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

FIRST PROGRESS REPORT

by

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THE PHARMACOLOGY OF EA 1476 AND RELATED COMPOUNDS

I. INTRODUCTION

The history and objective data available on marihuana-like compounds has been extensively reviewed and summarized by S. Loewe (7, 8, 11). It is believed that the tetrahydrocannabinol derivatives of the marihuana plant are responsible for the pharmacological activity of this plant. Loewe has stated that the psychic activity of the tetrahydrocannabinols can be correlated with their ability to produce ataxia in the dog. This ataxic activity has been used as a specific test for marihuana-like activity for many years and served as the official assay for the U.S.P. (7). Studies prior to 1950 were directed primarily to the actions of this class of compounds upon the central nervous system. New derivatives were synthesized and the most potent drug obtained with respect to ataxic activity (9) was assigned to us under the code name of EA 1476. The parent compound in this class of synthetic derivatives was designated as EA 1477. One other derivative with high activity was assigned to us with the code designation of EA 1465. These compounds had been evaluated by previous workers for their activity upon the central nervous system as well as upon other organ systems of mammalian species. Loewe (8) reported that the parent compound, EA 1477, and some of the more potent derivatives produced bradycardia and respiratory depression in unanesthetized dogs. On the basis of a small series of experiments they concluded that these drugs had no effect upon blood pressure in a wide range of dosage. The protocols for the latter experiments were not published so the important time factor could not be evaluated.

Our evaluation of the published reports on this class of compounds recommended that certain phases of the work should be repeated. Much of the published

material in this field is based upon strong emotional and subjective evaluations which appeared to be in sharp disagreement with the objective findings of Loewe and his coworkers (7).

Initially we directed our studies toward an evaluation of the toxicity and gross behavioral changes induced by EA 1476, EA 1477 and EA 1465 in several species of animals. These observations are recorded in a previous report (5). We were impressed with the marked pharmacological activity of this group of compounds and their relative lack of toxic effects. They possessed definite central nervous system activity which included a prolonged state of "sleep" that was not similar to sleep observed with barbiturates. In addition, the dogs' response to nociceptive stimuli was appreciably altered and bore some resemblance to the classical actions of morphine. When very low doses of the drugs were injected intravenously the gross behavior of the dog and monkey bore a marked resemblance to the actions of reserpine. In view of the similarity to reserpine we selected the most potent drug in this series, EA 1476, and began a systematic evaluation of its cardiovascular activity.

Our initial impression that EA 1476 possessed many of the pharmacological actions of reserpine has been substantiated by further investigation. At the present time our preliminary experiments indicate that EA 1476 is considerably more potent than reserpine with respect to inhibiting specific cardiovascular reflexes that are mediated over the autonomic nervous system. The details of this work and extensions of other phases of the problem are presented in the following pages.

II. TOXICITY

The majority of our studies in this category have been restricted to acute toxicity experiments in several species of animals. Some limited data on chronic

administration are available utilizing very small oral doses. The toxicity data are summarized in table #1 and must be regarded as incomplete. When more of the drugs, EA 1476, EA 1477, are made available for testing, these experiments will be completed.

An evaluation of oral toxicity is anticipated since these compounds are effective when administered orally. The ratio of intravenous to oral effective dose is approximately 1:10 as determined by preliminary acute experiments.

The chemical and physical properties of this class of compounds suggest that they may also be effective when administered by inhalation. Experiments to test this possibility are planned and will be executed when more of the compounds are made available.

III. PHARMACOLOGY

A. CARDIOVASCULAR SYSTEM

1. BRADYCARDIA

In barbitalized dogs this response is obtained with intravenous doses of 0.10 mgm./kgm. of EA 1476 or 1.0 mgm./kgm. of reserpine. EA 1477 produces a marked bradycardia with intravenous doses of 10.0 mgm./kgm. which is statistically significant within ten minutes after drug administration and persists for periods greater than one hour. Preliminary experiments suggest that the bradycardia produced by the above drugs is not blocked by prior atropinization.

EA 1476, EA 1477 and reserpine also produce a profound bradycardia when administered intravenously to unanesthetized dogs. When large doses of the above drugs are employed one often observes an initial tachycardia which is followed by the bradycardia. In the case of EA 1476 we suspect that the large doses may have a peripheral vasodilator action which initiates the tachycardia. This is supported by the

observation that large doses (1.0 mgm./kgm.) of EA 1476 administered to the denervated dog heart-lung preparation with a circulating blood volume of one liter had no effect upon heart rate or cardiac output. It appears, therefore, that this dose of EA 1476 has no immediate direct cardiac effects and a direct vasodilator action on peripheral vessels could easily explain the initial tachycardia which occurs concommitantly with a fall in mean arterial pressure.

2. HYPOTENSION

EA 1476, EA 1477 and reserpine are capable of producing a gradual prolonged fall in mean arterial blood pressure in barbitalized dogs. The intravenous threshold doses required to produce equivalent responses for each drug indicate marked quantitative differences in potency. A comparison of relative potency is presented in table #2.

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 administered. At this time some cardiovascular reflexes involving the sympathetic nervous system usually are significantly depressed. The sequence of these events suggests that the hypotension results from a decrease in central sympathetic outflow. In the case of reserpine Bein (1) and Trapold (17) have suggested that this drug is initiating the hypotensive response through its effect upon the hypothalamus. We do not have sufficient data at this time to be so specific in locating the site of the hypotensive action of EA 1476 or EA 1477. There is, however, considerable evidence to suggest that EA 1476 and EA 1477 do modify the normal activity of the hypothalamus. At present we entertain the possibility that EA 1476 and EA 1477 may modify the activity of the hypothalamus through an effect upon higher centers.

We have been unable to make a quantitative comparison between the hypotensive actions of EA 1476 and reserpine because of technical difficulties. Reserpine is only slightly soluble in the usual laboratory solvents. A 0.1 % solution can be obtained with the mixture of solvents employed by Trapold (17), however, this solvent was inadequate for our purposes. We utilized N-N dimethylacetamide as a solvent since reserpine is soluble in this solvent to the extent of 10.0%. Table #3 presents a comparison of the control series of dogs for EA 1476 and reserpine in which the solvents alone were tested for hypotensive activity. It is obvious that N-N dimethyl acetamide possesses considerable hypotensive activity per se, therefore, the reserpine experiments will have to be run again employing the water soluble reserpine phosphate which has just become available. When these experiments are completed, a quantitative comparison of the hypotensive activities of EA 1476 and reserpine can be made.

Both EA 1476 and reserpine are effective hypotensive agents when administered orally or by the intravenous route to unanesthetized dogs.

Figures #1 and #2 present the results obtained in two normotensive dogs who received the drugs orally for a period of twenty-four days. The broken lines indicate weekends when the animals did not receive the drugs. Blood presssure was determined by the cuff method and each point on the graph represents the average of six determinations as recorded by two observers. The animals were followed for two weeks before drug therapy was started so that a stable control blood pressure was obtained before any drugs were administered. Only the last seven days of the control readings are recorded for the purpose of convenience. We concluded that both drugs (EA 1476 and reserpine) in the doses employed have only minor effects upon the blood pressure of normotensive dogs. By the end of the experiment, however, we held the

subjective impression that the dogs were somewhat sedated with respect to their normal behavior pattern.

The experiments in which EA 1477 was administered intravenously to barbitalized dogs were followed for a period of one hour. The hypotensive response at this time was not statistically significant, however, the trend toward significance appears valid and we predict that this response will become significant at the two hour post injection interval.

At the present time we do not have adequate data on the effect of EA 1477 in the unanesthetized dog.

3. INHIBITION OF CARDIOVASCULAR REFLEXES

a. Common Carotid Occlusion Pressor Response

The common carotid occlusion pressor response is dramatically reduced in the barbitalized dog within thirty minutes after the injection of small doses (0.05 mgm./kgm.) of EA 1476. Much larger doses (1.0 mgm./kgm.) of reserpine are required to produce a similar response (17) and the latent period from injection to drug effect is about twice that for EA 1476.

EA 1477 in doses two hundred times greater than EA 1476 also produces a dramatic reduction in the common carotid occlusion pressor response. The onset of this reduction occurs more rapidly than with EA 1476, however, a quantitative comparison at the one hour post injection interval indicates that the doses employed have a comparable effect at this time. See table #4.

Table #5 presents an evaluation of the threshold doses required to produce a marked inhibition of the common carotid occlusion pressor response in the dog. It is of interest to note that once the threshold dose has been reached for a given animal larger doses of the drug have little additional effect upon inhibiting this

pressor reflex.

b. Central Vagal Stimulation Pressor Response

The pressor response elicited by unilateral central vagal stimulation when the contralateral vagus nerve is severed is greatly reduced and usually reversed when 1.0 mgm./kgm. of EA 1476 is administered to barbitalized dogs. Reserpine is reported to have the same qualitative effect in equivalent doses (17), however, it appears to be less potent quantitatively.

4. INCREASED VASCULAR RESPONSE TO EPINEPHRINE

Both EA 1476 (0.05 mgm./kgm.) and reserpine (1.0 mgm./kgm.) reduce peripheral vascular resistance in the barbitalized dog as manifested by a decrease in mean arterial blood pressure. A given dose of epinephrine will then produce a relatively greater pressor response than it did during the control period.

5. DIRECT CARDIAC ACTIONS

EA 1476 (1.0 mgm./kgm.) was administered to a denervated dog heart-lung preparation which was in a state of semifailure, C.I. = 0.5, as described by Hardman (6). This dose of the drug which was contained in a circulating blood volume of 1.0 liters had no effect upon heart rate or cardiac output. This experiment utilizing a large dose of the drug suggests that EA 1476 does not have any immediate direct effects upon the heart. We might, therefore, assume that the bradycardia produced by this drug in the intact anesthetized and unanesthetized dog 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 change in heart rate. It is possible that EA 1476 has a delayed direct effect upon the heart. This point will be investigated in the dog heart-lung preparation.

At present no comparable data are available for EA 1477 or reserpine.

B. CENTRAL NERVOUS SYSTEM

1. BEHAVIORAL CHANGES

It is our subjective impression that EA 1476 produces an equivalent degree of tranquilization in the dog and monkey as reserpine with $\frac{1}{4}$ to $\frac{1}{2}$ of the reserpine mgm./kgm. dose. Tranquilization is differentiated from sedation induced by barbiturates in that the animals may be readily aroused from their depressed state.

On a mgm./kgm. basis, however, the monkey (Rhesus) is definitely more resistant to the actions of reserpine and EA 1476 than is the dog. This statement also holds true for EA 1477 which is much less potent than either EA 1476 or reserpine, however, in adequate doses it possesses a qualitatively similar effect.

At the present time we have no objective method for quantitating tranquilization. We are attempting to devise such a method and some observations with the EEG appear promising with respect to this objective.

2. SLEEPING TIME IN MICE

Following the report by Shore and coworkers (15) that reserpine potentiated the sleeping time in white mice induced by intraperitoneal hexobarbital, we repeated this experiment utilizing EA 1476 in place of reserpine. The results obtained indicate that EA 1476 in doses of 1.0 and 3.0 mgm./kgm. can definitely potentiate the hexobarbital induced sleeping time.

Another series of experiments was devised in which d-amphetamine was employed to determine whether or not this central nervous system stimulant could antagonize the effects of EA 1476. The above experiments are summarized in tables #6 and #7.

The results obtained with d-amphetamine indicate that it can antagonize the potentiating effects of EA 1476 upon the hexobarbital induced sleeping time to an appreciable extent. In one series of experiments d-amphetamine was found to be as effective an antagonist as equivalent doses of LSD-25.

At times it was not possible to repeat the above experiments when different samples of EA 1476 were drawn from our stock of resinous EA 1476. In addition any given alcoholic solution of this drug which initially showed high activity appeared to lose this activity gradually over a period of time. One reason for these discrepancies may be related to the physical character of the drug which does not appear to be a homogenous entity.

3. ELECTROENCEPHALOGRAPHIC CHANGES

At the present time there is no clear cut agreement as to the effect of reserpine on the EEG pattern. Rinaldi and Himwich (14) report that intravenous doses of 0.05 to 0.5 mgm./kgm. of reserpine administered to rabbits produce no change in the electrical activity of the brain. With doses of 1.0 mgm./kgm. the cerebral electrographic picture began to change. Alerting responses to accidental stimuli occurred more often and their duration was longer lasting. Larger doses of reserpine (1.5 - 2.0 mgm./kgm.) caused the persistent presence of an electrographic picture of alertness with complete absence of the characteristics of sleep, such as the high voltage slow waves and spindle formation.

Monroe et al. (12) reported on 5.0 - 10.0 mgm. of reserpine administered intravenously to schizophrenic patients. The patients appeared to sleep, however, cortical EEG's taken during these periods of apparent sleep showed only a relaxed or drowsy record. Occasionally, a momentary run of very light sleep occurred for periods of no longer than ten seconds. Reserpine did not produce [illegible] spindling

characteristic of the barbiturates. Combinations of reserpine and Sodium Amytal induced deeper sleep than either drug alone. There was no difference, however, in the time of onset, amplitude, duration, or location of barbiturate spindling between the records of the patients receiving combinations of Amytal and reserpine, as compared with those receiving Amytal alone. In comparing the work of Rinaldi and Monroe it appears that there are some qualitative differences in their reports. These differences may be related to the species or doses of drug employed. We plan to repeat the above type of experiments in the near future utilizing the curarized dog as the test object.

We have examined the EEG response of the unanesthetized, curarized dog to 1.0 mgm./kgm. of EA 1476 administered intravenously. Within thirty minutes after injecting the drug the EEG pattern consists of generalized high voltage slow waves with some tendency to spindling. The animals show a clear cut arousal pattern to auditory or nociceptive stimuli. In this way the animals may be said to differ from the classical barbiturate response. In some ways the EEG pattern bears a subjective resemblance to that obtained after administration of morphine.

One dog was given 1.0 mgm./kgm. of d-amphetamine two hours after receiving 1.0 mgm./kgm. of EA 1476. Within five minutes there were periods of intermittent arousal of short duration followed by persistent low voltage fast activity for a period of one hour. At this time some spindling again appeared in all the leads. In view of the marked arousal or antagonism produced by d-amphetamine we decided to repeat this experiment on a non-curarized normal dog. The dog received 1.0 mgm./kgm. of EA 1476 intravenously and two hours later he appeared to be very depressed. Respiratory rate and heart rate were greatly reduced below control readings. The animal was then given d-amphetamine 1.0 mgm./kgm. intravenously. Within a few minutes

respiration was noticeably accelerated, the dog rose to his feet and ran around the room. He remained stimulated or hyperactive for about thirty minutes. The antagonistic action of this drug cannot be thought of as being complete in any sense of the word. The dog still exhibited marked ataxia and other responses characteristic of EA 1476. We should emphasize, however, that 1.0 mgm./kgm. is considered to be a very large dose of EA 1476 and is at least twenty times greater than the effective dose of this drug with respect to cardiovascular and behavioral responses. Our immediate attention has been directed to determining the threshold doses required to elicit EEG changes characteristic of this drug. When this is completed we shall return to studying the effects of antagonists upon this response. A recent experiment indicated that the characteristic EEG response to EA 1476 could be obtained with doses of 0.05 - 0.10 mgm./kgm.

C. MISCELLANEOUS

1. HYPOTHERMIA

Hypothermia is an outstanding sign following intravenous administration of 1.0 mgm./kgm. of EA 1476. The deep rectal temperature falls 4-7°C. within twenty-four hours and gradually returns to normal over a several day period in the unanesthetized dog.

Reserpine is reported to have a similar effect in the dog (17).

2. EYE SIGNS

Miosis is an inconstant finding with EA 1477 and EA 1476 in a wide range of doses. Often one may observe a conspicuous mydriasis after these drugs are administered intravenously. By contrast miosis is regarded as a constant finding after the administration of reserpine to many species of animals.

3. RELAXATION OF THE NICTITATING MEMBRANE

This structure in the dog has a pure sympathetic innervation and is readily relaxed by EA 1476 and reserpine when administered by intravenous injection. 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 is unlikely.

4. RESPIRATORY DEPRESSION

This response is most readily seen in the unanesthetized animal (dog, monkey) and often has a dramatic onset. In the case of EA 1476 the depression of respiratory rate usually appears with the onset of ataxia and just precedes the onset of bradycardia. Reserpine often produces an initial increase in respiratory rate lasting two to three hours, followed by a persistent, gradual decrease in respiratory rate (17).

5. DIARRHEA

The severe and usually bloody diarrhea in the dog is the outstanding toxic manifestation of reserpine. It occurs with intravenous doses of 0.10 mgm./kgm. Qualitatively EA 1476 possesses the same property, however, the amount of drug required to produce this effect is about one thousand times greater than the dose of reserpine on a mgm./kgm. basis.

See table #8 for a preliminary comparison of reserpine and EA 1476.

From the standpoint of the ratio of the LD_{50} to the effective dose in the dog the tetrahydrocannabinol derivatives have one of the widest margins of safety of any drug known to man.

IV. DISCUSSION

A. TOXICITY

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

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

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

$$\text{EA 1476 T.R.} = \frac{100.0 \text{ mgms./kgm. i.v.}}{0.05 \text{ mgms./kgm. i.v.}} = 2000.0$$

A comparison of the T.R. values for reserpine and EA 1476 indicates that reserpine is approximately 400 times more toxic than EA 1476 in the dog when administered by the intravenous route.

We have not determined the mechanism of death with intravenous doses of EA 1476 in the dog, however, Walton (18) has reported that purified cannabinol in toxic doses produces pulmonary edema and cerebral hyperemia with indications that death was due to cardiac rather than respiratory failure.

B. CARDIOVASCULAR SYSTEM

Prior to our findings (4) there have been no published reports on the extensive cardiovascular manifestations of the potent tetrahydrocannabinol derivatives. Bradycardia and hypotension have been observed by other workers in the field (7), however, no attempt had been made to determine the mechanism behind these responses.

The observation that EA 1476 in small doses could inhibit the pressor response to bilateral common carotid artery occlusion indicated that this drug 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 nervous 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 cardiovascular system continued to show its characteristic responses to exogenous epinephrine after the injection of EA 1476. Selective sympathetic ganglionic blockade has not been eliminated as a possible mechanism of action, however, the data accumulated to date do not favor this possibility. Experiments are planned in which the activity of the superior cervical ganglion will be studied so that this question may be answered directly.

The widespread inhibition of sympathetic activity suggests that the drug is acting upon central rather than peripheral regulating centers of the sympathetic nervous system. Since the cardiovascular reflexes studied have been shown to be significantly depressed before the hypotensive response becomes statistically significant we can suspect that the fall in blood pressure results from the inhibition of the efferent limbs of these reflexes which are mediated over the sympathetic nervous system. This general hypothesis fits the data that have been accumulated to date, however, additional studies are desired before a definitive hypothesis is presented.

It is of more than casual interest to note that mescaline, marihuana, LSD-25, reserpine and EA 1476 have been reported to produce a depressor response and bradycardia in several species with variable doses. The bradycardia observed is not blocked by atropine in all of the above cases, however, no data with respect to this

point could be found for LSD-25. There are additional signs common to the above drugs such as pupillary changes and salivation that suggest involvement of the autonomic nervous system. The cardiovascular responses per se could represent the effect of a decreased central sympathetic output of neural impulses. We suspect that this situation exists for EA 1476, reserpine, marihuana and to some extent mescaline although there are marked quantitative differences in the activity of these compounds. This type of activity could explain why some of the cardiovascular actions of these drugs are quantitatively exaggerated in the barbitalized dog in comparison with the unanesthetized normotensive dog. This explanation would be based upon the report by Gellhorn (3) in which he states that dogs under the influence of barbiturate anesthesia show a compensatory increase in sympathetic activity. Under these circumstances the response to any drug which inhibited sympathetic activity would be expected to be somewhat exaggerated.

It appears that most if not all of the known psychogenic drugs affect the cardiovascular system in adequate doses. Generally these responses are obtained with small doses and may indicate a primary action upon the central autonomic nervous system. If this correlation can be adequately established it will provide us with a new method for screening and further investigating the mechanism of action of psychogenic chemical agents.

At the present time the study of any one specific system in the body cannot in itself provide conclusive evidence as to the psychogenic properties of a given chemical agent. When one can put together several parts of the puzzle such as ataxia, hypesthesia, respiratory changes, alterations in body temperature, blood pressure, heart rate and specific cardiovascular reflexes in addition to gross

observations of the behavior pattern of the animal then a more intelligent prediction of psychogenic activity can be made. The more promising drugs selected through the above processing would then warrant a clinical investigation. We definitely favor the dog as a test animal in the above study especially when observing gross behavioral responses. In many ways the dog is more expressive than the monkey and also appears to be much more sensitive to the effects of this class of compounds.

C. CENTRAL NERVOUS SYSTEM

The behavioral changes observed in animals are the most difficult pharmacologic effects to evaluate. We have stated our preference for the dog as a test animal since he is relatively sensitive to the effects of this class of compounds and we are sufficiently familiar with his range of expressions to detect gross changes.

We have used the term tranquilization to indicate a type of depression which differs from that induced by barbiturates in that the animals may be readily aroused from their depressed state by adequate stimuli. The intensity of the stimulus required for arousal is proportional to the dose of EA 1476, EA 1477 or reserpine administered. With low doses of these drugs the dog may be readily aroused from apparent sleep by noise or movement towards him. After higher doses you may have to touch the animal in order to elicit arousal and finally with very high doses complete arousal to the point where the animal can rise and walk away is impossible.

It appears certain that EA 1476, mescaline, EA 1477 and reserpine have multiple types of action within the central nervous system. Even LSD-25 has been reported to produce ataxia, hypesthesia and increased tendon reflexes, all of which are seen with the other drugs in this class of compounds.

The studies to date utilizing the effect of EA 1476 and mescaline upon the dog EEG appear promising. EA 1476 in doses less than 0.10 mgm./kgm. produces a definite change in the pattern of activity which was not observed by Loewe (11). The typical picture obtained consists of high voltage slow wave activity with a tendency to spindling. Mescaline in a dose of 7.0 mgm./kgm. produces a similar response. Reserpine and LSD-25 by contrast have been reported to produce an arousal type EEG pattern with small doses.

The observation that d-amphetamine, 1.0 mgm./kgm., can antagonize the high voltage slow wave activity induced by EA 1476, 1.0 mgm./kgm., for a period of one hour or more is of interest since d-amphetamine is also an antagonist to the depressant effects of EA 1476 in the intact non-curarized dog. A correlation between EEG changes and comparable responses in the intact animal is not generally observed with many drugs. If this correlation holds at threshold dose levels of EA 1476 we may be able to quantitate tranquilization by the use of implanted EEG electrodes.

Even though EA 1476 is not a hypnotic per se it does potentiate the sleeping time in mice induced by hexobarbital. Loewe (7) 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 (11) he further limits the choice of barbiturates to Pernoston, a bromide containing barbital derivative. Loewe (7) states that 20.0 mgm./kgm. of cannabidiol prolongs the sleeping time of a barbitalized mouse by ninety per cent, in contrast to 100 mgm./kgm. of tetrahydrocannabinol which only prolonged the sleeping time by twenty per cent. Our data indicate that EA 1476 is about twenty times as potent as cannabidiol in this respect in specific cases since 1.0 mgm./kgm. prolongs the mouse sleeping time eighty per

cent when administered one hour before hexobarbital, 100 mgm./kgm. This response can also be antagonized by the administration of d-amphetamine or LSD-25 prior to the injection of hexobarbital. Since the similarity between the actions of reserpine and EA 1476 are so marked we plan to repeat the work presented by Shore and his coworkers (15) in which LSD-25 was shown to antagonize the potentiating effect of reserpine upon the hexobarbital sleeping time in the mouse. It seems quite possible that LSD-25 is not necessarily a specific competitive antagonist.

Another aspect of the central nervous system activity of EA 1476 and EA 1477 was reported by Loewe and Goodman (10). They observed that EA 1477 and EA 1476 were effective agents for abolishing the hindleg tonic extensor component of maximal electroshock seizures in the rat. The anticonvulsant ratios (EA 1477 = 1.0, EA 1476 = 200.0) 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. We might add that the potency ratios of these drugs with respect to their cardiovascular activities are of the same order of magnitude as the ataxia and anticonvulsant ratios.

Loewe and Goodman (10) 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 anticonvulsant.

Loewe (11) in a later publication reported that some experimental research by the group at Utah (2) indicates these compounds are 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 150 times more potent than diphenylhydantoin as an anti-epileptic agent against grand mal seizures and also can normalize the EEG pattern of the individual with grand mal epilepsy.

The reports by Loewe indicate that EA 1476 is capable of altering the response of the cerebral cortex to both internal and external stimuli. This serves as further evidence that EA 1476 can depress abnormal as well as normal cortical activity.

There is some unpublished evidence collected by Dr. Graham Chen of Parke Davis & Company which indicates that reserpine antagonizes the anticonvulsant actions of diphenylhydantoin. If this work is confirmed then an important qualitative difference in the actions of reserpine and EA 1476 will have been established.

D. MISCELLANEOUS

The prominent hypothermia observed with EA 1476, EA 1477 and reserpine is highly suggestive of a central mechanism of action. The pupillary alterations and diarrhea may possibly be attributed to a relative imbalance in the autonomic nervous system. The relaxation of the nictitating membrane by these drugs and the observation that none of the drug blocks the contraction of this structure following the injection of epinephrine is indirect evidence of a reduction in central sympathetic outflow.

No preliminary experiments have been run thus far which would permit us to speculate on the mechanism of the respiratory depression observed with EA 1476, EA 1477 and reserpine.

V. SUMMARY

1. EA 1476, EA 1477 and reserpine have numerous similar pharmacological effects in the dog when administered by the oral or intravenous route.

2. From the standpoint of the ratio of the LD_{50} to the effective dose EA 1476 has one of the greatest margins of safety of any drug known when administered intravenously to dogs.

3. EA 1476 and EA 1477 have qualitatively similar effects upon the cardiovascular system of the dog. There is, however, a marked quantitative difference with respect to potency. EA 1476 is approximately two hundred times more potent than EA 1477 in its actions upon the cardiovascular system.

4. The cardiovascular responses elicited by small doses of EA 1476 in the barbitalized dog after intravenous administration are: hypotension, bradycardia which is apparently not blocked by prior atropinization, inhibition of common carotid occlusion pressor response, inhibition of the central vagal stimulation pressor response, increased vascular response to injected epinephrine and no immediate direct cardiac effects in doses of 1.0 mgm./kgm.

5. EA 1476, EA 1477 and reserpine produce a state of tranquilization in the unanesthetized dog following intravenous administration. The term tranquilization is used to indicate a type of depression which differs from that induced by barbiturates in that the dogs may be readily aroused from their depressed state by adequate stimuli. This effect is seen in doses smaller than those required to produce obvious ataxia.

6. EA 1476 like reserpine can prolong the sleeping time of white mice induced by the intraperitoneal injection of hexobarbital.

7. The electroencephalographic response of the unanesthetized curarized dog to 0.10 mgm./kgm. of EA 1476 administered intravenously is characterized by high voltage slow waves with some tendency to spindling. The dogs exhibit a clear cut arousal pattern to auditory and nociceptive stimuli in contrast to dogs which have received a barbiturate.

8. The characteristic high voltage slow wave activity following intravenous EA 1476, 1.0 mgm./kgm., can be antagonized by intravenous d-amphetamine, 1.0 mgm./kgm. This antagonism is also observed grossly in the intact non-curarized dog.

9. Hypothermia is an outstanding sign following intravenous administration of 1.0 mgm./kgm. of EA 1476. The deep rectal temperature usually falls 4-7° C within twenty-four hours in the unanesthetized dog.

10. The pupillary changes observed following intravenous injections of EA 1476 and EA 1477 are variable. Both miosis and mydriasis have been observed frequently with a wide range of doses.

11. EA 1476 like reserpine induces relaxation of the nictitating membrane in the dog following intravenous administration.

12. EA 1476 as well as reserpine depresses respiratory rate following intravenous administration to unanesthetized dogs.

13. EA 1476 is capable of producing a bloody diarrhea in the dog, however, reserpine is one thousand times more potent than EA 1476 in this respect following intravenous injection.

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TABLE 1

TOXICITY DATA EA 1476

I. MOUSE	LD_{50} mgm./kgm.	Route of Administration	n	P value
	390.0	I.P.	40	<0.05

II. DOG	Effective Dose (Intravenous)	# Animals	Fatalities
	0.025 mgm./kgm.	2	0
	0.050 " "	5	0
	0.125 " "	2	0
	0.250 " "	4	1*
	1.0 " "	8	0
	5.0 " "	2	0
	10.0 " "	3	0
	50.0 " "	1	0
	100.0 " "	1	1

III. DOG	Effective Dose (Oral)	# Animals	Fatalities
	0.5 mgm./kgm.	1	0
	1.0 " "	2	0
	2.0 " "	2	0

III. MONKEY	Effective Dose (Intravenous)	# Animals	Fatalities
	0.5 mgm./kgm.	1	0
	1.0 " "	1	0
	2.0 " "	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 2

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

	CONTROLS					EXPERIMENTAL					
TIME	DRUG	DOSE	% CHANGE	S.E.	n	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.	"	"	- 1.40	± 2.8	5	"	"	- 7.4	± 3.0	6	>0.20
1 hour	"	"	- 0.60	± 3.3	5	"	"	- 15.1	± 5.6	6	>0.05
2 hours	"	"	+ 0.40	± 5.7	5	"	"	- 18.4	± 4.8	5	<0.05*
3 hours	"	"	+ 1.20	± 4.6	5	"	"	- 23.3	± 3.1	5	<0.01*
4 hours	"	"	+ 1.90	± 1.8	3	"	"	- 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.	"	"	- 0.1	± 1.5	6	"	"	- 11.2	± 5.8	6	>0.10
1 hour	"	"	- 6.4	± 4.7	6	"	"	- 21.3	± 5.7	6	>0.05
10 min.	NNDMA	0.1 ml./kgm.	- 2.5	± 1.7	6	Reserpine	1.0 mgm./kgm.	+ 6.5	± 2.3	5	<0.02*
30 min.	"	"	- 5.7	± 2.5	6	"	"	+ 3.7	± 4.4	5	>0.05
1 hour	"	"	- 17.7	± 5.9	6	"	"	- 12.1	± 3.2	5	>0.40
1½ hours	"	"	- 11.2	± 3.4	6	"	"	- 29.5	± 7.6	5	<0.05*
2 hours	"	"	- 15.8	± 2.6	6	"	"	- 25.4	± 2.3	5	<0.05*

S.E. = standard error.
EtOH = 95 % ethyl alcohol.
NNDMA = 100 % NN-dimethylacetamide.
n = number of animals.

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

TABLE 3

COMPARATIVE BLOOD PRESSURE RESPONSE TO EA 1476 AND RESERPINE SOLVENTS

	EA 1476 SOLVENT					RESERPINE SOLVENT					
TIME	DRUG	DOSE	% CHANGE	S.E.	n	DRUG	DOSE	% CHANGE	S.E.	n	P VALUE
10 min.	EtOH	0.1 mgm./kgm.	+ 0.04	± 1.2	5	NNDMA	0.1 ml./kgm.	- 2.5	± 1.7	6	>0.20
30 min.	"	"	- 1.40	± 2.8	5	"	"	- 5.7	± 2.5	6	>0.20
1 hour	"	"	- 0.60	± 3.3	5	"	"	- 17.7	± 5.9	6	<0.05*
2 hours	"	"	+ 0.40	± 5.7	5	"	"	- 15.8	± 2.6	6	<0.02*

* = statistically significant, group comparison (16).
Anesthesia = Sod. pentobarbital, 30.0 mgm./kgm.
Sepcies = dog.

S.E. = standard error.
EtOH = 95 % ethyl alcohol.
NNDMA = 100 % NN-dimethylacetamide.

TABLE 4.

INHIBITION OF COMMON CAROTID OCCLUSION (30 SECONDS) PRESSOR RESPONSE.
CORRECTED FOR CHANGES IN BLOOD PRESSURE ACCORDING TO PROCHNIK (13).

CONTROLS						EXPERIMENTAL					
TIME	DRUG	DOSE	%ᶜCHANGE	S.E.	n	DRUG	DOSE	%ᶜCHANGE	S.E.	n	P VALUE
10 min.	Alcohol	0.1 ml./kgm.	- 1.8	± 2.9	5	EA 1476	0.05 mgm./kgm.	- 4.7	± 3.0	6	>0.50
30 min.	"	"	+ 5.3	± 4.0	5	"	"	- 15.0	± 3.4	6	<0.01
1 hour	"	"	- 0.6	± 5.5	5	"	"	- 29.8	± 2.6	6	<0.01
2 hours	"	"	- 1.9	± 8.5	5	"	"	- 35.8	± 8.4	6	<0.05
3 hours	"	"	- 5.2	± 4.8	5	"	"	- 30.5	± 6.0	5	<0.02
4 hours	"	"	- 9.8	± 8.5	3	"	"	- 24.5	± 11.7	5	>0.30
TIME	DRUG	DOSE	%ᶜCHANGE	S.E	n	DRUG	DOSE	%ᶜCHANGE	S.E.	n	P VALUE
10 min.	NNDMA*	0.1 ml./kgm.	- 0.3	± 6.3	6	Reserpine	1.0 mgm./kgm.	- 13.0	± 2.1	5	>0.30
30 min.	"	"	- 7.3	± 6.7	6	"	"	- 18.0	± 6.5	5	>0.40
1 hour	"	"	- 16.0	± 9.1	6	"	"	- 15.0	± 9.6	5	>0.50
1½ hours	"	"	- 11.0	± 6.0	6	"	"	- 10.3	± 6.0	3	>0.50
2 hours	"	"	- 12.0	± 5.0	6	"	"	- 25.0	± 8.1	5	>0.20
TIME	DRUG	DOSE	%ᶜCHANGE	S.E.	n	DRUG	DOSE	%ᶜCHANGE	S.E.	n	P VALUE
10 min.	Alcohol	0.1 ml./kgm.	+ 2.5	± 2.4	6	EA 1477	10.0 mgm./kgm.	- 22.8	± 6.78	6	<0.01
30 min.	"	"	- 5.3	± 1.4	6	"	"	- 32.8	± 8.9	6	<0.02
1 hour	"	"	- 6.3	± 2.5	6	"	"	- 32.2	± 10.5	6	<0.05

* NNDMA = N,N-dimethylacetamide.
Alcohol = 95 % ethyl alcohol.
Species = dog.

Anesthesia - Sod. pentobarbital.
%ᶜChange - per cent change in pressor response corrected for changes in blood pressure.

TABLE 5

THRESHOLD DOSES OF EA 1476 REQUIRED FOR INHIBITION OF THE COMMON CAROTID ARTERY OCCLUSION PRESSOR RESPONSE.

EXP #	BLOOD PRESSURE mmHg.			% DECREASE IN PRESSOR RESPONSE TO C.C.O (30 sec.)	DOSE EA 1476 mgm./kgm.
	CONTROL	MINIMUM	% DECREASE		
6	153	106	31	100	0.010
7	154	143	7	72	0.090
8	134	104	22	88	0.030
9	117	92	21	80	0.015
10	142	95	33	100	0.010
			$\bar{x}$ = 23 %	$\bar{x}$ = 88 %	$\bar{x}$ = 0.031

Species = dog.
Anesthesia = Sod. pentobarbital (30.0 mgm./kgm.).
Time of recording % decrease in pressor response to C.C.O. was varied in order to obtain the maximal effect. This maximal value was recorded at the time of the minimum blood pressure listed above.

TABLE 6

MOUSE SLEEPING TIME POTENTIATION BY EA 1476.

	HEXOBARBITAL 100 mgm./kgm. i.p.						HEXOBARBITAL 100 mgm./kgm. + EA 1476+ i.p.							
GRP#	DATE	AVERAGE WEIGHT	AVERAGE SLEEPING TIME		RANGE	n.	GRP#	DATE	DOSE EA 1476 mgm./kgm.	AVERAGE WEIGHT	AVERAGE SLEEPING TIME		RANGE	n.	P VALUE
3	9-30	35 gms.	41 min.		30-54	10	4	9-30	1.0*	34 gms.	74 min.		50-89	10	<0.01
5	10-3	36 "	49 "		33-69	10	6	10-3	1.0	36 "	88 "		72-105	10	<0.01
7	10-5	35 "	49 "		36-65	10	8	10-5	3.0	36 "	123 "		80-180	10	<0.01
9	10-7	35 "	51 "		33-74	9	10	10-7	3.0	38 "	167 "		137-192	10	<0.01
13	10-10	18 "	50 "		40-59	10	12	10-10	5.0	18 "	114 "		82-136	8	<0.01
15	10-12	19 "	54 "		37-77	10	14	10-12	3.0	19 "	103 "		72-137	10	<0.01
20	10-14	19 "	47 "		39-54	5	17	10-14	3.0*	20 "	66 "		58-78	10	<0.01
21	10-26	25 "	61 "		56-67	10	22	10-26	1.0*	25 "	68 "		61-76	9	<0.01
21	10-26	25 "	61 "		56-67	10	23	10-26	3.0*	25 "	79 "		67-87	9	<0.01
24	10-28	20 "	57 "		42-66	9	26	10-28	Hexobarbital in 10% ethyl alcohol.	19 "	56 "		34-91	10	>0.50
25	10-28	36 "	56 "		46-75	8	27	10-28	"	36 "	57 "		48-63	8	>0.50

\+ EA 1476 was administered one hour prior to hexobarbital.

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

Groups 24 - 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 7

MOUSE SLEEPING TIME ANTAGONISM

	HEXOBARBITAL (A.)	HEXOBARBITAL EA 1476	HEXOBARBITAL EA 1476 d-AMPHETAMINE (B.)	HEXOBARBITAL	HEXOBARBITAL EA 1476	HEXOBARBITAL EA 1476 LSD-25
GROUP #	9	10	11	15	14	16
AVG. SLEEPING TIME	51 min.	167 min.	97 min.	54 min.	103 min.	91 min.
RANGE	33-74	137-192	60-139	37-77	72-137	65-130
AVG. WEIGHT	35 gms.	38 gms.	17 gms.	19 gms.	19 gms.	19 gms.
DATE	10-7	10-7	10-7	10-12	10-12	10-12
n.	9	10	10	10	10	10

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

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.

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

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

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

TABLE 7 (cont.)

MOUSE SLEEPING TIME ANTAGONISM

	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	58-78	46-109
AVG. WEIGHT	19 gms.	19 gms.	19 gms.
DATE	10-14	10-14	10-14
n.	5	10	10

	HEXOBARBITAL	HEXOBARBITAL EA 1476 d-AMPHETAMINE	HEXOBARBITAL EA 1476 LSD-25
GROUP #	20	18	19
AVG. SLEEPING TIME +	47 min.	72 min.	72 min.
RANGE	39-54	47-98	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

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. " "

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

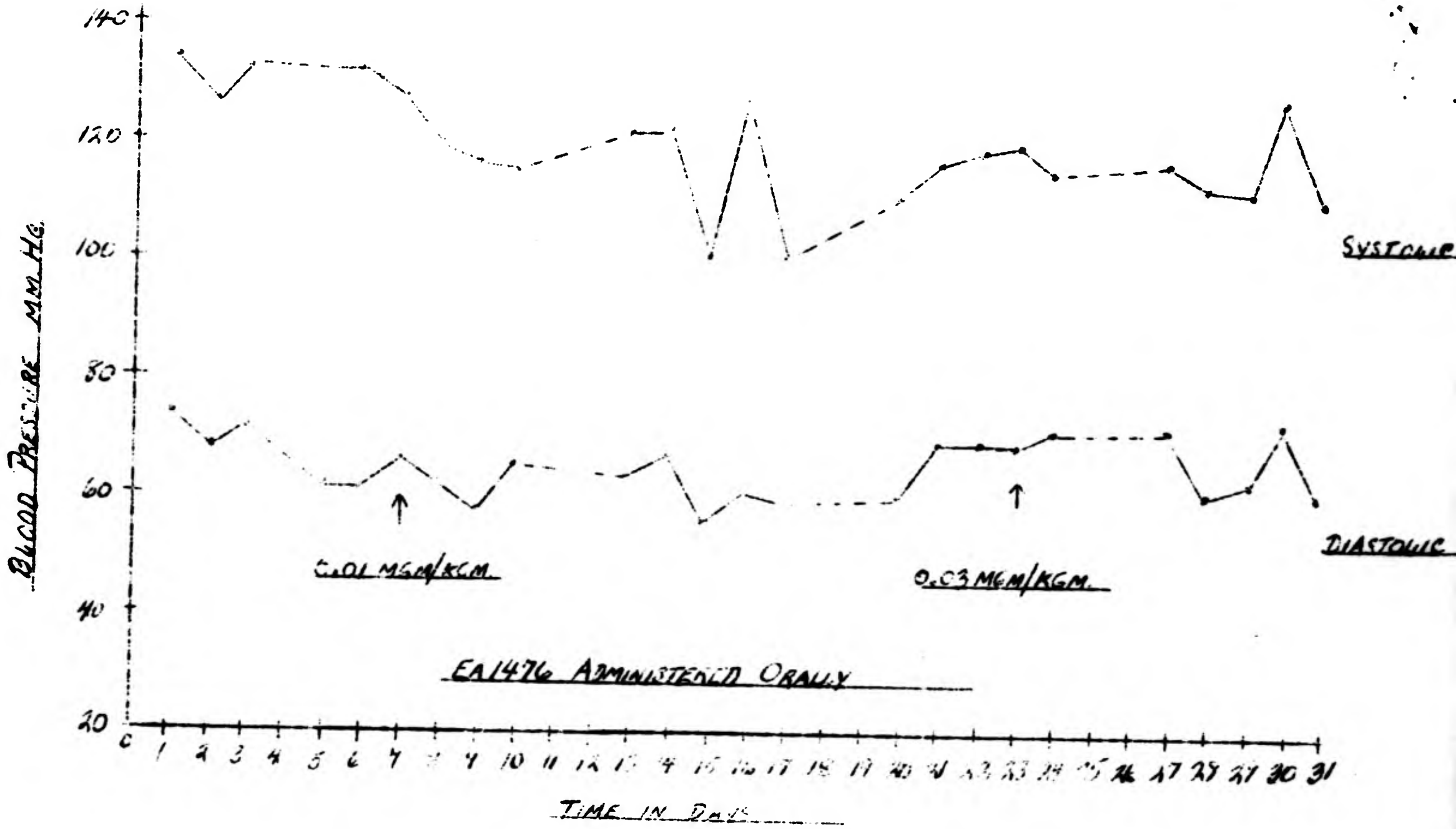
LSD-25 3.0 mgm./kgm. administered i.p. fifteen minutes prior to the hexobarbital.

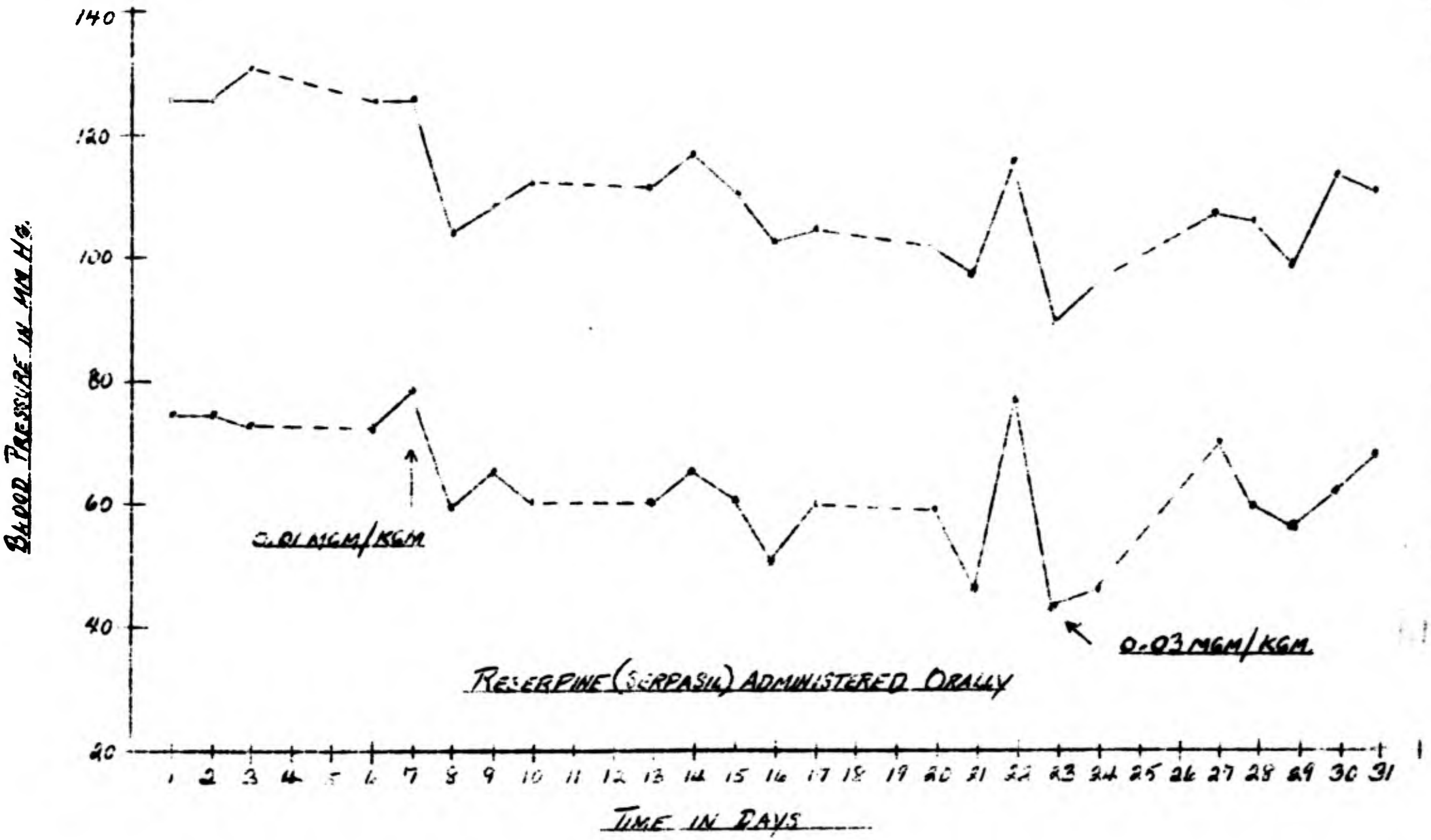
one hour prior to the hexobarbital.

TABLE 8

PRELIMINARY COMPARISON OF RESERPINE AND EA 1476

OBSERVATION	ANIMAL	DOSE (mgm./kgm.) RESERPINE	ROUTE	DOSE (mgm./kgm.) EA 1476	ROUTE
Toxicity LD_{50}	Dog	0.50	I.V.	100.00	I.V.
Toxicity LD_{50}	Mouse	20.00	I.P.	390.00	I.P.
Tranquilization	Monkey	0.50	I.V.	0.50	I.V.
Tranquilization	Dog	0.10	I.V.	0.025 - 0.050	I.V.
Miosis	Dog	0.10	I.V.	$\pm$ 0.050	I.V.
Bradycardia	Dog	0.10	I.V.	1.0 or less	I.V.
Hypothermia	Dog	1.0 or less	I.V.	100.00	I.V.
Diarrhea	Dog	0.10	I.V.	0.10	I.V.
Relaxation of Nictitating Membrane	Dog	0.10	I.V.	0.10	I.V.
Respiratory Depression	Dog	0.10	I.V.	0.05	I.V.
Inhibition of C.C.O. Pressor Response	Dog	1.00	I.V.	0.50	I.V.
Inhibition of C.V.S. Pressor Response	Dog	1.00	I.V.	0.05	I.V.
Increased Vascular Response to Epinephrine	Dog	1.00	I.V.		


140
120
100
80
60
40
20
BLOOD PRESSURE MM HG.
SYSTOLIC
DIASTOLIC
0.01 MGM/KGM.
0.03 MGM/KGM.
EA1476 ADMINISTERED ORALLY
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
TIME IN DAYS


140
120
100
80
60
40
20
BLOOD PRESSURE IN MM HG.
0.01 MGM/KGM
0.03 MGM/KGM
RESERPINE (SERPASIL) ADMINISTERED ORALLY
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
TIME IN DAYS