The effects of D-lysergic acid diethylamide tartrate (LSD-25) on the cholinesterases and monoamine oxidase in the spinal cord: a possible factor in the mechanism of hallucination

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Clinical investigators have reported that d-lysergic acid diethylamide tartrate (LSD-25), a psychotomimetic drug, produces various psychic symptoms such as different types of hallucinations, disturbances of thought, speech, and behaviour and irritability, as well as autonomic disturbances, namely, rise of body temperature, increased lacrimation, hypotension, slowing of pulse, pilomotor activity, dilatation of the pupils, and hyperpnoea. Numerous biochemical studies have been made on the drug regarding its site and mode of action and degree of inhibition of different enzymes in various parts of the central nervous system (Foldes, Zsigmond, Erdos, and Foldes, 1959; Zsigmond, Foldes, Foldes, and Erdos, 1959; Thompson, Tickner, and Webster, 1954; Rinz and Ogara, 1958). The review of the literature shows a wide variation in the technique employed and the results obtained, especially in regard to the effects of the drug on non-specific cholinesterase (Poloni and Maffezzoni, 1952; Thompson *et al.*, 1954; Foldes *et al.*, 1959; Goldberger, 1961).

Although the inhibitory effects of LSD-25 on the hydrolysis of acetylcholine in the brain have been studied previously by a number of workers, its effect on pseudocholinesterase and monoamine oxidase activity has not been recorded in any detail. While the effects of this drug on both cholinesterase and adrenaline metabolism have been studied biochemically, only limited histochemical studies have been made. Some workers (Shanthaveerappa, Nandy, and Bourne, 1963) have studied the histochemical effects of this drug in the cerebral cortex and basal nuclei. Studies of this type have not, however, been undertaken yet on the spinal cord and the spinal ganglia. The present study is a continuation of the work, using histochemical methods, on the action of hallucinogens such as LSD-25 on the latter structures.

MATERIAL AND METHODS

The present work deals with the sites of localization of specific cholinesterase, non-specific cholinesterase, and monoamine oxidase in the spinal cord and spinal ganglia in normal rats and following administration of LSD-25.

Young adult albino rats were used and were killed by decapitation or by carotid or jugular bleeding: pieces of spinal cord and spinal ganglia were removed by careful dissection. Fresh frozen sections, 15 μ thick, were cut in a Harris cryostat and mounted on pre-cleaned coverslips. The study was carried out in three series of experiments, with careful controls to standardize the results and to overcome the defects of any one particular technique.

1 The drug (LSD-25) was injected by intracardiac, intravenous, or intraperitoneal routes in the dose of 0.1 mg./kg. of body weight and the animals were killed within one hour, the time of sacrifice being determined by the changes in behaviour of the animals. The spinal cord and spinal ganglia of an un-injected rat of the same litter were studied as controls.

2 Sections of the spinal cord and spinal ganglia of normal rats were incubated with the respective substrate as well as the drug in the dose of 0.1 mg./10 ml. of the final incubating mixture, at 37°C., the sections incubated in a similar solution without the drug acting as controls.

3 While some sections were pre-treated with the drug in normal saline for one hour and finally incubated in the respective incubating mixtures others were pre-treated with normal saline without the drug, for the same period before incubation. The latter acted as controls in this case.

The localization of specific and non-specific cholinesterases was studied by Gerebtzoff's modification of Koelle's method (1959) and by the method of Coupland and Holmes (1957); that of Glenner, Burtner, and Brown (1957) was used for monoamine oxidase. The time of incubation for this study was two hours for specific cholinesterase, three hours for monoamine oxidase, and four hours for non-specific cholinesterase; longer periods caused diffusion of the enzymes in all cases and formation of needle crystals in the case of cholinesterases. In all cases the drug-treated tissues and the controls

were treated in an identical fashion, *e.g.*, period of incubation, thickness of the sections, and preparation of the incubation medium.

RESULTS

The present histochemical study on the effects of LSD-25 on the normal reactions for specific and non-specific cholinesterases and monoamine oxidase in the neurones of the spinal cord and spinal ganglia was carried out in the three series of experiments.

The most consistent and uniform results were obtained in our third series of experiments and it appears to be due to sufficient inactivation of the enzymes by the drug before the tissues were incubated in the appropriate substrate mixture.

The first series of experiments produced a less marked, though constant inhibition and this may be attributed to a smaller concentration of the drug in the blood as well as continuous and simultaneous reformation of the enzymes. The results of the second series were less uniform and the degree of inhibition was less pronounced. This may be due to simultaneous action of the drug and the substrate upon the enzyme systems and possibly the substrates acting faster than the drug during the period of incubation.

By the methods employed, a great variation in the reaction of specific cholinesterase was observed in the neurones of the spinal cord. The reaction was strong and finely granular and was located almost entirely on the cell membrane and cell processes of the motor neurones of the ventral horn. The reaction was exhibited as small clumps all round the cell border with thin strips of reaction in the intervening portions. The clumps of positive granules were more intense than the rest of the cell surface and appeared to be located in the synaptic areas on the surface of the cell (Figs. 1 and 2). The cells of the lateral horn (mostly medium-sized and multipolar) showed a moderately strong reaction which was not only located on the surface and synaptic regions of the cells but also extended to the peripheral part of the cytoplasm (Fig. 3). It is interesting to note that these neurones, from which originate the whole sympathetic system of the body, appeared from their reactions to be strongly cholinergic. The neurones of the dorsal horn also showed a strong reaction, coarsely granular in nature, located on the cell surface and synaptic regions (Figs. 4 and 5). The processes of the neurones, including their synapses in the grey matter also gave a positive reaction with a fair intensity (Figs. 1, 2, 4, and 5).

The reaction of non-specific cholinesterase, as studied by the methods employed, was limited entirely to the blood vessels of the spinal cord, the neural elements being completely negative (Fig. 8).

The spinal cord in general gave a poor reaction for monoamine oxidase. While neurones of the ventral and lateral horn, and the majority of the cells of the dorsal horn, including their processes, were completely negative (Fig. 10), a few neurones of the dorsal horn showed a faint homogeneous rim of peripheral reaction in their cytoplasm (Fig. 11). Although the cell processes were negative throughout, the myelin sheaths more or less constantly gave a moderately positive reaction both in grey as well as in the white matter (Figs. 10 and 11). It should be pointed out here that the neurones of the lateral horn, from which originate the whole sympathetic nervous system of the body, gave no sign of activity for monoamine oxidase.

A great variation was observed in the intensity and distribution of specific cholinesterase in the neurones of the spinal ganglia. In general, the small neurones exhibited a stronger and more extensive reaction than the larger ones. While most of the smaller neurones showed an intense reaction on their surfaces and in the cytoplasm, the larger neurones showed mostly a concentration of reaction towards the cell surface (Figs. 12 and 13).

The neurones of the dorsal root (spinal) ganglion showed no sign of activity for non-specific cholinesterase. The blood vessels showed a positive reaction.

The reaction for monoamine oxidase appeared in the form of fine granules with variable distribution and concentrations in the cytoplasm of the neurones of the spinal ganglion. While the majority of the cells showed a uniform distribution of the reaction of moderate intensity, others displayed a strong reaction distributed in the cytoplasm, and still others exhibited a tendency to a peripheral concentration of the enzyme toward the cell membrane. The satellite cells and the myelin sheaths of the nerve fibres also showed a positive reaction (Figs. 15 and 16).

The section of the spinal cord exhibited a general and profound inhibition of reaction for specific cholinesterase when treated with LSD-25. This effect was equally observed in cell membranes, the cytoplasm of the cells and their processes. In fact, in many sections the inhibition was so marked that practically no reaction could be detected (Figs. 6 and 7).

The drug also produced a marked inhibition of the reaction for non-specific cholinesterase in the spinal cord localized only in the blood vessels (Fig. 9).

The drug (LSD-25) produced inhibition of the reaction for specific cholinesterase in the neurones of the dorsal root ganglia. This inhibition was more profound over cell membranes than in the cytoplasm which showed some persistent reaction in many cells (Fig. 14).

The drug also inhibited the reaction of non-

FIG. 1. Specific cholinesterase reaction (Gerebtzoff's modification of Koelle's method, 1959). Large neurones of the ventral horn. Note that a strong reaction is located in the synaptic region of the cell border and cell processes in the form of round clumps (arrows) and in a thin strip between. $\times 600$.

FIG. 2. Specific cholinesterase reaction (Gerebtzoff's method, 1959). Large neurone of the ventral horn. Note the strong reaction in the synaptic areas on the cell border and cell processes in the form of clumps of granules (arrows). $\times 1,500$.

FIG. 3. Specific cholinesterase reaction (Gerebtzoff's method, 1959). Large neurone of the lateral horn. Note strong reaction also in the cytoplasm in addition to the cell border and cell processes. $\times 1,500$.

FIG. 4. Specific cholinesterase reaction (Gerebtzoff's method, 1959). Neurones of nuclear dorsalis. Note strong reaction in the synaptic areas, on the cell border and cell processes in the form of clumps of granules. $\times 1,500$.

FIG. 5. Specific cholinesterase reaction (Gerebtzoff's method, 1959). Dorsal horn neurones. Note the strong reaction on the cell border and cell processes (arrows). $\times 600$.

FIG. 6. Specific cholinesterases reaction (Gerebtzoff's method, 1959). Neurones of the dorsal horn. Sections pre-treated with LSD-25 before incubation. Note complete inhibition of the reaction in the neurones (arrows). $\times 600$.

FIG. 7. Specific cholinesterase reaction (Gerebtzoff's method, 1959). Neurone of the ventral horn. Section pre-treated with LSD-25. Note the almost complete absence of reaction in the neurone (arrow). $\times 600$.

FIG. 8. Non-specific cholinesterase reaction (Gerebtzoff's method, 1959). Note strong reaction in the blood vessels (arrows) in the grey matter. $\times 370$.

FIG. 9. Non-specific cholinesterase reaction (Gerebtzoff's method, 1959). Section pre-treated with LSD-25. Note profound inhibition of the reaction in the blood vessels (arrows). $\times 370$.

FIG. 10. Monoamine oxidase reaction (Glenner et al., 1957). The region of the ventral horn. Note that the reaction is absent in the cells (arrow) and its process (arrow). The myelin sheaths of the nerve fibres give a positive reaction. $\times 1,500$.

FIG. 11. Monoamine oxidase reaction (Glenner et al., 1957). Neurone of the dorsal horn. Note the thin peripheral rim of cytoplasmic reaction in the nerve cell (arrow). The myelin sheaths of the nerve fibres also show positive reaction. $\times 1,500$.

FIG. 12. Specific cholinesterase reaction (Gerebtzoff's method, 1959). Neurones of the dorsal root ganglion. Note that the positive reaction is localized mainly in the cell border (arrow) and also in the cytoplasm. $\times 600$.

FIG. 13. Specific cholinesterase reaction (Gerebtzoff's method, 1959). Large neurone of the dorsal root ganglion. Note that the reaction is mainly localized on the cell border. $\times 1,500$.

FIG. 14. Specific cholinesterase reaction (Gerebtzoff's method, 1959). Neurones of the dorsal root ganglion. Section treated with LSD-25 before incubation. Note the profound inhibition of the reaction in cells (arrows) by the drug. $\times 370$.

FIGS. 15 AND 16. Monoamine oxidase reaction (Glenner et al., 1957). Neurones of the dorsal root ganglion. Note the strong reaction in the cytoplasm of the nerve cells, myelin sheath of nerve fibres, and the satellite cells (arrows). $\times 600$.

FIGS. 17 AND 18. Monoamine oxidase reaction (Glenner et al., 1957). Neurones of the dorsal root ganglion. Section treated with LSD-25 before incubation. Note the profound inhibition of the enzymatic reaction in the nerve cells and satellite cells as well as in the myelin sheaths of the nerve fibres (arrows). $\times 600$.

specific cholinesterase in the spinal ganglia which was located in the blood vessels only.

The reaction of monoamine oxidase in the neurones of the spinal ganglia was also profoundly inhibited by the drug, although some reaction was still present in a few of the cells (Figs. 17 and 18).

DISCUSSION

Various workers have described different types of localization of specific cholinesterase in the neurones of the central nervous system (Ravin, Zacks, and Saligman, 1953; Gerebtzoff, 1956). While Gerebtzoff (1956) found this enzyme to be exclusively located on the neuronic and dendritic membranes, Koelle (1954) and Giacobini (1959) were of the opinion that the enzyme could be demonstrated in the perikaryon of the neurones as well as in the entire length of the axons. In the present study on spinal cord neurones the specific cholinesterase was found to be localized on the cell membrane and synaptic regions in the neurones of the ventral and dorsal horns (Figs. 1, 2, 4, and 5) and in the peripheral parts of the cytoplasm of the cells of the lateral horn (Fig. 3). The neurones of the spinal ganglia, however, showed a localization of the enzyme both on the cell membrane including the cell cytoplasm with variable intensity (Figs. 12 and 13). De Robertis, Pellegrino de Iraldi, Rodriguez de Lores Arnaiz, and Salganicoff (1961) and Shanthaveerappa *et al.* (1963) described two types of neurones in the central nervous system—cholinergic and non-cholinergic or adrenergic. Our present observations on the almost complete absence of the reaction for monoamine oxidase and strong activity of specific cholinesterase would tend to indicate that the neurones of the spinal cord and their synapses were cholinergic in nature.

It has been shown that in the central nervous system the areas which are rich in specific cholinesterase show a poor activity for monoamine oxidase (Koelle, 1954; Ishii, 1957), although Shimizu and Morikawa, (1959) also demonstrated some regions in the brain showing strong activity for both these enzymes. The present study shows strong activity for specific cholinesterase and the complete absence of the reaction for monoamine oxidase in the neurones of the spinal cord (Fig. 10) indicating that the activities of these two enzymes are inversely related to each other. This is also well shown by the cells of the spinal ganglia.

In a series of biochemical studies, Thompson *et al.* (1954) observed that the degree of inhibition by LSD-25 of specific cholinesterase was only 10% whereas that of non-specific cholinesterase was 50% in human plasma. In the present study, an equal degree of inhibition was noted for both the choline-

sterases, at least as far as could be subjectively assessed from our histochemical preparations. These preparations, however, are unlikely to distinguish even considerable variation in activity.

Eccles (1957) and Bremer (1957), the former opponents of cholinergic transmission of nerve centres, have admitted that acetylcholine is a central synaptic transmitter. It is well known that acetylcholine, which facilitates the synaptic transmission of impulses, is hydrolysed by cholinesterase and thereby controls the transmission of excessive impulses. Our studies suggest that the inhibitory effect of LSD-25 on specific cholinesterase is actually due to the interference with the hydrolysis of acetylcholine by the enzyme. Foldes *et al.* (1959) studied the inhibitory effect of the drug on the hydrolysis of acetylcholine in different parts of the brain in man and rabbit and they were of the opinion that LSD-25 can completely inhibit the hydrolysis of acetylcholine in a concentration of 3×10^{-5} M in most areas of human and rabbit brain. It has also been observed that LSD-25 caused a rise in the acetylcholine level of the brain (Poloni and Mafezzoni, 1952).

The present study indicates that the non-specific cholinesterase is located in the blood vessels only (Fig. 8) and similar observations were also made in the brain by Greig and Holland (1949), Paul, Marsol, and Coq (1957), Koelle, (1954), and Shanthaveerappa *et al.* (1963). While some authors (Greig and Holland, 1949) postulated that the enzyme might be concerned in the maintenance of the blood-brain barrier, others suggested that it acted as a neurohumoral scavenger. It has also been suggested that acetylcholine, which escapes splitting by specific cholinesterase at the synapses, on reaching the blood vessels is hydrolysed by non-specific cholinesterase. The profound inhibition of this enzyme caused by LSD might act as a contributory factor by depressing the barrier mechanism resulting in the rise of the acetylcholine level in the blood.

It has been observed that monoamine oxidase is located on the surface and in the cytoplasm of the neurones of the spinal ganglia and that the drug produces a profound inhibition of the reaction for this enzyme (Figs. 15, 16, 17, and 18).

As suggested by Costa and Zetler (1959), LSD, even in high doses (100 mg./kg.), potentiated the effects of adrenaline; according to Hoffer, Smith, Chivelos, Callbeck, and Mahon (1959) plasma adrenochrome levels and conversion of adrenaline to adrenochrome were increased by the drug. The latter group of workers suggested that the rise of adrenochrome in the blood might be due to the lowered ability of the blood to destroy adrenochrome.

Nandy and Bourne (1963), following administration of LSD to rats, observed a profound inhibition of monoamine oxidase in the cells of liver which plays an important part in the destruction of noradrenaline in the body, and suggested that the rise of the blood adrenochrome level might be due to the depression of the detoxication mechanism in the hepatic cells. Other workers are of the opinion that the psychosis produced by LSD-25 might be due to error in the adrenaline system (Rinkel, 1959) or due to disturbances of adrenergic or related cerebral neurohumoral mechanism (Merrazzi and Hart, 1955).

Hoffer *et al.* (1959) described at least five ways by which the body adrenaline may be detoxified: 1 excretion unchanged in the urine; 2 storage of active adrenaline in the cells; 3 by deamination of the side chain to form oxidizable aldehydes; 4 by esterification of the phenolic hydroxyls; 5 by quinone formation to adrenochrome and its derivatives.

The deamination is catalysed by the enzyme amine oxidase; esterification is catalysed by the enzyme sulphoesterase; and quinone formation is catalysed by the enzyme phenolase. Of these three main detoxicating mechanisms only phenolase forms adrenochrome. Therefore, if any drug blocks amine oxidase and sulphoesterase, most of the adrenaline in the body may be converted into adrenochrome. As shown by Axelrod and Laroche (1959), adrenaline injected intravenously rapidly disappears from the blood and is fixed in the tissues. It is inactivated chiefly through the action of the enzyme *o*-methyl

transferase to metanephrine which is oxidized by amine oxidase to vanillo-mandelic acid. The drug LSD-25 contains a grouping which can inhibit amine oxidase and it is possible it may raise the adrenochrome and adrenaline level of the blood in this way.

There is evidence to suggest that adrenochrome in contrast to adrenaline can cross the blood-brain barrier. It has also been observed that adrenochrome can rapidly cause epileptiform convulsion in man. It may be interesting to note that a substance which can occur in the human body has been found to be active in this way. It has also been demonstrated (Green and Richter, 1937) that adrenaline induces a vigorous oxygen uptake when added in low concentration to the reconstructed lactic and malic dehydrogenase systems of heart muscle and this effect has been found to be due to the formation of a red coloured oxidation product, adrenochrome, which can act as a respiration carrier.

It is well known that the neurones of the spinal ganglion represent the first relay station for various afferent impulses carrying exteroceptive (pain, temperature, and touch), proprioceptive (pressure, muscle, tendon and joint sensitivity), and interoceptive (visceral sensibility) sensations to the spinal cord. It may therefore be suggested that LSD by depressing the enzyme mechanisms controlling the entry of excess of impulses to the central nervous system may allow a greater stream of impulses to the central nervous system. The neurones of the dorsal

MECHANISM OF ACTION OF LSD-25 (Schematic representation)

horn are the second relay station in the pathway of the afferent impulses carrying pain, temperature, and crude touch sensations and an inhibition of the enzyme mechanism might facilitate the reaching of this excessive stream of impulses to the higher parts of the central nervous system. In this connexion it may be mentioned that Shanthaveerappa *et al.* (1963) observed in this laboratory that the drug causes a profound inhibition of cholinesterases and monoamine oxidase in the cerebral cortex and basal nuclei, including the thalamus, and suggested that the drug, by inhibiting the enzyme mechanisms, may set up a release phenomenon for a greater stream of impulses to the cerebral cortex from the subcortical centres to disturb the finer balance of cortical function, producing various psychic symptoms. Gaddum and Vogt (1956) observed that LSD-25 antagonized the stimulating action of serotonin in the central nervous system and suggested that the hallucinogenic action of drugs might also be due to its blocking the action of serotonin in the brain.

The profound inhibition of specific cholinesterase, monoamine oxidase, and also serotonin may also set up disturbances at different levels of the autonomic nervous system such as rise of body temperature, dilatation of pupils, pilomotor activity hypernoea, fall of blood pressure, and bradycardia.

It thus appears probable that LSD may act by depressing the enzyme mechanisms for the control and regulation of various impulses at different levels of the nervous system and thereby create a picture of overall imbalance of the various nervous mechanisms. It is also possible that disturbance in the nervous system itself may also be contributed to by other factors such as rise of acetylcholine and adrenaline and adrenochrome levels in the blood, which may be due to either depression of the mechanism of the blood-brain barrier or the disturbance of the adrenaline detoxification mechanism.

SUMMARY

In the present study the normal localization of specific and non-specific cholinesterases was studied by Gerebtzoff's modification of Koelle's method (1959) and Coupland and Holmes' method (1957) and monoamine oxidase by the technique of Glenner *et al.* (1957) in the spinal cord and dorsal root ganglia of the rat.

The neurones of the spinal cord gave a strong reaction for specific cholinesterase and almost none for monoamine oxidase while those of the spinal ganglion gave strong reactions for both. The reaction for non-specific cholinesterase was localized in the blood vessels only.

The effects of LSD-25 have been studied in three

series of experiments and a profound inhibition of all the enzymes was observed. It has been suggested that the drug may act by inhibiting the enzyme mechanism which controls the passage of excess of impulses across the synapses and also by raising the levels of acetylcholine, adrenaline, and adrenochrome in the blood.

It has also been suggested that the inhibition of specific cholinesterase and monoamine oxidase in the neurones of the spinal ganglion and dorsal horn may result in the entry of a greater stream of impulses to the central nervous system, which may finally reach the cerebral cortex to disturb the finer balance of cortical functions producing various psychic symptoms. The inhibition of the enzymes at different levels of the nervous system may disturb the activity of autonomic system producing various symptoms of autonomic disturbances.

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