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POTENTIATION OF SUBSTANCE P BY LYSERGIC ACID DIETHYLAMIDE IN VIVO

BY

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In doses of 10 μg/kg or more, lysergic acid diethylamide enhanced the fourth potential (DR IV) of the dorsal root potential complex in the cat. Smaller doses of lysergic acid diethylamide did not in themselves alter the DR IV, but revealed an enhancement of the potential by substance P, which by itself had no effect. 2-Bromolysergic acid diethylamide had no action on the dorsal root potentials, but prevented the actions of lysergic acid diethylamide.

The fact that posterior spinal roots contain large amounts of substance P and contain little or no acetylcholine led Lembeck (1953) to suggest that substance P is the chemical transmitter liberated by the first sensory neurone. If this were true, the administration of substance P might be expected to modify spinal reflexes. However, substance P is destroyed by an enzyme system which is found in various parts of the body (Gulbring, 1943), including spinal cord (Lembeck, 1953). The presence of this enzyme precludes examination of Lembeck's suggestion by simply determining the effect of injected substance P whilst examining reflex activity, unless extremely large amounts of substance P are used. Krivoy (1957) confirmed Gulbring's observations and also found that lysergic acid diethylamide was a specific antagonist to the enzyme responsible for destroying substance P. The purpose of the present experiments was to determine if, by using lysergic acid diethylamide as an enzyme inhibitor *in vivo*, it might be possible to unmask the actions of small amounts of substance P on transmission of impulses from the posterior spinal root to secondary and internuncial neurones of the spinal cord.

METHODS

The technique of Lloyd & McIntyre (1949) was used to evaluate the actions of substance P on transmission of nerve impulses along the intramedullary pathway of the primary afferent fibre to secondary neurones. These authors recorded and analysed five dorsal root potentials (DR I, II, III, IV, V) from a dorsal spinal rootlet adjacent to another stimulated rootlet (Fig. 1). DR I II, and III are due to intramedullary conduction of the nerve impulse along the primary afferent nerve. DR IV is mainly due to a residual negativity associated with supernormality in the nerve endings of the primary afferent fibre, as well as in the secondary neurone (see also Rudin & Eisenman, 1953).

Twenty-three decerebrate cats were used in this study: in addition to decerebration, four of these cats had spinal transections at L1. All operative procedures were performed under

ether anaesthesia. The last lumbar or first sacral spinal rootlets were used in all experiments. The entire area of exposed spinal cord and rootlets was covered with liquid paraffin contained in a trough constructed of the incised skin. The liquid paraffin was previously equilibrated with carbon dioxide and maintained at 37° C by radiant heat.

Stimuli used were biphasic (square waves of 50 μsec duration coupled through a 2 μF condenser) at a frequency of 0.5 or 2.5 cycles/sec. Intensity was adjusted so as to be either maximal or about 50% of maximal for DR IV. Stimulation, once started, was maintained without interruption for the duration of the experiment. Conventional electrophysiological techniques were used for amplification and display of the evoked potentials.

The drugs used in this study, lysergic acid diethylamide, 2-bromolysergic acid diethylamide and substance P, were injected via a polythene cannula in the femoral vein. No drug was given until at least 1 hr after the termination of ether anaesthesia, and then only after the size of the dorsal root potentials had remained constant for at least 30 min.

RESULTS

Stimulation at 2.5 cycles/sec. With submaximal stimuli, lysergic acid diethylamide, 5 μg/kg, enhanced DR IV in approximately half of the cats studied; 10 μg/kg produced enhancement in every cat. The augmentation of DR IV appeared within 1 min of the time of injection and reached a maximum approximately 5 min thereafter; if at this time the stimulus intensity was reduced so that DR IV was again submaximal, DR IV remained at this level, indicating that the action of lysergic acid diethylamide had in fact reached a plateau, and there had been no background of continued enhancement. The administration of large amounts of lysergic acid diethylamide did not alter the qualitative nature of the response, and, even with the administration of 120 μg/kg, only enhancement was seen.

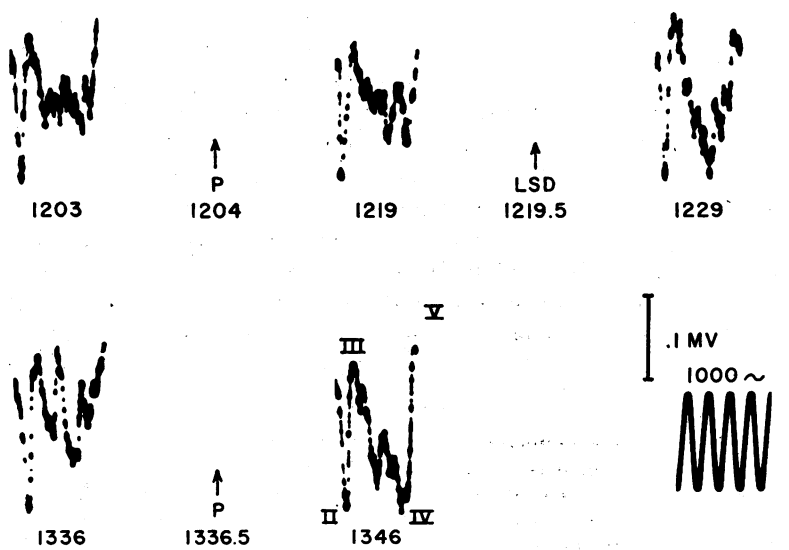

Fig. 1. Decerebrate cat. Dorsal root potentials evoked by biphasic stimuli (square waves of 50 μsec duration coupled through a 2 μF condenser). Injections of substance P (P) (30 u./kg at 1204 and 12 u./kg at 1336.5) and lysergic acid diethylamide (5 μg/kg) (LSD) were given via the femoral vein. The time is indicated under each tracing. The Roman numerals in the final trace indicate the designation of the potential sequence.

Substance P alone had no action on the dorsal root potentials when as much as 30 u./kg was given. On the other hand, when substance P, 12 u./kg, was given after 5 μg/kg of lysergic acid diethylamide, it produced enhancement of DR IV. This response to substance P was observed if the previous administration of lysergic acid diethylamide had not produced its own action, or if, after obtaining enhancement, 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 enhancement of DR IV produced by effective doses of lysergic acid diethylamide (Fig. 1).

Occasionally DR I, II, III and V were found to increase after DR IV had increased in response to lysergic acid diethylamide or to lysergic acid diethylamide followed by substance P. Bromolysergic acid diethylamide in doses up to 100 μg/kg had no action on dorsal root potentials ; lysergic acid diethylamide after bromolysergic acid diethylamide was found to have no action on dorsal root potentials in doses up to 100 μg/kg. There was no observable difference in response between decerebrate cats and decerebrate-spinal cats. With maximal stimuli, lysergic acid diethylamide in extremely large doses (70 μg/kg) produced enhancement of DR IV.

Submaximal or maximal stimuli at 0.5 cycles/sec. Lysergic acid diethylamide, substance P and combinations of these two drugs were found to have no clear-cut action on the preparation.

DISCUSSION

As the present results were obtained both in the decerebrate cat and in the decerebrate-spinal cat, the phenomena described must be mediated by an action directly on the spinal cord, rather than being secondary to events occurring more centrally. Lysergic acid diethylamide had no action at low frequencies of stimulation. This could be explained if lysergic acid diethylamide acts on the spinal cord by preventing the enzymatic degradation of a neurohormone, for its action should appear most clearly when that neurohormone is being produced in large amounts and rapid destruction is most critical, that is, during a period of rapid stimulation when the opportunity for temporal summation is greatest. In view of the enzymatic destruction of substance P, it is not surprising that the amounts of substance P used here had no action of their own (Kissel & Domino, 1959). The fact that large amounts of lysergic acid diethylamide produce the same phenomena as smaller doses of lysergic acid diethylamide and substance P would indicate a mechanism analogous to the phenomena one sees with the anticholinesterases and acetylcholine. One wonders if the potentiation bears any relation to the potentiation of ganglionic transmission by substance P reported by Beleslin, Radmanović & Varagić (1960).

The observation that substance P depresses spinal reflexes (Stern & Dobrič, 1957) may represent the second phase of a biphasic action of substance P on spinal transmission. Thus, substance P, like acetylcholine, may stimulate in small concentrations and depress in larger ones. Morphine antagonism by substance P (Zettler, 1956) can also be explained. Whereas substance P augments DR IV, morphine inhibits DR V (Krivoy & Huggins, unpublished), so that the polypeptide enhances the flow of sensory impulses which are antagonized at a later adjacent site by morphine.

The observation that 2-bromolysergic acid diethylamide does not change the dorsal root potentials but inhibits the action of lysergic acid diethylamide is additional evidence that bromolysergic acid diethylamide does not protect substance P from enzymatic destruction, but antagonizes the actions of lysergic acid diethylamide (Krivoy, 1957). Ginzel & Mayer-Gross (1956) also found that, in man, pretreatment with 2-bromolysergic acid diethylamide renders lysergic acid diethylamide ineffective.

Potentiation of DR I, II, III, in the cases where it was observed, was probably secondary to potentiation of DR IV (Grunfest & Magnes, 1951).

The data presented in this paper not only support the concept that substance P is a neurohormone, but indicate that it possesses a role in sensory transmission.

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