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

A PHARMACOLOGICAL COMPARISON OF EA 1476 (TETRAHYDROCANNABINOL) ISOMERS

PROGRESS REPORT

by

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In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences–National Research Council.

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A Pharmacological Comparison of EA 1476 Isomers

INTRODUCTION

The initial experiments in this study have been directed toward a comparison of the acute toxicity of two EA 1476 isomers in the dog. The drugs were administered intravenously using ethyl alcohol as a solvent. The concentration of the drug solution was such that 0.1 ml. of ethyl alcohol (95%) per kilogram represented the maximum amount of solvent employed. This procedure was used consistently in order to minimize the pharmacological actions of the solvent per se.

Our original sample of EA 1476 received in 1953 will hence forth be designated as EA 1476 (O S). The term "OS" representing original sample. The isomer selected for comparison is one recently submitted to us by Dr. Wills which has been subjected to some preliminary investigations in his laboratory. The sample has been designated EA 1476 (4018).

Our experiments were designed to observe behavioral changes and acute toxicity in the same dog. Two animals were employed in each experiment and subjected to exactly the same experimental conditions except for the drug administered. One dog received EA 1476 (OS) and the second animal received the same mgm./kgm. dose of EA 1476 (4018). This procedure provided an acceptable basis for comparing the two drugs with respect to behavioral effects and acute toxicity. It also provided a means by which we could review the acute toxicity and behavioral effects of EA 1476 (OS) and compare these effects with the responses obtained two years ago. In a previous report we referred to the early work of S. Loewe who reported that these compounds are very stable and may be stored in an open container on the laboratory shelf for several years without showing any marked change in biological activity.

On the recommendation of Dr. Wills we took special precautions in order

to detect any signs of hematuria in our experimental animals, especially those receiving EA 1476. (4018).

We have completed nine paired experiments (18 dogs) to date. The data indicate a slight difference in acute toxicity between EA 1476 (08) and EA 1476 (4018). In view of the relatively small number of animals employed thus far, we are forced to express our results as being tentative in nature.

RESULTS IN THE DOG:

I. DOSE = 1.0 mgm./kgm., i.v. (10 animals)

A. Heart Rate

1. EA 1476 (OS)

In a series of five dogs the range of the control heart rate varied from 80-120 beats per minute. A marked bradycardia was observed within one hour after drug administration and a maximum response was reached within 24 hrs. At this time the range of the recorded heart rates varied between 36-60 beats per minute. Dropped beats were frequently observed in these animals.

2. EA 1476 (4018)

In a series of five dogs the range of the control heart rate varied from 72-112 beats per minute. A maximum bradycardia response was reached within 24 hrs. The heart rate values at this time varied between 24-64 beats per minute. These animals also showed dropped beats.

B. Respiratory Rate

1. EA 1476 (OS)

In a series of five animals the control RR varied between 20-40 per minute. The maximum depression in RR occurred with 24 hrs. At this time the RR values varied between 2-10 respiratory excursions per minute.

2. EA 1476 (4018)

In a series of five animals the control RR varied between 16-40 per minute. Twenty four hours after drug injection the maximum depression was observed and the rates varied between 2-16 respiratory excursions per minute.

C. Rectal Temperature

1. EA 1476 (OS)

In a series of five animals the range for the control readings varied between 39.5 and 41.2°C. The maximum hypothermic response was observed within 24 hrs. At this time the temperature readings varied between 25.5 and 34.3°C.

2. EA 1476 (4018)

In a series of five animals the range for the control readings varied between 38.5 and 41.2°C. The maximum hypothermic response was observed within 24 hrs. At this time the temperature readings varied between 25.5 and 36.0°C.

D. Convulsions

1. EA 1476 (OS)

In a series of five dogs three animals exhibited convulsive episodes when forceful attempts were made to arouse them from their depressed state. The convulsions were classified as tonic extensor and flexor as well as the clonic variety. One dog in this series died following a tonic extensor convulsive episode 48 hrs. after receiving the drug. He appeared to be recovering from the drug effect at this time as evaluated by physical examination. His heart rate, respiratory rate and rectal temperature were approaching relatively normal values. We could not determine any extraneous reason for the cause of death, therefore, death was attributed to the drug by exclusion. Our subjective impression remains that other factors may have been involved.

2. EA 1476 (4018)

In a series of five dogs two animals exhibited convulsive

episodes upon forced arousal from their depressed state. One dog manifested a tonic extensor convulsion while the other dog exhibited a tonic flexor convulsion. Both of these animals died 23 hrs. after receiving the drug.

The convulsive episodes observed after administering either drug could always be terminated by the technique of "laying on of hands". If a dog convulsed on forced arousal, simply applying forceful pressure to his body terminated the convulsion within one minute.

E. Analgesia

Both drugs produced rapid "analgesia" within a few minutes after intravenous injection. The response to superficial pain (pinprick) was lost within 1-2 minutes after injection. The response to deep pain (stepping on paw and tail), however, was maintained for 10-30 minutes following intravenous injection. Once "analgesia" to deep pain was obtained we were unable to arouse the animals from their depressed state by any form of painful stimuli.

F. Ataxia

Both drugs produced a rapid onset of ataxia within 5-15 minutes following intravenous injection of the drug. There was no discernible difference in the onset of this effect between the two drugs evaluated.

II. DOSE = 0.5 mgm./kgm., i.v. (8 animals)

The results obtained with both drugs at this lower drug level did not differ significantly from the results described for the 1.0 mgm./kgm. dose. Quantitatively the bradycardia, decrease in respiratory rate, hypothermia and general CNS depression was less marked for both drugs. One animal in each of the two drug groups exhibited a tonic extensor convulsion. The dog who had received EA 1476 (4018) recovered uneventfully. The dog who had received

EA 1476 (OS) died 48 hrs. after intravenous administration of the drug. Just before death the following observations were made upon physical examination. The heart rate had decreased from a control value of 108 to 44 per minute. Rectal temperature had fallen from a control value of 39°C. to 24°C. Respiratory rate had decreased from a control value of 30 to 2 per minute. Death, therefore, appeared to result from an effect of the drug.

DISCUSSION

The experiments with EA 1476 (4018) at a dose of 1.0 mgm./kgm., i.v. suggest that it possesses the same order of acute toxicity as EA 1476 (OS) in comparable doses. The mechanism of death following i.v. EA 1476 (4018) has been definitely established as resulting from ventricular fibrillation. This diagnosis has been made on the basis of EKG tracings as well as upon direct observation. It is difficult to attribute this effect to a direct cardiac action in view of the prolonged interval between drug administration and cardiac arrest. It was observed that these dogs had rectal temperatures of 25.5 and 29.0°C. respectively just before cardiac arrest. They also exhibited tonic flexor and tonic extensor convulsions respectively just prior to death. It is entirely possible that ventricular fibrillation may have resulted in part or entirely from the extreme degree of hypothermia. The mechanism of death was not determined for the dog who died subsequent to receiving EA 1476 (OS) 1.0 mgm./kgm., i.v. The one animal who died in the lower dose range [0.5 mgm./kgm., i.v. of EA 1476 (OS)] also exhibited a marked hypothermia (24°C.) and manifested a tonic extensor convulsion shortly before death.

No evidence of hematuria was observed, although two dogs in each

group were observed to have blood streaked stools on occasion. These animals recovered uneventfully. They were sacrificed and autopsied 5 days after receiving the drug and no gross signs of internal bleeding were observed at that time.

We hold the impression that EA 1476 (OS) is more toxic now than when it was first evaluated by us in 1954*. We shall investigate this point further. EA 1476 (4018) may be slightly more toxic than EA 1476 (OS). A final conclusion cannot be drawn with respect to this point because of the limited number of animals observed. EA 1476 (4018), however, did exhibit a high incidence of ventricular fibrillation in the series of 5 dogs receiving 1.0 mgm./kgm., i.v. This effect may have been secondary to the extreme hypothermia observed. It should be noted that earlier experiments with EA 1476 (OS) were conducted during summer months. The higher ambient temperature possibly prevented. the extreme hypothermia and ventricular fibrillation. In general EA 1476 (4018) produced a more marked degree of hypothermia than EA 1476 (OS) although the series is too small to establish this on a statistical basis.

Both compounds have about the same latent period from injection to drug response and the duration of action is comparable. Both drugs may also produce convulsions which can be terminated by manual pressure exerted over the body of the dog.

Both compounds produce a marked enhancement of the deep tendon reflexes (knee jerk, ankle jerk, biceps and triceps). There is a prominent relaxation of the nictitating membrane with increased salivation suggesting a reduction in sympathetic tone and a relative enhancement of parasympathetic function. The eyes of the dogs also exhibit a prominent hypotropia following drug administration.

* See table #5, Second Summary Progress Report, 1956

SUMMARY.

1. EA 1476 (OS) and EA 1476 (4018) produce a similar behavioral response in the unanesthetized dog with comparable intravenous doses.
2. EA 1476 (4018) produced death by cardiac arrest resulting from ventricular fibrillation in two out of five dogs who received intravenous doses of 1.0 mgm./kgm.
3. EA 1476 (OS) appears to be more toxic now than when it was first evaluated in this laboratory two years ago.
4. EA 1476 (4018) may be slightly more toxic than EA 1476 (OS) with intravenous doses of 1.0 mgm./kgm.