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THE CHEMISTRY AND PHARMACOLOGY OF CERTAIN COMPOUNDS AFFECTING THE CENTRAL NERVOUS SYSTEM OF ANIMALS AND MAN

SECOND SUMMARY PROGRESS REPORT

by

Harold F. Hardman
Edward F. Domino
Maurice H. Seevers

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DEPARTMENT OF PHARMACOLOGY
SCHOOL OF MEDICINE
University of Michigan
Ann Arbor, Michigan

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THE PHARMACOLOGY OF EA 1476 AND RELATED COMPOUNDS

INTRODUCTION

The history and objective data available on marihuana-like compounds has been extensively reviewed by Loewe (1944, 1946, 1950). The major pharmacological activity of this class of compounds has been related to the tetrahydrocannabinol derivatives contained in the marihuana plant. Loewe and Adams (1947) designated EA 1476 as the most potent synthetic derivative tested with respect to producing ataxia in dogs. Loewe (1946) had previously examined the parent compound EA 1477, and related synthetic derivatives in dogs. In addition, the behavior of the dogs indicated definite central nervous system depression.

We were impressed with the outstanding pharmacological activity of this class of compounds and their relative lack of toxic effects. The series of marihuana derivatives examined produced a prolonged state of sleep that was not similar to sleep induced with the barbiturates. The animals also exhibited an indifference to nociceptive stimuli which suggested analgesia. In many ways this class of compounds resembled a new group of drugs being introduced as tranquilizing agents. These included reserpine and chlorpromazine. Reserpine was being investigated as a hypotensive agent at this time and we felt that a systematic investigation of the cardiovascular activities of the marihuana derivatives was warranted. The parent compound EA 1477 and the two most potent synthetic derivatives available, EA 1476 and EA 1465, were selected for evaluation.

RESULTS

1. CARDIOVASCULAR SYSTEM

A. BRADYCARDIA

A slowing of heart rate is observed in dogs anesthetized with sodium

pentobarbital (35 mgm./kgm) with intravenous administration of EA 1476 (0.10 mgm./kgm.), EA 1477 (10.0 mgm./kgm.) or reserpine (1.0 mgm./kgm.). Bradycardia is also a prominent feature when the above drugs are administered to unanesthetized dogs or monkeys. With large doses of these drugs an initial tachycardia may be observed which is followed by the characteristic bradycardia.

Prior atropinization of the dog reduces but does not eliminate the bradycardia in all the above cases. This observation does not negate the hypothesis that the above drugs produce bradycardia through decreased sympathetic activity and not by enhanced parasympathetic activity. Samaan (1935) has shown that the tachycardia following atropine administration is eliminated only by sympathectomy and adrenal gland denervation in addition to vagal section.

The initial tachycardia observed following large doses of EA 1476 and EA 1477 may result from peripheral vasodilator action, since neither drug appears to alter heart rate or cardiac output when examined in the dog heart-lung preparation. The reserpine tachycardia has been shown by Krayer and Fuentes (1956) to result from a direct action on the myocardium.

B. HYPOTENSION

EA 1476 and reserpine are capable of producing a significant fall in mean arterial pressure in anesthetized dogs. This effect occurs slowly with small doses of EA 1476 but large doses decrease the latent period considerably. EA 1477 in large doses produces a similar response but is relatively less potent than the other two drugs (table 1).

The hypotensive action of these drugs in threshold doses is characterized by a long latent period following intravenous

administration. In general the fall in blood pressure does not become significant until one to two hours after the drug is injected. Before this time, the carotid occlusion pressor reflex may be significantly depressed in the case of EA 1476 and EA 1477. The sequence of these events suggested that the hypotension results from a decrease in central sympathetic outflow.

Bein (1955) and Trapold (1953) have postulated that reserpine produced a fall in blood pressure through depressing sympathetic outflow at the level of the hypothalamus. A series of experiments were then planned in order to evaluate the role of the sympathetic nervous system in the depressor response initiated by EA 1476.

1. Common Carotid Occlusion Pressor Response

   This response is dramatically reduced in the pentobarbital anesthetized dog within thirty minutes after intravenous injection of EA 1476 (0.05 mgm./kgm.), EA 1477 (10.0 mgm./kgm.) and reserpine (1.0 mgm./kgm.). The results are tabulated in table 2, according to the method of Prochnik et al. (1950). This method corrects for the influence of blood pressure changes per se on the pressor reflex.

2. Central Vagal Stimulation Pressor Response

   The pressor response elicited by unilateral central vagal stimulation with the contralateral vagus sectioned is greatly reduced or even reversed following intravenous injection of EA 1476 (1.0 mgm./kgm.) to pentobarbital anesthetized dogs. Reserpine is reported to have the same qualitative effect in equivalent doses (Trapold 1953), however, it appears to be less potent.

3. The Effect of EA 1476 on Electrical Stimulation of Hypothalamic and Medullary Vasomotor Areas of the Cat.

The cat has been observed to respond to the hypotensive action of EA 1476 in the same manner as the dog. By means of stereotaxic technique, electrodes were placed in the hypothalamus and medullary vasomotor areas of cats anesthetized with pentobarbital (20-30 mgm./kgm.). Monopolar steel electrodes were utilized for electrical stimulation. They were insulated with varnish except for their tips which were exposed. Electrical stimulation of the posterior lateral hypothalamus produced a definite pressor response as shown in figure 1. The hypothalamus was stimulated for ten seconds as shown between the arrows of the upper left control tracing marked H In view of the varying threshold for eliciting these responses in anesthetized animals it was felt that optimal voltages of stimulation should be used. These voltages were definitely above threshold but were submaximal. The usual voltage range for stimulation was approximately two to three volts. The elicited pressor response was accompanied by marked pupillary dilatation and definite pilomotor erection of the dorsal hair. Occasionally urination occurred during the period of electrical stimulation. When the medullary vasopressor area was stimulated for ten seconds a pressor response as shown in fig. 1, lower left control tracing marked M, was observed. Within one half hour after 1.0 mgm./kgm. of EA 1476 the mean blood pressure was reduced. One hour after the administration of the above dose the pressor response to hypothalamic stimulation was still present (upper right tracing, fig. 1). Although the pressor response was still present, pilomotor erection was not

as prevalant. Similarly, seventy minutes after the administration of EA 1476 the medullary pressor response was still obtainable (lower right tracing, fig. 1). The pressor responses from hypothalamic and medullary stimulation appear to be relatively enhanced over control levels although the absolute rises in blood pressure were reduced. Upon completion of the experiment iron was deposited from the electrode tips electrolytically. The brain was sectioned and histological localization of the site of stimulation was visualized by means of the Prussian blue reaction according to Domino (1955). As illustrated in fig. 2 the sections taken through the hypothalamus (upper) and through the medulla (lower) are stained with thionin and show the location of the electrode tips. The hypothalamic electrode was located in the left posterior lateral hypothalamus and the medullary electrode was located in the right reticular formation at the floor of the fourth ventricle.

From several experiments of this type it was concluded that efferent vasomotor activity is not depressed per se when one stimulates the efferent side of the arc in the hypothalamus or in the medulla. This conclusion was strengthened considerably by the following experiment illustrated in fig. 3. One electrode was placed in the posterior hypothalamus and elicited a pressor response with electrical stimulation (see blood pressure tracing, upper left corner). The medullary response consisted of a biphasic blood pressure change. This was characterized by an initial fall and subsequent rise is blood pressure (lower left tracing). One hour following the administration of EA 1476 the absolute blood pressure was reduced and the pressor response from hypothalamic stimulation

was still present although altered in form (upper right tracing). As in previous animals a decrease in the piloerection response was now observed. The depressor response resulting from medullary stimulation was unaltered whereas the subsequent pressor response was practically eliminated (lower right tracing). Histologic localization of the electrode tips verified that the hypothalamic electrode was present in the posterior hypothalamus. The medullary electrode, however, had not been placed in the vasomotor center as anticipated, but was actually lateral to it intermingled with the medullary fibers of the vagus nerve. The biphasic blood pressure response obtained with medullary stimulation during the control period, therefore, resulted from stimulation of afferent vagal neurons. The elimination of the pressor component of the biphasic blood pressure response by E/. 1476 presumably resulted from an action between afferent and efferent neuronal mechanisms. The efferent side of this reflex is obviously not depressed as shown by previous experiments. This observation suggests that E/. 1476 does not have a major effect on the peripheral baroceptors in the mechanism of depressing the carotid occlusion pressor response. Presumably a central depressant action must be involved in the reduction of the cariovascular refloxes by E/. 1476. The results obtained in this medullary preparation strongly suggest that the central sympathetic system is the one primarily affected by the central depressant actions of E/. 1476. The central parasympathetic system appears to be relatively unaltered since the depressor response obtained before and after administration of E/. 1476 remains unaltered.

C. DIRECT CARDIAC ACTIONS

EA 1476 (1.0 mgm./kgm.) was evaluated in the denervated dog heart-lung preparation. The above dose was added to one liter of circulating blood when the heart was in a state of semifailure. The state of semifailure was determined by the "Competence Index" value of 0.5 recorded (Mollenberger, 1949). This dose of drug had no effect upon heart rate or cardiac output. The results obtained suggest that EA 1476 does not have any immediate direct effects upon the dog heart. We may, therefore, assume that the bradycardia produced by this drug in unanesthetized and anesthetized dogs results from a primary action on the central autonomic nervous system. The magnitude of the bradycardia, however, is such that inhibition of cardiac sympathetic tone alone may not offer a complete explanation of the observed changes in heart rate. It is possible that EA 1476 has a delayed direct effect upon the heart.

E. VASCULAR RESPONSE TO EPINEPHRINE

EA 1476 (0.05 mgm./kgm.) and reserpine (1.0 mgm./kgm.) reduce the mean arterial pressure significantly within two hours after intravenous administration to dogs anesthetized with pentobarbital (table 1). It has been assumed that the fall in blood pressure results from a decrease in peripheral resistance due to reduced sympathetic outflow. This assumption is based upon the lack of any evidence to suggest that either drug directly depresses cardiac output as determined in the heart-lung preparation. In addition, neither drug significantly lowers the blood pressure of spinal cats transected at cervical vertebra #1. The pressor response to graded doses of intravenous epinephrine is not altered significantly either in anesthetized dogs or spinal cats following administration of EA 1476 or reserpine in the doses mentioned above. The

relative rise in blood pressure to epinephrine appears to be enhanced when the mean arterial pressure has been reduced significantly by EA 1476 or reserpine. However, the absolute pressure attained is less than that observed during the control readings. These results are consistent with the hypothesis that EA 1476 and reserpine reduce peripheral resistance by decreasing sympathetic arterial tone. The results also eliminate the possibility that either drug possesses any appreciable adrenergic blocking activity.

II. CENTRAL NERVOUS SYSTEM

A. BEHAVIORAL CHANGES.

The outstanding behavioral changes in dogs and monkeys resulting from administration of EA 1476, EA 1477 and EA 1465 consists of profound central nervous system depression lasting from several hours to several days depending upon the dose utilized. During this period the animals do not respond in a normal manner to painful stimuli. The degree of apparent analgesia is outstanding. With very small doses of the above drugs one can achieve a selective CNS depression of the dog or monkey that is free from signs of central excitation or analgesia. The animals appear to prefer to lie quietly and passively resent being disturbed. This effect is observed in the dog with intravenous doses of 0.1 to 0.2 mgm./kgm. of the three drugs mentioned above. Higher doses are required to produce a similar response in the monkey. With larger doses of these drugs, a period of initial central nervous system stimulation is observed followed by prolonged depression and finally the period of recovery is heralded by the return of the signs of stimulation. This sequence of events follows the classical pattern of induction and emergence from general anesthesia. The animals, however, are not anesthetized

in the usual sense of the word. They may show a reduction or absence of response to pain; however, it is almost always possible to arouse them from the depressed state through adequate stimuli. The simple procedure of righting the dog or monkey and placing him in the normal position of locomotion usually produces arousal of short duration. This procedure may in fact induce a period of hyperexcitation characterized by a very brief return to an almost normal state. If the dose of drug was sufficient to cause initial excitation characterized by tremors or convulsions, these may return when the animal is subjected to forced arousal during the period of marked central nervous system depression.

EA 1476 is the most potent of the three drugs. EA 1465 has an almost identical spectrum of activity and is nearly as potent as EA 1476. EA 1477, the parent compound of this class of drugs, is considerably less potent; however, in adequate doses it possesses all the qualitative actions of the other two drugs.

These drugs as a group possess several additional interesting properties. They are effective orally in doses less than 0.5 mgm./kgm. The duration of CNS depression is roughly proportional to the magnitude of the administered dose. With large doses of EA 1476 and EA 1477 dogs have been maintained in an unconscious state for five to six days and recovered uneventfully. There is a marked reduction in body temperature which is maintained during the phase of CNS depression. Elevating the body temperature by placing the dog in warm water can initiate arousal during the phase of CNS depression. We have been unable to show any correlation between CNS depression and the hypotensive action of EA 1476. The blood pressure returned to near normal levels several days before a series of dogs recovered from the CNS depressant effects.

detailed description of behavioral changes is contained in a previous report by Hardman et al. (1954).

B. POTENTIATION OF SLEEPING TIME IN MICE

Following the report by Shore et al. (1955) that reserpine potentiated the sleeping time in white mice induced by intraperitoneal hexobarbital, we repeated their experiments utilizing EA 1476 in place of reserpine. The results obtained indicate that EA 1476 administered intraperitoneally in doses of 1.0 and 3.0 mgm./kgm. can significantly potentiate the hexobarbital sleeping time. Another series of experiments was run in which d-amphetamine was employed to antagonize the potentiating effect of EA 1476 upon the hexobarbital sleeping time. The results are summarized in tables 3 and 4.

Even though EA 1476 is not a hypnotic per se in the manner of the barbiturates it does potentiate the sleeping time in mice induced by hexobarbital. Loewe (1944) has reported that the synergistic hypnotic action of marihuana and barbiturates is primarily due to the cannabidiol content of the marihuana. In a later report (Loewe, 1950) he further limits the choice of barbiturates to Pernoston, a bromide containing barbital derivative. Loewe (1944) states that 20.0 mgm./kgm. of cannabidiol prolongs the sleeping time of barbitalized mice by ninety per-cent, in contrast to 100 mgm./kgm. of tetrahydrocannabinol which only prolongs the sleeping time by twenty per-cent. Our data indicates that EA 1476 is approximately twenty times as potent as cannabidiol in this respect. In specific cases 1.0 mgm./kgm. of EA 1476 prolongs the mouse sleeping time by eighty per-cent when administered one hour before hexobarbital (100 mgm./kgm.). This response can be antagonized by the administration of d-amphetamine prior to the injection of hexobarbital.

C. ELECTROENCEPHALOGRAPHIC CHANGES

There are few published studies on the electroencephalographic effects of cannabis and its derivatives. Straus (1944) in a study of fifteen patients noted an increase in alpha activity of the human electroencephalogram (EEG) which was associated with euphoria. Two control patients who received no cannabis showed a similar increase in alpha activity. Davis and Ramsey (1949) reported that EA 1477 and EA 1476 were effective in the treatment of grand mal and petit mal epilepsy. A diphenylhydantoin refractory group of grand mal epileptics showed considerable improvement with a lack of toxic side effects after several months use of EA 1476. The report suggests that EA 1476 is about one hundred and fifty times more potent than diphenylhydantoin as an anti-epileptic agent against grand mal seizures and also can normalize the EEG pattern of such patients. The dose of drug employed was not stated in the reports by Straus or Davis and Ramsey. Loewe and Goodman (1947) observed that EA 1476 and EA 1477 were effective agents for abolishing the hind leg tonic extensor component of maximal electroshock seizures in the rat. They concluded that the pattern of high anticonvulsant potency in the maximal electroshock test and the absence of protection against Metrazol aligns the marihuana congeners with the diphenylhydantoin type of anticonvulsants. The anticonvulsant ratios (EA 1477 = 1,0, EA 1476 = 200) are similar to the ataxia potency ratios in the dog, thus suggesting that anticonvulsant and ataxia activity are closely related. Threshold doses for anticonvulsant activity, however, were below those causing ataxia and other neurological signs.

Chronic administration of marihuana and related derivatives has been reported to cause a slowing of alpha activity and the appearance of

activity in the dog (Williams et al, 1946, Hikler and Lloyd, 1945).

1. Method. Mongrel dogs were immobilized by decamethonium (250 microgm./kgm., i.v.) or Flaxadil (500 microgm./kgm., i.v., p.r.n.) and placed on continuous artificial respiration with a Palmer pump. Surgery was performed under local anesthesia with either ethyl chloride or one per cent procaine hydrochloride. Insulated steel nails were placed through the scalp into the calvarium for recording the electrical activity of the cerebrum and care was taken to avoid passing through the posterior lamella. Electrical activity was recorded with a Grass electroencephalograph. The electrocardiogram, lead I, and the femoral arterial blood pressure were recorded simultaneously with the same instrument.

Seven dogs were utilized to obtain control EEG data. The dogs received either one ml. of a 95% solution of ethyl alcohol or a comparable volume of isotonic sodium chloride solution and were observed for several hours. The usual EEG pattern observed consisted of low voltage, fast frequency waves characteristic of an awake, unanesthetized dog. Over a two hour period slow wave bursts would appear occasionally. These were quickly converted by noise or other afferent stimuli to a low voltage, fast frequency EEG. Control animals, therefore, show electrical correlates of drowsiness or sleep that are quickly reversed to a state of wakefulness by certain afferent stimuli.

2. EEG After EA 1476

Seven dogs received varied doses of EA 1476 administered intravenously in 95% ethyl alcohol. With doses of 50 microgm./kgm., occasionally sleep spindles were observed. However, such spindling was also

shown in the control animals. After 100 microgm./kgm., the spindle bursts were prolonged and more frequent than in controls. The EEG record of one such experiment is shown in fig. 4. The upper tracing A represents the control EEG of the dog one hour after surgery. One hour after 100 microgm./kgm. of EA 1476 the spindle bursts were prolonged and alternated frequently with low voltage fast frequency activity (see B, fig 4). The observed slow wave bursts of twelve cycles per second could readily be converted to a low voltage, fast frequency EEG pattern by afferent stimuli such as noise (see C, fig. 4) Since increased afferent activity resulted in EEG arousal, it was postulated that drugs which stimulate the brain stem activating system would antagonize the EEG effects of EA 1476. As shown (see D, fig. 4) d-amphetamine (100 microgm./kgm.) does antagonize the slow wave activity induced by EA 1476. The EEG pattern obtained is almost indistinguishable from the control (compare A and D).

The effect of large doses of EA 1476 (2.0 mgm./kgm.) is illustrated in fig. 5. Tracing A represents a normal low voltage fast frequency control pattern. The frequency of spindling increased about one half hour after drug administration. One hour after the drug, generalized slow wave activity with frequent spindle bursts was observed. Auditory stimuli were completely ineffective in producing EEG arousal during this period (see B, fig.5). Exceedingly painful stimuli, however, such as pinching the testicles resulted in a barely effective EEG arousal. Within a minute the spontaneous electrical activity returned to a high voltage, slow wave pattern (see C, fig. 5). The same animal is presented again in figure 6. The control tracing is labeled A. The response to EA 1476 and lack of arousal to auditory

stimuli is labeled B. Part C represents the response to d-amphetamine (1.0 mgm./kgm.). There was a definite EEG antagonism. However, the response after amphetamine was not the same as the very fast frequency activity observed in the control tracing.

3. EEG After EA 1465

Four dogs received EA 1465 intravenously in doses of 0.5 to 1.0 mgm./kgm. A typical response is illustrated in figure 7. Tracing A represents the normal control activity. Occasionally alpha-like waves are observed but the predominant character of the EEG is a low voltage, fast frequency pattern. Tracing B represents the response to EA 1465 (1.0 mgm./kgm.) one hour after drug administration. At this time auditory stimuli were barely able to produce EEG arousal. Fewer high frequency waves were observed at this time than during the control tracing. In addition, there was a delay in the onset of arousal. Tracing C, represents the response to d-amphetamine (1.0 mgm./kgm.) and suggests an incomplete antagonism of the slow wave activity induced by EA 1465.

4. EEG After Atropine

Several groups of investigators have described the changes in cerebral activity due to atropine. Typical sleep-like patterns are described in the cat (Hescoe et al., 1949; Funderburk and Case, 1951; Bradley and Elkes, 1953; and Longo, 1956). Sleep-like waves have also been observed in the EEG of the normal dog given atropine although the behavior of the dog suggests excitation (Wikler, 1952). In the present investigation six dogs received d-l atropine intravenously in doses of 0.1 to 1.0 mgm./kgm. The 0.1 mgm./kgm. dose had no apparent effect upon the EEG of the normal awake dog. There was,

however, an obvious tachycardia as recorded by the EKG. A subsequent dose of 0.5 mgm./kgm. of atropine produced some slow wave activity in the EEG. Doses of 1.0 mgm./kgm. of atropine characteristically produced slow wave, sleep-like activity in the dog EEG. The administration of d-amphetamine in doses of 0.5 to 1.0 mgm./kgm. resulted in a much less effective EEG arousal than the arousal observed when EA 1476 was substituted for atropine. In dogs who had previously received EA 1476 (1.0 mgm./kgm.) the subsequent administration of atropine (1.0 mgm./kmg.) appeared to enhance the slow wave activity. Spindle bursts were still present but less discrete than prior to the administration of atropine. Generalized slow wave activity persisted throughout the remainder of the experiment. This impression of an additive or potentiating action of atropine upon cannabis derivatives has been described previously in a report of the Indian Hemp Drugs Commission, 1893-1904 (See Osmond, 1956, on the behavioral effects of Indian hemp combined with Datura).

5. EEG After Morphine

The effects of morphine on the EEG of animals and man has been reviewed by Wikler (1950). Morphine in doses of 5-10 mgm./kgm. produces irregular high voltage random slow waves. Large doses (200 mgm./kgm.) produce high voltage, fast activity which tends to appear in bursts. These resemble petit mal seizure discharges of the spike-dome variety and occasionally sustained spike discharges.

We examined the effects of morphine on three dogs. They received intravenous injections of 1-5 mgm./kgm. In a dose of 1.0 mgm./kgm. morphine produced enhanced sleep-like activity in the EEG. Enhanced slow wave activity was observed with frequent delta wave bursts

after doses of 5,0 mgm./kgm. Amphetamine (1,0 mgm./kgm.) administered intravenously was found to be less effective in antagonizing the slow wave activity due to morphine than that resulting from administration of E/. 1476.

6. EEG After Reserpine

The EEG changes elicited by reserpine (0,5 - 1,0 mgm./kgm.) administered intravenously to the monkey have been described by Schneider and Earl (1954). They observed no change from the normal alpha activity of the control tracings. There were periods of slow wave activity of higher voltage indicating drowsiness. The monkeys, however, could be readily aroused by a variety of afferent stimuli. In contrast to the above report, Rinaldi and Himwich (1955) observed an EEG arousal pattern in the rabbit following intravenous reserpine (1,0 - 3,0 mgm./kgm.).

In the present investigation seven dogs were given intravenous doses of reserpine phosphate or acetate (1,0 mgm./kgm.). The EEG was observed for two hours following drug administration and consisted of normal low voltage, fast frequency patterns. Fewer periods of sleep-like spindles were observed in these dogs than in the control series. Our results are, therefore, in agreement with the observations of Rinaldi and Himwich (1955).

7. Discussion of EEG Observations

The electroencephalographic changes produced by E/. 1476 in the dog are most similar to the changes produced by small doses of morphine (1-5 mgm./kgm.). These changes consist primarily of sleep-like activity, spindle formation and continuous delta bursts with large doses. Of considerable interest is the fact that one can

arouse animals under the influence of EA 1476 or EA 1465 through efferent stimuli. As is true for animals under light Dial anesthesia, auditory induced EEG arousal is depressed first while pain induced EEG arousal is more resistant to depression by Dial (Gellhorn, 1953), EA 1476 or EA 1465. After a full anesthetic dose of Dial painful stimuli no longer produce EEG arousal. Similarly doses of EA 1476 and EA 1465 that produce a profound behavioral depression (1.0 mgm./kgm.) permit only a very transient EEG arousal to painful stimuli. These observations correlate quite well with observations in the intact dog, in which intense efferent stimuli can arouse a previously prostrate animal sufficiently so that it can briefly rise to its feet and stand without support. The EEG changes produced by atropine also bear some superficial similarity to those produced by EA 1476. Although EA 1476 and reserpine produce approximately similar effects upon behavior they produce different responses on the dog EEG pattern. The observation that d-amphetamine partially antagonized the slow wave bursts produced by EA 1476 and EA 1465 suggests that other agents which stimulate the brain stem activating system and/or cerebral cortex would be effective in partially antagonizing the behavioral effect of these drugs. The results of such a study are reported in section III, 2., of this report.

D. EFFECT OF EA 1476 ON SPINAL REFLEXES.

Two acute spinal cats were prepared according to the technique of Henneman et al. (1946). In this preparation stimulation of the ipsilateral sciatic nerve produced monosynaptic inhibition of the patellar reflex. The patellar reflex is also mediated over a monosynaptic arc. Stimulation of the contralateral sciatic nerve produced facilitation or

crossed extension which is mediated over a polysynaptic arc. The intravenous injection of EA 1476 (1.0 mgm./kgm.) reduced slightly the response to contralateral sciatic nerve stimulation. A slight reduction in the amplitude of the knee jerk occurred over a period of one hour. The slight changes observed were well within the normal variation to be expected in control animals subjected to this technique. It was concluded that the spinal actions of EA 1476 were indeed minor if at all present.

We were impressed by the fact that an enhancement of the knee jerk was not observed. One of the outstanding features demonstrable in the unanesthetized dog who has received EA 1476, 1477 or 1465 is the greatly exaggerated tendon reflexes. We must, therefore, attribute the enhanced spinal reflexes to an action of EA 1476 which occurs at supraspinal levels.

E. MISCELLANEOUS ACTION

1. Hypothermia

Hypothermia is an outstanding sign following intravenous administration of 1.0 mgm./kgm. of EA 1476. The rectal temperature falls 4-7° C. within twenty four hours and gradually returns to normal over a several day period in the unanesthetized dog. Trendold et al. (1953) have reported that reserpine has a similar effect in the dog.

2. Eye Signs

Miosis is an inconstant finding after administration of EA 1476 or EA 1477. By contrast miosis is regarded as a constant finding after the administration of reserpine to several animal species. In general a conspicuous mydriasis was observed in dogs following intravenous administration of EA 1476 or EA 1477.

3. Relaxation of the Nictitating Membrane

In the dog this structure is innervated only by the sympathetic nervous system. It is readily relaxed following the intravenous administration of EA 1476 or reserpine. These observations suggest that both drugs effectively reduce the normal central sympathetic outflow since neither drug exhibits a characteristic adrenergic blocking action, and ganglionic blockade in an unlikely explanation.

4. Respiratory Depression

This response is most readily observed in the unanesthetized dog or monkey and often has a dramatic onset of action. With EA 1476 the depression of respiratory rate usually appears with the onset of ataxia and just precedes the development of bradycardia. Reserpine often produces an initial increase in respiratory rate lasting two to three hours. This is followed by a persistent gradual decrease in respiratory rate (Trapold et al. 1953). With large doses of either drug, the acute toxicity and death appears to be of respiratory origin.

5. Diarrhea

The severe and usually bloody diarrhea in the dog is the outstanding toxic manifestation of reserpine alkaloid. In our studies bloody diarrhea occurred with intravenous doses of reserpine as low as 0.10 mgm./kgm. Some recent data utilizing the phosphate and acetate salts of reserpine suggest that larger doses may be tolerated without producing bloody diarrhea. Qualitatively EA 1476 possesses this property also; however, the amount of drug required to produce an equivalent degree of bloody diarrhea is about one thousand times greater than the dose of reserpine on a mgm./kgm. basis.

III. TOXICITY

A. GENERAL

The majority of our studies in this category have been restricted to acute toxicity experiments in several species of animals. The toxicity data now available are summarized in table 5 and must be regarded as preliminary.

$$\text{Therapeutic Ratio (T.R.)} = \frac{LD_{50}}{\text{Effective Dose}}$$

Effective dose is defined as that dose required to produce tranquilization in the unanesthetized dog.

$$\text{Reserpine T.R.} = \frac{0.50 \text{ mgm./kgm., i.v.}}{0.10 \text{ mgm./kgm., i.v.}} = 5.0$$

$$\text{EA 1476 T.R.} = \frac{100.0 \text{ mgm./kgm., i.v.}}{0.05 \text{ mgm./kgm., i.v.}} = 2000$$

A comparison of the T.R. values for reserpine and EA 1476 indicates that reserpine is approximately four hundred times more toxic than EA 1476 in the dog when administered by the intravenous route.

B. ANTAGONISM OF EA 1476 BY OTHER DRUGS

1. EA 1476 Plus Cocaine

Four control dogs received 4.0 mgm./kgm. of cocaine intravenously. They exhibited mild signs of hyperactivity for several hours after receiving the drug. A brief period of marked excitement was observed immediately after drug administration which was accompanied by hyperpnea. This effect was replaced by mild excitation within thirty minutes after drug administration. Recovery was uneventful.

One dog was given EA 1476 (1.0 mgm./kgm., i.v.) and four hours later appeared to be sleeping. He could be aroused by handling. Cocaine (0.5 mgm./kgm., i.v.) was administered and the dog appeared to recover his alertness immediately. He responded to calling

and scretched his ~~floss vigorously.~~ Some degree of analgesia was still present. Recovery from drug effects was recorded on the fourth day after receiving the drug.

A second dog received cocaine (1.0 mgm./kgm., i.v.) four hours after EA 1476 (1.0 mgm./kgm., i.v.) However, no marked change in behavior was observed.

Four dogs received cocaine (2.0 mgm./kgm., i.v.) four hours after EA 1476 (1.0 mgm./kgm., i.v.). One dog exhibited tonic and clonic convulsions accompanied by continuous yelping immediately after the administration of cocaine. This was followed by well coordinated running movements for several hours with intermittent rest periods. The dog died eight hours after receiving the cocaine. The other three dogs in this group were markedly stimulated following the injection of cocaine but they did not convulse. Increased running activity, mild ataxia and some disorientation was observed. Respiration was greatly stimulated and frequent periods of panting were observed. The three dogs appeared to have recovered completely three days after cocaine administration.

Four dogs received cocaine (4.0 mgm./kgm., i.v.) four hours after EA 1476 (1.0 mgm./kgm., i.v.). Three of the animals convulsed immediately after receiving the cocaine. Two dogs exhibited strychnine-like convulsions with opisthotonus and the third dog underwent a clonic convulsion with continuous yelping. The degree of ataxia was greatly reduced in all four dogs after cocaine. The symptoms of excitation disappeared within one hour after which the dogs were depressed and slept quietly. Three days later two of the four dogs were found dead. The remaining dogs recovered uneventfully.

2. EA 1476 plus d-amphetamine

A control dog received amphetamine (1.0 mgm./kgm., i.v.) and exhibited signs of hyperactivity characterized by pacing the floor. Piloerection and mydriasis were also observed in this animal. Recovery was complete in twenty four hours.

Two dogs received amphetamine (1.0 mgm./kgm., i.v.) three hours after EA 1476 (1.0 mgm./kgm., i.v.). One dog was roused from a state of deep depression characterized by marked lethargy, depressed respiratory rate and complete indifference to handling following the injection of amphetamine. He rose to his feet, walked around, and appeared to be aware of his surroundings. The ataxia produced by EA 1476, however, was not completely antagonized by the amphetamine. The second dog reacted in a similar manner and the amphetamine arousal persisted for several hours.

When the response to EA 1476 (1.0 mgm./kgm., i.v.) was antagonized by amphetamine (2.0 mgm./kgm., i.v.) definite signs of hyperactivity were observed. This included rapid pacing of the floor with sudden bursts of running and falling. Respiratory rate was also greatly accelerated. Three hours later the dog appeared alert and more restful although he still exhibited a definite hyperpnea.

Twenty four hours after the depressant effects of EA 1476 (1.0 mgm./kgm., i.v.) were antagonized by d-amphetamine (1.0 - 2.0 mgm./kgm., i.v.) all three animals were found dead. A post mortem examination of the duodenal mucosa failed to reveal any signs of internal bleeding so that shock resulting from the sympathomimetic amine appears unlikely as the cause of death.

A parallel series of three dogs received only EA 1476 (1.0 mgm./kgm., i.v.). Twenty four hours after this they still showed a characteristic drug response. At this time they were lethargic and showed some response to calling and petting. They were able to stand and walk around the cage. Three days later recovery appeared to be complete.

Four dogs received EA 1476 (1.0 mgm./kgm., i.v.) which was followed three hours later by d-amphetamine (0.25 - 0.50 mgm./kgm. i.v.). The antagonism observed was marked. Analgesia, exaggerated tendon reflexes and ataxia were still present. All the animals recovered uneventfully in three days.

The antagonism of EA 1476 by d-amphetamine is outstanding; however, it is incomplete. Ataxia may still persist although the behavior of the dogs is suggestive of marked improvement. It is apparent that d-amphetamine greatly enhances the toxicity of EA 1476.

3. EA 1476 Plus Caffeine

Two dogs received only caffeine alkaloid (10.0 mgm./kgm., i.v.). Their behavior suggested hyperactivity and was characterized by pacing the floor, excessive playfullness and minor muscle tremors. They appeared to be normal three to four hours later.

Two dogs received caffeine (5.0 mgm./kgm., i.v.) four hours after EA 1476 (1.0 mgm./kgm., i.v.). A slight decrease in respiratory rate was the only effect observed.

Three dogs received caffeine (10.0 mgm./kgm., i.v.) four hours after EA 1476 (1.0 mgm./kgm., i.v.). Two of the animals showed an immediate arousal from their depressed state. They exhibited a tonic extensor position, ataxia, hyperactivity, and hyperpnea. The

third dog did not exhibit an arousal response to caffeine.

One of the two dogs that responded to caffeine died twenty four hours later. The second dog was still ataxic and exhibited analgesia at this time. He was found dead five days after receiving the caffeine.

4. EA 1476 Plus Nalorphine

One dog received only nalorphine (6.0 mgm./kgm., i.v.). One hour after receiving the drug the dog appeared to be sedated and preferred to lie quietly. Considerable prodding was necessary to induce the animal to walk. A previous report by Unna (1943) indicated that nalorphine had little effect upon the CNS and respiration of dogs in doses of 10-30 mgm./kgm. The effect of lethal doses in the monkey is the production of tremors, periodic clonic convulsion, simultaneous respiratory arrest and cardiovascular collapse as the terminal event (Irwin and Seevers, 1954). It appears that CNS stimulation is the primary factor in the lethal effects of nalorphine although certain CNS depressant effects are undoubtedly involved to some degree (Woods, 1956).

Two dogs received nalorphine (6.0 mgm./kgm., i.v.) one hour after EA 1476 (0.25 mgm./kgm., i.v.). Prior to injection of nalorphine the dogs were very depressed but exhibited ataxia and tonic spasticity with forced arousal. There was marked arousal immediately after the injection of nalorphine. The dogs were very stimulated and alert. They were able to jump from a table to the floor in a well coordinated manner and were almost free from signs of ataxia. No signs of analgesia were present at this time in contrast to definite analgesia demonstrated prior to the nalorphine injection.

On the following day both dogs still exhibited slight sedation and ataxia. Recovery was uneventful.

Four dogs received nalorphine (6.0 mgm./kgm., i.v.) one hour after EA 1476 (1.0 mgm./kgm., i.v.). Prior to the injection of nalorphine the animals were very depressed and analgesia was demonstrable. Tonic extensor movements were observed with forced arousal at this time. After receiving nalorphine all dogs exhibited marked arousal characterized by spastic running movements and convulsions. They also reacted to painful stimuli at this time. Twenty four hours later the animals were depressed but could easily be aroused. Seventy two hours after drug administration one dog was found dead.

One dog received nalorphine (12.0 mgm./kgm., i.v.) in two equally divided doses one and three hours after EA 1476 (1.0 mgm./kgm. i.v.). A marked arousal was observed following the injections of nalorphine similar in character to that described in the previous paragraph. The animal died seventy two hours after the last injection of nalorphine.

5. EA 1476 Plus Dextrose

On one occasion we had injected a concentrated solution of dextrose into a dog depressed by the previous administration of EA 1476. A marked arousal was observed. However, we were not sure that the procedure of handling the dog had not precipitated the arousal rather than the injected dextrose.

Five dogs received dextrose (125 mgm./kgm., i.v.) one hour after the administration of EA 1476 (1.0 mgm./kgm., i.v.). No appreciable change in behavior was observed following the injection of dextrose.

Five additional dogs received EA 1476 (1.0 mgm./kgm., i.v.) and dextrose (250 mgm./kgm., i.v.) in rapid succession. An impression was formed that this procedure delayed the onset of the typical response to EA 1476. The dextrose did not appear to alter the usual qualitative signs resulting from injection of EA 1476 in any other way than to delay the onset of drug action.

We concluded that dextrose had no significant effect as an antidote with respect to the typical EA 1476 drug response.

C. POTENTIATION OF EA 1476 BY OTHER DRUGS

1. EA 1476 Plus Atropine

Two control dogs received only atropine (2.0 mgm./kgm., i.v.). The dogs appeared to be slightly depressed but were easily aroused. Heart rate and respiratory rate increased significantly and mydriasis was prominent. Eleven hours later the dogs were alert and active and mydriasis was still observed.

Two dogs received only EA 1476 (0.125 mgm./kgm., i.v.). Definite depression was observed but the animals were easily aroused and exhibited hyper-reflexia at this time. Two hours after receiving the drug the heart rates were 36 and 80/min. while the respiratory rates were 36 and 20/min. Eleven hours later the dogs were still depressed and ataxic but easily aroused from their depressed state.

Two dogs received atropine (0.5 mgm./kgm., i.v.) two hours after EA 1476 (0.125 mgm./kgm., i.v.). The degree of CNS depression appeared to be enhanced following atropine administration; however, the animals could be readily aroused. Paradoxically, hyperalgesia was observed in both dogs at this time. Twelve hours later both animals were sleeping, could be readily aroused, and resented being

disturbed. The dogs recovered uneventfully.

Two dogs received atropine (2.0 mgm./kgm., i.v.) two hours after EA 1476 (0.125 mgm./kgm., i.v.). Their behavior was characterized by CNS depression, ataxia, analgesia and ease of arousal. Twelve hours later they were still depressed and ataxic.

All the dogs in this series recovered uneventfully. We offer only the subjective impression that atropine appeared to slightly enhance the CNS depressant action of EA 1476.

2. EA 1476 Plus Morphine Sulfate.

Two dogs received only EA 1476 (1.0 mgm./kgm., i.v.). Marked CNS depression was observed one hour later. The dogs could not be aroused at this time and showed no response to painful stimuli. Three hours after receiving the drug the dogs exhibited tonic extensor convulsions on arousal forced by violent shocking. A similar response was observed six hours after drug administration. Twenty four hours later CNS depression and ataxia on forced arousal was observed. A period of uneventful recovery began at this time.

Two dogs recived only morphine sulfate (2.0 mgm./kgm.). One hour later CNS sedation was the prominent feature of their behavior. Emesis was also observed. Three hours after drug administration the dogs appeared to be restless and apprehensive. Their heart rates were 60 and 54 per minute at this time. The presence of analgesia was questionable. Six hours after receiving the drug the animals appeared to be normal.

Four dogs received morphine (2.0 mgm./kgm.) subcutaneously one hour after EA 1476 (0.1 mgm./kgm., i.v.). Prior to the injection of morphine the dogs appeared to be sedated but were easily aroused

from this state. Thirty minutes after administration of morphine ataxia, emesis, and hind limb weakness were the prominent signs observed. Three hours later bradycardia, mydriasis, marked analgesia and a normal respiratory rate were observed. Ataxia and exaggerated tendon reflexes were also present at this time. Six hours after drug administration CNS depression was still prominent, analgesia was present and respiratory rate was normal. The onset of an uneventful recove y began twenty four hours after the injection of morphine.

No fatalities were observed in this series of dogs. It was our subjective impression that morphine enhanced the CNS depressant effects of EA 1476. This generalization also includes an enhancement of the analgesic properties of the combined drugs. The observation of a normal respiratory rate was unexpected since either drug alone usually has a definite respiratory depressant effect in the doses employed.

IV. STRUCTURE ACTIVITY RELATIONSHIP

EA 1477 has been designated as the parent compound of a large series of potential derivatives. A detailed description of the behavioral changes and cardiovascular effects induced by this drug in the dog and monkey has appeared in previous reports. We have considered EA 1477 to be our standard and all the derivatives of this series have been compared to it with respect to qualitative and quantitative activity. EA 1465 and EA 1476 are approximately two hundred times as potent as EA 1477 in their ability to inhibit the carotid occlusion pressor reflex. They are also approximately one hundred times more potent that EA 1477 in producing comparable behavioral changes in the dog. A series of five additional

derivatives were received for evaluation. They were examined for their effect upon the behavior of unanesthetized dogs and their ability to inhibit the carotid occlusion pressor reflex in anesthetized dogs. Observations upon blood pressure, heart rate, respiratory rate and body temperature changes were also recorded (see table 6).

Drugs #EA 1465, EA 1476, and EA 1545 differ from EA 1477 in that a different aliphatic chain is present in position #3. EA 1465 and EA 1476 are the two most potent derivatives of EA 1477 studied to date. Both of them contain a longer side chain than EA 1477 and in addition their side chains are branched. EA 1545 has a shorter side chain than EA 1477 and is practically inactive. The only other compound examined that approached EA 1477 in potency was EA 1542. This drug differed chemically, from EA 1477 in that a nitrogen atom was substituted for the oxygen atom in position #5.

The remaining drugs examined, EA 1507, EA 1543, and EA 1544 were sufficiently less potent than EA 1477 to discourage any immediate further studies. They differed chemically from EA 1477 in having substitutions in the #1, 1-3, and 3-5 positions.

The results obtained suggested that other substitutions in the #3 position of EA 1477 may provide even more potent derivatives than EA 1465 or EA 1476. Relatively minor changes in these positions can greatly alter potency in this class of compounds.

V. DISCUSSION

CARDIOVASCULAR SYSTEM

Prior to our observations there have been no published reports on the extensive cardiovascular actions of the synthetic tetrahydrocannabinol derivatives. Bradycardia and hypotension had been observed by another worker (Loewe, 1944). However, no attempt was made to formulate

these observations into an hypothesis to explain the underlying mechanism of action. The observation that EA 1476 could inhibit the pressor response to carotid artery occlusion suggested it was capable of altering the normal activity of the autonomic nervous system. The additional observations of hypothermia, relaxation of the nictitating membrane, inhibition of the pressor response to central vagal stimulation and bradycardia that was not blocked by atropinization further implicated the autonomic system as an important site of action of EA 1476. The possibility that EA 1476 might be acting as an adrenergic blocking agent was considered since the drug appeared to affect primarily those functions under the control of the sympathetic nervous system. This possibility was eliminated when it was shown that the pressor response to injected epinephrine was not appreciably altered after administration of EA 1476. Ganglionic blockade can be ruled out as a primary site of action since electrical stimulation of the efferent sympathetic pathways in the hypothalamus or vasomotor center of the cat produced similar pressor responses before and after EA 1476 (see figure 1). When medullary vagal afferent neurons leading into the vasomotor center of the cat are stimulated electrically with the stereotaxis apparatus, a biphasic blood pressure response is obtained. Following EA 1476 the initial depressor response to electrical stimulation of these afferent neurons is retained; however, the subsequent expected pressor response did not materialize. These observations strongly suggest that the mechanism of depression of sympathetic outflow is located centrally and interposed between the afferent and efferent components of this arc. The widespread inhibition of sympathetic activity involving several different organs also suggests that the drug is acting upon a central rather than peripheral regulating centers of the sympathetic nervous system. In view of the definite

alteration in electrical activity of the cerebral cortex following administration of EA 1476 and the close relationship of the limbic system and hypothalamus in controlling autonomic nervous system reactions one might profitably explore the limbic system as a possible site of action for EA 1476. The work of Kaada (1951) demonstrated that all viscera having autonomic innervation have representation in the limbic complex and its related medial structures, and that the limbic system is to be regarded as the primary autonomic area of the forebrain.

CENTRAL NERVOUS SYSTEM

The behavioral changes resulting from administration of EA 1476 and related compounds are outstanding. Central nervous system depression is the most characteristic response obtained. Analgesia and ataxia are also prominent signs observed with these drugs.

Joel (1925) was one of the first workers to attempt a localization of the site of action of marihuana. He compared the effects of the drug in normal, decorticated and decerebrated cats. Normal cats showed swaying, decreased motility, cataleptic perserverance and general depression after the drug. Swaying was the only sign observed in decorticated cats and no effect was observed in decerebrate cats. Joel interpreted his results as indicating that marihuana acts predominantly upon the cerebral hemispheres. The ataxia observed with this drug and derivatives probably results from a central action, since we were unable to demonstrate any significant cord effects in spinal cats. This is in contrast to the greatly enhanced tendon reflexes obtained in unanesthetized cats and dogs after receiving comparable doses of EA 1476.

The mechanism of analgesia is undoubtedly a central action of the drug in which the animal may feel the pain although his normal reaction to it is depressed or absent. An EEG arousal to pain has been observed after

1.0 mgm./kgm. doses of EA 1476. The arousal is maintained for a few seconds after which the EEG pattern returns to the pre-pain state.

Our experiments indicate that EA 1476 potentiates the sleeping time in mice induced by hexobarbital. These results are in contrast to the observations of Loewe (1944). However, they are in agreement with the earlier work of Burgi (1924) who found that marihuana exerts a synergistic hypnotic action in rabbits in combination with other hypnotic agents.

The characteristic EEG response to EA 1476 and EA 1465 consisted of high voltage slow wave activity with a tendency to spindling. We were unable to observe a similar response to comparable doses of reserpine despite many other common actions of these two drugs. We observed an EEG pattern of arousal with reserpine in doses of 1.0 mgm./kgm. of the phosphate or acetate salt. Monroe et al. (1951) reported that reserpine (5-10 mgm., i.v.) produced a state of sleep in schizophrenic patients. The cortical EEG's taken during this period consisted of periods of light sleep interspersed in a drowsy or relaxed record. The typical spindling characteristic of the barbiturates was not observed. In comparing the work of Monroe (1951) and Rinaldi (1955) a qualitative difference in the EEG response to reserpine was obtained. The difference may be related to the different species or doses employed in their studies. Schneider and Earl (1954) utilizing the monkey as a test animal observed an EEG response to reserpine that was similar to that described by Monroe (1951) in humans. Our results in the dog, however, agree with the observations of Rinaldi and Himwich (1955) in the rabbit. Further work is indicated before one can describe the typical EEG response to reserpine and compare it with our observations obtained with EA 1476 and EA 1465.

The observation that EA 1476 (1.0 mgm./kgm.) has no appreciable

effect upon the patellar reflex in spinal cats whereas this dose in an intact cat or dog greatly enhances this reflex was surprising. Evidently EA 1476, in the intact cat and presumably the dog also, acts to supress inhibitory impulses of supraspinal origin that are relayed to the spinal cord. Further studies on this mechanism are indicated.

MISCELLANEOUS

The prominent hypothermia observed with EA 1476, EA 1477, and reserpine is highly suggestive of a central mechanism of a tion. The pupillary alterations and diarrhea observed with these drugs may possibly be attributed to a relative imbalance in the autonomic nervous system, however a direct action on these structures has not been eliminated. The relaxation of the nictitating membrane by these drugs and the observation that none of the drugs blocks the contraction of this structure following the injection of epinephrine is indirect evidence of a reduction in central sympathetic outflow.

The mechanism of respiratory depression observed with EA 1476, EA 1477, EA 1465, and reserpine remains unknown. Loewe (1946) observed pulmonary edema in a series of dogs who died following the intravenous administration of a specific tetrahydrocannabinol derivative.

TOXICITY

The lethal doses of EA 1476, EA 1477, or EA 1465 are extremely high by comparison with the small doses required for their pharmacodynamic effects. Loewe (1946) states that albino mice can withstand chronic administration of EA 1477 better than comparable doses of sodium chloride. Walton (1938) reported that purified cannabinol in toxic doses produced pulmonary edema and cerebral hyperemia with indications that death resulted from cardiac rather than respiratory failure. Loewe (1946) estimated that the intravenous

LD_{50} for marihuana in mice, rabbits, and dogs lay between 100-230 mgm./kgm. Our limited results in the dog with EA 1476 would support this estimate. Our results in mice, however, would place the intraperitoneal LD_{50} closer to 400 mgm./kgm.

A few words of caution with respect to the toxicity of EA 1476 under special circumstances are indicated. Our results obtained with cocaine, caffeine, and d-amphetamine as antagonists of EA 1476 indicate that despite a gross appearance of antagonism there is a marked increase in subsequent fatalities. Neither drug alone in the doses employed approached the LD_{50} value for the individual drug. However, when given in the sequence employed there was a high incidence of fatalities. Death occurred during the depressed state which followed the stimulation induced by the antagonist. Adequate nursing care appears to be the best method available for survival after administration of the potent tetrahydrocannabinol derivatives.

VI. SUMMARY

1. The extensive actions of tetrahydrocannabinol derivatives upon the cardiovascular system suggest that these agents act by inhibition of the afferent to efferent areas of the central components of the sympathetic nervous system.

A marked similarity to the actions of reserpine has been demonstrated. However, qualitative and quantitative difference exist between reserpine and the tetrahydrocannabinols.

2. Behavioral changes characterized by CNS depression, ataxia, analgesia and enhanced tendon reflexes have been described. These effects appear to originate at supraspinal levels. Alterations in cortical electrical activity have been described resulting from administration of the tetrahydrocannabinol derivatives. The outstanding similarities and differences observed on the EEG with related drugs have been described.

A potentiation of hexobarbital induced sleeping time in mice has been described for EA 1476.

Miscellaneous actions of EA 1476 which may be related to its effects upon the autonomic nervous system are discussed.

3. The general toxicity of the tetrahydrocannabinol derivatives is discussed as well as the effects of drugs which antagonize or potentiate their pharmacological actions.

4. An analysis of structure activity relationship is presented for the tetrahydrocannabinol derivatives evaluated in this study.

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TABLE #1

COMPARATIVE BLOOD PRESSURE RESPONSE TO INTRAVENOUS EA 1476, RESERPINE AND EA 1477

POST INJECTION TIME	Controls DRUG	DOSE	% CHANGE	S.E.	N	Experimental DRUG	DOSE	% CHANGE	S.E.	N	P VALUE
10 min.	EtOH	0.1 ml./kgm.	+0.04	± 1.2	5	EA 1476	0.05 mgm./kgm.	- 2.1	± 1.4	6	> 0.20
30 min.	EtOH	0.1 ml./kgm.	-1.40	± 2.8	5	EA 1476	0.05 mgm./kgm.	- 7.4	± 3.0	6	> 0.20
1 hr.	EtOH	0.1 ml./kgm.	-0.60	± 3.3	5	EA 1476	0.05 mgm./kgm.	-15.1	± 5.6	6	> 0.05
2 hrs.	EtOH	0.1 ml./kgm.	+0.40	± 5.7	5	EA 1476	0.05 mgm./kgm.	-18.4	± 4.8	5	< 0.05*
3 hrs.	EtOH	0.1 ml./kgm.	+1.20	± 4.6	5	EA 1476	0.05 mgm./kgm.	-23.3	± 3.1	5	< 0.01*
4 hrs.	EtOH	0.1 ml./kgm.	+1.90	± 1.8	5	EA 1476	0.05 mgm./kgm.	-22.4	± 3.2	5	< 0.01*
10 min.	EtOH	0.1 ml./kgm.	+1.0	± 0.15	6	EA 1477	10.0 mgm./kgm.	- 6.7	± 3.7	6	> 0.05
30 min.	EtOH	0.1 ml./kgm.	-0.1	± 1.5	6	EA 1477	10.0 mgm./kgm.	-11.2	± 5.8	6	> 0.10
1 hr.	EtOH	0.1 ml./kgm.	-6.4	± 4.7	6	EA 1477	10.0 mgm./kgm.	-21.3	± 5.7	6	> 0.05
10 min.	NMDMA	0.1 ml./kgm.	-2.5	± 1.7	6	reserpine	1.0 mgm./kgm.	+6.5	± 2.3	5	< 0.02*
30 min.	NMDMA	0.1 ml./kgm.	-5.7	± 2.5	6	reserpine	1.0 mgm./kgm.	+3.7	± 4.4	5	> 0.05
1 hr.	NMDMA	0.1 ml./kgm.	-17.7	±5.9	6	reserpine	1.0 mgm./kgm.	-12.1	± 3.2	5	> 0.40
1½ hr.	NMDMA	0.1 ml./kgm.	-11.2	±3.4	6	reserpine	1.0 mgm./kgm.	-39.5	± 7.8	5	< 0.05*
2 hrs.	NMDMA	0.1 ml./kgm.	-15.8	±2.6	6	reserpine	1.0 mgm./kgm.	-25.4	± 2.3	5	< 0.05*

S.E. = standard error
EtOH = 95% ethyl alcohol
NMDMA = 100% N1-dimethylacetamide

* = statistically significant, group comparison, (16)
Anesthesia = Sod. Pentobarbital 30.0 mgm./kgm.
Species = Dog

TABLE #2

INHIBITION OF COMMON CAROTID OCCLUSION (30 SECONDS) PRESSOR RESPONSE

CORRECTED FOR CHANGES IN BLOOD PRESSURE ACCORDING TO [redacted] (1950)

TIME	DRUG	DOSE	%[c]CHANGE	S.E.	N	DRUG	DOSE	%[c]CHANGE	S.E.	N	P VALUE
10 min.	EtOH	0.1 ml./kgm.	- 1.8	± 2.9	5	EA 1476	0.05 mgm./kgm.	- 4.7	± 3.0	6	>0.50
30 min.	EtOH	0.1 ml./kgm.	+ 5.3	± 4.0	5	EA 1476	0.05 mgm./kgm.	-15.0	± 3.4	6	<0.01
1 hr.	EtOH	0.1 ml./kgm.	- 0.6	± 5.5	5	EA 1476	0.05 mgm./kgm.	-29.3	± 2.6	6	<0.01
2 hr.	EtOH	0.1 ml./kgm.	- 1.9	± 8.5	5	EA 1476	0.05 mgm./kgm.	-35.8	± 8.4	6	<0.05
3 hr.	EtOH	0.1 ml./kgm.	- 5.2	± 4.8	5	EA 1476	0.05 mgm./kgm.	-30.5	± 6.0	5	<0.02
4 hr.	EtOH	0.1 ml./kgm.	- 4.9	± 5.1	3	EA 1476	0.05 mgm./kgm.	-24.5	±11.7	5	>0.30
TIME	DRUG	DOSE	%[c]CHANGE	S.E.	N	DRUG	DOSE	%[c]CHANGE	S.E.	N	P VALUE
10 min.	DMA	0.1 ml./kgm.	- 0.3	± 6.3	6	reserpine	1.0 mgm./kgm.	-13.0	± 2.1	5	>0.30
30 min.	DMA	0.1 ml./kgm.	- 7.3	± 6.7	6	reserpine	1.0 mgm./kgm.	-18.0	± 6.5	5	>0.40
1 hr.	DMA	0.1 ml./kgm.	-16.0	± 9.1	6	reserpine	1.0 mgm./kgm.	-15.0	± 9.6	5	>0.50
1.5 hr.	DMA	0.1 ml./kgm.	-11.0	± 6.0	6	reserpine	1.0 mgm./kgm.	+103.0	±75.0	5	>0.30
2.0 hr.	DMA	0.1 ml./kgm.	-12.0	±5.0	6	reserpine	1.0 mgm./kgm.	-25.0	± 0.1	5	>0.40
TIME	DRUG	DOSE	%[c]CHANGE	S.E.	N	DRUG	DOSE	%[c]CHANGE	S.E.	N	P VALUE
10 min.	EtOH	0.1 ml./kgm.	+ 2.5	±24.0	6	EA 1477	10.0 mgm./kgm.	-22.8	±67.8	6	<0.01
30 min.	EtOH	0.1 ml./kgm.	- 5.3	± 1.4	6	EA 1477	10.0 mgm./kgm.	-32.8	± 0.9	6	<0.02
1 hr.	EtOH	0.1 ml./kgm.	- 6.3	± 6.3	6	EA 1477	10.0 mgm./kgm.	-32.2	±105.3	6	<0.05

[c] DMA = N,N-dimethylacetamide EtOH = 95% Ethyl alcohol Species = Dog Anesthesia = Sodium Pentobarbital
%[c]CHANGE = Per cent change in pressor response corrected for changes in blood pressure.

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TABLE #3

MOUSE SLEEPING TIME POTENTIATION BY E.I. 1476

HEXOBARBITAL 100 mgm./kgm. i.p.

GRP#	DATE	AVG. WGT.	AVG. SLEEPING TIME	RANGE	n
3	9-30	35 gms.	41 min.	30-52	10
5	10-3	36 "	49 "	33-69	10
7	10-5	35 "	49 "	36-65	10
9	10-7	35 "	51 "	33-74	9
13	10-10	18 "	50 "	40-59	10
15	10-12	19 "	54 "	37-77	10
20	10-14	19 "	47 "	39-54	5
21	10-26	25 "	61 "	56-67	10
21	10-26	25 "	61 "	56-67	10
24	10-28	20 "	57 "	42-66	9
25	10-28	36 "	56 "	46-76	8

HEXOBARBITAL 100 mgm./kgm. +E.I. 1476+ i.p.

GRP#	DATE	DOSE E.I. 1476 mgm./kgm.	AVG. WGT.	AVG. SLEEPING TIME	RANGE	n	P value
4	9-30	1.0*	34 gms.	74 min.	50-89	10	<0.01
6	10-3	1.0	36 "	88 "	72-105	10	<0.01
8	10-5	3.0	36 "	123 "	80-160	10	<0.01
10	10-7	3.0	28 "	167 "	137-192	10	<0.01
12	10-10	5.0	16 "	114 "	82-136	8	<0.01
14	10-12	3.0	19 "	103 "	72-137	10	<0.01
17	10-14	3.0*	20 "	68 "	58-78	10	<0.01
22	10-26	1.0*	25 "	68 "	61-76	9	<0.01
23	10-26	3.0*	25 "	79 "	67-87	9	<0.01
26	10-28	Hexobarbital in 10% ethyl alcohol	19 "	56 "	36-91	10	>0.50
27	10-28	"	36 "	57 "	45-63	8	>0.50

+ E.I. 1476 was administered one hour prior to hexobarbital.

* Fresh solutions of E.I. 1476 were made up on these days.

Groups 4-27 represent a comparison of hexobarbital (100 mgm./kgm.) when saline or a ten per cent solution of ethanol is used as the drug solvent.

TABLE #

MOUSE SLEEPING TIME ANTAGONISM

(A)	HEXOBARBITAL	HEXOBARBITAL E/. 1476	HEXOBARBITAL E/. 1476 d-AMPHETAMINE
GROUP #	9	10	11
AVG. SLEEPING TIME	51 min.	167 min.	97 min.
RANGE	33-74	137-192	60-139
AVG. WEIGHT	35 gms.	38 gms.	17 gms.
DATE	10-7	10-7	10-7
n.	9	10	10

Group #	9 vs 10	P value	<0.01
	10 vs 11	"	<0.01
	9 vs 11	"	<0.01

HEXOBARBITAL	HEXOBARBITAL E/. 1476	HEXOBARBITAL E/. 1476 d-AMPHETAMINE
15	14	16
54 min.	103 min.	91 min.
37-77	72-137	65-130
19 gms.	19 gms.	19 gms.
10-12	10-12	10-12
10	10	10

Group #	15 vs 14	P value	<0.01
	15 vs 16	"	<0.01
	14 vs 16	"	>0.20

HEXOBARBITAL 100 mgm./kgm. administered i.p.

E/. 1476 3.0 mgm./kgm. " " one hour prior to the hexobarbital

d-AMPHETAMINE 3.0 mgm./kgm. " " " " " " " "

ISS-25 3.0 mgm./kgm. " " fifteen minutes prior to the hexobarbital

TABLE 44 (cont.)

MOUSE SLEEPING TIME (MINUTES)

(3)

	HEXOBARBITAL	HEXOBARBITAL EA 1476	HEXOBARBITAL EA 1476 LSD-25
GROUP #	20	17	19
AVG. SLEEPING TIME	47 min.	68 min.	72 min.
RANGE	39-54	50-78	46-109
AVG. WEIGHT	19 gms.	19 gms.	19 gms.
DATE	10-14	10-14	10-14
n.	5	10	10

Group # 20 vs 17	P value		<0.01
17 vs 19	"		>0.50
20 vs 19	"		<0.02

HEXOBARBITAL	HEXOBARBITAL EA 1476 d-AMPHETAMINE	HEXOBARBITAL EA 1476 LSD-25
20	18	19
47 min.	72 min.	72 min.
39-54	47-98	46-109
19 gms.	19 gms.	19 gms.
10-14	10-14	10-14
5	10	10

Group # 20 vs 18	P value		<0.02
18 vs 19	"		>0.50
20 vs 19	"		<0.02

HEXOBARBITAL 100 mgm./kgm. administered i.p.

EA 1476 3.0 mgm./kgm. " " one hour prior to hexobarbital

d-AMPHETAMINE 3.0 mgm./kgm. " " " " " " "

LSD-25 3.0 mgm./kgm. " " fifteen minutes prior to the hexobarbital

TABLE #5

TOXICITY DATA E.I. 1476

	LD_{50} mgms./kgm.	Route of Administration	n
	390.0	I.P.	40

II. [illegible]	Effective Dose (intravenous)	# Animals	#Fatalities
	0.025 mgms./kgm.	2	0
	0.050 mgms./kgm.	5	0
	0.125 mgms./kgm.	2	0
	0.250 mgms./kgm.	4	1*
	1.0 mgms./kgm.	8	0
	5.0 mgms./kgm.	2	0
	10.0 mgms./kgm.	3	0
	50.0 mgms./kgm.	1	0
	100.0 mgms./kgm.	1	1

III. Dog	Effective Dose (oral)	# Animals	#Fatalities
	0.5 mgms./kgm.	1	0
	1.0 mgms./kgm.	2	0
	2.0 mgms./kgm.	2	0

IV. Monkey	Effective Dose (intravenous)	# Animals	#Fatalities
	0.5 mgms./kgm.	1	0
	1.0 mgms./kgm.	1	0
	2.0 mgms./kgm.	1	0

Effective Dose is defined as the dose required to produce obvious tranquilization

* Dog died ten days after receiving the drug. Possible distemper.

TABLE #6

A. GROSS OBSERVATIONS IN THE INTACT ANIMAL.

EA 1507

SPECIES	DOSE	ROUTE	OBSERVATIONS	#/ANIMALS
Dog	0.125 mgm./kgm.	I.V.	No effect	1
Dog	1.00 mgm./kgm.	I.V.	No effect	1
Dog	5.00 mgm./kgm.	I.V.	Some sedation	1

EA 1542

SPECIES	DOSE	ROUTE	OBSERVATIONS	#/ANIMALS
Dog	0.125 mgm./kgm.	I.V.	No effect	1
Dog	1.00 mgm./kgm.	I.V.	Some Dyspnea	1
Dog	5.00 mgm./kgm.	I.V.	No effect	1
Dog	10.00 mgm./kgm.	I.V.	Ataxia, Sedation	1

EA 1543

SPECIES	DOSE	ROUTE	OBSERVATIONS	#/ANIMALS
Dog	0.125 mgm./kgm.	I.V.	No effect	1
Dog	1.00 mgm./kgm.	I.V.	No effect	1
Dog	5.00 mgm./kgm.	I.V.	No effect	1

EA 1544

SPECIES	DOSE	ROUTE	OBSERVATIONS	#/ANIMALS
Dog	0.125 mgm./kgm.	I.V.	No effect	1
Dog	1.00 mgm./kgm.	I.V.	No effect	1
Dog	5.00 mgm./kgm.	I.V.	Possible sedation	1

EA 1545

SPECIES	DOSE	ROUTE	OBSERVATIONS	#/ANIMALS
Dog	0.125 mgm./kgm.	I.V.	No effect	1
Dog	1.00 mgm./kgm.	I.V.	No effect	1
Dog	5.00 mgm./kgm.	I.V.	No effect	1

TABLE #6 (cont.)

B. OBSERVATIONS IN THE ANESTHETIZED ANIMAL

Dogs anesthetized with sodium pentobarbital 30.0 mgm./kgm.

EA 1507 (10.0 mgm./kgm.) administered I.V. to 3 dogs.

1. Respiratory rate: a slight increase one hour after drug.
2. Blood pressure: a slight decrease one hour after drug.
3. Heart rate: a slight decrease one hour after drug.
4. Rectal temperature: no significant change one hour after drug.
5. Common carotid occlusion pressor response reduced about 30%.

EA 1542 (10.0 mgm./kgm.) administered I.V. to 2 dogs.

1. Respiratory rate: a slight decrease one hour after drug.
2. Blood pressure: a slight decrease one hour after drug.
3. Heart rate: a slight decrease one hour after drug.
4. Rectal temperature a slight decrease one hour after drug.
5. Common carotid occlusion pressor response reduced about 50%.

EA 1543 (10.0 mgm./kgm.) administered I.V. to 2 dogs.

1. Respiratory rate: no appreciable change one hour after drug.
2. Blood pressure: no appreciable change one hour after drug.
3. Heart rate: a slight decrease one hour after drug.
4. Rectal temperature: a slight decrease (0.5°C) one hour after drug.
5. Common carotid occlusion pressor response reduced about 25%.

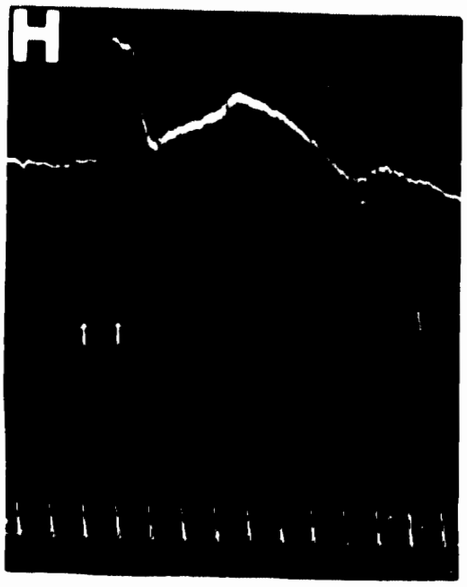
EA 1544 (10.0 mgm./kgm.) administered I.V. to 2 dogs.

1. Respiratory rate: a slight decrease one hour after drug.
2. Blood pressure: a slight decrease one hour after drug.
3. Heart rate: no appreciable change one hour after drug.
4. Rectal temperature: no appreciable change one hour after drug.
5. Common carotid occlusion pressor response reduced about 10%.

E/. 1545 (10.0 mgm./kgm.) administered I.V. to 2 dogs.

1. Respiratory rate: no appreciable change one hour after drug.
2. Blood pressure: no appreciable change one hour after drug.
3. Heart rate: a slight decrease one hour after drug.
4. Rectal temperature: no appreciable change one hour after drug.
5. Common carotid occlusion pressor response reduced about 5%.

Of the five compounds examined only E/. 1542 appears to possess appreciable activity in large doses. It is roughly comparable to E/. 1477 in its effect upon the common carotid occlusion pressor response. The outstanding property of this drug noted on gross observation was that its characteristic effect comes on rapidly after injection and has a short duration of action.



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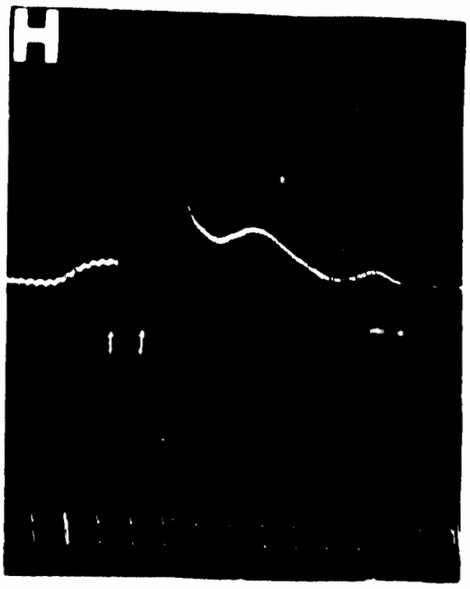

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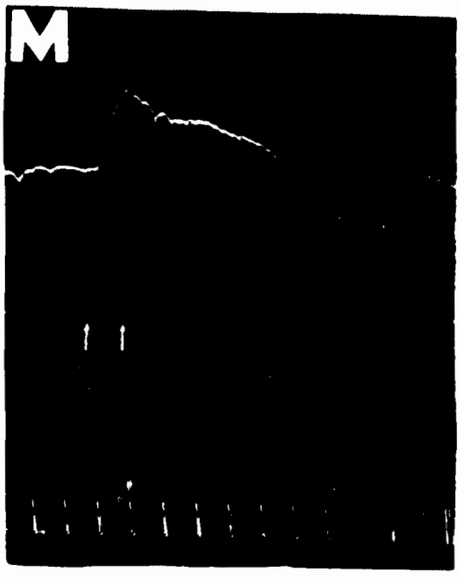

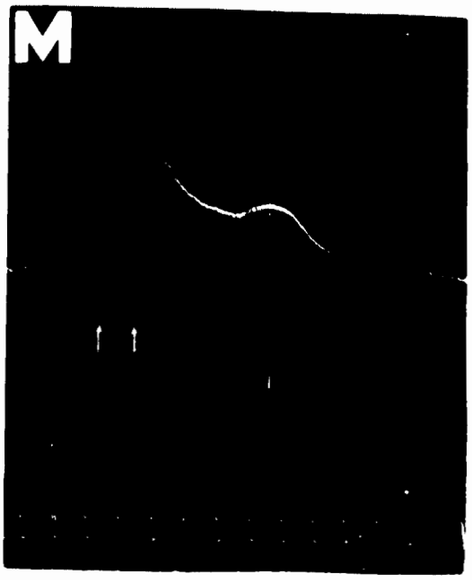

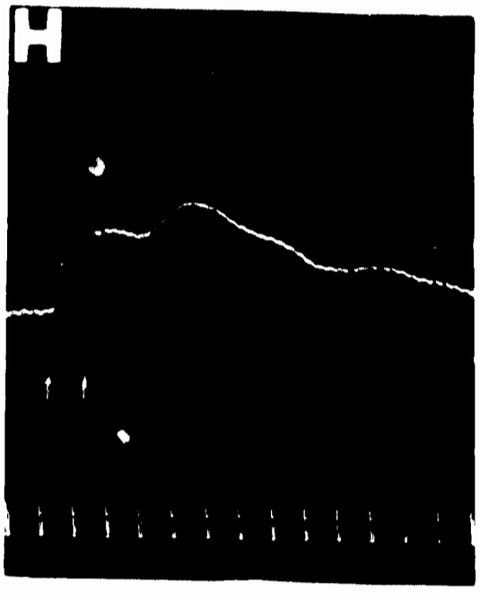

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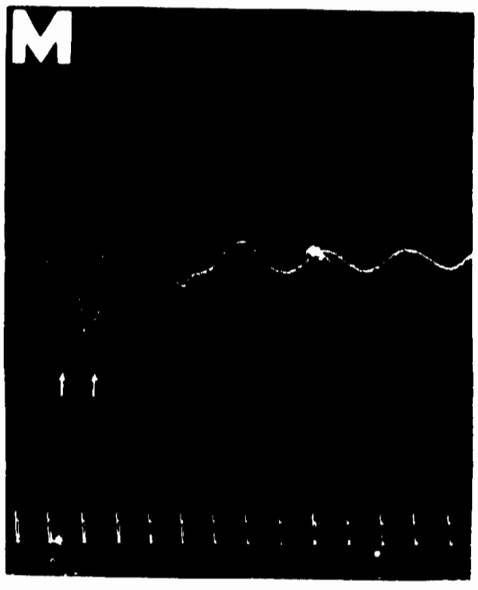
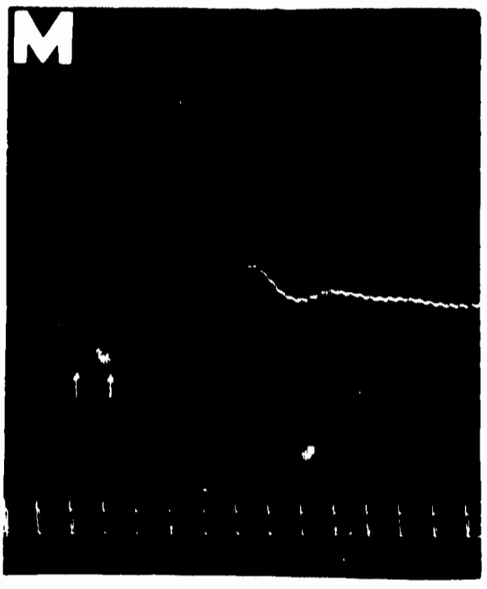
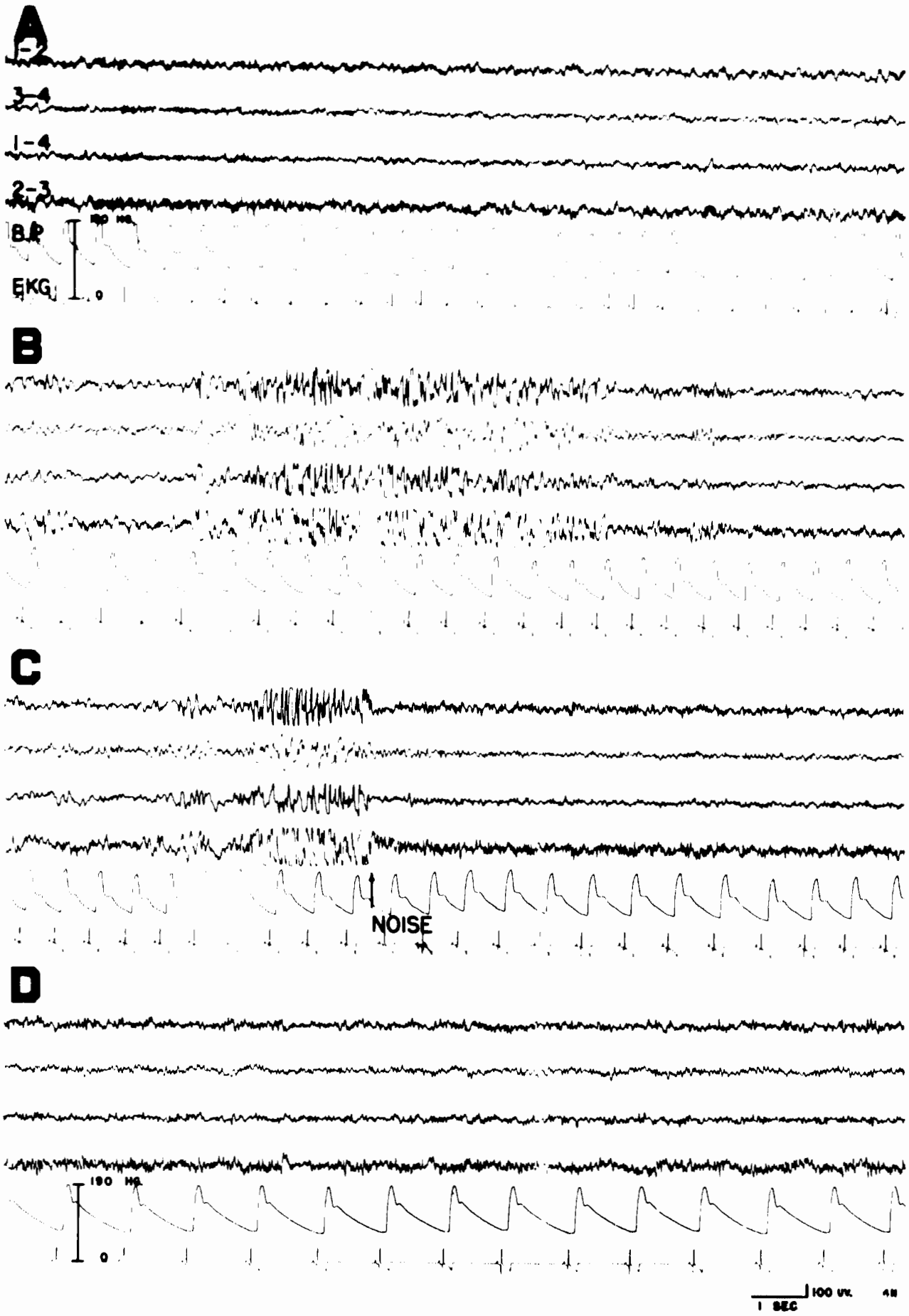
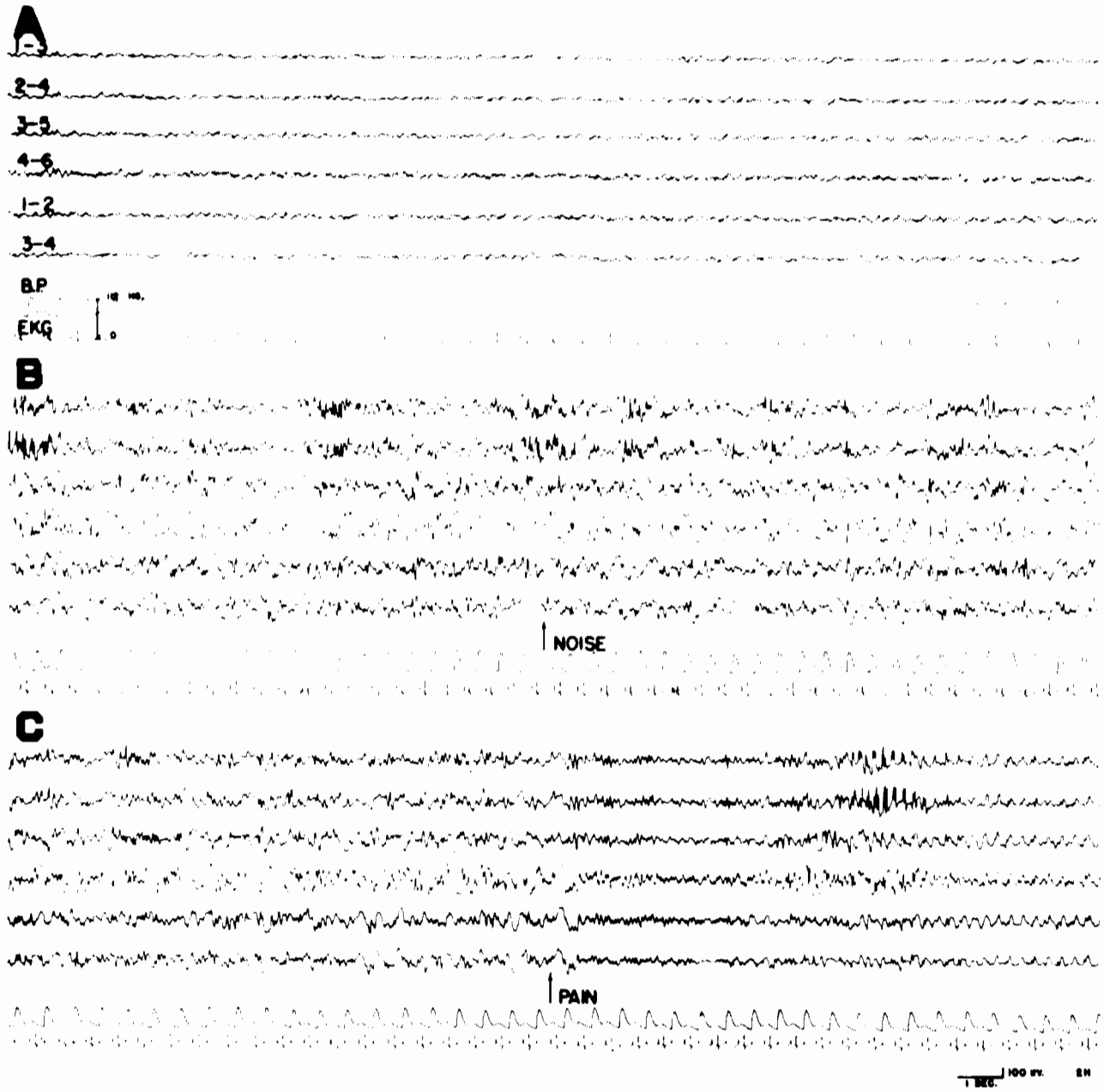
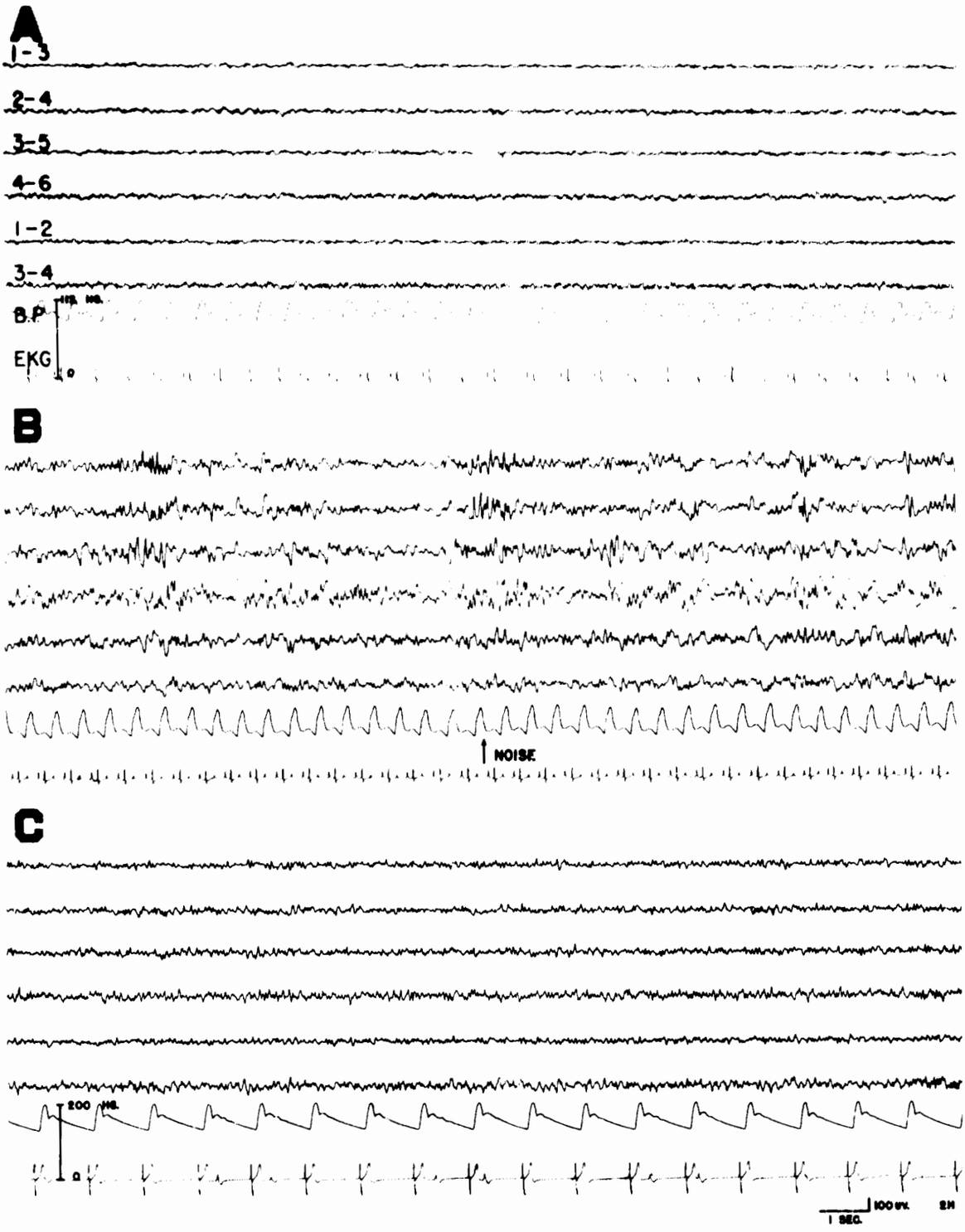





A
2-2
3-4
1-4
2-3
B.P
190 MM.
EKG
0
B
C
NOISE
D
190 MM.
0
100 UV.
1 SEC


A
1-2
3-4
4-5
1-2
3-4
B.P.
EKG
10 MM.
0
B
NOISE
C
PAIN
1 SEC.
100 MV.
EH


A
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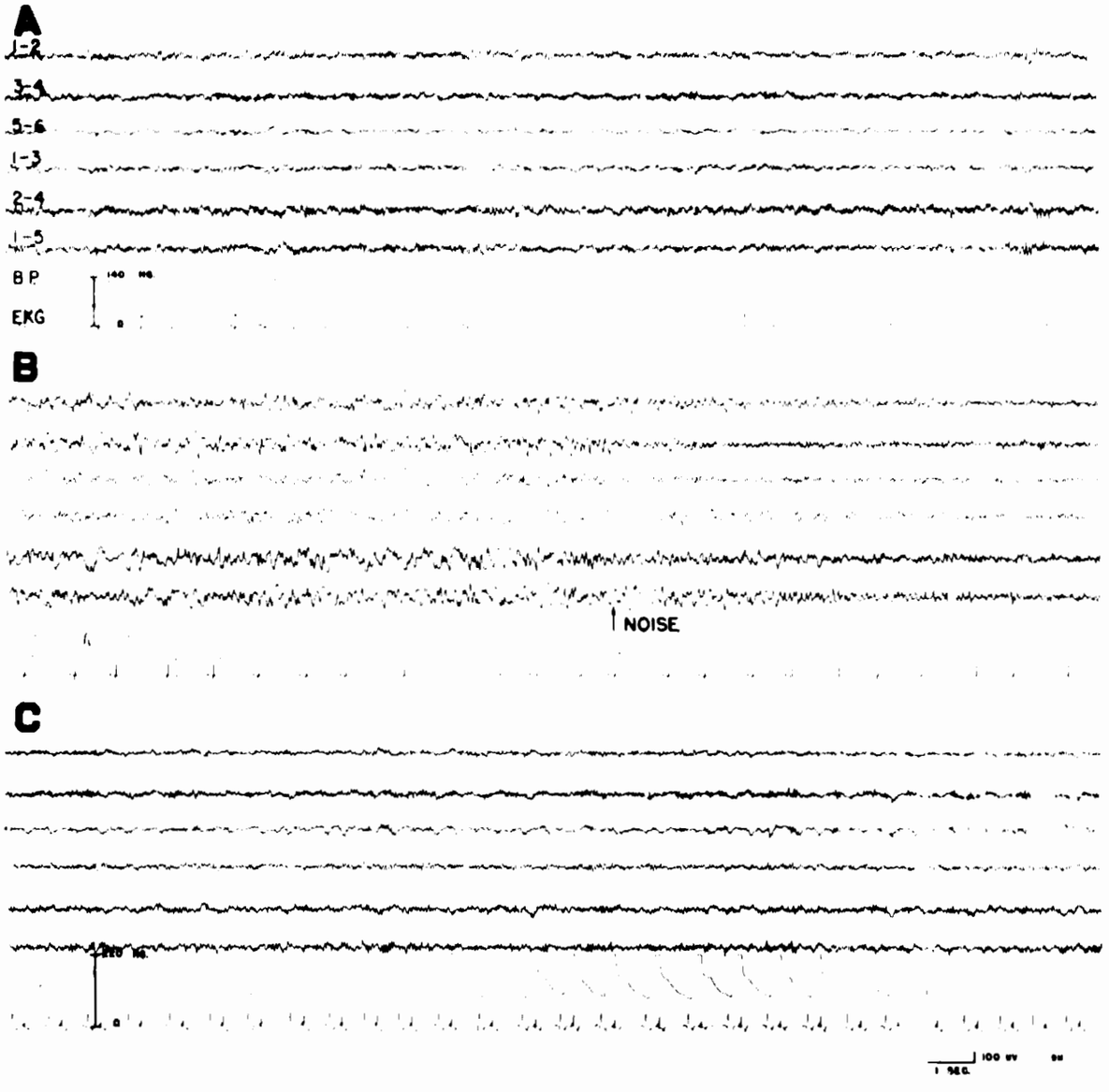
2-4
3-5
4-6
1-2
3-4
B.P
EKG
B
NOISE
C
200 MS.
1 SEC.
100 UV.
2H


A
1-2
3-4
5-6
1-3
2-4
1-5
B P
EKG
B
NOISE
C
100 mV
1 SEC.