

ACETYLCHOLINESTERASE ACTIVITY IN THE NUCLEI OF THE AMYGDALOID COMPLEX IN THE RAT

Liliana NITECKA, Olgierd NARKIEWICZ and Helena ZAWISTOWSKA

Department of Anatomy, School of Medicine, Gdańsk, Poland

The amygdaloid complex is composed of nuclei of various cellular structure, different connections and differentiated functions. Delimitation of separate amygdaloid nuclei on the basis of cytoarchitectonic or myeloarchitectonic principles exclusively is very often difficult. Therefore it seems, that in addition to purely morphological methods some histochemical techniques have to be elaborated. The methods showing acetylcholinesterase (AChE) activity are especially suitable in this respect (Olivier et al. 1970).

It is assumed (*see* Koelle 1969) that acetylcholine is a transmitter not only in the peripheral but also in the central nervous system. Therefore, it seems probable, that high AChE activity in some portions of the central nervous system is related to specific functions. Some authors (Shute and Lewis 1963, 1967, Krnjević and Silver 1965, 1966, Lewis and Shute 1967, Krnjević 1969) have even suggested that in the telencephalon there are separate systems composed of ascending cholinergic neurons and tracts.

The results of previous investigations concerning AChE activity in amygdala of the rat as well as of other animals are contradictory (Giacomo 1962, according to Friede 1966, Krnjević and Silver 1965, Friede 1966, Ishii and Friede 1967, Girgis 1969), very often due to misleading nomenclature and lack of comparative, histochemical studies of this portion of the brain. So far there is no definite and from the phylogenetic point of view justified subdivision of the amygdaloid complex in the rat. Brodal's (1947) subdivision agrees with our observations only partly. In spite of this we applied it to our work though with far-going modifications taking into account the fact that this nomenclature is in general use.

MATERIAL AND METHODS

Material consists of six rats Wistar strain. Gerebtzoff's modification of Koelle's acetylthiocholine method was used (Gerebtzoff 1959). To obtain a rapid fixation of the tissues, the animals were perfused intravitaly with cooled Baker's fluid. After the brain was removed slices of tissues 3 mm thick were fixed in Baker's fluid for 4 hr in 4°C temperature. 12 μ m thick frozen sections were cut and removed into the incubation medium and then kept there for 2 hr in 30°C temperature.

The incubation medium was composed of:

- a) 0.4 ml of the substrate (15 mg acetylthiocholine iodide, 0.78 ml distilled water, 0.26 ml 0.1 M (CuSO_4),
- b) 2.5 ml acetate buffer (pH 6.8),
- c) 1.9 ml distilled water,
- d) 0.1 ml glycine solution (3.75 ml glycine + 100 ml distilled water),
- e) 0.1 ml CuSO_4 .

Some sections were kept in the incubation medium containing butyrylthiocholine iodide instead of acetylthiocholine iodide. The sections were washed with distilled water and transferred into an aqueous solution of ammonium sulphide. After being washed the sections were mounted on slides with Canadian balsam.

Sections stained with cresyl violet were used for the comparison of AChE activity of the amygdaloid complex with its cellular structure.

RESULTS

The amygdaloid complex has been generally subdivided into two main parts — basilateral and corticomедial. The basilateral part contains two basal nuclei — nucleus basalis dorsalis and nucleus basalis ventralis, as well as the lateral nucleus. All other nuclei: central, medial, cortical, area amygdaloidea anterior and nucleus of the lateral olfactory tract are considered to be parts of the corticomедial group.

The butyrylcholinesterase activity in the amygdaloid complex is rather weak and its localization will be not discussed here in details. Contrary to that the AChE activity is in some nuclei of the amygdala very high. The most evident AChE reaction which is comparable to that of striatum was found in the basal dorsal nucleus.

Nucleus basalis dorsalis is a distinct group of large neurons. It is limited on the dorsolateral side by the lateral and from the below by the basal ventral nucleus. The AChE reaction is positive in the whole area occupied by the basal dorsal nucleus. The nerve cells of this area

are partly visible as a limbus of dark cytoplasm surrounding white spots corresponding to the nuclei of the neurons. High AChE activity of the cytoplasm suggest that those neurons may be cholinergic.

The above mentioned histochemical data make possible a more precise delimitation of the basal dorsal nucleus. Comparing our histochemical and cytoarchitectonic results with Brodal's description (Brodal 1947) we found striking differences. The basal dorsal nucleus of our subdivision comprises Brodal's rostral portion of nucleus lateralis posterior and the caudal one of the nucleus basalis lateralis. This area is in some respect comparable to nucleus principalis intermedius of Koikegami (1963).

Nucleus basalis ventralis is a less distinct group of more sparsely arranged neurons situated below the basal dorsal nucleus. This area was not distinguished by Brodal as a separate unit and it seems impossible to identify it as any nucleus mentioned by him. It corresponds probably to nucleus principalis medialis of Koikegami (1963) and to nucleus basalis ventralis described by Miodoński (1965) in the dog.

The AChE activity of the basal ventral nucleus is moderately high. Rostrally (Fig. 1B) AChE activity of the basal ventral nucleus is clearly higher than in the area amygdaloidea anterior and in other neighbouring nuclei of the corticomедial group. More caudally (Fig. 1C) the difference in AChE activity between the basal ventral and cortical nucleus becomes less apparent.

The *nucleus lateralis* occupies a large area limited laterally by the external capsule. AChE activity is here moderately high, comparable to that found in the basal ventral nucleus. In the rostral portion of the lateral nucleus AChE activity is somewhat higher, especially in the dorsolateral area, on the borderline with the external capsule (Fig. 1AB). More caudally (Fig. 1C) this area of higher AChE activity is larger and occupies a significant portion of the lateral nucleus. But caudally the intensity of enzyme reaction becomes weaker and the difference between both portions of the lateral nucleus is less clear but it still exists.

The distribution of AChE activity in the lateral nucleus is diffuse as in other nuclei of the basilar complex; but there is no positive AChE reaction in the neuron cell bodies.

The *nucleus centralis* of the rat is composed of two parts — a medial and a lateral one. In the lateral part a clearly negative AChE reaction was found to occur. This part of the central nucleus is visible on cross-sections as a white, oval structure. Contrary to that the medial part of the nucleus shows an AChE activity of moderate intensity. The boundary between both parts of the central nucleus based on AChE activity is in agreement with Brodal's (1947) cytoarchitectonic subdivision.

The *nucleus medialis* is situated laterally from the optic tract. It

shows a negative or low AChE activity similar to that of the lateral part of the central nucleus.

The *nucleus corticalis* is a poorly delimited structure. It was found that its AChE activity is different in the rostral and caudal portion. The rostral portion shows a weak AChE activity. More caudally AChE activity becomes higher and even resembles that in the basal ventral nucleus.

Area amygdaloidea anterior. By this term the authors designated rostrally situated portions of the corticomедial nuclei in which it was difficult to differentiate the various groups of neurons. AChE activity is generally very low in almost all the described area.

Nucleus tractus olfactorii lateralis is a well separated group of nerve cells situated in the rostral portion of the amygdala (Fig. 1A). AChE activity is almost as high here as in the basal dorsal nucleus and is also diffuse. In this area many neurons with cytoplasm showing positive AChE reaction were found to occur. The dorsal part of the nucleus of the lateral olfactory tract which has the shape of a crescent exhibits a lower AChE activity than the larger ventral part.

The most medially situated portion of the prepiriform cortex lying close to the area amygdaloidea anterior and to the nucleus of the lateral olfactory tract also shows a strikingly high AChE activity, much higher than all the rest of the paleocortex (Fig. 1A).

DISCUSSION

In the amygdala of the rat, according to Koelle (1954, 1963), the highest AChE activity has been found in: nucleus centralis, nucleus lateralis and nucleus tractus olfactorii lateralis. Our investigations confirm these results only partly in respect to the nucleus of the lateral olfactory tract, in which also a very strong AChE reaction was found to occur. The reason for different results in respect to the central and lateral nucleus is not clear due to lack of photograms in Koelle's papers mentioned above.

The investigations carried out on human brains (Giacomo 1962, according to Friede 1966) have shown that the highest AChE activity is localized in the basal dorsal nucleus. Ishii and Friede (1967) found a high AChE activity in all basal nuclei of the amygdala. In other nuclei the AChE reaction becomes weaker in the following sequence: nucleus lateralis, nucleus corticalis and nucleus medialis.

Krnjević and Silver (1965) in a study on AChE localization in the telencephalon of the cat found a very high AChE activity in the central and basal dorsal nucleus of the amygdala. Girgis (1969) studying brains of *Galago senegalensis* found the highest AChE activity in the basal

nucleus as well as in the nucleus of the lateral olfactory tract. The data shown above pertaining to the localization of AChE activity in the amygdala of the cat, *Galago senegalensis*, and man are generally similar to our results found in the amygdaloid complex of the rat. These results seem to confirm the data from the literature (Gerebtzoff 1959, Koelle 1963, Silver 1967) that except for the cerebellum there is generally a great similarity of AChE activity in homologous structures of the brain in various species.

As butyrylcholinesterase activity in the amygdaloid complex is rather weak it seems highly probable that the positive reaction obtained here by means of the acetylthiocholine iodide method is virtually due to presence of AChE. This results are in agreement with Shute and Lewis (1963) who found that neurons of the amygdaloid complex contain AChE without non-specific cholinesterase activity being present.

It should be stressed that the quantitative, biochemical data concerning AChE activity in various portions of the central nervous system are generally in agreement with histochemical results. Therefore in spite of all imperfections of the histochemical methods of AChE activity localization, they are of value especially in the study of such a differentiated structure like the amygdaloid complex. In this case they made possible a more precise understanding of the structure and function of the amygdala and provided the opportunity of determining better the homology of amygdaloid nuclei in various species.

Though the role of the AChE in the central nervous system is not clear it is probable that high AChE activity appearing only in some nuclei and pathways is connected with specific functions of those structures.

The strongly positive AChE reaction in the basal dorsal nucleus of amygdala and in the nucleus of the lateral olfactory tract suggest that these nuclei may belong to the so-called cholinergic brain system (Shute and Lewis 1967, Krnjević 1969).

SUMMARY

1. AChE activity in the different nuclei of the rat's amygdala varies widely.

2. The highest AChE activity has been found to occur in the basal dorsal nucleus as well as in the nucleus of the lateral olfactory tract, a much lower one — in the lateral, basal ventral nucleus and in the medial part of the central nucleus. Lack of AChE activity or very little activity occurs in the medial nucleus, area amygdaloidea anterior and in the lateral part of the central nucleus.

3. These results suggest that the basal dorsal nucleus and nucleus of the lateral olfactory tract may belong to the so-called cholinergic brain system and play a specific role in the amygdaloid complex.

This investigation was partially supported by Foreign Research Agreement No. 05-275-2 of U.S. Department of the Health Education and Welfare under PL 480.

REFERENCES

- BRODAL, A. 1947. The amygdaloid nucleus in the rat. *J. Comp. Neurol.* 87: 1-16.
- FRIEDE, R. L. 1966. Topographic brain chemistry. Acad. Press, New York 543 p.
- GEREBTZOFF, M. A. 1959. Cholinesterases. A histochemical contribution to the solution of some functional problems. Pergamon Press, London. 3 vol.
- GIACOMO, 1962. *Quoted*, Friede 1966.
- GIRGIS, M. 1969. Distribution of cholinesterase in the basal rhinencephalic structures of the Senegal bush baby (*Galago senegalensis senegalensis*). *Acta Anat.* 72: 94-100.
- ISHII, T. and FRIEDE, R. L. 1967. A comparative histochemical mapping of the distribution of acetylcholinesterase and nicotinamide adenine dinucleotide diaphorase activities in the human brain. *Int. Rev. Neurobiol.* 10: 231-275.
- KOELLE, G. B. 1954. The histochemical localization of cholinesterases in the central nervous system of the rat. *J. Comp. Neurol.* 100: 211-228.
- KOELLE, G. B. 1963. Cytological distribution and physiological function of cholinesterases. *Handb. Exp. Pharmacol.* 50: 197-298.
- KOELLE, G. B. 1969. Significance of acetylcholinesterase in central synaptic transmission. *Fed. Proc.* 28: 95-100.
- KOIKEGAMI, H. 1963. Amygdala and other related limbic structures; experimental studies on the anatomy and function. *Acta Med. Biol.* 10: 161-277.
- KRNJEVIĆ, K. 1969. Central cholinergic pathways. *Fed. Proc.* 28: 113-120.
- KRNJEVIĆ, K. and SILVER, A. 1965. A histochemical study of cholinergic fibres in the cerebral cortex. *J. Anat.* 99: 711-759.
- KRNJEVIĆ, K. and SILVER, A. 1966. Acetylcholinesterase in the developing fore-brain. *J. Anat.* 100: 63-89.
- LEWIS, R. R. and SHUTE, C. C. D. 1967. The cholinergic limbic system: projection to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system, the subfornical organ and supraoptic crest. *Brain* 90: 521-540.
- MIODONSKI, R. 1965. Myeloarchitectonics of the amygdaloid complex of the dog. *Acta Biol. Exp.* 25: 263-287.
- OLIVIER, A., PARENT, A. and POIRIER, L. J. 1970. Identification of the thalamic nuclei on the basis of their cholinesterase content in the monkey. *J. Anat.* 106: 37-50.
- SHUTE, C. C. D. and LEWIS, P. R. 1963. Cholinesterase-containing systems of the brain of the rat. *Nature* 199: 1160-1164.
- SHUTE, C. C. D. and LEWIS, P. R. 1967. The ascending cholinergic reticular system neocortical, olfactory and subcortical projections. *Brain* 90: 497-520.
- SILVER, A. 1967. Cholinesterases of the central nervous system with special reference to the cerebellum. *Int. Rev. Neurobiol.* 10: 57-109.

Received 12 December 1970

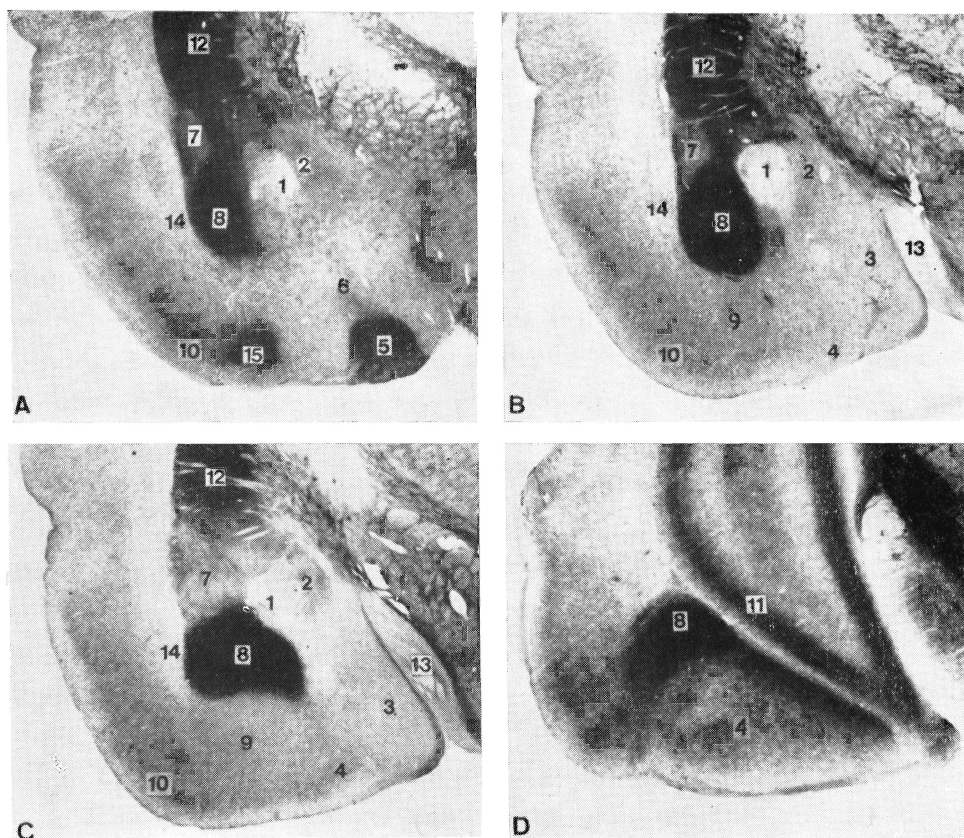


Fig. 1. Acetylcholinesterase activity in amygdaloid complex of the rat. A-D, microphotographs of frontal sections; the various levels are set out in rostrocaudal order. Thiocholine techniques. $\times 20$. 1, nucleus centralis pars lateralis; 2, nucleus centralis pars medialis; 3, nucleus medialis; 4, nucleus corticalis; 5, nucleus tractus olfactorii lateralis; 6, area amygdaloidea anterior; 7, nucleus lateralis; 8, nucleus basalis dorsalis; 9, nucleus basalis ventralis; 10, cortex prepiriformis; 11, hippocampus; 12, striatum; 13, tractus opticus; 14, capsula externa; 15, portion of the prepiriform cortex with high AChE activity.