RELATIONSHIP BETWEEN CHANGES IN THE CONTENT OF ACETYLCHOLINE AND THE ACTIVITIES OF ACETYLCHOLINESTERASE AND CHOLINE ACETYLTRANSFERASE IN THE HIPPOCAMPUS OF THE RAT AFTER SEPTAL LESIONS

Barbara ODERFELD-NOWAK and Anna POTEMPSKA

Department of Biochemistry of Nervous System and Muscle
Nencki Institute of Experimental Biology, Warsaw, Poland

Abstract. Changes in acetylcholine (ACh) content and acetylcholineesterase (AChE) and choline acetyltransferase (ChAc) activity in the hippocampus were compared after various septal lesions. With the use of a multiple regression model it was shown that the time course of the ACh and enzymic level changes is concordant after total destruction of the septum, but not always after partial damage. Septal lesions produced an effect mainly on bound ACh. The results suggest that the activity of AChE and ChAc is not the rate-limiting factor of the ACh level in the hippocampus. The involvement of other hippocampal neurotransmitters acting through the high-affinity uptake of choline is supposed to play an important role in ACh content regulation.

INTRODUCTION

The existence of a septo-hippocampal cholinergic pathway has been demonstrated in a great variety of experiments (4, 6, 14, 15, 30). A dramatic decline in AChE and ChAc activities in the hippocampus following septal lesions, which has been reported (13, 18), is accompanied by a decrease in the level of ACh (13, 17). However, the data concerning the relations between the enzymic and ACh level changes are controversial. Sethy et al. (27) reported that ACh changes follow closely
those of the enzymes and depend only on the degeneration of septo-
hippocampal cholinergic endings in the hippocampus. Our data (17)
and those of Pepeu et al. (20) suggest, however, that the changes in ACh
level depend on some other factors besides the disruption of cholinergic
connections between the septum and hippocampus.

In view of that apparent discrepancy and because of the important
functional aspects of the problem, further investigations of the relation
between enzymic and ACh level changes in the hippocampus after septal
damage have been undertaken. Our unpublished data, in which a cor-
relation between ACh and both enzymes changes was studied on a “left–right”
model described earlier (17) indicated that the relations
between these two variables depend highly both on the type of the
lesion and on the time after the surgery. In the present study the time
course of the changes in the hippocampal ACh after various types of
septal lesions were compared with the recently described (19) cor-
responding changes in the enzymic activities. The model of multiple
regression, used for analysis of the time course in enzymic activities,
was here adopted to analyse ACh content changes. The advantages of
using this type of regression have been discussed in detail in the pre-
vious paper (19).

For a better understanding of the mechanisms of regulation of the
ACh level in the hippocampus after septal lesions — the response to
lesion of three different ACh pools: free, labile-bound and stable-bound,
deriving, according to Whittaker (33), respectively from pericaria and/or
axons, synaptoplasm and synaptic vesicles, was also studied.

**METHODS**

*Animals and surgery.* Experiments were performed on male Wistar
rats weighing about 250 g. Animals were anesthetized with sodium
pentobarbital (50 mg/kg i.m.) and various types of bilateral, electro-
coagulation lesions in the septum were made as described previously
(18). As controls, unoperated anesthetized rats were used. A sham opera-
tion was not made because it had been shown before that it does not
affect the ACh amount in the hippocampus (17). After the operation
each animal was placed in an individual cage.

Rats were decapitated at various time intervals after the operation
(2 days to 1 year). All animals were killed approximately at the same
time of day (9:00–10:00 a.m.), in order to avoid the well known cir-
cadian variations of neurotransmitter level. The brains were quickly
removed from the skull and a dissection of hippocampi was made on ice. The hippocampi were weighed and stored in 0 °C for some minutes. The frontal part of the brain was taken out to perform a histological verification of the extent of the lesion in the septum after staining the slices with the use of Klüver–Barrera method.

Analytical methods. ACh was extracted totally with a mixture of acetone–1 N formic acid (85:15) according to Toru and Aprison (32). ACh of different pools was extracted from both the hippocampi according to Beani et al. (2) as modified by Schwarzenfeld et al. (26). Free ACh was extracted with 0.30 M glucose in water containing $5 \times 10^{-5}$ M eserine, labile-bound — with water containing $5 \times 10^{-5}$ M eserine and stable-bound — with 20% trichloracetic acid. ACh amount in the samples was assayed on the dorsal muscle of the leech.

In some experiments the activity of AChE was also determined. The Ellman method (7) with slight modifications, as described previously (18), was used for the purpose.

RESULTS

Time course of changes in hippocampal ACh level after various types of septal lesions. The multiple regression model of a general formula: $A = a + \beta t^{-1} + \gamma t^{-2}$, where $A$ denotes ACh content in percent of control values $a$, $\beta$ and $\gamma$, regression coefficients, and $t$, time after the lesion, was used to describe the changes in the level of hippocampal ACh on time after various septal lesions. Estimation of the regression coefficients was standard (23). The time course of ACh changes was evaluated within each of the four types of septal lesions (shown on diagrams in Fig. 1): type 1, total, which involved all the nuclei of the septum; type 2, destroying the vertical limb of the diagonal band, nucleus fimbriatus and partially nucleus lateralis; type 3, small medio-ventral — involving part of the vertical limb of the diagonal band and nucleus fimbriatus, and type 4, lateral — damaging only the nucleus lateralis.

Figure 1 shows the regression lines of ACh level changes on time corresponding to the respective equations. As it is seen, after each type of septal lesion, ACh content in the hippocampus is diminished and the changes are observed as early as 2 days after the operation. After total lesions a profound decrease in ACh amount was observed in the first postoperative week. ACh level dropped to about 25% of control value and remained unchanged during the whole investigated period (up to
Fig. 1. Time course of changes in hippocampal ACh level after four types of septal lesions: total (I), extensive medioventral (2) small medioventral (3), and lateral (4). The extent of the lesions is shown in black on the cross-sections (above the respective regression curves). Abbreviations used: CA, anterior commissure; CC, corpus callosum; D, nucleus dorsalis; L, nucleus lateralis; NA, nucleus accumbens; NB, nucleus tractus diagonalis of Broca; NF, nucleus fimbriatus; S, striatum; ST, stria terminalis and its nucleus. The regression lines of ACh amount on time
are given by the respective equations of general formula: \( A = a + \beta t^{-1} + \gamma t^{-2} \), where \( A \) denotes ACh content in per cent of control values; \( a, \beta \) and \( \gamma \) regression coefficients, and \( t \), time after the lesion (days). Each point represents mean experimental values of two to four determinations. In a few cases single values are also shown. Broken parts of the regression lines indicate the intervals obtained by extrapolation. \( n \), number of investigated animals. Control level of ACh content — 24.0 ± 1.6 nmol/g wt.
In contrast, after all types of partial lesions the decrease in ACh content was much smaller and the character of the changes on time was distinctly different from the one seen after the total lesions.

To see whether the changes in ACh content after all types of partial lesions (2, 3 and 4) differ among themselves, the corresponding regression lines were compared.

The appropriate Fisher–Snedecor tests were used and the two null hypotheses were tested: the first,

$$H_0: \left(\frac{\beta_2}{\gamma_2}\right) = \left(\frac{\beta_3}{\gamma_3}\right) = \left(\frac{\beta_4}{\gamma_4}\right),$$

that the corresponding “vectors” of regression coefficients are equal, therefore the courses of changes are concordant, and the second,

$$H_0^{\prime \prime}: a_2 = a_3 = a_4,$$

that the constant values are equal, meaning that the average levels of ACh in each case are of the same order. The results of $H_0$ testing show that the time courses of the ACh level do not differ significantly ($F = 0.30; F_{crit}(0.05,4,57) = 2.54$) and the verification of $H_0^{\prime \prime}$ shows, that they no not differ in the mean value of the level ($F = 2.61; F_{crit}(0.05,2,61) = 3.15$).

A comparison between the time courses of changes in hippocampal ACh level and AChE and ChAc activities after various types of septal lesions. The relationship between the changes in hippocampal ACh level and enzymic activities at time was evaluated within each of the above described types of septal lesions. The corresponding regression lines were compared, testing the null hypotheses

$$H_0: \left(\frac{\beta_A}{\gamma_A}\right) = \left(\frac{\beta_B}{\gamma_B}\right) = \left(\frac{\beta_C}{\gamma_C}\right)$$

and

$$H_0^{\prime \prime}: a_A = a_B = a_C,$$

where $A$, $B$ and $C$ denote ACh, AChE and ChAc respectively. All regression coefficients for AChE and ChAc activities after types 1, 2 and 3 were estimated previously (19). In the type 4 the regression coefficients for AChE activity ($n = 19$) were estimated at the time of the experiment ($a = 60.1; \beta = 92.6; \gamma = -147$).

The results of the testing are shown in Table I. For type 1, i.e., total lesions — the time course for all three compounds can be regarded as concordant, differing only in the mean level. For type 2 — the time course for particular compounds differs significantly, and for types 3 and 4 there are no grounds to reject both null hypotheses, i.e., it can be accepted that the time courses of ACh and its enzymes are the same.

Effect of septal lesions on different ACh pools in the hippocampus.

The effect of total (type 1) and partial damage (mainly type 3) of the
TABLE I

Testing hypotheses on the time courses of changes of ACh level and enzymes activity within four types of lesions. \( F \), computed values; \( F_{\text{crit}} \), critical values at 5% level of confidence; in parentheses, degrees of freedom of the Fisher's ratio; \( a \), \( \beta \), \( \gamma \), coefficients of the multiple regression equations; \( A \) (or \( B \), or \( C \)) = \( a + \beta t^{-1} + \gamma t^{-2} \), where \( A \) denotes ACh content; \( B \), AChE activity, and \( C \), ChAc activity in % of control values; \( t \), time after operation (days)

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>Hypotheses</th>
<th>( F )</th>
<th>( F_{\text{crit}} )</th>
<th>( F )</th>
<th>( F_{\text{crit}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \frac{\beta_A}{\gamma_A} ) = ( \frac{\beta_B}{\gamma_B} ) = ( \frac{\beta_C}{\gamma_C} )</td>
<td>0.43**</td>
<td>2.48 (4; 79)</td>
<td>5.84*</td>
<td>3.11 (2; 83)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>9.04**</td>
<td>2.47 (4; 88)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.58**</td>
<td>2.57 (4; 45)</td>
<td>2.25**</td>
<td>3.19 (2; 49)</td>
</tr>
<tr>
<td>4</td>
<td>( H'_{0}: \frac{\beta_A}{\gamma_A} = \frac{\beta_B}{\gamma_B} )</td>
<td>0.36**</td>
<td>3.25 (2; 37)</td>
<td>0.29**</td>
<td>4.09 (1; 39)</td>
</tr>
</tbody>
</table>

* Significant; ** highly significant: significant also at 1% level of confidence; — not significant.

September upon the distribution of ACh among its three pools: free, labile-bound and stable-bound was studied on the 2nd and 7-12th postoperative day. These time points have been chosen after considering the character of the time courses of ACh changes (see Fig. 1). Two days after total destruction of the septum (Fig. 2A) the amount of hippocampal ACh decreased to about 60-70% of control values in all pools. Subsequently, the ACh content of the pools diminished and 7-12 days postoperatively reached 27, 14 and 18% of control values in free, labile-bound and stable-bound pools, respectively. Partial damage of the septum (Fig. 2B) did not evoke such a fall in hippocampal ACh content lowering the amount of the transmitter in all three pools to about 75% of control values 2 days after the lesion. One week to 12 days after the operation the ACh level of the free pool still amounted to 75%, labile-bound ACh fell to 59% and stable-bound to 43% of respective control values. The effect of time was therefore seen particularly in the group of the total lesions.

In both groups of the lesions — total and partial — ACh in all three pools was diminished, but the bound fractions were most seriously affected. This is especially seen in the case of partial lesions (Fig. 2B) for the stable-bound ACh. As a consequence, the proportions between the various pools of ACh in hippocampi of the lesioned animals changed as compared with the normal hippocampi.
Fig. 2. Changes in ACh pools in the hippocampus after septal lesions. A, ACh after total septal lesion; B, ACh after partial septal lesions — mainly type 3. Columns indicate mean ACh content in the three pools: diagonally lined fields, free; empty, labile bound; and checked, stable bound; n, number of investigated animals; bars, SEM. Control levels of the respective ACh pools were $4.8 \pm 0.2$, $12.7 \pm 0.4$ and $6.5 \pm 0.3$ nmol/g wt.

DISCUSSION

Our results support previous suggestions (17) that the regulation of the amount of ACh in the hippocampus after septal lesions is a complex process, which, contrary to the suggestions of Kuhar et al. (13), does not depend only on the degeneration of septal cholinergic nerve endings.

Although ACh and enzymic declines are significantly correlated when the effect of septal lesions was analysed in toto (unpublished data), which indicates a connection between these two phenomena, the present studies on the time courses of hippocampal ACh and its enzymes performed on
the multiple regression model show that the relations between their changes are highly dependent on the type of the septal damage (Table I). After small lesions, both lateral and medioventral, as well as after total destruction of the septum the time courses of the transmitter and its enzymes were concordant, differing for the total lesions only in the mean value. The discrepancy in the time course of ACh and that of the enzymes was drastically seen after extensive medioventral lesions. In that case the initial almost complete decrease of enzymic activity, as well as the subsequent recovery in enzymic activity due probably to the collateral sprouting phenomenon (19), were not followed by comparable changes in the transmitter level.

Only total lesions of the septum caused a very substantial decrease in the amount of ACh, all the partial lesions — independently of their size and location — evoked a small decrease: the ACh content in the hippocampus was then maintained practically at the same steady level of about 70% of the control value (Fig. 1) which might be important for some functional reasons. This fact is in line with the existence of other mechanisms, besides the action of the enzymes AChE and ChAc, which may account for the regulation of the ACh level in the hippocampus. Obviously, these mechanisms cannot operate in the hippocampus when the whole septal input is cut off; they are also probably not so much engaged when, as in the case of very small lesions, the ACh content is not seriously affected.

There are several indications that, in general, ChAc as well as AChE are not the rate-limiting steps for regulation of the metabolism of ACh (1, 11), although the involvement of ChAc in the regulation of ACh synthesis has been suggested (8, 10). The lack of parallel between the changes in ACh content and enzyme activities in the brain in different experimental conditions has been described in other studies (2, 12, 29). Another possibility of regulation of ACh level is the availability of acetylcoenzyme A (AcCoA) and choline; while there are no data on AcCoA changes in the hippocampus in the course of septal lesions, it is not excluded that they may be of significance for ACh amount (24). It is not known if the choline content has an important role in the regulation of ACh level in the hippocampus. But it has been shown that after septal lesions, where cholinergic nerve terminals have degenerated, the choline amount in the hippocampus was reduced only slightly or not at all (27).

Various recent findings (9, 29, 34) suggest that the most important rate-limiting factor of the regulation of biosynthesis and of the release of ACh is the high-affinity uptake of choline which seems to be coupled with the neuronal impulse flow (28). It is plausible that the high-affinity choline uptake remains the rate-limiting factor also in the case of hip-
Pocampal ACh. As a consequence of septal lesions it is reduced to about 40% of the control value (13), which corresponds rather to ACh changes than to its enzymic changes. It was recently shown that the high-affinity choline uptake is stimulated by other neurotransmitters, like norepinephrine (5). Various interactions between ACh and other neurotransmitter systems are known (eg. 3, 21, 25). We tend to favor the hypothesis that the regulation of ACh content in the hippocampus might — through the high-affinity choline uptake — depend on the level of other neurotransmitters in this structure. The serotonin and a substantial part of norepinephrine innervation seem to reach the hippocampal formation largely from the fornix, fimbria and cingulum (16, 31). Therefore, the total septal lesions could influence profoundly the level of these neurotransmitters. In that case, the regulation of the ACh level would not be possible; when, however, their projections are not much affected, i.e., in the case of other septal lesions, a mobilization of the mechanisms regulating the ACh content in the hippocampus with the contribution of norepinephrine and/or serotonin seems possible. This assumption can account for the discrepancy between the results reported by Kuhar et al. (13), Sethy et al. (27) and the data provided by Oderfeld-Nowak et al. (17) and Pepeu et al. (20) as far as the absolute amount of hippocampal ACh decreased after septal lesions is concerned. Namely, that discrepancy might result from a difference in the degree of destruction of other neurotransmitter systems input to the hippocampus in the course of the respective septal lesions. The verification of the above hypothesis would require an experimental proof.

The free ACh pool, which represents probably mainly the intrinsic cholinergic hippocampal system (22), was — among all ACh fractions — the least changed in result of septal lesions (Fig. 2). This was especially seen after partial septal lesions (Fig. 2B) when a considerable proportion of the septo-hippocampal fibers was still preserved. When, as in the case of total lesions (Fig. 2A), almost the whole septal input was cut off, that pool was released from the hippocampal store to make up for the overall diminished quantity of ACh. On the other hand, the free ACh pool remained unchanged when bound ACh pools content rose markedly as a result of acute septal lesions (22).

Both the weak response of the free ACh pool to the septal lesions, and the postulated regulation of ACh content, which most probably would concern mainly the fraction of the bound ACh — as the high-affinity choline uptake is connected with cholinergic nerve endings (34) — might account for maintaining the total ACh content at a rather steady level after partial septal lesions.
We still must not forget that the septal lesions cause a widespread alteration of the ACh content in the whole brain (20) and it is possible that the level of ACh in the hippocampus after septal lesions may be regulated also by some other factors.

We thank Professor J. Oderfeld for his suggestion to use the multiple regression model and his continuous interest in this study, Professor S. Niemierko for her valuable remarks and help in the preparation of the manuscript and Professor O. Narkiewicz for allowing to perform part of the histological verifications in his Laboratory. The aid of W. Kupść, M. Math. in statistical calculations and Mrs. B. Heiler's skillful technical assistance are greatly appreciated. The investigation was supported by Project 10.4.1.01 of the Polish Academy of Sciences.

REFERENCES

6. DUDAR, J. D. 1975. The effect of septal nuclei stimulation on the release of acetylcholine from the rabbit hippocampus. Brain Res. 83: 123–133.


Accepted 20 January 1977

Barbara ODERFELD-NOWAK and Anna POTEMPSKA, Nencki Institute of Experimental Biology, Pasteura 3, 02-093 Warsaw, Poland.