Acta anat. 46: 185–191 (1961)

Department of Anatomy, Emory University, Atlanta, Georgia

THE EFFECTS OF LYSERGIC ACID DIETHYLAMIDE (LSD-25) UPON THE HISTOCHEMICAL REACTIONS FOR CHOLINESTERASE IN THE CENTRAL NERVOUS SYSTEM

By MICHAEL GOLDBERGER

(With Plate I)

Introduction

Lysergic acid diethylamide (LSD-25) is known to be a psycho-mimetic drug. When injected into the human subject it produces a temporary psychotic state characterized by hallucinations, dissociation, disturbance of thought and perception and depersonalization as well as characteristic autonomic changes (e.g. increased heart rate and body temperature); (*Abramson* [1956]). The action of this drug is as yet undetermined although many investigators are attempting to determine the specific site of action using *in vitro* techniques. It was noted (*Mayer-Gross* [1953]) that treatment with LSD was followed by a fall in blood sugar and the effect of LSD on various enzymes concerned with carbohydrate metabolism has been studied. It was also found (*Poloni and Mafezzoni* [1952]), that LSD caused a rise in the acetyl choline level in the brain. It was thought that some of the psychic effects of LSD might be explained by an interference with the cholinesterase enzymes by the drug. Pseudocholinesterase was found to be inhibited 50 % and acetyl cholinesterase 10 % by LSD (*Thompson* et al. [1955]).

If LSD inhibits cholinesterase in the brain, thus causing some of the above mentioned symptoms, it is important to know not only the degree of inhibition by the drug, but since the symptoms are specific, the specificity of the inhibition as well. Does LSD inhibit cholinesterase in all areas of the brain, in all parts of the nerve cells, or does it cause inhibition at specific sites?

The present studies attempt to examine these questions by performing histochemical tests on cholinesterase in the brain in which the sites of enzyme activity as well as enzyme inhibition can be observed and in which LSD was added to some substrate solutions and not to others.

Method

Rat brains were dissected out of freshly killed rats and cut transversely into 5 mm blocks which were fixed in neutral formol saline from 4 to 5 hours. After fixation the blocks were washed in distilled water and cut at 25 μ on a freezing microtome. Then whole brain sections were cut into distilled water in order to wash off any remaining formalin which is known to inhibit cholinesterases. The frontal sections were cut from different areas of the brain i.e. from frontal to occipital ten consecutive sections were cut and saved and the next ten were discarded so that a sample of different areas of the brain was used. Thus all cortical plus underlying brain stem areas were sampled. Only the cerebellum and spinal cord were not examined. About 50 sections from each of 3 brains were stained and studied. The incubation mixture was prepared according to Gerebtzoff's modification of Koelle's technique [1953] for the histochemical demonstration of cholinesterases.

The floating sections were incubated in 40 ml of incubation mixture at 37° C; 4 hours for acetyl choline, 16 hours for butyryl choline. For experimental sections 1 mg of lysergic acid diethylamide (LSD) was added to the incubation mixture (8×10^{-5}M). The sections were then washed in distilled water and visualized in a dilute solution of ammonium sulfide for 5 minutes; washed again and placed in a 10 % solution of formol saline. A dilute solution of toluidine blue was used for counterstaining. The sections were mounted on albuminized slides, dehydrated in alcohols (2 changes of absolute) taken through two toluenes and mounted in Canada balsam. Equivalent sections were examined with a light microscope to compare controls with those treated with LSD. Since serial sections were cut it was possible to compare neighbouring sections; control with experimental. There were thus two sets of sections for each brain studied. The intensity of the cholinesterase reaction was estimated by the investigator, comparing different areas in a section or different sections.

Results

The non-specific cholinesterase reaction was limited to non-nervous tissue. The endothelium of the capillaries and particularly the nuclei were positive (fig. 1) as were many of the neuroglial cells. An occasional neuron showed a reaction in the cytoplasm when butyryl choline was used as the substrate. The smooth muscle of arterioles was also positive.

Different areas of the brain showed varied degrees of intensity in the cholinesterase reaction. The specificity of the various tracts and nuclei of the brain stem has been given elsewhere and our findings are in accord with those of *Koelle* [1954]. In areas of greatest cholinesterase activity, the copper sulphide precipitate (produced by the enzyme activity) was so heavy that it was difficult to distinguish the reaction in the cell body and cell prolongations from the intercellular, background reaction which is due probably to fibres cut in cross section, synapses and the fibres of glial cells. In

the neo-cortex however the different layers of cortex showed characteristic patterns of reactivity. A general account of the reaction in this area of the brain has been described by *Gerebtzoff* [1959] but more detailed information on the distribution of the reaction is given below.

Layer I — The lamina zonalis contains some glial cells, but no neurons. Shows background reaction of moderate intensity.

Layer II, III — The laminae granularis interna and pyramidalis contain many large pyramid cells. The superficial layers show very light background reaction while the deeper layers show moderate intensity between the cells which here are less densely packed.

Layer IV — The lamina granularis interna shows moderate reaction between the cells.

Layer V — The lamina ganglionaris shows large pyramids most of which give a positive reaction in the cells as well as a moderate reaction between them. The intracellular reaction is of three types:

1. Cytoplasm and prolongations are negative except for a positive area along the nuclear membrane.
2. Positive reaction for cholinesterase limited to the cytoplasm of bodies (fig. 3).
3. Cytoplasm of cell body is negative but cell prolongations (mostly directed outwards) are filled with a granular reaction positive for cholinesterase (fig. 5).

Although there are variations between types 2 and 3, the positive ring around the nucleus is not present when there are granules in the cytoplasm and/or axoplasm. The positive intracellular reaction was seen only in Layer V (and some of the large cells in Layer IV which probably belong to Layer V) of the neocortex.

Layer VI — The lamina multiformis contains some large positive neurons but the reaction in this area is light and sparse for the most part.

When LSD was added to the substrate, there was no change noted in the non-specific cholinesterase preparations (fig. 2). In the acetyl cholinesterase preparations, the changes were very specific. The hallucinogen manifests no difference in intensity in the cholinesterase reaction in the non-cortical areas of the brain. In the cortex however, in homologous regions, the intensity of reaction between the cells was markedly reduced and, even more striking was the fact that the cell bodies which were positive in the control preparations were negative in the experimental preparations of similar cortical areas (fig. 4), only a few cell prolongations showed positive reactions in the form of granules (fig. 6).

Discussion

The three types of cholinesterase reactivity observed in the slides are of some interest. It is not known where cholinesterase is manufactured although the endoplasmic reticulum has been suggested (*Koelle and Steiner* [1956]). Koelle's examination of cholinesterase in the brain [1954] does not report intracellular localization similar to that found in the present study, but he found cholinesterase activity distributed uniformly throughout the cytoplasm. Koelle's method (*Koelle and Friedenwald* [1949]) is, as previously mentioned, somewhat different from the one used in the present study. It was found that Koelle's technique allowed more diffusion to take place than that of *Gerebtzoff*, although the two techniques are basically similar. *Koelle* feels that cholinesterase activity is associated with the cellular membrane whereas our findings demonstrate activity within the cytoplasm, in the form of granules and as a ring around the nuclear membrane as well as around the outside of the cell. It is difficult to tell whether the background reaction i.e. between the cells is associated with boutons terminaux at synaptic regions or if it is also in supporting tissue between the cells. Comparing these results of LSD inhibition of cholinesterase with those of *Thompson* et al. [1955] who studied it biochemically rather than histochemically, a disparity is seen. *Thompson* found 50% inhibition of the non-specific esterase at 5×10^{-6} M of LSD and 10% inhibition of acetyl cholinesterase at 5×10^{-5} M LSD. In the present study, no inhibition of the non-specific cholinesterase could be detected. We may guess then that since only very strong inhibition may be observed with the limited sensitivity of the histochemical method for demonstrating cholinesterase, the inhibition of the non-specific esterase although rather strong is a general one. The inhibition of the specific esterase is only 10%, but this may be because it is very greatly inhibited in the neo-cortex but only very slightly or not all inhibited by LSD in other areas of the brain. Furthermore, since non-specific cholinesterase is limited to the non-nervous elements in the brain, it is probably not active in synaptic transmissions; but it may be concerned with the permeability of the capillaries rather than of the nerve cell membrane. By comparing the LSD and acetyl choline molecules one may suggest that LSD acts on the acetyl choline-cholinesterase reaction by competitive inhibition (Diagram 1).

It is already known that tertiary and quaternary amines are anti-cholinesterase agents (*Koelle and Isaac* [1959]) and it may be hypothesized that the LSD molecule forms an enzyme-substrate complex with the esterase thus inhibiting the hydrolysis of the choline ester. Although the inhibition was found, in this study, to be specific to the cerebral cortex, how such a specificity is accomplished has not been determined but is simply observed. That the action of LSD does take place in the cerebral cortex however is suggested by the types of symptoms characteristic of the LSD psychosis, since they are changes in cortical phenomena (i.e. thought processes). The autonomic changes with LSD however, may be due to effects on adrenergic or amine oxidase systems (*Rinkel* et al. [1955]). There arises also a difficulty in comparing human and rat brains. The frontal cortex, very large in man is rather insignificant in the rat (*Craigie* [1925]). Human cholinesterase is more inhibited by LSD than is rat cholinesterase (*Thompson* et al. [1955]). The specificity of LSD for the cerebral cortex cholinesterase may still however be the same in the human brain as in that of the rat. The strong inhibition of acetyl cholinesterase in layer V is particularly interesting since the cells in this layer are largely motor and LSD injected into the intact animal inhibits various motor feats. The sensory effects of LSD in the rat unfortunately cannot be or have not yet been determined.

Acetyl choline may effect nervous transmission by changing the permeability of the nerve cell membrane. Excesses of acetyl choline may be accumulated at synaptic junctions when cholinesterases do not act normally thus causing perhaps excessive nervous stimulation and transmission. If LSD inhibits cholinesterase in the cerebral cortex, the LSD psychosis may be interpreted, at least in part as an uncontrolled, diffuse stimulation of the cells of the cerebral cortex which may account for some of the mental effects of the drug.

Acknowledgements

This work was carried out with the aid of grant B–1914 from the National Institute for Neurological Diseases and Blindness, U.S.P.H.

The author would like to thank Dr. *Geoffrey H. Bourne* for his direction and encouragement and Dr. *Evelyn B. Beckett* for her help with technical details.

Summary

An attempt was made to determine the localization of inhibition of cholinesterases by lysergic acid diethylamide, a psycho-mimetic drug, in rat brain.

Gerebtzoff's modification of Koelle's method for histochemical demonstration of cholinesterases was used. Acetyl choline was used as a substrate to demonstrate specific cholinesterase; butyryl choline as a substrate for non-specific cholinesterase. Non-specific cholinesterase activity was found only in the capillary endothelium, some glial cells and smooth muscle of arterioles. The specific esterase was found to have different degrees of intensity depending on the region of the brain with which it was associated. Types of localization were found to be associated with different layers of the cerebral cortex; varying degrees of intensity of intercellular reaction are found in different layers as well. LSD inhibition of non-specific cholinesterase. was not observed in the preparations. Inhibition of specific cholinesterase was found to occur only in the cerebral cortex and was most marked in the lamina ganglionaris.

Résumé

L'auteur a essayé de préciser les points du cerveau du rat où la cholinestérase est inhibée sous l'influence d'un médicament psychomimétique, le diéthylamide de l'acide lysergique (LSD). Il a utilisé dans ses recherches la modification de la méthode de Koelle (proposée par Gerebtzoff) pour la démonstration histochimique de la cholinestérase. L'acétylcholine fut utilisée comme substrat pour la cholinestérase non-spécifique. Une activité de cet enzyme fut démontrée seulement au niveau des endothélia capillaires, dans certains éléments de la névroglie et dans les muscles lisses des artérioles. L'activité de l'estérase spécifique présente une intensité différente selon les régions du cerveau où elle se trouve. Les types de localisation présentent des relations avec les diverses couches de l'écorce cérébrale; des réactions intercellulaires d'intensité variable peuvent se voir également dans les différentes couches. Une inhibition de la cholinestérase non-spécifique par le LSD n'a pas été mise en évidence dans les préparations étudiées. Une inhibition de la cholinestérase spécifique fut observée seulement dans l'écorce cérébrale; son intensité était la plus forte au niveau de la lamina ganglionaris.

Zusammenfassung

Der Ort der Cholinesterasehemmung im Rattengehirn wurde mit Hilfe des Lysergsäurediaethylamids, einer psychomimetischen Droge, zu eruieren versucht. Zur histochemischen Darstellung der Cholinesterasen wurde die Koellesche Methode in der Modifikation nach Gerebtzoff verwendet. Zur Darstellung der spezifischen Cholinesterase wurde Acetylcholin als Substrat verwendet, Butyrylcholin für die nicht-spezifischen Cholinesterasen. Eine nicht-spezifische Cholinesteraseaktivität wurde nur im Endothel der Kapillaren, in einigen Gliazellen sowie in den glatten Muskelzellen der Arteriolen gefunden. Die spezifische Esterase wies verschiedene Intensitätsgrade je nach der entsprechenden Hirnregion auf. Verschiedene Lokalisationstypen sind an verschiedene Hirnrindenschichten gebunden. In verschiedenen Schichten finden sich ebenfalls verschiedene Intensitätsgrade einer interzellulären Reaktion. In den Präparaten konnte keine LSD-Hemmung der nicht-spezifischen Cholinesterasen beobachtet werden. Eine Hemmung der spezifischen Cholinesterase konnte nur in der Hirnrinde, am ausgeprägtesten in der Lamina ganglionaris, festgestellt werden.

REFERENCES

Cerletti, A.: Lysergic acid diethylamide (LSD) and related compounds in neuropharmacology; pp. 9–82, ed. Abramson, H.A. (Josiah H.Macy Jr. Foundation, 1956).

Craigie, E.H.: An introduction to the finer anatomy of the central nervous system based upon that of the albino rat. Univ. of Toronto, Toronto (1925).

Gerebtzoff, M.: Recherches histochimiques pour les acetylcholine et choline esterases. Acta anat. *19:* 366–379 (1953). – Cholinesterases. (Pergamon Press, New York 1959).

Koelle, G.B.: The histochemical localization of cholinesterases in the central nervous system of the rat. J. comp. Neurol. *100:* 211–235 (1954).

Koelle, G.B. and Friedenwald, J.S.: A histochemical method for localizing cholinesterase activity. Proc. Soc. exp. Biol., N.Y. *70:* 617–622 (1949).

Koelle, G.B. and McIsaac, R.: Comparison of the effects of inhibition of external, internal and total acetylcholinesterase upon ganglionic transmission. J. Pharm. exp. Ther. *126:* 9–20 (1959).

Koelle, G.B. and Steiner, E.C.: The cerebral distributions of a tertiary and a quaternary anticholinesterase agent following intravenous and intraventricular injection. J. Pharm. exp. Ther. *118:* 420–434 (1956).

Mayer-Gross, W.; McAdam, W. and Walker, J.W.: Further observations on the effects of lysergic acid diethylamide. J. ment. Sci. *99:* 804–808 (1953).

Salvatore, S. and Hyde, R.W.: Progression of effects of lysergic acid diethylamide (LSD). Arch. Neurol. Psychiat., Chicago *76:* 50–59 (1956).

Poloni, A. and Maffezzoni, G.: Le variazioni dell'Attività Colinergica del tessuto cerebrale per effetto della bulbocapnina, della mescalina e della dietilamide dell'acido lisergico. Sist. Nerv. Milano *4:* 578–581 (1952).

Thompson, R.H.S.; Tickner, A. and Webster, G.R.: The action of lysergic acid diethylamide on mammalian cholinesterases. Brit. J. Pharmacol. *10:* 61–65 (1955).

Received May 18th, 1961

Author's address: Dr. M. Goldberger, Dept. of Anatomy, Emory University, *Atlanta 22.* Ga. (USA).