lines of evidence on various mutants and viral infections have correlated loss of granule cells with a specific reduction in glutamate content. Thus it appears that the granule cells are glutamergic, but not glutamoceptive. Since kainate selectively spares the granule cells, the claim that it acts via glutamate receptors is strengthened.

Schwarcz and Coyle (54) reported that when they injected the striatum with kainate (10 mM, 1 µl) they also found that the CA3/4 pyramidal neurons of the hippocampus underwent degeneration. This selective degeneration was studied directly by Nadler, Perry and Cotman (69). They injected 0.1 - 3.0 µg (0.5 - 15 nmol) of kainate into or near the hippocampus. As little as 1.5 nmol (0.3 µg) caused many neurons of CA3/4 to degenerate, but CA1 and dentate granule cells were spared. CA3/4 neurons differ from the others in one respect by virtue of their innervation by the mossy fibre bundle. Earlier it was suggested that the evidence is sufficient to claim that mossy fibers are glutamergic, as is the perforant pathway.

At higher doses, and correlated with total loss of CA3/4 neurons, CA1 neurons degenerate. The dentate granules degenerate if the injection is made directly into the hippocampal formation, but not by ventricular injection. The results indicate that it is possible to selectively destroy CA3/4 of the hippocampus at concentrations which are not known to be toxic to any other neurons. The results also show that very low concentrations of kainate are selectively toxic to certain CA3/4 neurons. Injection of 0.1 µg (0.5 nmol) induced degeneration of CA3a pyramidal neurons only (69).
DeGubareff and Olney (70) injected 20 nmol of kainate into the general ventricular circulation. The volume was unspecified. They reported that the subjects (mice) died within one hour. Degeneration was reported in primary sensory-receiving neurons of the spinal cord, some cerebellar, and some hippocampal neurons. The fact that kainate spread so far so quickly suggests that a large volume was injected. This report indicates the sensitivity of the hippocampus and cerebellum which contain major fiber tracts suspected to be glutamergic.
DOSE-RESPONSE STUDY OF BEHAVIORS INDUCED BY KAINATE

Introduction

The behaviors reported in these studies of kainate that were just reviewed result from administration of neurotoxic doses of kainate. It is unclear whether they tell us much about behaviors in which glutamate normally participates. However, injection of agonists of other suspected transmitters have resulted in enhancement or reduction of particular behaviors. One example are the turning behaviors induced by unilateral striatal injection of various agents. Such agonist-induced behaviors can be useful for examining neural circuits and may also indicate neural mechanisms underlying behavioral problems. Thus, it seemed useful to determine if kainate would induce a particular constellation of behaviors, especially at nontoxic doses.

Materials and Methods

The subjects for this study were 56 male, Long-Evans hooded rats and six male Sprague-Dawley albino rats. All weighed from 200-300 grams.

Surgical procedures were performed while the subjects were anesthe-
tized by sodium pentobarbital (55 mg/kg), administered intraperitoneally. The head was shaved and the animals secured in a stereotaxic device. The head was tilted such that the nose pointed upward five degrees. This allowed the use of the atlas of the rat brain by Pellegrino and Cushman (71). The skin was split with a scalpel and the fascia reflected from the skull with a blunt scalpel handle. The skin and fascia were maintained
in a retracted position by means of S-shaped pins attached by rubber bands to the stereotaxic machine.

The stereotaxic was used to locate the part of the skull above the left lateral ventricle. The coordinates, relative to bregma, used were 2.4 mm posterior and 4.7 mm lateral. A hand-held drill with a one millimeter diameter bit was used to drill through the skull. The bit extended one millimeter out of the drill chuck which allowed it to drill through the skull without slipping into cortex and possibly damaging cortex. Three holes were drilled, one at the coordinates given above and two more, one on each side of the skull to hold supporting screws. After the holes were drilled, bone chips were removed with the aid of microdissecting forceps and a dissecting microscope until the dura matter was unobstructed and flat across the bottom of the hole. The dura and pia matters, in the hole placed above the lateral ventricle, were slit using the beveled edge of a 27 gauge needle.

Two small screws were attached to the skull and then a polyethylene cannula guide, riding on the electrode holder of the stereotaxic instrument, was lowered through the hole placed above the left lateral ventricle to a depth of five millimeters from the dorsal surface of the skull. This placement was calculated to put the tip of the guide into the lateral ventricle just antero-lateral to the posterior portion of the hippocampus. The guide was constructed from polyethylene tubing that had an outer diameter of about 0.7 millimeter. A metal wire which fit snugly into this size tubing was threaded through a length of the tubing. This combination was placed over a low flame until the plastic softened. This softened plastic was then compressed so that it bulged out and formed a ridge around the tubing. This process was repeated every few centimeters along the tubing. The tubing was then cut into pieces such that five
millimeters of tubing was left below the ridge and the total length of the piece was 11 millimeters long. The ridge served as a guard against the guide slipping too far into the brain.

Once the guide was in place in the brain, it and the two screws were covered by cranial cement to form a smooth, solid cap. The loose skin was pulled together around the cap and held together by wound clips. A stylet made from a 27 gauge needle was placed in the guide to keep it from becoming clogged prior to use. Bicillin (60,000 U) was administered intramuscularly to reduce the possibility of infection. The subject was then removed from the stereotaxic and allowed to recover in its home cage for seven to ten days before testing.

Just prior to testing, the appropriate solution of kainate was prepared by dissolving it in saline. The solution was brought up to a pH of 7.4 with sodium hydroxide. The subjects were transported to the observation area in their home cages. Injections were performed by gently hand-restraining the subjects and, after removing the stylet, inserting a 27 gauge needle into the cannula guide.

Eleven and one-half millimeters of this needle were allowed to pass into the guide so that a small portion extended past the guide itself. The 27 gauge needle was attached to one end of a length of polyethylene tubing which was filled with kainate solution. The other end of this tubing was attached to a microliter syringe.

Two and one-half microliters of kainate solution, of different concentrations, was injected over a 30-second period. Kainate solutions used had concentrations of 0.006, 0.03, 0.1, 0.2, 0.8, 2, 20 and 200 millimolar. After the injection was complete, the needle was removed and the subject was immediately placed in an individual observation box.
(30 x 30 x 20 centimeters). Each box was illuminated by a 7.5 watt white light bulb. The rats were observed from an adjacent room through a one-way mirror. White noise (65 decibels) was introduced into the experimental room to mask extraneous noise. The subject's behaviors were observed for one hour and any unexpected behaviors recorded and, where possible, counted.

At the end of behavioral testing, the subjects were given an overdose of sodium pentobarbital and two and one-half microliters of India ink was injected in the same manner as kainate had been. The animals were then intracardially perfused with 0.9% saline, followed by 10% formalin. The brain was removed, sectioned and the presence of ink in the various ventricles was determined.

**Results**

Rats receiving large concentrations of kainate (20 or 200 millimolar) underwent fits of leaping and running, convulsions and death. Injection of lower concentrations (0.8 and 2 millimolar) of kainate was followed by convulsions, barrel-rolls and contralateral turning. Concentrations from 0.006 - 0.2 millimolar induced 'wet-dog' shaking (WDS) and diarrhea. At the 0.2 millimolar concentration of kainate, wet-dog shaking was overshadowed by myoclonic jerks, especially prominent in the front legs. The magnitude of these convulsive bodily reactions made it difficult to identify 'wet-dog' shaking apart from the other convulsive movements and therefore WDS was not quantified at this dose. Following injections of the 0.1, 0.03, and 0.006 millimolar kainate solutions, WDS were not accompanied by clonic jerks. Kainate at a concentration of 0.006 millimolar induced a mean of 5.5 'wet-dog' shakes during the one-hour observation period. Rats injected with 0.03 millimolar kainate produced a mean of 40 WDS in the hour, while 0.1 millimolar kainate
resulted in a mean of 75 WDS in one hour. Diarrhea became very pronounced at this last dose level. Excessive salivation, chewing and teeth chattering were also seen at these three concentrations, but their occurrence was not as consistent as WDS and diarrhea. Also, especially with 0.1 millimolar kainate, some rats seemed to expel a large amount of moisture which manifested itself in the fogging up of the observation boxes. Injection of pH-corrected saline (the vehicle solution) into the lateral ventricle produced a consistent pattern of effects: short bouts of exploration and grooming for 20-30 minutes, followed by sleep. Very few WDS were observed, mean of 0.9 in the hour period, in vehicle-injected animals. The three groups receiving the lowest concentrations of kainate differed significantly from each other and from vehicle-injected rats in terms of the number of WDS produced (p's < .05 or less; Mann-Whitney U-test). These data are summarized in Table 1.

Six Sprague-Dawley albino rats were injected with 0.25 nmol of kainate. Their behavior was quantitatively and qualitatively similar to the Long-Evans hooded rats at the same dose.

Study of the distribution pattern of India ink showed that 2.5 μl of ink injected intraventricularly generally filled most of the left lateral ventricle, but only rarely entered the body of the right ventricle. It was present in the intraventricular foramen of Munro, the third ventricle, cerebral aqueduct, and fourth ventricle. In some animals it was present in the cisterna magna, in the area above the colliculi, and around the lateral edge of the thalamus.
Table 1

Behavioral Changes Found After Various Doses of Kainate

<table>
<thead>
<tr>
<th>Kainate (nmol)</th>
<th>Number of Subjects</th>
<th>Distinctive Behaviors</th>
<th>Mean WDS in one hour</th>
<th>Statistical Comparisons (Mann-Whitney U test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>1</td>
<td>wild leaping and running fits, death in 1 min.</td>
<td>not counted (NC)</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>3</td>
<td>periodic leaping and running fits, death within 1 hr.</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>tonic-clonic convulsions; some contralateral turning; and posturing</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>nearly continuous contralateral turning; barrel rolls; clonic jerks</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>4</td>
<td>wet-dog shakes; frequent clonic jerks; especially front legs</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>17</td>
<td>wet-dog shakes; rarely, clonic jerk of front legs; diarrhea</td>
<td>75</td>
<td>.005 vs. saline, .05 vs. .075nM</td>
</tr>
<tr>
<td>0.075</td>
<td>6</td>
<td>wet-dog shakes; diarrhea; active longer than normals</td>
<td>40</td>
<td>.01 vs. saline, .05 vs. .015nM</td>
</tr>
<tr>
<td>0.015</td>
<td>8</td>
<td>some wet-dog shakes; active longer than normals</td>
<td>5.5</td>
<td>.05 vs. saline</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>slight grooming for 15-20 min, then sleep</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>saline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The results indicate that kainate is a very potent behaviorally active agent. As little as 1.3 ng/μl of injection solution induced a significant modification of behavior. The doses of kainate used here induced three concentration-dependent sets of behavior. At the high concentrations examined, kainate administration resulted in fits of leaping and running, convulsions, and death. The middle range of concentrations induced contralateral turning, barrel-rolls and convulsions. Administration of the lower doses was associated most consistently with wet-dog shaking and diarrhea.

Leaping and running fits and death, as seen after large doses of kainate, are behaviors which are associated with general neurotoxins such as quabain (72), copper (72) and high doses of glutamate (28). Since these concentrations of kainate are much higher than those necessary to destroy neurons in any area tested, it is suggested that these doses, associated with toxic behaviors and death, are acting as general neurotoxins.

Contralateral turning and convulsions have been reported by Schwarcz and Coyle (53) and McGeer and McGeer (55) following intrastriatal injection of kainate. The effective concentrations are in the same range as those which induce the same behaviors following intraventricular injection. The distribution of India ink suggests that kainate solution reached the ventricular surface of the striatum. Thus, the occurrence of these behaviors after intraventricular injection of kainate may be a consequence of direct striatal stimulation.

Low concentrations of kainate induce a set of behaviors which appears to be very similar to morphine withdrawal (the morphine abstinence syndrome) (73). Jumping is a behavior commonly observed during withdrawal in
rats dependent on large amounts of morphine (73). It was not observed in rats administered kainate and observed in small, closed-top boxes. However, jumping was seen when a couple of kainate-injected subjects were transferred to a large, open-top field. Thus, many of the symptoms of morphine withdrawal can be elicited by acute administration of kainate in opiate-naive rats.

The capability of kainate to induce WDS places it among a rapidly growing list of substances which can induce WDS in opiate-naive animals. Natural situations such as skin contact with cold water or xylene are followed by wet-dog shaking. Chemicals which can bring on this kind of shaking include TRH (thyrotropic hormone) (given intraperitoneally, ip, or intracerebroventricularly, icv) (74,75), 5-HTP (given ip) (76), \( \alpha \), \( \beta \) and \( \gamma \)-endorphin (given icv) (77), met- and leu-enkephalin (given icv) (78), high doses of morphine (given icv) (79), AG-3-5 (1-\([-\text{hydroxyphenyl}]-4-[3-\text{nitrophenyl}]-1,2,3,6 \text{tetrahydropyrimidine}-2\text{-one}) (given ip) (80), sodium valproate (given ip) (81), Sgd 8473 (\( \alpha \)-[(4-chlorobenzylideneamino)-oxy]-iso-butyric acid) (given ip) (82), somatostatin (given icv) (83), RX 336-M (7,8-dihydro-5,6'-dimethylcyclohex-5'-eno-[1',2',8,14]-codeinone) (given ip) (84) and theophylline (given ip) (85).
DOSE-RESPONSE STUDY OF LESIONS INDUCED BY KAINATE

Introduction

The aim of the dose-response studies was to discover behavioral manifestations of nonneurotoxic doses of kainate. Therefore it is necessary to examine the brain itself to determine what consequences various doses of kainate have. Concentrations of kainate of two nanomoles/microliter, or more, result in potently convulsive behaviors and often in death. These toxic behaviors represent abnormal neuronal functioning and so histological examination was restricted to lower concentrations.

Methods and Materials

Histological alterations produced by unilateral injection of kainate were studied in 24 male, Long-Evans hooded rats. Cannulas were implanted in the same manner as previously described and the animals were allowed to recover seven to ten days before receiving kainate. Kainate solutions were prepared and injected in the manner already described.

Seven rats were injected with a 1 nmol/μl solution into the left lateral ventricle as described above. Two of the seven received 1 μl, two received 2 μl, and two were injected with 5 μl of the kainate solution. The other member of each group was sacrificed 24 hrs after injection. The seventh rat was administered 0.5 μl and it was sacrificed 24 hrs after injection. Seven other rats received 2 μl containing a total of 0.2 nmol of kainate. Two of these were sacrificed 24 hrs afterwards, while the other five were sacrificed after one week. Ten rats were injected with 2 μl of 0.05 nmol/μl kainate solution and sacrificed one week later.
All subjects were sacrificed by an overdose of sodium pentobarbital (Nembutal). This was followed by intracardial perfusion with 0.9% saline and then a 10% formalin solution. The brains were removed and placed in 10% formalin. The brains were subsequently embedded in celloidin and cut into 30 μm coronal sections. Every fifth section was saved, mounted on slides and stained with thionin for microscopic examination.

Results

Examination of the sections revealed that a 1 nmol/μl solution is consistently neurotoxic to some areas of the brain within reach of the injection. Most prominent of the affected areas is the CA3 and CA4 pyramidal neurons of the hippocampus. Just three hours after injection these neurons on the injected side appear deflated and twisted. By 24 hrs degeneration is quite evident, involving all the neurons in these two fields of Ammon's horn on the side of the injection.

The CA3 and CA4 fields of the contralateral hippocampus appear unaffected. CA1 neurons on the injected side appear pale compared to the contralateral CA1 neurons, but structurally well-formed. Neurons of the dentate gyrus seem unaltered. A small cluster of large pyramidal neurons in the dorso-lateral extreme of Ammon's horn also appear to be insensitive to this concentration of kainate. These neurons may represent the CA2 field of the hippocampus.

At the larger injection volumes, neuronal degeneration also appeared in the cortico-medial division of the ipsilateral amygdala. There is also loss of deep pyramidal neurons of the neocortex near the injection site, but whether this is due to the kainate or the cannula itself was not determined.

A lower concentration of kainate (0.2 nmol in 2 μl) produced neuronal degeneration in three out of seven rats. The loss of neurons was
restricted to area CA3a in dorsal hippocampus. This area is immediately adjacent to the tip of the implanted cannula. In most cases the degeneration extended only a short distance from the level of the cannula in the anterior-posterior plane. The appearance of degeneration in these animals did not correlate with the number of wet-dog shakes observed, nor with the appearance of convulsive behavior.

The lowest dose of kainate used (0.1 nmol in 2 µl) produced no observable neuronal alterations in the hippocampus or any other structure in the brain.

**Discussion**

As reported in many studies (53,55), kainate is a neurotoxic agent. The neurotoxicity appears to be both selective and dose-dependent. The CA3-4 region of the hippocampus appears to be extremely sensitive to kainate, even at a concentration of 0.1 nmol/µl. The extreme sensitivity of the CA3-4 region was first noted by Coyle and Schwarcz (52) who reported destruction of the neurons in this hippocampal area following intrastriatal injection of kainate. The CA3 and CA4 fields of the hippocampus were the only extrastriatal damage observed by these investigators. This selective neurotoxicity was also investigated by Nadler, Perry, and Cotman (69). They reported that intraventricular injection of 0.5 nmol/µl - 2 nmol/µl kainate resulted in the selective destruction of the CA3-4 region of the brain. No disturbances were seen in any other parts of the brain when examined in the presence of a Nissl body stain or silver stain for degenerating fibers, by these investigators.

The present study indicates that this part of the hippocampus is even more sensitive to the neurotoxic effects of kainate than previously reported (69). A 0.1 nmol/µl solution was found to destroy some neurons in this region in about half the animals examined, suggesting that by this
method of administration, 0.1 nmol/μl represents an LD₅₀ (lethal dose, half the time) for these neurons. Injection of kainate in a lower concentration seemed to be without neurotoxic effect, as examined by a Nissl body stain, even though this dose was effective in inducing WDS and other behaviors associated with the injection of kainate.

The number of WDS which occurred following the injection of a 0.1 nmol/μl solution did not appear to be correlated to the presence or absence of degenerating neurons. This may further indicate that WDS and neurotoxicity are not directly correlated phenomena.

The primary hippocampal neurons destroyed by these neurotoxic doses of kainate are large pyramidal neurons. However, a small cluster of large pyramidal neurons in the dorsolateral extreme of Ammon's horn appear to be insensitive to these doses. Since the CA1 field of the hippocampus consists primarily of medium-size pyramidal neurons, this kainate-insensitive cluster of large pyramidal neurons may represent the CA2 field of the hippocampus.
CHOICE OF KAINATE-INDUCED BEHAVIORS FOR INTENSIVE STUDY

Administration of 2.5 μl of kainate solution into the lateral ventricle of rats results in changes in their ongoing behaviors. There seem to be three sets of dose-dependent kainate-sensitive behaviors. Concentrations of 0.006 - 0.1 nmol/μl induce a constellation including wet-dog shakes, diarrhea, etc. that is quite similar to morphine withdrawal (73) or endorphin- or enkephalin-induced behavior (77). Higher concentrations, up to about 5 nmol/μl, induce myoclonic jerks and contralateral turning, while even higher concentrations manifest themselves as running fits and death.

Simply because of the problem of keeping subjects alive, further investigation of the highest concentrations of kainate does not seem advantageous. Furthermore, leaping and running fits are a highly abnormal behavior which makes them, at least to this investigator, intrinsically less interesting. Since lower doses are neurotoxic, it is likely that these doses are at least as toxic, adding to the feeling that this is a very abnormal situation and less directly related to the mundane activities of the animal.

The histological evidence indicates that the intermediate doses, associated with contralateral turning and convulsions, are neurotoxic. A 1 nmol/μl solution consistently obliterates the CA3-4 field of the hippocampus, while even 0.1 nmol/μl resulted in more restricted destruction in about half the animals examined. This belies that the effects of these doses are also correlated with highly unusual neuronal functioning.
It is interesting that similar behaviors can be obtained from direct intrastriatal injection of similar concentrations, but the fact that other groups are working on this type of injection suggests that work on some other behaviors would be useful and possibly most helpful.

Kainate can induce wet-dog shakes, etc. at doses which do not appear to be neurotoxic by light microscopic examination of brains stained with Nissl-substance stains (to allow examination of cell bodies) by either this investigator or Nadler, Perry and Cotman (69) or in material stained with silver to identify degenerating axons (Nadler, Perry and Cotman). Even though some structural or functional alterations may have occurred that were not identified by these techniques, the behaviors induced by these doses stand as the most likely possibility for a behavior normally mediated by glutamate.

The constellation of behaviors is interesting in its own right because it appears to be very similar to the constellation of behaviors observed in opiate withdrawal in rats. Any chance to shed some light on the mechanisms of opiate dependence and withdrawal is worth following up.

Therefore, the behaviors resulting from administration of kainate in concentrations of 0.1 nmol/μl or less will be intensely investigated. Several questions will be emphasized.

1) Is kainate-induced behavior glutamate-mediated behavior?
2) Where does kainate act to induce wet-dog shakes, etc.?
3) How does kainate-induced behavior relate to similar behaviors induced by other agents and situations, especially morphine withdrawal?
4) Does this behavioral constellation represent a response to normally encountered environmental situations?
Of the behaviors in this constellation, wet-dog shakes appear to be the most consistent response. It is also the most quantal response and the frequency of the occurrence of wet-dog shakes seems to be directly related to the concentration of kainate (within a range of 0.006 - 0.1 nmol/µl). Therefore, wet-dog shakes will most often be the behavior used to test the efficacy of experimental manipulations, though the presence of the other associated behaviors will be recorded when possible.
TIME COURSE OF KAINATE-INDUCED WET-DOG SHAKES

Introduction

In the dose-response studies, behavior was observed for one hour. This time period was selected simply because many of the obvious alterations in the behavior of the subjects had disappeared by that time and the subjects had begun to sleep. However, in order to manipulate a specific behavior, in particular kainate-induced WDS, it is useful to know the exact time course of the behavior of interest. This is important in order to devise the most effective test period.

Methods and Materials

The subjects for this study were eight male, Long-Evans hooded rats weighing 200-250 grams. Surgical and injection procedures were the same as already described. A dose of 0.2 nmol of kainate (in 2 μl), the most effective concentration in inducing WDS, was administered to six rats. Two rats served as controls and were injected with 2 μl of the vehicle solution (pH-corrected saline). The observation period was extended to 3.5 hrs.

Results

The majority (79%) of kainate-induced WDS occurred during the first hour following its administration. Another 18% occurred during the second hour and by 2.5 hrs, all WDS had occurred. At this time, all subjects were asleep and except for brief periods of wakefulness, remained asleep for the remainder of the observation period. One saline-
injected rat emitted one shake during the second half-hour. The other control subject showed no WDS. The data are shown graphically in Figure 2.

Discussion

Most wet-dog shakes induced by kainate occur in the first hour after injection. This suggests that a one-hour observation period is probably sufficient for testing. This result also suggests that the effects of this concentration of kainate do not persist or progress into toxic behaviors as might be expected if permanent changes in the brain were occurring.
Figure 2

Time Course of Kainate-Induced Wet-Dog Shakes
SUSCEPTIBILITY OF KAINATE-INDUCED WET-DOG SHAKES TO GLUTAMATE RECEPTOR BLOCKADE

Introduction

It appears from the data examined above that kainate can result in alterations in behavior at doses which are not neurotoxic. Tentatively, then, we may hypothesize that this change in behavior is due to neuronal stimulation and furthermore to glutamate receptor stimulation. This hypothesis may be tested by studying the effect of a glutamate receptor antagonist on kainate-induced behavior.

Glutamic acid diethyl ester (GDEE) has been reported to antagonize glutamate-induced neuronal excitation (86,89). Furthermore, it has been shown, by iontophoretic and systemic administration, to reduce the effects of stimulation of fiber tracts thought to release glutamate (87,89). However, there have been reports that GDEE is not particularly effective or specific (90). In general though, the most recent reports (e.g., 88) show GDEE to be a very effective antagonist, which is selective to aspartate and glutamate, but which shows little ability to separate these two amino acid putative neurotransmitters. A large part of the earlier controversy probably stems from the speed with which GDEE undergoes hydrolysis to form glutamate itself (87). Therefore, if GDEE is properly stored (dry and cold) and used soon after being placed in aqueous solution, it appears to be a very effective antagonist of glutamate and aspartate.

Segal (89) reported that systemic administration of 200 mg/kg (1 mmol/kg) moderately reduced the field potential associated with
perforant pathway stimulation—thought to be mediated by glutamate. Stone (87) reported that 30Q and 60Q mg/kg reduced the effects of corticostriatal tract stimulation. This tract also contains fibers believed to release glutamate.

Methods and Materials

Eighteen male, Long-Evans hooded rats were used as subjects for this study. Surgical and intraventricular injection procedures were the same as in preceding studies. GDEE was removed from a freezer and dissolved in saline just prior to each use. Ten rats were injected with 2 mmol/kg GDEE (2 ml/kg), intraperitoneally, 5 min prior to intraventricular injection of kainate (0.25 nmol in 2.5 µl). Eight animals received saline (2ml/kg) instead of GDEE. All subjects were observed for 1 hour, but for six animals in each group separate determinations of the WDS in the first and second 30-min periods were made.

Results

Rats pretreated with 2 mmoles/kg of GDEE had an overall reduction of 56% of WDS relative to their controls (p <.02). As evaluated by those subjects where separate evaluations of the first and second 30 min periods were made, this effect occurred because of a reduction of WDS in the first 30-min period. For these GDEE-treated animals the median WDS for the first 30-min period was 21, while their controls exhibited 42 (p <.005). During the second 30-min period the median WDS of the GDEE-treated group was 27 compared to the median of the control animals of 36. This difference was not significant.

Discussion

Stone (87) and Segal (89) reported that GDEE was effective when administered intraperitoneally. This route was chosen for this study
because the time to effect had been reported and there was concern about injecting two volumes of liquid into the ventricles. On the other hand, this meant allowing GDEE to spread throughout the central nervous system.

The result from this experiment indicates that blockade of glutamate and aspartate receptors reduces the effect kainate has on overt behavior. This is consistent with the expectation that kainate affects behavior by stimulating glutamate receptors. However, it is also possible that GDEE blocks excitatory amino acid receptors at some point on the output side of the site of kainate's action. Thus, this result can only be taken as suggestive, though it does indicate that a hypothesis in which kainate does not act via glutamate receptors will not gain easy support from the data.
EFFECTS OF REPEATED INJECTIONS OF KAINATE

Introduction

There exist doses of kainate which induce WDS, but do not produce noticeable neural degeneration. Degeneration is seen in some animals injected with a 0.1 nmol/μl solution, but the occurrence of degeneration does not appear to be related to the number of wet-dog shakes recorded. These findings suggest that WDS induced by kainate are not a result of degenerative changes in neurons.

The actions of kainate in inducing WDS appear, at the light microscopic level, to be dissociable from kainate's neurotoxic effects. However, it is possible that permanent subcellular or functional alterations are produced by the WDS-inducing actions of kainate. This possibility may be examined by giving kainate on multiple occasions and examining if consistent alterations in the response to kainate administration are found as a consequence of prior administrations. The dose of kainate employed should be in the nonneurotoxic range.

The elicitation of a unique and consistent set of behaviors by low doses of kainate suggests that kainate may be stimulating a particular part of the nervous system at these doses. Slightly higher concentrations of kainate have been found to induce neural degeneration in a specific field of neurons. It is possible that these two events are linked.

It has been suggested that prolonged, excessive depolarization is the means by which glutamate and kainate induce neuronal death--excitotoxic effects (24). Depolarization mediated by stimulation of glutamate...
mate receptors would also be expected to be the mechanism by which kainate induces behavior. It is possible that the concentrations of kainate which induce wet-dog shakes do so by depolarizing a particular group of sensitive neurons and that raising the concentration of kainate increases and/or prolongs that depolarization to a toxic level. If this were the case, then the specific neurotoxicity induced by minimally toxic doses of kainate may be on the same neural field by which kainate induces WDS. This possibility can be tested by first inducing a lesion with kainate itself and subsequently examining the ability of kainate to produce WDS.

Methods and Materials

The subjects for this experiment were 23 male, Long-Evans hooded rats. They all weighed 200-250 grams at the beginning of the experiment. Surgical and injection procedures were again the same as described earlier. Five rats made up Group 1 and were not implanted. They were used to measure the behavior of intact animals in the experimental situation. Five other rats (Group 2) were implanted with a cannula in the left ventricle and were injected only with the vehicle solution on each test day. The test days for all groups were four days apart. Group 3 consisted of five rats implanted with a cannula. This group of rats was treated according to the following schedule:

Test Day 1 1.5 nmol kainate (in 1.5 μl solution)
Test Days 2-4 0.25 nmol kainate (in 1.5 μl solution)
Test Day 5 vehicle (1.5 μl)

Eight rats made up Group 4. They were cannulated in the same manner as groups 2 and 3 and were tested according to the following schedule:

Test Days 1-6 0.25 nmol kainate (in 1.5 μl solution)
Test Day 7 vehicle (1.5 μl)
Test Days 8,9 0.25 nmol kainate (in 1.5 μl solution)
Six weeks separated Test Days 7 and 8, but all other Test Days were separated by four days.

In addition to wet-dog shakes (WDS), the occurrence of stretching and yawning was also recorded for all the groups during the one-hour observation periods. Observation began immediately after the injections were completed.

**Results**

Very few WDS were seen at any time in either intact or saline-injected rats. The average median score for the intact animals was 1.0, while it was 1.17 WDS in an hour for the saline-injected rats. The median number of WDS did not change significantly across days for either group, nor were there any significant differences between the two control groups. The mean and median number of wet-dog shakes for each group is shown in Table 2.

The number of wet-dog shakes was not recorded on the first test day for those animals administered 1.5 nmol of kainate (Group 3). All rats given 1.5 nmol manifested numerous myoclonic jerks and some tonic-clonic convulsions. On Test Days 2-4, when 0.25 nmol of kainate was injected, the number of wet-dog shakes stayed at a relatively consistent, low level. The mean median number of WDS for all four groups is given in Table 2. The number of wet-dog shakes evoked on Days 2-4 were not significantly different from either control group on the corresponding test day. Furthermore, the number of wet-dog shakes produced on Days 2-4 were not different from that produced by vehicle injection on Test Day 5.

The median number of wet-dog shakes produced by injection of 0.25 nmol kainate on Day 1 (Group 4) was 74.5. This was significantly more than produced by intact, uninjected animals or by vehicle injection
On the second test day the median number of WDS was 89. The Group 4 scores on Day 2 were not significantly different from their Day 1 scores. They were significantly different from the control groups (ps < .01). The number of wet-dog shakes produced on Day 2 in Group 4 was also significantly higher than that induced by similar injection in Group 3 (p < .01). On Test Days 3-6 the number of WDS evoked steadily decreased, though it always remained significantly higher than the control groups (ps < .05). In order to examine whether the decrease was due to tolerance or habituation, or due to neuronal destruction, six weeks were allowed to pass without the subjects being exposed to injection or the observation apparatus. Two more test sessions, four days apart, were then carried out. The median WDS produced on these days was 2 and 3 respectively, which was not significantly different from the number produced by the saline injection on Test Day 7.

The number of stretches and yawns evoked by either control group was never significantly different from the other on any day. The number of stretches and yawns recorded for all four groups is compiled in Table 3. As can be seen, the number of stretches and yawns rises quickly and reaches a plateau of about 8 by the third test day.

Stretches and yawns in Group 3 rats were not counted on Test Day 1. On Test Days 2-4, when they received 0.25 nmol of kainate, they did not differ from either control group, and also reached a plateau of about 8. Injection of vehicle solution on Test Day 5 did not induce more or less SY than the kainate injection of Test Day 4.

On all test days when they received kainate injections, except Day 6, Group 4 animals evoked significantly less SY than either control group (ps < .05, or less). On Test Day 7 when they were injected with the
### Table 2
Median Number of Wet-Dog Shakes Induced with Repeated Tests

<table>
<thead>
<tr>
<th>Test Days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group 2</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group 3</td>
<td>N.C.</td>
<td>1</td>
<td>6</td>
<td>7</td>
<td>1(veh.)-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Group 4</td>
<td>74.5</td>
<td>89</td>
<td>47</td>
<td>12</td>
<td>32.5</td>
<td>17</td>
<td>0(veh.)2*</td>
<td>3**</td>
<td></td>
</tr>
</tbody>
</table>

N.C. not counted; *7 Ss; **5 Ss

### Table 3
Median Number of Stretches and Yawns Recorded with Repeated Tests

<table>
<thead>
<tr>
<th>Test Days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Group 2</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>8</td>
<td>10</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Group 3</td>
<td>N.C.</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>8(veh.)-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group 4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2.5</td>
<td>9(veh.)</td>
</tr>
</tbody>
</table>

N.C. not counted
vehicle they produced a median of 9 SY during the observation period, well within the plateau established by the other groups.

**Discussion**

Doses of kainate which have been shown to be neurotoxic to the CA3-4 field of the hippocampus almost totally prevent subsequent doses of kainate from inducing WDS. Thus, the effects of kainate in inducing this behavior and producing neuronal degeneration are linked. This data indicates that these two effects are mediated by the same neural circuit and possibly even the same neurons, though this cannot be determined from this data.

The ability of the kainate-induced lesion to prevent kainate-induced behavior is further confirmed by the occurrence of a normal amount and pattern, across days, of stretching and yawning following kainate administration in lesioned subjects. In animals given only the lower dose of kainate, the normal appearance of stretching and yawning is suppressed until Test Day 6 when the number of WDS induced by kainate is much reduced relative to earlier administrations. To summarize, the ability of kainate to suppress stretching and yawning is lost when the animal is pretreated with a neurotoxic dose of kainate.

The number of WDS induced by injection of kainate after one previous injection of kainate, also at the lower dose (Group 3), was not significantly different from the number produced by the first injection. This could suggest that no significant alterations in function of the nervous system had resulted from the first application of kainate. Furthermore, stretching and yawning were effectively suppressed by the second application indicating that no loss of potency had occurred in regard to this behavior as a consequence of the previous dose.
However, subsequent injections of kainate resulted in reductions in the amount of WDS produced and a reduced ability to suppress stretching and yawning. The reduction could be the result of many factors such as tolerance or habituation. It could also be the result of destruction. In order to separate these possibilities, the subjects in Group 4 were not exposed to the experimental situation, the injection procedure, or kainate for six weeks. Retesting indicated that the ability of kainate to induce WDS was still reduced. This suggests that a permanent change in the nervous system had occurred.

To try to increase the chances of selectively affecting the hippocampus the volume of injection solution had been reduced to 1.5 μl. However, the amount of kainate used to induce WDS, i.e., 0.25 nmol, was kept the same. This raised the concentration as compared to previous studies, from 0.1 to 0.167 nmol/μl. Furthermore, at the time this study was begun, the ability of a 0.1 nmol/μl solution to lesion some neurons had not been recognized. These facts suggest that the low dose used here was probably capable of inducing partial destruction of CA3 and CA4 each time it was injected.
LOCALIZATION OF KAINATE-INDUCED WDS

Intraventricular versus Intracisternal Injection

Introduction

Since intraventricular administration of low doses of kainate induce specific constellations of behaviors, it is probably reasonable to suspect that it is acting on specific neural circuits. Thus, it may be possible to define specific anatomical loci of kainate's action which result in behavioral change. In particular, the structure by which kainate induces WDS should be identifiable.

Bloom, Segal, Ling, and Guillemin (77) reported that intraventricular (lateral ventricle) injection of the endorphins or enkephalins resulted in the occurrence of wet-dog shakes in rats. However, intracisternal (cisterna cerebellomedullaris) injection of these agents did not induce wet-dog shaking. The ventricular space of the brain is comprised of four ventricles, the cisterna magna (or cisternacerebellomedullaris), several interconnecting foramen and the cerebral aqueduct. Most endogenous cerebrospinal fluid (CSF) is produced in the two lateral ventricles and from there flows through a large foramen (the foramen of Munro) underneath the septal area, into the third ventricle. From the third ventricle, the CSF passes through the narrow cerebral aqueduct, in the midbrain, and enters the fourth ventricle. At the juncture of the pons and medulla the fourth ventricle loses its lateral walls which include the cerebellar peduncles. Posterior to this point the ventricular space is known as the cisterna magna. These above-named spaces comprise
the most commonly used ventricular spaces. Flow in these spaces is most restricted in the cerebral aqueduct. Drugs injected into the cisterna cerebellomedullaris diffuse easily into the fourth ventricle. However, given a reasonable volume of injection fluid and reasonable injection pressure,* it is less likely that the drug will pass forward through the cerebral aqueduct. Similarly, solutions administered in either of the lateral ventricles should flow freely into the third ventricle, but will encounter resistance at the cerebral aqueduct. Thus, the data of Bloom et al. (77) suggests that endorphins induce WDS by actions on forebrain structures.

However, their intracisternal injections were performed while the subjects were under the influence of ether, while intraventricular injections were performed in unanesthetized animals. It was suggested that the ether may have been responsible for the inability of endorphins to induce WDS when injected via the intracisternal route, but no control was performed.

This possible interaction with ether can be controlled for, at least to the extent of giving ether to animals injected intraventricularly. Since kainate also results in WDS after intraventricular injection, this same experiment, using kainate, could provide evidence on the general locus of kainate's action and also provide a comparison of kainate- and endorphin-induced WDS.

*The parameters of reasonable volume and pressure are unanswered questions. However, Bass (91) has estimated total CSF volume in the rat to be 250 µl and its production rate to be about 1 µl/min and therefore injection volumes should be kept small in relation to 250 µl and injection rates around 1 µl/min.
In order to try to strengthen the belief that the injected solution is hindered in its passage by the cerebral aqueduct, India ink was injected in the same way as kainate and its localization examined.

**Methods and Materials**

Male, Long-Evans hooded rats weighing 200-250 grams were permanently implanted with plastic cannula guides stereotaxically guided to end in the left lateral ventricle. The stereotaxic coordinates of the cannula guide were -2.5AP, 4.5L, and 5.5V, Pelligreno and Cushman (71). Kainate (0.25 nmoles) in 2.5 μl of saline or 2.5 μl saline was injected through this guide via a 27-gauge hypodermic needle. Intracisternal injections (2.5 and 5 μl) of kainate (0.1 mM) were performed in rats anesthetized with ether. The rats were held by the ear bars of a stereotaxic instrument with their noses downward to gain most effective access to the cisterna magna. Injection was made through a 27-gauge hypodermic needle attached to a microliter syringe. The needle had a ball of solder attached to it about 4-5 mm from the tip to insure that the needle could not slip beyond the ventricular space. The solutions used for intracerebral injection were adjusted to a pH of 7.4.

When behavioral testing was completed, subjects were anesthetized with sodium pentobarbital and injected with India ink in the same amount and location as the kainate. They were then intracardially perfused with 0.9% saline followed by 10% formalin. The brains were removed, sliced, and examined for the presence of ink in the ventricular system, and gross destruction of neural structures.

Seven rats were injected with 0.25 nmoles of kainate (in 2.5 μl saline) icv. Six rats received 0.25 nmoles of kainate in 2.5 μl and 12 rats 0.5 nmoles in 5 μl, ic. Five additional rats were injected
intraventricularly with 0.25 nmoles of kainate while anesthetized with ether.

Results

In previous work we observed WDS in rats injected with saline, icv. Saline produced less than 1 per hr which did not differ from WDS observed in uninjected rats. Data from these saline-injected rats were used for the determination of baseline levels of WDS in this study.

Icv injections of 0.25 nmoles of kainate in unanesthetized rats induced WDS at a mean rate of 102.6 per hr. Icv injection in ether-anesthetized rats induces WDS at a mean rate of 95.6 per hr. These were both different from saline-injected animals (ps < .001), but not different from each other.

Intracisternal injections of 0.25 and 0.5 nmoles produced means of 7.2 and 7.8 WDS in an hour and medians of 3.5 and 6, respectively. The number of WDS seen in those rats administered 0.25 nmoles of kainate was not significantly different from saline-injected rats. Those animals receiving 0.5 nmoles of kainate did show more WDS than the saline-injected animals (p < .01).

The two icv-injected groups were combined into one and this was used for comparison with the ic-injected groups. Statistical tests confirmed that icv injections produced more WDS than either ic injection (ps < .001). These results are shown in Figure 3.

Discussion

There was no significant difference in the number of WDS occurring following intraventricular administration of kainate with or without ether anesthesia. Thus, at least by this route of administration, ether
Effectiveness of Injection Route in Inducing Wet-Dog Shakes

(a) kainate, icv; (b) kainate, icv with ether; (c) kainate, ic 0.25nmol; (d) kainate, ic 0.5 nmol. Shaded area represents range of WDS in saline-injected rats.
does not seriously affect kainate-induced WDS. On the other hand, intracisternal injection of kainate was virtually ineffective in inducing WDS. These results suggest that kainate produces WDS by actions on forebrain structures.

This conclusion is strengthened by the examination of India ink injections. Intraventricular injections of 2.5 µl of ink filled the injected lateral ventricle and the whole third ventricle. On some occasions, intraventricular injections of ink resulted in its presence in the cerebral aqueduct and the fourth ventricle. Ink in these areas was diluted (lighter in color) compared to that found in areas rostral to the cerebral aqueduct.

On the other hand, intracisternal injection of India ink resulted in the blackening of the fourth ventricle and adjacent portions of the cisterna magna. In a few cases, when 5 µl was injected, the cerebral aqueduct was colored. In one case, ink was also present in the posterior third ventricle. These injections indicate that the cerebral aqueduct forms a partial barrier to the flow of substances injected into the CSF and strengthens the suggestion that the WDS seen after administration of kainate are the result of actions on forebrain structures.

Ink did not enter the body of the contralateral lateral ventricle. This distribution is not surprising since the exogenous flow of CSF is from each lateral ventricle into the third ventricle. If the ink had tried to flow into the opposite lateral ventricle it would have had to turn nearly 360° and flow against the exogenous current, both of which it is unlikely to do.

**Kainate-induced Lesion versus Kainate WDS**

**Introduction**

The hippocampus appears to be extremely sensitive to the neurotoxic
properties of kainate. Coyle and Schwarcz (52) reported that following intrastriatal injections of kainate, they found selective destruction of the CA3 and CA4 fields of the hippocampus. Nadler, Perry, and Cotman (69) found that intraventricular injections of less than 3 nmol/μl induce lesions which appear, in both Nissl- and silver-stained material, to be selective for the CA3 and CA4 fields of the hippocampus. This neurotoxic effect was observed after as little as 0.5 nmol/μl (1μl) kainate. We have confirmed this selective lesion using Nissl-stained material and observed that as little as 0.1 nmol/μl (2μl) kainate induced neural destruction in about half the animals so injected. Other reports (70, 92) also attest to the extreme sensitivity of the hippocampus.

Low doses of kainate induce a highly specific behavioral response. Slightly higher doses result in a highly specific lesion. These two occurrences may be linked. It was hypothesized that kainate induces WDS by its action on neurons of the CA3 and CA4 fields of the hippocampus. Since the hippocampus lines the lateral ventricle, this hypothesis is consistent with the results of the study just reported. This hypothesis was investigated by injecting neurotoxic doses of kainate into rats to destroy the CA3 and CA4 neurons and subsequently examining the ability of kainate to induce WDS. This experiment was reported earlier in Chapter VII.

Discussion

The result of this test was that the initial dose of 1 nmol/μl kainate, a dose associated with total destruction of CA3 and CA4, prevented the appearance of WDS following subsequent injection of the lower dose. This result is consistent with the hypothesis that kainate acts
on CA3 and CA4 neurons to induce WDS. However, it does not fully address the question. It is possible that kainate acts on other neurons, e.g., entorhinal or septal neurons, and in this case the CA3 and CA4 neurons form the output pathway. It is also possible that the initial kainate altered, but did not destroy, neurons other than CA3 or CA4 neurons and that this alteration was responsible for the observed reduction in WDS.

Selective Lesions of Hippocampal Subfields

Introduction

The previous study strongly suggests that the CA3 and CA4 fields of the hippocampus are part of the neural circuit by which kainate induces WDS.

The aims of this study are 1) to show that the destruction of CA3-4 by itself can prevent kainate-induced WDS, 2) to investigate the importance of various inputs to CA3 and CA4 to kainate-induced WDS, and 3) to investigate the importance of various outputs from CA3 and CA4 to kainate-induced WDS.

First, it is necessary to selectively lesion CA3 and CA4 to insure that the reduction of WDS following kainic acid-induced lesions is attributable to the destruction of CA3 and CA4.

Then, assuming that CA3 and CA4 lesions do prevent WDS following injection of kainate, it is most important to determine if kainate-induced behavior is a result of kainate's actions on neurons afferent to regio inferior and not directly on the pyramidal neurons themselves.

Finally, it is useful to try to understand what pathway out of the hippocampus is necessary for the realization of WDS. This data would help suggest how kainate-induced opiate withdrawal-like behavior is related to true opiate withdrawal.
In order to carry out this study it is necessary to identify the input and output pathways of the hippocampus. The hippocampus is comprised of two interlocking lobes, the dentate gyrus and Ammon's horn. Ammon's horn has been further divided into four fields, CA(Cornu Ammonis) 1-4 (93). The primary bases for these distinctions are the size of the principal neurons (the pyramidal neurons), their packing density (or how restricted they are to lamellar organization) and the mixture of different size pyramidal cells. In particular, CA1 is comprised only of medium-size pyramidal neurons tightly organized into one layer. On the other hand, CA3 is composed only of large pyramidal neurons organized into one thin layer, while CA4 is composed of a scattered pattern of large pyramidal neurons. Field CA2 is an area between CA1 and CA3 where there is a mixture of medium and large pyramidal neurons. These fields can also be examined in terms of input and output connections. On this basis it is difficult to distinguish between CA3 and CA4, and CA2 appears simply to be an overlapping of CA1 and CA3 (94). It has been suggested that at our present level of understanding it is sufficient to divide Ammon's horn into two fields, regio superior and regio inferior (95). These correspond to CA1-2 and CA3-4, respectively. Since they also correspond to kainate-insensitive and kainate-sensitive areas, in regard to the toxic effects of a 1 nmol/µl solution, this simplified description will be used in looking at input and output connections of the hippocampus.

Since the focus of this study has already become set on the regio inferior we can narrow our original goals to affecting its input and output. Input to CA3-4 pyramids comes from two directions, anterio-lateral
and postero-medial. The antero-lateral intrusion comes in the form of the fornix which contains fibers from the septal area, hypothalamus and other areas (96). The fornix just ahead of the hippocampus is a fiber bundle separate from any of the structures itinnervates or whose axons make it up. Thus, at this level the fornix can be discretely destroyed. Two complications arise, one of which can be rectified. The fimbria, which contains the axons of CA3-4 neurons (that is, the output of regio inferior), also travels in the fornix (96). Thus, destruction of the fornix destroys both input and output of CA3-4. However, the ability to discretely destroy a fiber bundle is too good an opportunity to pass up, and much easier than trying to destroy all the structures which make up, or are innervated by, the fornix.

Axons of CA3-4 neurons in the fimbria also pass through the hippocampal commissure (psalterium) into the contralateral fimbria (96). This occurs just anterior to the hippocampus. Since this constitutes an output pathway it is important to destroy it. To accomplish this it is necessary to destroy the fimbria-fornix just anterior to the hippocampus. To do this without damaging cell bodies of the hippocampus is probably most easily accomplished by cutting the fornix-fimbria with a knife.

The other major inputs to CA3-4 are the mossy fibre bundle, the perforant pathway and the dorsal fornix/cingulum (96, 98) The mossy fibre bundle arises from the dentate gyrus and synapses exclusively with CA3-4 neurons. The perforant pathway, which originates in entorhinal cortex, terminates on the dentate gyrus granule cells and also sends fibers over and past the dentate gyrus to synapse with the apical dendrites of the CA3 and CA4 pyramidal neurons. The dorsal fornix carries fibers from various locations including the locus coeruleus and the
raphe nuclei (97, 98). These fibers enter the hippocampus from the back taking a pathway between CA1 and the dentate gyrus. They distribute to all regions of the hippocampus.

To eliminate the mossy fibre bundle the dentate gyrus must be destroyed. This will also strongly reduce the effect the perforant pathway has on the CA3-4 neurons because a large number of perforant path fibers terminate on neurons of the dentate gyrus and excite those neurons which, in turn, excite the CA3-4 neurons. If the lesion used to damage the dentate gyrus is allowed to extend slightly dorsal to the dentate gyrus into the superficial molecular layer of CA1 almost all the perforant path fibers can be destroyed. This manoeuvre will also eliminate many of the fibers from the dorsal fornix that would have terminated in CA3 or CA4. Thus, an extensive lesion of the dentate gyrus can destroy most of the input to the CA3-4 neurons from the postero-medial direction.

Regio superior acts as an output pathway for regio inferior. Axon collaterals of regio inferior neurons, the Schaeffer collaterals, synapse on CA1-2 neurons (95). The CA1-2 neurons send axons out to synapse in the subiculum. Destruction of CA1-2 can provide information about the pathway out of the hippocampus necessary for the realization of kainate-induced WDS. It can also help suggest the function of the fornix. If destruction of either the fornix or regio superior prevents kainate-induced WDS, then the most reasonable explanation would be that the fornix is important as an input to regio inferior. This is so because CA1-2 appears to act only as an output pathway for CA3-4, not as an input (99).

Therefore, four kinds of lesions should suffice to indicate how kainate-induced WDS are produced. In summary these are:
Methods and Materials

The subjects for this study were 31 male, Long-Evans hooded rats. At the time of surgery they weighed from 250-375 grams. Surgical procedures were performed while the subjects were anesthetized by chloral hydrate (400 mg/kg; 500 mg/ml) which was administered intraperitoneally.

The head was shaved and the animals secured in a stereotaxic device. The head was tilted upward five degrees to allow the use of the atlas of the rat brain by Pelligreno and Cushman. The skin was split with a scalpel and the fascia retracted from the skull with a blunt scalpel handle. The skin and fascia were maintained in a retracted position by means of S-shaped pins attached by rubber bands to the stereotaxic machine.

The stereotaxic was used to locate the areas of the skull above the structures of interest. A hand-held drill with a one mm diameter bit was used to drill through the skull. The bit extended only one mm out of the drill to protect against damaging cortex. After the holes were drilled, bone chips were removed with the aid of microdissecting forceps and a dissecting microscope until the dura matter was unobstructed and flat across the bottom of the hole. In those holes where electrodes or cannulae were to be lowered, the dura and pia matter were split using the beveled edge of a 27 gauge needle.

Lesion devices were directed at four areas. In ten animals the fornix-fimbria was cut. A small knife, 2.5 mm wide, made from a razor
blade was used to make the cut. This knife was directed at the fornix-fimbria just anterior to the hippocampus and posterior to the psalterium. The knife was placed at the following coordinates of the Pelligreno and Cushman atlas--P0.2mm, L 0.5mm, V 4.5 mm.

In seven animals, destruction of CA3 and CA4 was attempted. In these animals, a 400 μm stainless steel wire insulated except for the final 0.5 mm, was inserted into the hippocampus at the following locations: P1.5, L2.5, V4; P2.8, L3.7, V4; P4, L5.2, V5; P4, L5.2, V7. At each of these locations 1.5 mA of DC current, produced by a Grass lesion maker, was passed for 15 sec. A banana plug inserted into the rectum formed the other pole of the circuit.

Electrolytic lesion of the dentate gyrus was attempted in seven rats. Current (1.5 mA) was passed for 15 sec at the following sites: P1.5, L1, V3.6; P2.8, L2.3, V3.6; P4, L3.5, V4.3; and P4, L5, V7.5.

Lesions were directed at the CA1 field of the hippocampus in seven rats by passing 1.5 mA of current for 10 sec at each of the following locations: P1.8, L1, V2.8; P2.8, L1.2, V2.8; P2.8, L2.5, V2.3; P4, L2.7, V215; and P4, L5, V4.2.

Immediately following the creation of lesions, two small screws were placed in holes drilled into the skull. A stainless steel cannula guide was then lowered through a hole drilled at P2.4, L4.75 to a depth of 5 mm from the skull. This placement left the tip of the guide in the left lateral ventricle just dorso-lateral to the ventral hippocampus. The cannula guide was constructed from a 23 gauge (0.61 mm dia.) syringe needle. The needle was broken 5 mm down from the hub by bending it to cause metal fatigue. The hub, which is larger than the 1 mm skull hole, served as a guard to prevent the guide from going too far into the brain.
Once the guide was in place, it and the two screws were surrounded by dental cement to form a solid cap. The skin was then placed over this cap, but around the hub, and closed with wound clips. Bicillin, im, (60,000U) was administered. The subject was removed from the stereotaxic and allowed to recover in its home cage for seven to ten days before testing.

Just prior to testing, a 50 µM solution of kainate was prepared by dissolving it in saline. The solution was brought up to a pH of 7.4 with NaOH. The subjects were transported to the observation area in their home cages. Injections were performed by gently hand-restraining the subject and inserting a 30 gauge needle into the cannula guide. When the guides are constructed the implanted end becomes slightly constricted. Because of this, only the beveled tip of the 30 gauge needle (< 1mm) entered the lateral ventricle. The close fit also ensured the absence of backflow of solution into the guide. The 30 gauge needle was attached to one end of a length of polyethylene tubing which was filled with kainate. The other end was attached to a microliter syringe. Two microliters of the kainate solution (0.2 nmol, 43 ng) was injected over a 30 sec period. The rats were observed, as described in Chapter II, for one hour. The number of wet-dog shakes was recorded, as well as the number of tonic-clonic convulsions and the general deportment of the subject.

At the end of behavioral testing, the subjects were given an overdose of chloral hydrate, and intracardially perfused with saline followed by 10% formalin. The brain was removed and stored in cold 10% formalin. At a later time, the brains were frozen and cut into 30 µm sections. Brains possessing electrolytic lesions were cut coronally and every eighth section saved and mounted. Brains in which a knife cut was made
were cut horizontally and every fourth section through the fornix retained and mounted.

**Results**

The fornix-fimbria knife cuts proved almost impossible to verify histologically. Therefore these animals will not be presented. In regard to animals receiving electrolytic lesions, those in which the cannula obviously ended up outside the ventricle were thrown out prior to analysis ($n = 2$). One additional animal was not subjected to the analysis because of widespread, unexplained damage throughout the third ventricle.

The results from the remaining subjects were analyzed in two ways. In the first, all those subjects sustaining any CA3 damage at all were put into one group. Other groups consisted of the subjects in which hippocampal damage was restricted to the dentate gyrus, the CA1 field or to a combination of these fields. Another group was comprised of subjects sustaining damage only in the cortex overlying the hippocampus. These groups were compared to 14 nonlesioned rats. The second method for examining the data consisted of determining the lesions sustained by those subjects showing less WDS following application of kainate than any of the nonlesioned subjects.

There were eleven rats which sustained at least some damage to CA3 or CA4 along with varying amounts of damage to other structures. The number of WDS induced in these animals by kainate ranged from zero to 143. The mean number of WDS was 19.8, while the median was one. Two rats sustained hippocampal damage restricted to CA1. These two subjects produced 17 and 88 WDS in response to kainate administration for a mean (and median) of 52.5. One rat had hippocampal damage limited to the ventral blade of the dentate gyrus. That rat was observed to evoke 14 WDS and
three tonic-clonic convulsions. Two subjects sustained damage to both CA1 and the dentate gyrus. These lesions, combined with kainate, resulted in 125 and 173 WDS. Two rats were found to have damage limited to the overlying cortex and were observed to respond to kainate administration with 30 and 75 WDS. This works out to be a mean of 52.5 WDS. Finally, injection of kainate into 14 nonlesioned animals produced a range of five to 112 WDS—a mean of 45.5 and a median of 42.

Statistical analysis was possible only to compare rats with at least some CA3 damage to those having no lesions at all. The result of a Mann-Whitney U test revealed a significant change (reduction) in the WDS produced by the group sustaining CA3 damage (p < .01). While no analysis can be performed for the remaining groups it is worth noting that either cortical or CA1 damage alone yielded means of 52.5 WDS, not far from the 45.5 of the nonlesioned rats. Combined damage of CA1 and the dentate gyrus yielded a mean of 149, far above that seen in nonlesioned animals.

The smallest number of WDS observed after kainate application in nonlesioned rats was five. Nine rats given electrolytic lesions produced four or less WDS. All nine sustained damage of CA3. The damage was generally extensive to the cellular field and/or the fimbria. Damage to any other particular region within or outside the hippocampus was not consistently seen in these nine subjects.

Discussion

The results of the lesion experiments are anything but clean. However, both in terms of the number of WDS produced by CA3-damaged rats and the lesions sustained by animals showing subnormal numbers of WDS, damage to regio inferior is strongly correlated with a reduction in the ability of kainate to induce WDS.
Animals not sustaining CA3 damage showed no obvious trend toward a reduced number of WDS. Any such trend was, in fact, in the opposite direction—towards increased numbers of WDS.
COMPARISONS WITH OTHER WDS-INDUCING SITUATIONS

Introduction

In Chapter II it was stated that WDS are a symptom of morphine withdrawal in the rat. Furthermore, as the name suggests, WDS are a common response of dogs and most other mammals, including rats (73), to immersion in water. More recently, a number of chemicals have been found to induce WDS in opiate-naive, dry rats. These were listed in Chapter II. In Chapter VIII it was reported that kainate-induced WDS were similar to endorphin- and enkephalin-induced WDS in at least one respect. They are all induced by intraventricular, but not intracisternal administration.

It is useful to further compare agents which will induce WDS. It is possible that all of these agents act on the same site or the same neural circuit, but this hypothesis should be subject to testing. Since kainate-induced WDS can be prevented by pretreatment with a selective neurotoxic dose of kainate, this same pretreatment can be used to compare kainate-induced WDS to those induced by other agents. If all WDS-inducing agents are acting on the same neural circuit, then this manipulation should also prevent WDS induced by agents acting on neurons afferent to, and including, those destroyed by the pretreatment. Similarly, manipulations which affect the production of WDS by various agents should have similar effects on kainate-induced WDS. These possibilities were examined in the following series of studies.
Opiate Withdrawal

Naloxone

Introduction. Naloxone is recognized as an effective opiate antagonist, with little or no opiate agonist properties at commonly used doses. In animals made dependent upon morphine, administration of naloxone (by various routes) will result in the appearance of the morphine abstinence syndrome (73). The intensity of this syndrome, when induced by naloxone, may be greater than that seen during simple morphine abstinence. However, naloxone, administered to an opiate-naive animal does not induce the withdrawal behaviors (73).

If kainate were acting by stimulating the endogenous mechanism for the abstinence behaviors, then naloxone should have either no effect or enhance kainate-induced WDS.

Methods and materials. Surgical and intraventricular injection procedures were the same as described in Chapter VIII. Either naloxone or saline was injected i.p., 5 min prior to icv injection of 0.25 nmoles of kainate in 1.5 μl saline. Three groups of naloxone-treated animals receiving different doses were used along with separate saline control groups. Ten animals received 1 mg/kg naloxone (2.5 ml/kg), and seven received a similar volume of saline. Nine animals received 2 mg/kg naloxone (5 ml/kg), while six received saline and served as controls. Six rats were injected with 4 mg/kg naloxone (5 ml/kg) and six animals were saline controls.

Results. The two lower doses of naloxone (1 and 2 mg/kg) did not alter the number of WDS produced by the icv injection of kainate. The 4 mg/kg dose of naloxone reduced the kainate-induced WDS by 88%. The mean WDS of the 4 mg/kg naloxone-treated group was 12.2 and the mean of the control group was 103 per hr.
**Discussion.** Naloxone antagonizes kainate-induced WDS. This suggests that kainate-induced WDS are mediated by opiate receptor stimulation. This is in contrast to morphine withdrawal where loss of opiate receptor stimulation appears to be the precipitating factor. However, naloxone prevents endorphin-induced WDS (77), again indicating that kainate and endorphins have similar actions. Furthermore, kainate- and endorphin-induced WDS were only antagonized by rather high doses, greater than 1 mg/kg, of naloxone.

**Morphine**

**Introduction.** Once the morphine abstinence syndrome has been precipitated, it can be antagonized by administration of morphine. Thus, if kainate directly induces the morphine abstinence syndrome, morphine would be expected to reduce the behavioral manifestations of kainate.

At a moderate dose, morphine will induce active behaviors like grooming and quick, darting movements in rats. The effect of kainate on this morphine action can be examined at the same time and provide additional information about the relationship between morphine and kainate.

**Methods and materials.** Morphine sulfate 10 μmoles/kg (0.4 ml/kg) i.p., was injected 30 min prior to icv injection of kainate. Nine animals were injected with both systemic morphine and icv kainate; eight received systemic saline (0.4 ml/kg) and kainate icv; and seven received systemic morphine with saline icv. WDS, grooming behaviors, and the presence of quick, darting movements and turns were recorded for 1 hr. Grooming was counted every 15th sec. Since observations were made for 1 hr, a maximum grooming score of 240 was possible.

**Results.** The results are summarized in Figure 4. Systemic injection
of morphine along with saline, icv, induced a mean grooming score of 69. Quick, darting movements were observed in six of the seven animals treated in this way. WDS occurred infrequently (4 WDS per hr).

Icv kainate and systemic saline induced WDS, but little grooming and no quick darting and turning. Grooming occurred at a mean rate of 10 per hr. Icv kainate and systemic morphine also induced WDS and a small amount of grooming (mean = 9). Only one of these rats showed quick, darting movements. This one rat produced only 1 wet-dog shake and, on post-mortem examination, no evidence of ink was found in the ventricles. This indicated that the intraventricular injection was not successful. The animal acted as if it had only received systemic morphine.

The number of WDS produced following icv kainate injection did not differ between the groups receiving morphine or saline systemically. The group receiving kainate icv and systemic morphine had more WDS than the one administered saline icv and systemic morphine (p < .01). The group injected with kainate icv and systemic saline also had more WDS than the group receiving intraventricular saline and systemic morphine (p < .001).

Grooming scores for both kainate-injected groups did not differ, but both groomed significantly less than the one administered saline with systemic morphine (ps < .02). A smaller number of the rats injected with kainate icv and systemic saline presented quick, darting movements than those administered saline icv plus systemic morphine (p < .01). Fewer of the rats receiving kainate icv with systemic morphine showed quick, darting movements than those receiving saline icv and systemic morphine (p < .05). The two groups receiving kainate injections did not differ from each other.
Figure 4

Competition between Kainate and Morphine Behaviors

KS, kainate icv and saline ip; KM, kainate icv and morphine ip; SM, saline icv and morphine ip. Shaded area represents range of WDS in saline-injected rats.
Discussion. Morphine, at this dose, does not prevent kainate-induced WDS. This is not consistent with the hypothesis that kainate activates the morphine abstinence syndrome. Thus, in regard to acute administration of morphine and naloxone, kainate-induced WDS do not respond as morphine abstinence. In fact, kainate-induced WDS react in an opposite manner. On the other hand, kainate did reduce morphine-induced behavior suggesting an interaction between the two chemicals at some level.

Serotonin

5-Hydroxytryptophan

Introduction. Bedard and Pycock (76) reported that systemic administration of 5-HTP, following pretreatment with a peripherally acting decarboxylase inhibitor, resulted in the occurrence of WDS. 5-HTP is the metabolic precursor of the putative neurotransmitter, serotonin. This response lasted for several hours. The maximum rate of WDS was observed 2 hr after administration of 5-HTP.

The result of this pharmacological manipulation might be expected to be due to increased amounts of serotonin in the brain. Consistent with this expectation, Bedard and Pycock (76) reported massive increases in cerebral serotonin content following systemic 5-HTP injection.

If kainate were inducing WDS by increasing serotonin receptor stimulation, then a kainate-induced lesion should not affect 5-HTP-induced WDS unless the kainate-sensitive neurons were the serotonergic ones. In the latter case, the loss of serotonergic presynaptic endings could result in a lowered rate of conversion of 5-HTP to serotonin and a lowered rate of WDS. On the other hand, if 5-HTP application resulted in WDS because of increased glutamate (kainate) receptor stimulation, then a lesion which reduces kainate-induced WDS should equivalently reduce 5-HTP-induced WDS.
Methods and materials. General surgical procedures were the same as used in previous studies. Holes at the coordinates for the lateral ventricles (P2.5, L4.7) were drilled on both sides of the skull. A 30 gauge needle, attached to a microliter syringe by polyethylene tubing, was lowered into each lateral ventricle--one at a time. A volume of 1.5 µl of a 1nmol/µl solution of kainate, or the vehicle solution, was injected over a 90 sec period into each lateral ventricle. The needle was left in place an additional 45 sec and then slowly removed. Eight rats received kainate solution and seven were administered the vehicle solution. Following these injections, the scalp wound was closed and the animals allowed to recover. The animals were tested 7 to 10 days later.

Carbidopa (25 mg/kg), a peripherally acting decarboxylase inhibitor, was injected 30 min prior to 5-HTP (100 mg/kg, i.p.). Ninety min after 5-HTP was injected, the subjects were placed in the observation boxes and wet-dog shakes were recorded for 1 hr. Bedard and Pycock (76) reported that the maximum rate of 5-HTP-induced wet-dog shakes occurred during this period of time.

Following behavioral testing, the animals were given an overdose of chloral hydrate, and perfused, intracardially, with saline followed by 10% formalin. The brains were removed and later prepared for microscopic analysis.

Results. Seven of the eight kainate-injected rats were found to have extensive bilateral lesions of CA3 and CA4. The other subject showed only a unilateral lesion which did not destroy all of CA3 or CA4. Control injections produced no lesions beyond the cannula track.

Injection of 5-HTP in control animals produced a behavioral constellation consisting of wet-dog shakes (Mn = 35.3; Mdn = 44), scratching,
stretching and yawning. These animals were also very active for the entire observation period.

In animals pretreated with kainate the number of WDS induced by 5-HTP was less (Mn = 12; Mdn = 3.5) than in control animals. This difference was significant (p < .03). The one kainate-treated rat showing only a partial lesion accounted for 65% of the WDS seen in the experimental group. If he is removed for the statistical analysis, the difference between groups is significant at the p < .005 level.

Discussion. Lesions of the CA3-4 region of the hippocampus strongly reduce 5-HTP-induced WDS. After removing the one rat showing only a partial lesion, the total reduction was about 90%. This is about the same as the amount of reduction in kainate-induced WDS following the same kind of lesion. This data suggests that 5-HTP acts on neurons afferent to kainate-sensitive neurons or that the kainate-sensitive neurons are serotonergic.

Parachlorophenylalanine

Introduction. If kainate induces WDS by releasing serotonin, then blockade of serotonin synthesis should reduce the effects of kainate administration. PCPA (parachlorophenylalanine) has been reported to competitively block tryptophan hydroxylase, an essential enzyme for the synthesis of serotonin (100). Administration of 100 mg/kg daily for three consecutive days has been shown to lead to a 90% decrease in the total brain concentration of serotonin at 48 hr after the third injection (100).

Methods and materials. Thirteen male, Long-Evans rats were implanted with a permanent 23 gauge cannula guide as described in Chapter VIII. One week later, seven of these rats were injected with 100 mg/kg of
PCPA, given intraperitoneally, once a day for three consecutive days. The six remaining subjects acted as controls and were injected with the vehicle for PCPA (saline plus 0.01% HCl) only. Forty-eight hours after the third injection all subjects received 0.2 nmol of kainate (2 µl) injected into the lateral ventricle in the manner previously described. They were immediately placed in observation boxes and wet-dog shakes counted for one hour.

Results. Injection of kainate in vehicle-treated rats induced WDS (mean = 120.4; median = 92). This same injection in the seven PCPA-treated animals induced a mean of 119.6 WDS (median = 129). This difference was not significant by either nonparametric tests or the t-test.

Discussion. Administration of PCPA to rats did not significantly affect the number of WDS induced by 2 µl of a 0.1 nmol/µl solution of kainate. Since it is expected that the PCPA strongly reduced the amount of serotonin in the brain, this result suggests that kainate is not inducing WDS by releasing serotonin.

In relation to the preceding study, this result suggests that a kainate-induced lesion does not reduce 5-HTP-induced WDS by destroying serotonergic neurons.

A schema in which serotonergic neurons are afferent to kainate-sensitive neurons would be consistent with both results. Since kainate induces WDS via a neural mechanism including the CA3-4 region of the hippocampus, this serotonergic influence might be terminals of raphe nuclei on the CA3-4 pyramidal neurons.

Methionine-Enkephalin

Introduction

The endorphins and enkephalins induce WDS following intraventricular, but not intracisternal administration (77). This manifestation of
their administration is prevented by naloxone (77), but not morphine (Bloom, personal communication). The number of WDS induced by kainate are affected in a similar manner by these same manipulations. The similarity of kainate- and endorphin-/enkephalin-induced WDS was further examined by testing the ability of kainate-induced lesions to prevent endogenous opiate-induced WDS. Such lesions do prevent kainate-induced WDS.

Methionine-enkephalin (met-enkephalin) was used because it was most readily available. Urca, Frenk, Liebeskind and Taylor (101) reported that 100 µg of met-enkephalin (in 10 µl) resulted in WDS in rats.

Methods and Materials

Seven rats were bilaterally injected with 1.5 µl of a 1 nmol/µl solution of kainate one week before testing to induce lesions of the CA3-4 regions. Nine more male, Long-Evans hooded rats received the vehicle for kainate alone. Following injection of kainate or its vehicle, a 23 gauge cannula guide was implanted as described in Chapter VIII.

On the test day, met-enkephalin was dissolved in saline shortly before use. A solution containing 14 µg/µl (approximately 20 nmol/µl) was prepared. Five microliters of this solution was administered to each rat. Immediately after the injection, the subjects were placed in individual observation boxes and observed for one hour. Following behavioral testing, the subjects were sacrificed and their brains prepared for microscopic examination.

Results

Histological examination of the brains revealed that kainate pre-treatment resulted in complete, but selective lesions of the CA3-4 area of the hippocampus. The only lesion seen in vehicle-pretreated subjects was that associated with the cannula itself.
In vehicle-pretreated rats, enkephalin induced a mean of 11.9 WDS in an hour. They also spent considerable time engaged in maintenance behaviors. Pretreatment with kainate reduced the number of WDS resulting from administration of met-enkephalin by 88%. This was significant at the \( p < .02 \) level (Mann-Whitney \( U \) test).

**Discussion**

Pretreatment of rats with a neurotoxic dose of kainate reduced the number of WDS induced by met-enkephalin. The reduction in terms of percent of control behavior was similar to that seen for 5-HTP- and kainate-induced WDS. This result further indicates a similarity between kainate and endogenous opiate-induced WDS.

**Ketocyclazocine**

**Introduction**

Wet-dog shaking in rats can be induced by endogenous opiates and this action can be prevented by naloxone. In pharmacological terms this indicates that WDS can be induced by stimulation of opiate receptors. However, the dose of naloxone required to block this opiate behavior is higher than required to block most other opiate-induced behaviors. For example, \( \beta \)-endorphin will induce WDS followed by catalepsy. A dose of 1 mg/kg of naloxone will reverse the catalepsy, but the WDS reappears. A dose of 2 mg/kg of naloxone will prevent the occurrence of WDS (77). This might suggest that a specific type of opiate receptor is responsible for WDS.

Etorphine, a potent morphine-like agonist, has been reported not to induce WDS (102) and morphine will only induce WDS at doses far beyond those necessary to induce analgesia (79). These data could also be taken to imply that endogenous opiate-induced WDS is mediated by a
second type of opiate receptor, one that is relatively insensitive to morphine or naloxone.

The presence of such second opiate receptors has been suggested (103,104) and ketocyclazocines have been suggested as selective agonists (103). It has been found that intraventricular injection of small amounts of ketocyclazocine itself (2µl of 1 nmol/µl solution) will induce WDS. This response was prevented by 10 mg/kg of naloxone, but not 1 mg/kg of naloxone. (Lanthorn, Smith and Isaacson, submitted).

These results strongly suggest that endogenous opiate-induced WDS is mediated by a second opiate receptor known as the kappa (K)-opiate receptor. If this is true, then kainate-induced lesions should reduce ketocyclazocine-induced WDS as they reduce kainate- and met-enkephalin-induced shaking.

Methods and Materials

Surgical and injection procedures were identical to those of the preceding experiment. Seven rats were treated with kainate, while eight received vehicle only. Ketocyclazocine (Sterling-Winthrop) was dissolved in saline containing 2 mM HCl. The ketocyclazocine solution was prepared shortly before each use. Two µl of a 1 nmol/µl (0.29 µg/µl) solution of ketocyclazocine was injected into each subject. Observation began immediately after the injection.

Results

All seven kainate treated rats showed extensive CA3 and CA4 lesions. No lesions were evident in control animals except for the cannula track.

In saline-injected animals, ketocyclazocine produced a behavioral constellation consisting primarily of intensive grooming and wet-dog shakes. The number of WDS induced was reduced 86% in rats pretreated
with kainate. This change was significant at the \(p < .01\) level.

**Discussion**

The appearance of WDS as a consequence of administration of ketocyclazocine can be largely prevented by pretreatment with a neurotoxic dose of kainate. This result strengthens the hypothesis that endogenous opiate-induced WDS is mediated by the K-opiate receptor by showing that endogenous opiates and a K agonist utilize the same neural circuit. At the same time it strengthens the belief that opiates and kainate act on the same neurons to induce WDS.

**Sodium Valproate**

**Introduction**

It has been reported that intraperitoneal administration of sodium valproate, or dipropylacetate, will induce WDS and other morphine withdrawal-like behaviors in rats (81, 84). Sodium valproate is used as an antiepileptic agent and, at least at high doses, increases the concentration of GABA in the brain. This increase in GABA is probably a result of competitive inhibition of succinic semialdehyde dehydrogenase (SSA-DH), an enzyme in the catabolic pathway of GABA.

The induction of WDS by sodium valproate can be blocked by low doses of morphine (1 mg/kg) and were released by naloxone (1 mg/kg). This is similar to how morphine withdrawal responds to these agents, but is opposite to how kainate- and endorphin-induced WDS respond to these chemicals.

**Methods and Materials**

A neurotoxic amount of kainate solution or an equal volume of vehicle solution was administered (as described in the section on 5-HTP) to six and thirteen male, Long-Evans hooded rats, respectively. Valproic acid (300 mg/kg) or sodium valproate (345 mg/kg) was injected intra-
peritoneally immediately prior to observation.

Results

Valproic acid (300 mg/kg) was injected into six rats pretreated with the vehicle for kainate. These rats became very inactive and were ataxic for 30-45 min. Following this period a couple of the subjects produced sporadic wet-dog shakes. Such severe motor depression was not reported by deBoers et al. (81) following similar doses of sodium valproate. Therefore, the valproic acid was converted to its sodium salt and an equimolar dose (345 mg/kg) was injected.

Sodium valproate was injected into six rats pretreated with kainate's vehicle. They became somewhat inactive, took on a hunchback posture, urinated excessively and produced wet-dog shakes (mean = 18.8; median = 15). In seven animals pretreated with neurotoxic doses of kainate, sodium valproate induced increased locomotion and exploration, excessive grooming, excessive urination and wet-dog shakes (mean = 21; median = 21).

The difference in the number of wet-dog shakes produced by the two groups was not statistically significant. All shaking in both groups occurred within 25 min of sodium valproate administration.

Discussion

The wet-dog shake response to sodium valproate does not appear to be affected by kainate-induced lesions. This indicates that not all factors which induce WDS do so via kainate-sensitive neurons. The data does not suggest whether sodium valproate acts on neurons directly downstream from kainate-sensitive neurons or on a parallel circuit.

It is intriguing that sodium valproate- and kainate-induced WDS react differently to naloxone and morphine and also to kainate-induced lesions.
Ice-Water

Introduction

Rats immersed in cold water will produce WDS and diarrhea (105). This response is most consistently seen in moderately anesthetized rats in which most escape behaviors are inoperable. This response to cold water can be antagonized by morphine (10 mg/kg) (105) and prevented by a lesion at the medial parts of the diencephalic-mesencephalic juncture (105), a lesion which also prevents naloxone-induced WDS in morphine-dependent rats (106). Thus this response to an environmental alteration reacts to certain manipulations in the same manner as morphine withdrawal.

It has been reported that normal rats, anesthetized with 40 mg/kg of sodium pentobarbital, dunked in cold water (4°C) and then held in the water for 5 min with their heads raised out of the water, exhibited an average of 10-15 shakes (105).

Methods and Materials

The subjects for this study were those which comprised Group 3 in Chapter VII. Four days after receiving their last injection of test solution, they were anesthetized with sodium pentobarbital (40 mg/kg). Thirty minutes later they were briefly completely immersed in ice-water (4°C). Then the heads were raised out of the water and the animal held in that position for 5 min. The number of WDS occurring in that time was recorded.

Results

All the rats subjected to this regimen exhibited WDS. A range of 7-20 WDS was observed (median = 10).
Discussion

A neurotoxic amount of kainate does not abolish the shaking response of rats to immersion in cold water. In fact, the number of shakes seen in these rats appeared to be the same as the number reported by others in normal rats (105).
GENERAL DISCUSSION

Summary

These results indicate that kainate, an analogue of glutamate, is a behaviorally active substance of tremendous potency. As little as 3 ng injected through the lateral ventricle will induce a statistically significant alteration in the ongoing behavior of a rat. Concentrations of kainate of less than 0.1 nmol/μl were most consistently correlated with increased numbers of wet-dog shakes (WDS) exhibited by the rats and were not correlated with obvious neurotoxicity. Higher concentrations of kainate were correlated with convulsions and with neuronal degeneration. A concentration of 1 nmol/μl induced selective destruction of the CA3 and CA4 fields of the hippocampus.

The WDS-inducing effect of kainate appears to be localized to forebrain structures since intraventricular, but not intracisternal, injection of kainate resulted in WDS. This effect can be further localized to some circuit including CA3 and CA4 because kainate-induced lesions of CA3 and CA4 prevent the induction of WDS by subsequent administration of kainate. Electrolytic lesions of this same area also reduce kainate's ability to induce WDS. Lesions not involving CA3 and CA4, but involving CA1 or the dentate gyrus did not appear to decrease the effect of kainate, but statistical confirmation was not possible because the groups were too small.

WDS is a conspicuous component of morphine withdrawal in the rat. The WDS induced by kainate were not affected by a moderate dose of
morphine and were blocked by a high dose of naloxone. This suggests that an opiate receptor may be involved in kainate-induced WDS, but not in the same way that morphine withdrawal is.

Substances which induce WDS in opiate-naive rats include 5-HTP (the metabolic precursor to serotonin), met-enkephalin (an endogenous opiate), ketocyclazocine (agonist of the kappa opiate receptor) and sodium valproate (an anticonvulsant with influence on GABA metabolism). Lesions, of the type which prevent kainate-induced WDS, were used to test the similarity of these agents to kainate in the manner in which they induce WDS. Destruction of CA3 and CA4 prevented 5-HTP, met-enkephalin and ketocyclazocine from inducing WDS. The WDS-inducing ability of sodium valproate was not affected by this lesion. The wet-dog shake response of rats to immersion in cold water is not affected by a lesion which blocks kainate-induced WDS.

**Implications**

There are several points of interest which these studies can be used to speak to. First is whether or not kainate-induced behavior is directly related to morphine withdrawal. The evidence presented here suggests that kainate-induced behavior is not synonymous with morphine withdrawal. In particular, it would be expected that morphine should counter the signs of withdrawal while a morphine antagonist, such as naloxone, should enhance these behavioral symptoms. In sharp contrast to expectation, morphine had no effect on kainate-induced behavior while naloxone, albeit at a high dose, reduced kainate-induced behavior. Thus, pharmacologically, kainate-induced behavior is not identical to withdrawal as it is normally encountered.

Anatomically, also, morphine withdrawal and kainate-induced behavior can be distinguished. Withdrawal can be elicited in morphine-dependent
animals by administration of naloxone or other antagonists (73). Injection of opiate antagonists into the brain itself is effective when the antagonist is allowed to interact with structures lining the fourth ventricle and cerebral aqueduct (107). Injections restricted to the lateral and third ventricle or application directly into the hippocampus are not effective (107,108). On the other hand, kainate appears to induce WDS by its action on the hippocampus. It would not be surprising if more than one part of the brain is involved, but this data does indicate that kainate-induced behavior does not involve all of the neural circuit employed by morphine withdrawal.

Coronally-oriented knife cuts of the medial part of the brain abolish withdrawal induced by systemically injected naloxone only when those cuts are made in the caudal brainstem (106), caudal to the periaqueductal region. This suggests that the withdrawal effects arising from this naloxone-sensitive region are caudally directed. Destruction of the CA3 and CA4 fields of the hippocampus, preventing kainate-induced behavior, does not affect ice-water induced shaking. However, ice-water induced shaking is prevented by morphine and by lesions similar to those which prevent withdrawal (105). These results suggest that if kainate-induced and morphine withdrawal-induced behavior are wired in series, the kainate influence must be upstream from morphine's site of action. In such a scheme, kainate's action should be subject to modulation by opiates in the same way they influence withdrawal. This, however, is not the case, suggesting that kainate- and withdrawal-induced behaviors are on parallel circuits at these levels.

However, kainate-induced behavior is sensitive to opiate antagonists. This suggests that opiates act downstream from kainate's site of action, in the expression of kainate-sensitive behavior. Furthermore, it has
been reported that endogenous opiates will induce these same behaviors, thus confirming the involvement of opiates in these behaviors. The site of this opiate-induced action appears to be a kappa opiate receptor because it is relatively insensitive to morphine or naloxone, but quite sensitive to ketocyclazocine (103,104). In the studies presented here, evidence turns up which suggests a close relationship between kainate- and endogenous opiate-induced behavior. Specifically, both occur after intraventricular, but not intracisternal injection. Lesions of the CA3 and CA4 fields of the hippocampus prevent either from inducing WDS. Finally, relatively high doses of naloxone are required to block kainate-induced WDS just as relatively high doses are required to block endogenous opiate- or ketocyclazocine-induced WDS. Thus, both anatomically and pharmacologically, kainate and kappa opiate receptor agonists show strikingly similar behavioral characteristics.

The evidence from these studies can be seen as suggesting two different, but both opiate-sensitive, mechanisms for inducing WDS. One of these mechanisms is also kainate-sensitive. This suggested scheme appears to be consistent with more of the evidence. Some of the substances which induce WDS are sensitive to blockade of their behavioral manifestations by naloxone. These include kainate, endogenous opiates and ketocyclazocine. The behavioral responses to other WDS-inducing agents are not blocked by naloxone, but are blocked by low doses of morphine. These include TRH, theophylline, sodium valproate, AG-3-5 and RX 33G-M. In the pharmacological studies presented here, the ability of kainate, met-enkephalin or ketocyclazocine to induce WDS was prevented by lesion of regio inferior. The ability of sodium valproate to induce WDS was not affected by similar lesions. Even though this is a small
sample of the available substances, those substances sensitive to
the lesion are sensitive to naloxone antagonism, while the one agent
insensitive to the lesion is insensitive to naloxone antagonism, but
is sensitive to morphine antagonism. Such evidence indicates that the
available data may be characterized as being mediated by two pharmaco-
logically and anatomically distinct mechanisms. One mechanism, sensi-
tive to kainate, utilizes the hippocampus and kappa opiate receptors.
The other utilizes the periaqueductal region and the classical mu (mor-
phine) opiate receptor.

The relationship of the hippocampus to situations in which animals,
rats in particular, exhibit wet-dog shaking is unknown. This is largely
because the behavior has not been widely described outside of two situ-
atations--immersion in water and morphine withdrawal. That the hippocampus
is capable of inducing this behavior is strongly suggested by the present
set of studies and confirmed by MacLean (109) who reported that electric-
ally stimulated afterdischarge of the hippocampus of rats was accompanied
by wet-dog shaking.

In order to further study the role of kainate-sensitive elements
of the hippocampus in normal behavior it will be necessary to discover
situations in which rats exhibit WDS. By accident, I have found that
rats exhibit WDS when repeatedly rebuffed in their attempts to achieve
sexual intercourse. Thus, it appears that rats may exhibit WDS in more
situations than were previously studied and it is possible that examina-
tion of such situations may provide a test behavior for kainate and also
reveal more of the subtleties of hippocampal modulation of behavior.
REFERENCES

40. Stephanson and G. A. R. Johnston (unpublished observation), reported in ref. 36.


BIOGRAPHICAL SKETCH

Thomas Lanthorn was born in St. Louis, Missouri, on November 4, 1950. He received his elementary and secondary education in Grand Rapids, Michigan, and was graduated from Ottawa Hills High School in June, 1968. He attended Lyman Briggs College at Michigan State University and received the degree of Bachelor of Science in June, 1972. He began work as a graduate student at the University of Florida in the summer of 1973 under the direction of Professor Robert L. Isaacson. He is presently a candidate for the degree of Doctor of Philosophy at the University of Florida.
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert L. Isaacson, Chairman
Professor of Psychology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Carol Van Hartesveldt
Associate Professor of Psychology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Marc N. Branch
Associate Professor of Psychology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Charles J. Viscek, Jr.
Professor of Neuroscience
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Adrian J. Dunn
Associate Professor of Neuroscience

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Luther J. Willmore
Affiliate Assistant Professor of Neuroscience

This dissertation was submitted to the Graduate Faculty of the Department of Psychology in the College of Liberal Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1978

Dean, Graduate School