ACETYLCHOLINE LEVELS INCREASE IN RAT HIPPOCAMPUS FOLLOWING ACUTE SEPTAL LESIONS: EVIDENCE FOR INTERACTIONS BETWEEN CHOLINERGIC AND NONCHOLINERGIC NEURONS

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Abstract—Acute septal lesions in rat brain resulted in elevation of the amount of particle-bound acetylcholine in the hippocampus irrespective of the extent of damage to the cholinergic septohippocampal projection. Changes in the high affinity choline uptake in the hippocampus were, however, proportional to the degree of destruction of this projection. The results are discussed in terms of possible interactions between the cholinergic and noncholinergic pathways in the system investigated.

EXPERIMENTAL PROCEDURES

Experiments were performed on three-month-old albino Wistar rats. Bilateral septal lesions were made by electrocoagulation under ether anaesthesia as described previously (Oderfeld-Nowak et al., 1974). Control animals were subjected to exactly the same degree and extent of ether anaesthesia, since ether affects both ACh content (A. Potwpska & B. Oderfeld-Nowak, unpublished) and high affinity choline uptake (Simon et al., 1976). In several cases, sham operations (craniotomy and electrode placement into the septum in the absence of current) were performed. Hippocampi were dissected 1 min to 20 h after the lesions. Anterior parts of brains were examined histologically to verify the extent of the lesion, using the method of Klüver & Barrera (1953).

Matched pairs of control and operated animals were examined in parallel. Acetylcholine in various pools (free, labile-bound and stable-bound) was determined by the method of Beani, Bianchi, Megazzini, Balotti & Bernardi (1969) as modified by Strasburger & Fischer-Kunkel, Kautzleben & Oelzner (1972). Synaptosomal (bound) ACh was measured according to the subcellular fractionation scheme of Wittsaker (1959) and the ACh content of the extracts was assayed on the dorsal muscle of the leech. High affinity choline uptake was measured in the synaptosomal fraction (P1) by the method of Roskoski (1978) or of Simon et al. (1976) as specified. Protein was determined according to Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Figure 1 (A,B) shows the 3 types of septal lesions studied, and the effect of these lesions on the ACh
content of the pools and on the high affinity choline uptake in hippocampus 30 min after surgery. The present confirmatory experiments indicate that the free ACh pool, which corresponds to neuronal perikarya and fibres and constitutes about 20% of the total ACh content (i.e. 5.12 ± 0.80 nmoles g⁻¹) is not affected by either the large medial, dorsomedial or lateral lesions (Fig. 1B). Changes in ACh are confined to the bound form including synaptosomal acetylcholine (labile-bound), which amounted to 12.80 ± 1.60 nmoles g⁻¹ and the vesicular pools (stable-bound) which amounted to 7.68 ± 0.65 nmoles g⁻¹ (50 and 30%, of the total ACh content respectively). We found the increase in bound ACh content to be independent of the degree of damage to the cholinergic projections to the hippocampus: the labile-bound content of ACh increased to 245-280% of control values and the stable-bound to 170-240%. In contrast, changes in high affinity choline uptake (control values amounted to 4.37 ± 0.39 pmoles Ch min⁻¹ mg prot.⁻¹) are proportional to the degree of destruction of septal cholinergic projections to the hippocampus. Whereas an extensive lesion of the medial area caused a decrease of 50%, the lateral lesion produced no change.

In a separate series of experiments, bound ACh and high affinity choline uptake were measured in the same synaptosome fractions from the same animals (operated and controls) at various times after small

TABLE 1. HIGH AFFINITY CHOLINE UPTAKE AND SYNAPTO-SOMAL ACh CONTENT AFTER SMALL LATERAL SEPTAL LESIONS

<table>
<thead>
<tr>
<th>Time after the lesion</th>
<th>Choline uptake (%)</th>
<th>Synaptosomal ACh content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>98</td>
<td>146</td>
</tr>
<tr>
<td>20 min</td>
<td>99</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>97</td>
<td>142</td>
</tr>
<tr>
<td>90 min</td>
<td>102</td>
<td>117</td>
</tr>
</tbody>
</table>

Choline uptake and acetylcholine content were measured in the same synaptosome-containing fractions (P₂) synaptosomes (bound) ACh according to Whittaker (1959), and high affinity choline uptake by the procedure of Simon et al. (1976). Results are expressed as percent of values obtained from control animals.
Acetylcholine pools and choline uptake after acute septal lesions

Fig. 2. Time course of change in hippocampal bound acetylcholine after large medial (●) or lateral (▲) septal lesions. Each point represents the mean from 2-6 experiments. Bound acetylcholine represents a sum of labile-bound and stable-bound pools.

The results indicate that the increase in bound ACh in the hippocampus after acute septal lesions is independent of the degree of destruction of the cholinergic projection (from almost total to none). On the other hand high affinity choline uptake is correlated with the extent of damage to the septohippocampal cholinergic projection. These observations led us to assume that the rise in bound ACh is due to more than one mechanism (see also ROMMELSPACHER & KUHAR, 1975a) and probably interactions between other neurotransmitters and the cholinergic system in the hippocampus are involved in the phenomenon. The effects of a change in one neurotransmitter on the release, level and turnover of another have been described in various experimental conditions (BUTCHER, BUTCHER & CHO, 1976; LADINSKY, CONSOLO, FORLONI & TIRELLI, 1980; ROBINSON, CHENEY & COSTA, 1980; ROBINSON, MALTHE-SORENSEN, WOOD & COMMISSIONG, 1979; ROMMELSPACHER & KUHAR, 1975b; ROTH & BUNNEY, 1976; SAMANIN, QUATTORNE, PERI, LADINSKY & CONSOLO, 1978).

The functional control of the rat septohippocampal cholinergic pathway by other neurotransmitter systems has been much discussed recently (LADINSKY et al., 1980; POTEMPSKA et al., 1977; ROBINSON et al., 1979; ROBINSON et al., 1980). Various neurotransmitter substances have been identified in the septal area. Noradrenaline, serotonin and dopamine-containing afferent fibres are present in the lateral septal nucleus (LINDVALL, 1975; MOORE, 1975). STORM-MATHISEN & GULDBERG (1974) have reported that transection of the dorsal hippocampal afferents, including fimbria and fornix, i.e. the septohippocampal pathways caused degeneration of noradrenaline and serotonin systems in the hippocampus. Acute lesions of serotoninergic afferents change serotonin metabolism in the hippocampus (HERR & ROTH, 1976). Furthermore, it has been found that the pyramidal cells of the hippocampal formation which project through fimbria and fornix superior to the doro-silateral part of the septum release glutamate (FONNUM, KARLSEN, MALTHE-SORENSEN, SKREDE & WALAAS, 1979). Therefore all lateral septal lesions would alter noradrenaline, serotonin and dopamine metabolism, and the doro-silateral lesions would, in addition, affect glutaminergic mechanisms. These neurotransmitters may modulate cholinergic mechanisms either directly at the level of septum or indirectly at the nerve endings in the hippocampus. Pharmacological data obtained by ROBINSON et al. (1979) with selective manipulation of the septal dopaminergic system suggest that dopamine exerts an inhibitory effect on acetylcholine metabolism of the septohippocampal pathway and that this effect is mediated via interneurons containing γ-aminobutyric acid (ROBINSON et al., 1980).

Based on the data mentioned above we conclude that the direct cessation of firing of cholinergic neurons is responsible for the rise in ACh content only in the case of large medioventral lesions (see POTEMPSKA et al., 1975; SETHY et al., 1973). In the case of lateral septal lesions, not encroaching upon cholinergic centers, the changed balance between various neurotransmitters could be responsible for the increase in hippocampal ACh level. In the case of small dorsomedial lesions, only slightly invading cholinergic centers, the rise may be due to a summation of the loss of afferent impulses and an interaction between cholinergic and noncholinergic neurons. The mechanisms leading to the increase in ACh content in the hippocampus, not coupled directly to the neuronal
impulse flow, were suggested by Rommelspacher & Kuhar (1975a).

Interesting, but not clear so far, is the fact that in each of the above situations the increase in the bound ACh is very similar, indicating that some mechanisms are operating which prevent the further increase, independently of the reason causing it. In each case, some compensating neuronal mechanisms within the hippocampus and/or septum cause the ACh levels to revert to their normal values after a longer time (Fig. 2).

Also of interest is the fact that an inverse relation between ACh content and the high affinity choline uptake changes has been found only in the case of the large medioventral lesions. Although in most described experimental conditions, the situation is similar (Jope, 1979; Roskoski, 1978; Simon et al., 1976) some recent findings indicate that there are some treatments which although affecting one of the two parameters do not evoke changes in the other one, and various mechanisms are offered for regulation of high affinity choline uptake (Jope, 1979). It has been reported that various neurotransmitters and/or their agonists and antagonists exert stimulating or depressing effects on high affinity choline uptake. In our experimental conditions the altered balance between various neurotransmitters may maintain unchanged high affinity choline uptake (lateral lesions) or only slightly changed (dorsomedial lesions). It has been recently reported (Jope, 1979; Marchbanks, 1980) that there is no direct coupling of choline uptake to the acetylation of choline, i.e. to ACh synthesis and that the high concentration of ACh competing with choline for binding to the carrier leads to the lowering of high affinity choline uptake instead (Marchbanks, 1980). It may be the case therefore, that in our experimental condition i.e. after the lesions not encroaching into cholinergic areas in the septum, the changed balance between various neurotransmitters not only maintain the high affinity choline uptake level, but affect other important steps involved in ACh synthesis leading to the increase in the amount of ACh.

Experiments are in progress to test which neurotransmitters may be involved and by which mechanisms they affect the cholinergic activity in the septo-hippocampal pathway.

Acknowledgements—We thank Professor E. M. Gál, the University of Iowa for the hospitality of his laboratory where part of this work has been done, and Professor J. A. Harvey and his collaborators, the University of Iowa, for their help with the surgery and histological verification. This work was supported in part by Fogarty Fellowship (NIH) PHS FO5 TW 2570-01 to BON.

REFERENCES


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(Accepted 5 May 1980)