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W. A. KRIVOY

A COMPARISON OF THE ACTIONS OF SUBSTANCE P AND OTHER NATURALLY OCCURING POLYPEPTIDES ON SPINAL CORD

Introduction

Lysergic acid diethylamide (LSD) has been shown to inhibit the enzymatic destruction of SP »in vitro« (Krivoy, 1957; Smith and Walaszek, 1961). This communication represents an attempt to determine if LSD potentiates the actions of SP »in vivo«, thereby providing information on the physiology of SP on the one hand, and the pharmacology of LSD on the other.

Lembeck (1953) suggested that SP is a neurohumor associated with primary afferent transmission in the spinal cord. Although this suggestion was predicated entirely upon the distribution of SP, it seemed most reasonable to initiate this investigation by looking for some interaction between LSD and SP at the level of the spinal cord.

Methods and materials

To evaluate the actions of SP on transmission of nerve impulses along the intramedullary pathway of the primary afferent fiber to secondary and internuncial neurons, use was made of the technique described by Lloyd and McIntyre (1949). These authors described and analysed a series of five potentials which were recorded from a dorsal rootlet adjacent to another rootlet which was stimulated. For the sake of clarity these potentials will be designated according to the nomenclature of these authors (see Fig. 1). The first three dorsal root potentials, DR I, II and III are due to intramedullary conduction of the nerve impulse along the primary afferent nerve. The fourth, DR IV, is in large measure due to a residual negativity associated with supernormality of the nerve endings of the primary afferent fiber, as well as in the secondary neurons. The fifth response, DR V, is associated with supernormality in internuncial neurons (Gasser and Graham, 1933; Rudin and Eisenman, 1953; Eccles and Krnjević, 1959).

Forty-two decerebrate cats were used in this study. All experiments were duplicated on decerebrate cats which had spinal transections at L1. Decerebration and subsequent laminectomy were performed under

ether anesthesia. After exposure of the spinal cord the last lumbar or first sacral spinal roots were dissected and split into two rootlets, each of which was mounted on silver or chlorided silver electrodes. One rootlet was used for stimulating, the other for recording. The entire area of exposed spinal cord and rootlets was covered with mineral oil contained in a trough constructed of the incised skin. The mineral oil was previously equilibrated with CO_2 and maintained at 37°C by radiant heat.

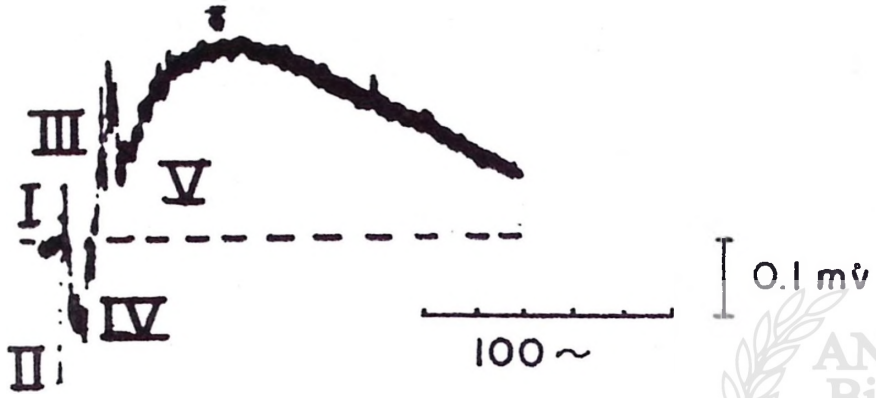


FIG. 1
DR I-V of the dorsal root potential.

Stimuli used were biphasic and either maximal or about 50% of maximal for DR IV. Once stimulation had been started it was maintained at a constant frequency of 0.5 or 2.5 cycles per second (cps) for the duration of the experiment. Stimulation was provided by means of a Grass S4 stimulator isolated from ground by a Schmitt-type stimulus isolation unit (Grass). Preamplification of potentials was accomplished by a Grass P6A DC preamplifier. A Tektronix 502 cathode ray oscilloscope was used for further amplification and display of the evoked potential.

Drugs used in this study were LSD[1], BrLSD[1], SP[2,3], chlorpromazine, synthetic bradykinin[1] and homogenous β-melanocyte stimulating hormone (β-MSH). All drugs were administered intravenously via a polyethylene cannula fixed in the femoral vein. No drug was administered until at least one hour after the termination of ether anesthesia, and then only after the size of the dorsal root potentials had been observed to remain constant for at least thirty minutes.

Blood pressure was monitored in some preparations by means of a mercury manometer connected to the femoral artery. No anticoagulant was used. Respiration was noted by observation.

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[1]) Kindly provided by Dr. R. P. Bircher, Sandoz Pharmaceutical Co., Hanover, New Jersey, U. S. A.
[2]) Kindly provided by Dr. J. H. Gaddum, Institute of Animal Physiology, Babraham, England.
[3]) Kindly provided by D. Graham Chen, Parke Davis and Company, Ann Arbor, Michigan, U. S. A.

Results

A. Substance P. — When submaximal or maximal stimuli of 0.5 cps were used, LSD, SP and combinations of these two drugs were found to have no consistent action on the preparation. When maximal stimuli of 2.5 cps were used, LSD in extremely large doses (70 μg/kg) produced enhancement of DR IV.

When submaximal stimuli were used at a frequency of 2.5 cps, LSD 5 μg/kg was found to enhance DR IV in approximately half of the cats studied, and 10 μg/kg produced enhancement in every cat. When augmentation of DR IV appeared, it was observed within one minute of the time of injection and reached a maximum approximately five minutes thereafter; if at this time the stimulus intensity was reduced such that DR IV was again submaximal, DR IV remained at this new level indicating that the action of LSD had reached a stable level, and that there was no background of continued enhancement.

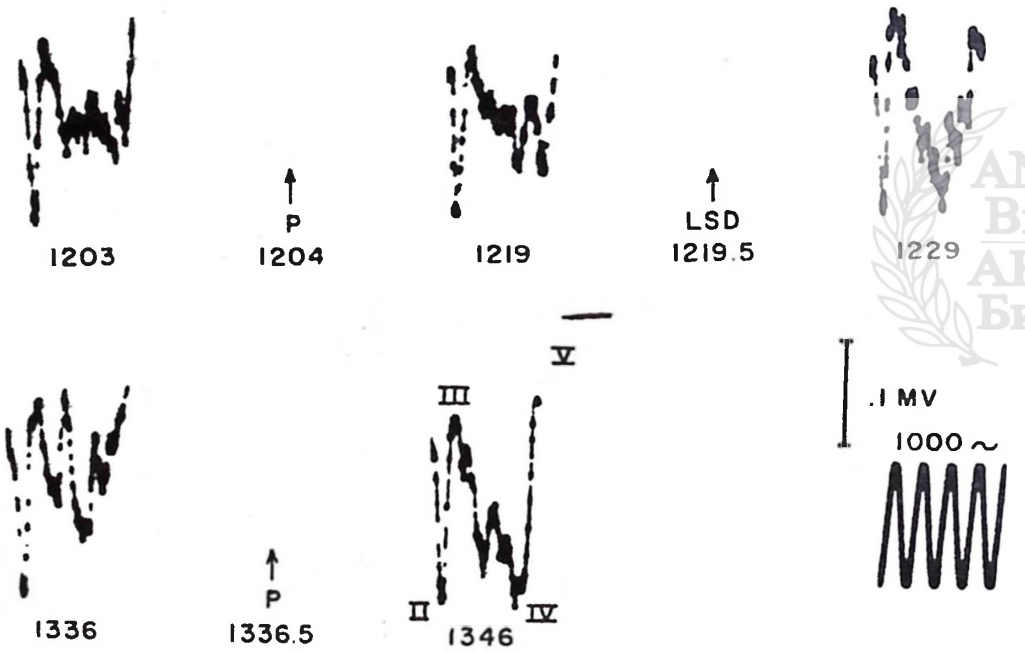


FIG. 2

Dorsal root potentials from a single experiment. The times each record was obtained is indicated below each tracing. Drugs and times of administration are indicated. Injections of substance P (P) (30 U./kg at 1204 and 12 U./kg at 1336.5) and LSD (5 μg/kg) were given via the femoral vein. The Roman Numerals in the final trace indicate the designation of the dorsal root potential sequence. The amplification factor in this experiment was so high that DR V did not appear on the oscilloscope screen. (This figure is reproduced with permission of the Editors of The British Journal of Pharmacology and Chemotherapy).

SP alone was found to have no action on the dorsal root potentials when doses as high as 30 U./kg were injected. On the other hand, under the conditions of submaximal stimulation at a frequency of

2.5 cps, SP was found to have a pronounced action in the presence of LSD, such that when SP, 12 U./kg, was given after 5 μg/kg of LSD, it produced enhancement of DR IV. This response to SP was observed if the previous administration of LSD had not produced its own action, or if, after enhancement was obtained, the stimulus intensity was reduced so as to render DR IV approximately equal to its control value relative to maximal. The enhancement of DR IV under these conditions could not be distinguished from the actions of effective doses of LSD (see Fig. 2). Occasionally DR I, II, III and V were found to increase after DR IV had increased in response to LSD or to LSD followed by SP.

BrLSD in doses up to 100 μg/kg had no action on dorsal root potentials; LSD after BrLSD was found to have no action on dorsal root potentials in doses up to 100 μg/kg.

B. Other Polypeptides. — Because of the observations on interaction between LSD and SP, it became important to know if other polypeptides might modify the dorsal root potentials. Bradykinin was injected and found to have no action. Since Rocha e Silva, Corrado and Ramos (1960) reported that chlorpromazine potentiates the actions of bradykinin and Krivoy and Kroeger (in preparation) reported that chlorpromazine inhibits DR V, it was of interest to determine if bradykinin had any action on the dorsal root potentials in the presence of chlorpromazine. The results of these experiments are indicated in Fig. 3. It was found that bradykinin alone, or in the presence of ineffective amounts of chlorpromazine, had no action on the dorsal root potentials. However, if an effective dose of chlorpromazine was injected, i. e., one which produced depression of DR V, then the subsequent injection of bradykinin was followed by further depression of DR V. In a series of experiments it was found that chlorpromazine induced depression of DR V lasted more than thirty minutes, and the bradykinin-chlorpromazine induced depression lasted more than fifty minutes.

It had been shown previously (Krivoy and Guillemin, 1961) that β-MSH stimulates spinal reflexes. This prompted an attempt to determine if β-MSH had any influence on the depression produced by chlorpromazine or by the combination of chlorpromazine and bradykinin. As can be seen in Fig. 3, the injection of β-MSH was followed by a return of DR V toward the control level. It was particularly interesting that the time course of this recovery followed the time course of spinal stimulation produced by β-MSH (Krivoy and Guillemin, 1961), i. e., the onset of recovery appeared approximately four minutes after injection of the drug, and the maximal action appeared 15—20 minutes thereafter. It was also notable that the dose of β-MSH required to produce this action was approximately 200 μg/kg, whereas in the untreated cat spinal stimulation was seen when doses as low as 2 μg/kg were used.

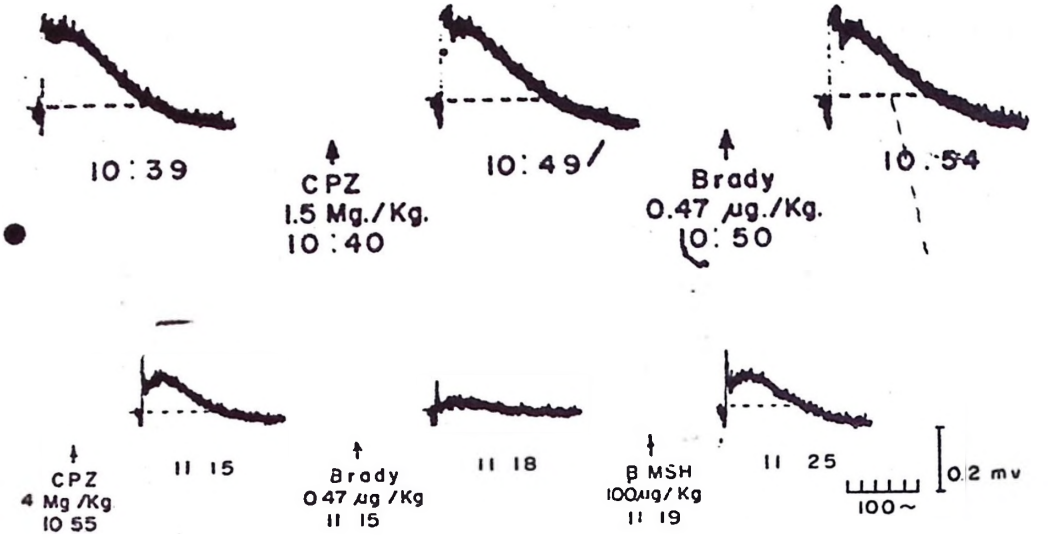


FIG. 3

Dorsal root potentials taken from a single experiment. The time appears below each trace. Injections of Chlorpromazine (CPZ) (1.5 mg/kg at 10:40 and 4 mg/kg at 10:55), bradykinin (Brady) (0,47 µg/kg at 10:50 and again at 11:15), and β-MSH (100 µg/kg at 11:19).

Bradykinin given after LSD had no action on DR IV or DR V. SP, given after chlorpromazine had no action on DR IV or DR V.

There were no observable differences in the responses of decerebrate cats compared with decerebrate-spinal cats. Furthermore, there was no consistant change in blood pressure or respiration which would account for any of the phenomena observed.

Discussion

In view of Gulbring's discovery (1943) of the enzymatic destruction of SP, it is not too surprising that the amounts of SP used here had no action of their own. These data confirm those of Kissel and Domino (1959) that SP has no action on spinal reflexes. Unfortunately, insufficient quantities of SP were available to attempt confirmation of the observation that large amounts of SP inhibit spinal reflexes (Stern and Dobrić, 1957). It is possible that in the spinal cord SP has a diphasic action, stimulating in small concentrations and depressing in larger ones. A diphasic action of SP on ganglia has been observed by Beleslin, Radmanović and Varagić (1960).

The observation that concomitant use of sub-effective concentrations of SP and of LSD produce enhancement of DR IV is predictable, if one accepts Lembeck's conclusion that SP is a neurohumor and at the same time applies »in vivo« the »in vitro« observation that LSD preserves SP from enzymatic destruction (Krivoy, 1957). The fact that large amounts of LSD produce the same phenomena as smaller doses

of LSD plus SP would indicate a system which is analogous to the phenomena observed with the anticholinesterases and ACh.

The finding that LSD was not observed to have an action at low frequencies is in keeping with the concept that it is an enzyme inhibitor. It is known that SP is rapidly destroyed. If LSD acts by preventing the destruction of a neurohumor, then its action would appear most dramatically at a time when the rapid destruction of the neurohumor is most critical, i. e., during a period of rapid stimulation.

Morphine antagonism by large doses of SP (Zetler, 1956) can be explained by physiological antagonism of morphine and SP. We have seen here that SP enhances the flow of sensory impulses (augmentation of DR IV). Krivoy and Huggins (in preparation), have shown that morphine inhibits sensory impulses at a later adjacent site (inhibition of DR V).

BrLSD had no action on the dorsal root potentials. This is in keeping with the finding that BrLSD does not modify the rate of destruction of SP (Krivoy, 1957). On the other hand, the observation that BrLSD inhibits the actions of LSD and of combinations of LSD and SP is of interest because of the report that BrLSD antagonizes the hallucinogenic properties of LSD (Ginzel and Mayer—Gross, 1956).

Neurogenic specificity among the polypeptides as a specific property is borne out by the differential actions of these substances. Under appropriate conditions SP enhances DR IV, bradykinin depresses DR V and β-MSH enhances DR V whereas no neurogenic activity could be demonstrated for α-MSH, vasopressin, oxytocin or ACTH (Krivoy and Guillemin, 1961). Furthermore, since the phenomena observed could be obtained in both the decerebrate cat and in the decerebrate-spinal cat, it would appear that SP, bradykinin and β-MSH act directly on the spinal cord, rather than on some more centrally located nervous tissue. The concept that these drugs act directly on the spinal cord is further substantiated by the fact that there was no correlation between the changes in dorsal root potentials and alterations in respiration or in blood pressure.

The data presented in this paper would tend to support Lembeck's concept that SP is a neurohumor in the primary afferent pathway of the spinal cord. They would also tend to support the concept that if the neurogenically active polypeptides are neurohumors, they are most likely modulators rather than detonators.

Summary

LSD, 10 μg/kg, was found to enhance DR IV of the dorsal root potential sequence of the cat spinal cord. SP alone had no action. Sub-effective amounts of LSD followed by sub-effective amounts of SP resulted in enhancement of DR IV. BrLSD had no observable action on the dorsal root potentials, but antagonized the actions of subsequently administered LSD and combinations of LSD and SP.

Bradykinin was found to have no action on the dorsal root potentials. Chlorpromazine, in adequate concentrations, depresses the dorsal root potentials. Combinations of chlorpromazine and bradykinin produce a depression of the dorsal root potentials which is greater than the action of chlorpromazine alone.

β-MSH is capable of antagonizing the actions of chlorpromazine and of combinations of chlorpromazine and bradykinin.

USPOREDBA DJELOVANJA SP I DRUGIH PRIRODNIH POLIPEPTIDA NA KIČMENU MOŽDINU

LSD u dozi od 10 μg/kg pojačava DR IV-sekvencije potencijala dorzalnog korijena kičmene moždine mačke. SP, sama, bila je nedjelotvorna. Subefektivne doze LSD i, poslije njih, subefektivne doze SP pojačavaju DR IV. Brom-LSD nije imala vidljivog efekta na potencijale dorzalnih korjenova, ali je djelovala antagonistički u odnosu na LSD i kombinaciju LSD—SP, ako su ove aplikovane poslije brom-LSD. Bradikinin ne djeluje na potencijale dorzalnih korjenova. Klorpromazin, u pogodnim dozama, djeluje depresivno na potencijale dorzalnih korjenova. Kombinacija klorpromazina i bradikinina također djeluje depresivno na potencijale dorzalnih korjenova, ali u jačoj mjeri nego sam klorpromazin. Hormon koji stimuliše β-melanocite djeluje antagonistički u odnosu na klorpromazin i bradikinin.

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DISCUSSION

GADDUM: Have you tried giving SP by close arterial injection?

KRIVOY: No.

HUKOVIC: We have seen tachyphylaxis to SP after LSD. What explanation do you think could there be for this?

KRIVOY: The tachyphylaxis to SP can be explained on the basis of professor Gaddum's findings.