DIFFERENTIAL RESPONSE OF HIPPOCAMPAL MUSCARINIC CHOLINERGIC RECEPTORS TO VARIOUS DEAFFERENTATIONS OF THE HIPPOCAMPUS IN THE RAT

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Abstract. The influence of bilateral electrolytic lesions of different parts of the septum on muscarinic receptor binding in the hippocampus was studied within 14-21 days after operation. The effect of total septal lesion upon receptor binding was also investigated separately in the dorsal and ventral hippocampus and in five consecutive hippocampal parts along the septotemporal axis of the structure. The data indicate that: (i) differential response of muscarinic receptors, as revealed by a decrease, increase or lack of changes in the \textsuperscript{3}H\textsubscript{QNB} binding depends on the site and extent of the lesion, (ii) lack of changes in muscarinic receptor binding can be spurious when the investigation is performed on the whole hippocampus, and masked by regional response differences, (iii) differential response of \textsuperscript{3}H\textsubscript{QNB} binding sites in distinct parts of the hippocampus to total septal lesion may depend on the preexisting differences in the density of cholinergic innervation and of muscarinic receptors.

A considerable evidence has already been accumulated indicating that damage to cholinergic and non-cholinergic hippocampal afferents results in serious alterations of the cholinergic system in the hippocampus (11, 13). Whereas changes in the activity of two cholinergic marker enzymes, choline acetyltransferase (ChAT) and acetylcholine-
sterase (AChE) are well established (6, 7, 15), information concerning the response of hippocampal muscarinic receptors to various deafferentations is still contradictory (3, 16, 20, 23, 24).

Paradoxically, most studies have failed to detect any changes in muscarinic receptor binding of the whole hippocampus following various lesions of the cholinergic input to the hippocampus (16, 24), although it has been well documented that muscarinic receptors are capable of regulation in response to the pharmacologically altered level of their activation (1).

The first aim of the present study was to reinvestigate the response of muscarinic receptors in the whole hippocampus, as measured by changes in [3H]QNB binding, to the total lesion of the septum — the major source of hippocampal cholinergic innervation, as compared with that caused by the lesions performed in its distinct parts. The second aim was to study the response of muscarinic receptors to the total lesion of the septum separately in the dorsal and in the ventral hippocampal parts. This investigation was undertaken in view of the biochemical data indicating an uneven distribution of muscarinic receptors throughout the hippocampus (9, 17).

All the investigations were made within 14-21 days after the operation — a sufficient time to develop a postlesion receptor adaptive response, e.g., evoked by denervation upregulation of postsynaptic binding sites (23). To assess the degree of degeneration of cholinergic fibers, AChE activity was estimated.

Male Wistar rats weighing 220-250 g were used. They were individually housed and received standard food and water ad. lib. The animals underwent surgery under sodium pentobarbital anesthesia (50 mg/kg, i.m.). Bilateral electrolytic lesions of the septum were performed as described previously (18). Briefly, an anodal current (2-3 mA) was passed for 20-30 s through a tungsten electrode 0.1-0.3 mm in diameter, insulated except for the tip (0.4 mm). Conditions varied according to the required size of the lesion. The rats were killed between the 14th and the 21st day after the operation. A histological verification of the lesion was performed for each operated rat. Frozen coronal sections of 25 μm were cut on a cryostat and stained with cresyl violet.

The rats were killed by decapitation. The hippocampal formation, when needed, was arbitrarily divided into a dorsal part (two-thirds of the whole structure from the septal end) and a ventral part (the remaining part of the structure) or sliced into five equal parts along the septotemporal hippocampal axis. Following dissection, the tissue was homogenized in 0.32 mol/l sucrose solution to produce 5% (w/v) homogenate. All estimations were made in triplicate.
Muscarinic receptor binding was determined by using \[^3\text{H}\]-quinuclidinyl benzilate (QNB) (S.A. 33.1 Ci/nmol, Amersham, UK) as described previously (20). AChE activity was estimated in the homogenate (1 mg of tissue/ml) by using a modification of the method of Ellman et al. (5), as described by Srebro et al. (18). Protein content was estimated according to the method of Markwell et al. (12). Statistical analysis was performed by means of Student's t-test.

Four groups of animals (see Fig. 1) were distinguished according to the septal area destroyed: three groups of animals with partial lesion of the septum (type A, B, C) and a group of animals with total lesion of the septum (type D).

The type A lesions (Fig. 1) were placed in the dorsal part of the septum. Although the AChE activity in the hippocampus was not substantially reduced (77% of control), indicating only a very moderate destruction of the cholinergic pathway, there was a 17% increase \((P < 0.05)\) in the \[^3\text{H}\]QNB binding. In contrast, the type B lesions (Fig. 1), destroying totally the dorsal nucleus and encroaching upon the nucleus fimbriatus while reducing AChE activity to the same extent (75% of control), did not produce any changes in the \[^3\text{H}\]QNB binding.

The type C lesions (Fig. 1) destroyed the dorsal nucleus and a substantial part of nucleus fimbriatus and the vertical limb of diagonal band. This type of lesion was followed by a 54% decrease in the AChE activity and a small, but statistically significant, fall of 10% in \[^3\text{H}\]QNB binding. In the case of total lesion of the septum (type D, Fig. 1), the damage involved nearly all the nuclei of the septum, causing a decrease by 77% in the AChE activity in the hippocampus. Nevertheless, no changes in the \[^3\text{H}\]QNB binding were found.

When analogous estimations of \[^3\text{H}\]QNB binding after a total lesion of the septum (type D) were performed on the hippocampus divided along its septotemporal axis into two or five consecutive parts, small but significant changes in the distribution of \[^3\text{H}\]QNB binding sites with respect to the controls were revealed (Fig. 2a and b).

Confirming our earlier results (17), it was found that the \[^3\text{H}\]QNB binding prevailed in the dorsal hippocampal part (Fig. 2A, left panel). Twenty one days after the total septal lesion this difference was much more pronounced (Fig. 2A, right panel). At the same postlesion time, AChE activity, which in unoperated rats prevailed in the ventral part (Fig. 3A, left panel), decreased dramatically in both hippocampal parts (Fig. 3A, right panel), indicating almost a total destruction of the cholinergic input to the hippocampus.

These results were confirmed in an experiment in which the post-lesion changes in the muscarinic receptor binding (Fig. 2B) and in the
Fig. 1. Effect of various lesions of the septum upon the $[^3]$H-QNB binding and AChE activity in the whole hippocampus 14-21 days after operation. The diagram represents frontal sections of the septum from the rostral (left) to the caudal (right) part. The extent of the lesion is shown as a dotted area. CA, commissura anterior; CC, corpus callosum; D, nucleus dorsalis; F, fornix; L, nucleus lateralis; NA, nucleus accumbens septi; NB, nucleus tractus diagonalis of Broca; NF, nucleus fimbriatus; S, striatum; ST, stria terminalis and its nucleus. Data (mean ± SEM) from 5-10 rats in each operated group are expressed as a percentage of the respective control values. The average values for hippocampi of unoperated control rats were: $[^3]$H-QNB binding — 0.698±0.022 pmol/mg protein, AChE activity — 4.21±0.193 μmol AcThCh/mg prot/h. *P < 0.05; **P < 0.01 compared with control.

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<th>Type</th>
<th>Example of lesion</th>
<th>% of control</th>
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<td>$[^3]$H-QNB binding</td>
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<tr>
<td>A</td>
<td><img src="image1" alt="Image" /></td>
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<td>B</td>
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<td>C</td>
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AChE activity (Fig. 3B) were monitored, separately, in five consecutive parts of the hippocampus. In operated animals (Fig. 2B, right panel), the descending gradient of [³H]QNB binding sites was again more pronounced, since all the parts (I-III) corresponding approximately to the dorsal hippocampus exhibited an elevation of the muscarinic receptor binding in comparison with the unoperated controls (Fig. 2B, left panel).

The present study has contributed new information about the response of [³H]QNB binding sites in distinct parts of the hippocampus to its denervation. Indirectly, it also throws some light on the question of pre- and postsynaptic localization of muscarinic receptors in the

Fig. 2. Changes in the distribution of [³H]QNB binding sites A, in the dorsal and ventral hippocampal parts, B in five septotemporal (s - t) hippocampal parts 21 days after total septal lesion. The data (mean ± SEM) from 4-7 rats are expressed as a percentage of the value calculated for the whole hippocampi of unoperated control rats. The average value (i.e. 100%) for the whole control hippocampus was: 0.711 ± 0.024 (Fig. 2A) and (0.729 ± 0.018) pmol/mg protein (Fig. 2B). The width of bar corresponds to the length of the hippocampal part, measured along the septotemporal axis, taken for biochemical estimations. *P < 0.05; **P < 0.01 compared with value for the whole control hippocampus.

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Fig. 3. Changes in AChE activity A, in the dorsal and ventral hippocampal parts; B, in five septotemporal (s — t) hippocampal parts 21 days after total septal lesion. The data (mean ± SEM) from 4–7 rats are expressed as a percentage of the value calculated for the whole hippocampi of unoperated, control rats. The average value (i.e. 100%) for the whole control hippocampus was: 4.43 ± 0.151 nmoles AcThCh/mg protein/h (Fig. 3A) and 4.53 ± 0.122 nmoles AcThCh/mg protein/h (Fig. 3B). *P < 0.05; **P < 0.01 compared with value for the whole control hippocampus. Other explanations as in Fig. 2.

hippocampus (preliminary results of Scatchard analysis for operated rats indicate that changes in the [³H]QNB binding are confined to the B$_{max}$ without significant alterations in the K$_d$). However, some reservations in drawing definite conclusions about receptor localization have to be made, considering the possibility of the occurrence of transsynaptic degeneration (see 8) and/or spontaneous reinnervation phenomena at the investigated postlesion period (15).

A 10% drop in [³H]QNB binding found in the hippocampus 14–21 days after the partial septal type C lesion (Fig. 1) may reflect the presence of a small population of muscarinic receptors on the cholinergic
septohippocampal fibers, which were severely damaged, as revealed by the 50% decrease in AChE activity. This explanation seems to be likely in view of some pharmacological and biochemical data (3, 14, 19). On the other hand, the data obtained for the whole hippocampus after the total septal lesion (type D, Fig. 1) and some other authors' results which followed lesions of cholinergic input to the hippocampus, failing to reveal any significant changes in the muscarinic receptor binding in the hippocampus, seem to contradict the existence of presynaptic muscarinic receptors on the septohippocampal cholinergic fibers (16, 24). The apparent lack of changes in the [3H]QNB binding after nearly total cholinergic denervation of the hippocampus could be explained if we presume that the degeneration of presynaptic terminals subsequent to the lesion causes a compensatory increase of the postsynaptic muscarinic binding sites, which can obscure the primary decrease of the presynaptic sites. Such adaptive response of the postsynaptic receptors to alterations in the level of transmission is at present widely recognized. This assumption seems to be supported by the present findings, showing a significant increase of the [3H]QNB binding in the dorsal part of the hippocampus 21 days after the total lesion of the septum (Fig. 2). The increase in the [3H]QNB binding was found in those hippocampal parts which normally exhibit the highest density of muscarinic receptors (B\text{max}: dorsal — 0.812 ± 0.032, ventral — 0.684 ± 0.029 pmol/mg protein; K\text{d}: dorsal — 0.030 ± 0.03, ventral — 0.033 ± 0.002 nM, Ulas et al., in preparation) and the lowest AChE activity. This suggests the dependence of the local response upon the degree of impairment of cholinergic impulsion and the density of the muscarinic binding sites. The regional differences in response of hippocampal muscarinic receptors to various deafferentations of the hippocampus were recently reported also by other authors (20, 23).

The response of the muscarinic receptors in the hippocampus might also be the result of the damage and interactions of various neurotransmitter systems in the septum and/or hippocampus (see 4, 22). In line with the above suggestion seem to be the data obtained on the whole hippocampus, when lesions of different areas of the septum (compare lesions type A and B, Fig. 1), resulting 14-21 days later in a similar decrease of the hippocampal AChE activity, induced different changes in the [3H]QNB binding. The possibility of differential response of various subtypes of muscarinic receptors to septal lesions should also be taken into account (2).

Considering the response of the muscarinic receptors in the hippocampus to its denervation, one should not neglect the possibility that in such a response the muscarinic receptors located on non-neuronal
elements might be engaged, e.g., on becoming reactive and proliferating in response to brain injury astrocytes (10). This possibility is reinforced by our recent findings, indicating the presence of muscarinic receptors on the astrocytes of the rat hippocampus and suggesting a similarity of astroglial receptors to neuronal ones, as far as their binding properties are concerned (21).

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